Behavioural and ecological dynamics in competitive pollination networks

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Live as if you were to die tomorrow. Learn as if you were to live forever.

Mahatma Gandhi (disputed)

ABSTRACT

Flowers interact with several flower-visitors that remarkably differ, among others, in their morphologies, foraging strategies, and nutritional requirements, and thus in their efficiency to act as pollinators. In order to forage as effective as possible, flower-visitors rely on specific floral traits to detect and handle flowers in the heterogeneous environment they inhabit. Classical pollination syndromes comprise the identity and combination of specific floral traits, e.g. long corolla tubes or sweet scent. These flower traits are traditionally assumed to bear an adaptive value in relation to those flower-visitors that act as pollinators. However, the network between flowers and flower-visitors includes mutualistic as well as antagonistic relationships. Hence, floral traits may have not solely evolved to attract pollinators, but rather evolved to diminish antagonistic relationships with non-pollinating flower-visitors, and thus structure competitive pollination networks. Due to striking differences in colour vision systems, neural processing, and colour choice behaviours across flower-visitor taxa, floral colouration may have developed private communication channels between flowers and selective flower-visitors.

Focusing on bees and birds as flower-visitors and on bee- and bird-pollinated flowers, the present study reveals that floral colouration partially acts as such a floral filter structuring pollination networks. Choice experiments show that specific colour parameters selectively attract bees. Thereby bees prefer colours of high bee-subjective spectral purity, whereas flower-visiting birds do not show any preferences for colours or specific colour parameters. Consequently, the colour choice behaviour of flower-visitors affects the colouration of flowers pollinated by them. The comparison of flower colours reveals marked differences between those flowers pollinated by either bees or birds. We found that the floral colouration is well adapted to the visual capabilities of pollinators, but, at times, in addition negatively affects the visitation frequency of antagonistically operating visitors due to comparably less attractive colours. This is true for red and white flowers, which differ in their spectral reflectance depending on the pollinator guild, i.e. bees or birds. We show that the main differences in spectral reflectance arise in ultraviolet (UV)-reflectance properties. Here, the amount of UV-light determines bee-subjective spectral purity, making red and white bee-pollinated flowers more attractive for bees than red and white bird-pollinated ones, respectively. Red and white colours of bird-pollinated flowers display such a low spectral purity and low colour contrast to the background that the flowers are difficult to detect for the bees' eye. Hence, the red and white colouration of bird-pollinated flowers acts as a sensorial floral filter, almost exclusively attracting the beneficial visitors. The attractive function for birds has not arisen from colour-

preferences per se, but has rather result from learning in birds that associate these colours with higher amounts of nectar rewards, which are not depleted by bees. In contrast, for yellow flowers we show that the main colour does not differ between those pollinated exclusively by either bees or birds, and that yellow bird-pollinated flowers do not create a private niche for birds via a colour-based communication channel. However, we show that intra-floral colour patterns exclusively appear in yellow bee-, but not in yellow bird-pollinated flowers. Nonetheless, bees are known to prefer colour patterns as they guide them to the site of reward and thus, enhance their foraging efficiency due to decreased handling time. Hence, we conclude that yellow bee-pollinated flowers and their pollinators are well adapted to each other and that yellow bird-pollinated flowers hamper bees as antagonistically operating non-pollinating visitors from effective foraging.

Furthermore, the present study investigates the impact of pigment chemistry and cell morphology on floral colouration. We show that an increase of pigment concentration causes a parallel increase of the bees' subjective spectral purity. However, this is true only up to intermediate concentrations, with even higher concentrations the spectral purity decreases. In accordance, choice experiments show that bees prefer colours of intermediate rather than of low or high pigment concentration. In addition, the flowers' epidermal cell structure may affect floral colouration and structure the network of flowers, their mutual pollinators and antagonists. We show that bee-pollinated flowers on average possess more often conical epidermal cells, whereas bird-pollinated ones possess flat epidermal cells. Conical epidermal cells are assumed to act as light traps, enhancing the strength of the flowers' colour signal. However, we show that the ambiguity of epidermal cell shape depends on the main pollinator as well as on the flower part, but does not affect the petals' colouration as predicted. Nevertheless, epidermal cell shape affects mechanical properties in respect of floral grip, which influence the handling by bees that land on flowers, but not that by hovering birds. It is known that the possession of conical epidermal cells facilitates the bees' grip, whereas flat epidermal cells cause a slippery surface. As grip is furthermore important for nectar robbing bees, the possession of flat epidermal cells on those flower parts that are vulnerable to nectar robbing is assumed to represent a mechanical floral filter. In fact, this is the case for bee- as well as bird-pollinated flowers.

TABLE OF CONTENTS

Chapter 1 General Introduction

Chapter 12 Appendix

CHAPTER 1

GENERAL INTRODUCTION

Competitive Pollination Networks

Nowadays, more than 350,000 species of angiosperms are known worldwide and are a result of evolution and adaptive radiation involving, beside others, interactions between their flowers and flower-visitors (Lunau 2004; Ollerton *et al*. 2011). Flowering plants possess a large number of different lifestyles and reproductive strategies, but 90% of the todays' present angiosperms are pollinate by animals (Tepedino 1979; Buchmann and Nabhan 1996; Barrett 1998; Bascompte and Jordano 2007; Ollerton *et al*. 2011). Plants and animals are in direct relationships, and thus form networks of interdependences (Memmott 1999; Ings *et al.* 2009). Two-mode pollination networks include interactions between flowers as reproductive organs of plants on the one hand and pollinators on the other hand (Jordano 1987; Jordano *et al*. 2003; Bascompte and Jordano 2006, 2007; Olesen *et al*. 2006; Olesen and Jordano 2002). The relationships between these two partners are mutualistic in respect of the flowers' as well as the pollinators' reproductive success (Jordano 1987; Olesen and Jordano 2002). On the one hand, zoophilous flowers rely on pollinators ensuring their reproductive success. The pollinators effectively deposit pollen from conspecific flowers on the flowers' stigma and remove the flowers' own pollen from stamina in order to transfer the paternal reproductive material to other conspecifics. In doing so, pollinators ensure recombination of the genetic material and promote genetic variability of the offspring (Bawa and Beach 1981, and references within). The degree of adaptability of these plant species towards changing environmental conditions is enhanced compared to other plant species with an asexual reproduction strategy (Bawa and Beach 1981). Moreover, pollinators that promote cross-pollination, i.e. the pollination with pollen from other plant individuals, rather than self-pollination, i.e. the pollination with conspecific pollen from the same flowers or from other flowers of the same individual, additionally enhance the genetic variability of the offspring (Bawa and Beach 1981). On the other hand, pollinators rely on food sources supplied by appropriate flowers. Adult bees for example rely on nectar as energy-supply and their offspring need in addition pollen as protein source (for review, see Nicolson 2011). Moreover, some bee species collect essential resins, fragrances or oils offered as reward by specialized flowers and perform pollination service in doing so (Vogel 1971; Dressler 1982; Armbruster 1984; Buchmann 1987). Hummingbirds likewise rely on floral nectar as energy source in regular time intervals (Wolf *et al*. 1972; Hainsworth and Wolf 1972). Other flower-visiting species gather also floral rewards and perform pollination, but mainly feed on other sources than flowers like fruits (Kevan and Baker 1983, and references within). Different flower-visitors remarkably differ, among others, in their morphological properties, habitat requirements, foraging strategies, and nutritional requirements. Hence, different flower-visitors also differ in their effectivity as pollen vectors for specific plant species (Schemske and Horvitz 1984). For example, plant species in habitats of rainy, cold conditions rely on pollinators which forage during appropriate weather conditions (Totland 1994; Dalsgaard *et al*. 2009; Ortega-Jimenez and Dudley 2012), and plant species whose conspecifics grow far away rely on pollinators, which are able to overcome long distances during their foraging activities (Gill 1988). However, the degree of specialisation of flowers towards selective flower-visitors varies among different plant species. Generalist plant species are frequently visited by many different flower-visitors and are quite common (Waser *et al*. 1996; Waser 2006). Thus, within a plant-pollinator network generalist plant species are highly linked with many flower-visitors (Olesen *et al*. 2007). However, other plant species are assumed to be highly specialized towards a restricted set of flower-visitors, and possess flowers, which show high degrees of specialization to one or a few specific flower-visitors or a guild of flower-visitors with similar characteristics crucial in determining their effectiveness as pollinators (Bastolla *et al.* 2009).

The specialization of flowers towards specific flower-visitors is achieved by characteristic flower traits which promote the transfer of pollen between the flowers' reproductive organs and the pollinators' body parts (van der Pijl 1961; Stebbins 1970; Faegri and van der Pijl 1979; Crepet 1983). Flower traits include morphological parameters like corolla length or landing platforms as well as chemical parameters like floral colouration and floral scent bouquets (Faegri and van der Pijl 1979). Specific flower-visitors are commonly associated with a characteristic set of flower traits within a single flower, because specific flower-visitors selectively visit a few but not all flowering plants in their environment. The combinations of different floral characteristics, which are adapted to specific flower-visitors lead to the classical view of pollination syndromes in ecology (Vogel 1954; Faegri and van der Pijl 1979; Fenster *et al*. 2004). For example flowers with a melittophilous pollination syndrome are frequently visited by bees and share characteristic traits including a zygomorphic shape, structures serving as landing platforms, nectar guides, the emission of medium-strong fresh scents, within corolla concealed sexual organs, and moderate quantities of not deeply hidden nectar (Faegri and van der Pijl 1979). In contrast, flowers with an ornithophilous pollination syndrome, i.e. flowers pollinated by birds, have in general a long medium-wide corolla tube or are hanging and zygomorphic, no landing platforms, no nectar guides and no scent, the distance between reproductive organs and nectar is quite large that anthers and stigmas are by times exerted from the corolla tube, and bear abundant dilute nectar (Faegri and van der

Pijl 1979). However, different species of bees as well as different species of birds at times account for different characteristic flower traits within the same pollination syndrome and by times even the foraging behaviour of individual bees, i.e. the collection of pollen or nectar, account for different syndromes (Faegri and van der Pijl 1979; Rocca and Sazima 2010). The strong association of flower-visitors with specific flower traits and a specific set of flower traits within a single flower has been longstanding assumed to promote interactions between flowers and their pollinators (Sprengel 1793; Darwin 1862; Knuth 1908; Baker 1963; Faegri and van der Pijl 1979; Stebbins 1970; Crepet 1983). This assumption includes that flower traits evolved solely in order to attract pollinators (Sprengel 1793; Darwin 1862; Knuth 1908; Crepet 1983). In other words, floral traits are assumed to represent an evolutionary adaptation by flowers to a specific pollinator guild, which bases on convergent evolution of specific flower traits among different plant species (Baker 1963; Faegri and van der Pijl 1979; Stebbins 1970; Johnson and Steiner 2000; Schistl and Johnson 2013). Thus, the evolution of flower traits is assumed to be due to selective pressure exerted by the pollinators, only (Baker 1963; Faegri and van der Pijl 1979; Stebbins 1970; Johnson and Steiner 2000; Schistl and Johnson 2013). Stebbins (1970) complements this common mind by claiming that only pollinators that are most effective and most abundant will exert the most important selective force on floral traits. However, the classical view of pollination syndromes has been criticized in several studies in the last years due to the disagreement of prediction and factual field-observations of pollinators and due to a large number of arising studies assessing widespread generalization across pollination systems (Waser *et al*. 1996; Ollerton 1998; Kingston and McQuillan 2000; Thomson *et al*. 2000; Lázaro *et al*. 2008; Ollerton *et al*. 2009). Moreover, several other non-pollinating flower-visitors are linked within the interactions between flowers and pollinators, and might affect the evolution of flower traits (Herrera 1996; Ollerton 1996; Waser *et al*. 1996; Memmott 1999; Strauss and Irwin 2004; Strauss and Whittall 2006; Santamaría and Rodríguez-Gironés 2007). In fact, once adapted to a specific pollinator, flowers might suffer from being visited by other flower-visitors than their pollinators (for review, see Irwin *et al*. 2010). For example, due to concomitant changes in the flowers' morphology promoting contact between the plants' reproductive organs and pollinators' body parts, other visitors with differing morphology might fail to transfer pollen. This might have indirect or direct negative effects on the plants' fitness, if these visitors remove pollen, which is no longer available for pollination (Westerkamp 1991; Waser and Price 1983) or remove floral rewards, which are no longer available for pollinators, which, in turn, might negatively affect the visitation-behaviour of pollinators (Irwin and Brody 1998; Irwin and Brody 2000). Inouye (1980) categorized non-mutualistic operating flower-visitor behaviours into two groups of floral larceny. Behavioural "robbers" are those flower-visitors, which pierce flowers, most often basal parts of corollas, in order to extract nectar (Müller 1873; Darwin 1876; Inouye 1980; Irwin *et al*. 2001). In contrast, "thieves" are those flower-visitors that enter flowers in the normal way but also provide little or no pollination service, as their body is not in contact with reproductive organs of the flowers (Inouye 1980; Irwin *et al*. 2001). However, the impact of robbers or thieves on the flowers' reproductive success remarkably vary and can be negative, neutral or, by times, even positive (Inouye 1983; for review, see Maloof and Inouye 2000). Moreover, the same flower-visiting species can act as mutualist or antagonist depending on the plant species (for review, see Maloof and Inouye 2000). At times, if a flower-visitor act as an mutualist or antagonist even depends on the nectar- or pollen-collecting strategy of the flower-visitors (i.e. nectar- or pollen-collecting; Waser 1979), or on the anthesis of a given plant species (Morris 1996). Moreover, there is variation in robbing rates on an annual basis, on a seasonal basis, among different sites, and within sites of specific plant species (Irwin and Maloof 2002).

Recent research focus on floral traits, which provide defence strategies of flowers against antagonistic flower-visitors, which raise the question if the selection on flower traits is always driven by pollinators alone, but rather by robbers or thieves (Brown 2002; summarized in Strauss and Whittall 2006). In fact, flower traits represent a compromise between attraction of pollinating visitors on the one hand and defence of non-pollinating ones on the other hand (Brown 2002; Irwin *et al*. 2004; Santamaría and Rodríguez-Gironés 2007), and thus influence competitive pollination networks. For example, Galen and Cuba (2001) show that floral traits represent a conflict between pollinator attraction and avoidance of flower predation, and influences the evolution of flower shape in *Polemonium viscosum*. Here, the formation of tubular flowers reduces the risk that nectar-thieving ants visit the flowers, but at the same time reduces the pollination effectivity of bumblebees (Galen and Cuba 2001). Strauss and Agrawal (1999) discuss the tolerance of flowers towards antagonists and give further examples that the selective pressure on flower traits, which is exerted by antagonists as well as pollinators leads, at times, into the same direction. In contrast, Johnson *et al*. (2006) show that the coloured nectar of the South African shrub *Aloe vryheidensis* filters selectively pollinators as visitors, but not antagonists. Here, phenolic compounds of the nectar reduce the visitation frequency by antagonistically operating flower-visitors due to a repelling effect, but at the same time do not affect the visitation rate by pollinators (Johnson *et al*. 2006). As the phenolic compounds colourise the nectar into dark-brown, the nectar colouration communicates the bitter taste for experienced antagonists before visiting the flowers again (Johnson *et al*. 2006). Junker and Blüthgen (2008) likewise show that floral traits by times rather filter out the flowers' enemies than solely attract pollinators. This study gives evidence that floral scents repel nectar-thieving ant species from visiting specific flowers (Junker and Blüthgen 2008). At times, floral traits adopt simultaneously the function of attraction of pollinators on the one hand and defence of non-pollinating visitors on the other hand (Herrera *et al*. 2002; Irwin *et al*. 2004; Strauss and

Whittall 2006; Hanley *et al.* 2009; Junker and Blüthgen 2010). Thus, considering the complex combined effects of mutualistic and antagonistic interactions within competitive plant-pollinator networks is essential to understand the evolution of flower traits. Ornithophilous flowers are a good study system to investigate these questions.

In ornithophilous flowers, the petals are often confused to long but narrow corolla tubes (Faegri and van der Pijl 1979). These long corolla tubes are assumed to constitute floral adaptations for the pollination by long-billed or long-tongued pollinators guiding the pollinators' mouthparts to the floral reward located at the corollas' basal part (Nilsson 1988). However, at the same time long corollas denying the access for other flower-visitors with inappropriate body morphometry, i.e. for example bees with body sizes exceeding the corollas' width or short-billed hummingbird species (Inouye 1980; Pleasants and Waser 1985; Lara and Ornelas 2001). If bees visit hummingbird-pollinated flowers with long corolla tubes, they often rob the nectar by biting holes in the basal parts of the corolla, and thereby remove the floral reward without performing pollination service for the plant (Roubik 1982; Irwin and Brody 1998, 2000). Nectar robbing can lead to direct competition between bees and birds, if bees aggressively defend nectar sources (Roubik 1982), or to indirect competition, if birds avoid to visit flowers frequently visited by bees due to not sufficient amounts of reward left in the flowers by bees (Roubik 1985; Irwin and Brody 1998, 2000; but see Irwin 2009). Thus, although several floral characteristics seem to be well adapted to capabilities of birds as flower-visitors, maladaptation of the same floral characteristics towards non-pollinating visitors received growing attention (Castellanos *et al.* 2004; Rausher 2008). For instance, on the one hand the lack of scent in ornithophilous flowers might be explained by the underdeveloped olfactory sense of flower-visiting birds, but on the other hand might hamper bees from detecting these flowers (Faegri and van der Pijl 1979; Goldsmith and Goldsmith 1982; Pleasants and Waser 1985; Knudsen *et al.* 2004). In this case, probably both, pollinators and non-pollinating antagonists, exert selective pressure (Knudsen *et al.* 2004). That the selective pressure on floral characteristics is exerted exclusively by non-pollinating visitors rather than by pollinators is more obvious for other floral traits of ornithophilous flowers. Hummingbirds prefer concentrated nectar over dilute one (Hainsworth and Wolf 1976; Stiles 1976), and if selective pressure is exerted by the pollinator hummingbird-pollinated flowers should be associated with concentrated nectars (Bolten and Feinsinger 1978). Instead, the sugar concentration of nectars in hummingbird-pollinated flowers is low (Baker 1975). Hence, the prevalence of low nectar concentrations in hummingbird-pollinated flowers is assumed to evolve under selective pressure exerted by nectar robbing bees, which likewise prefer concentrated nectar sources, rather than exerted by pollinating hummingbirds (Bolten and Feinsinger 1978). Moreover, the nectar concentration of flowers pollinated by hummingbirds acquires the function of a floral trait evolved to deter non-pollinating visitors rather than to attract the pollinators Bolten and Feinsinger (1978). For example in *Penstemon*, evolutionary adaptations of flowers towards hummingbirds as pollinators simultaneously appear with maladaptations to bees (Castellanos *et al*. 2004). Maladaptations include the degree of exposition of reproductive organs, the presence of a lower corolla lip, the width of the corolla tube, and the angle of flower inclination (Castellanos *et al*. 2004), but hummingbirds are at the same time still able to effectively pollinate bee-pollinated *Penstemon* species (Castellanos *et al*. 2003).

The investigation of floral traits in respect of their evolutionary drivers helps to understand whether and how the structure of an ecological competitive pollination-network affects its dynamics. More precisely, understanding the function of specific floral traits in their degree of attraction of pollinators and degree of defence function against non-mutualistic flower-visitors at the same time, aids to understand behavioural and ecological dynamics in competitive pollination networks. This thesis deals mainly with floral colouration of bee- and bird-pollinated flowers and investigates whether and how floral colouration act as a filter that selectively attracts flower-visitors. The following paragraph provides an overview of an ongoing debate whether and how the colouration of bird-pollinated flowers is involved in the attraction of pollinators and in the prevention of damaging by non-mutualistic operating flower-visitors.

Visual Ecology in Competitive Pollination Networks

Colour is probably the most striking attribute of flowers for humans and many flower-visitors as well, and occurs in an enormous diversity among the plant kingdom. The associations between flower colours and specific flower-visitors in the literature is longstanding (Vogel 1954; Faegri and van der Pijl 1979). Harborne (1977, p. 38) noted, “there is clear evidence of natural selection for particular colours in different environments, according to the most active pollinators which are present”. It is commonly assumed that many flowers appear yellow when primarily visited by unspecialized insects like flies (Kevan and Baker 1983; Lázaro *et al*. 2008), white when primarily visited by nocturnal moths or bats (Faegri and van der Pijl 1979; Grant 1992), red when primarily visited by birds (Porsch 1931; Grant 1966), red or pinkish when primarily visited by butterflies (Faegri and van der Pijl 1979; Johnson and Bond 1994), and yellow or blue when primarily visited by bees (Faegri and van der Pijl 1979). It is noteworthy that the ultraviolet range of wavelength, although visible for most flower-visitors, has not been included into the hypothetical associations.

The conventional wisdom about flower colours is that it serves as an attractant for pollinators (Sprengel 1793; Darwin 1876; Fenster *et al*. 2004). However, it is possible that the floral colouration is under selective pressure by pollinators and non-pollinating, antagonistic flower-visitors as well (Strauss and Whittall 2006; Rausher 2008). Several studies show that flower colour transitions are concomitant with transitions in pollination syndromes, and reveal that the plants' pollinator drives the evolution of flower colour (Waser and Price 1981; Melendez-Ackerman and Campbell 1998). On the other hand, in many other plant species pollinators do not drive the transition of flower colour, but the main driver is often unknown (summarized in Rausher 2008). The role of pollinators and non-pollinating visitors on the selection of flower colouration is however, still unknown (Fenster *et al*. 2004). Due to striking differences in colour vision systems and neural processing across animal species, flower colours might evoke specific behavioural responses by different flower-visitors. In turn, floral colouration might structure competitive pollination networks in a still unexplored manner. Studying interactions between flowers and mutualistic as well as antagonistic flower-visitors, might aid particular understanding of the impact of floral colouration in competitive pollination networks. Bird-pollinated flowers are particular suited as study objects as several flower traits of the ornithophilous pollination syndrome are suspected to represent maladaptation to non-pollinating agents.

"Ornithophilous flowers are predominantly red". This is a common statement in the literature, and Porsch described this phenomenon 1931 for 370 genera in 75 plant families. The common explanation for this prevalence focuses on observed preferences of wild hummingbirds for red flowers as evolutionary driver (Raven 1972; Sutherland and Vickery 1993). That the eyes of flower-visiting birds are comparably more sensitive in the red wavelength range supported this rationality (Kühn 1929; Herrera et al. 2008). However, other studies reveal that birds do not show innate preferences for red, but rather associate flower colours with rewards due to superior memory performances (Bené 1941; Miller and Miller 1971; Stiles 1976; Goldsmith and Goldsmith 1979; McDade 1983; Miller *et al.* 1985; Delph and Lively 1989; Hurly and Healy 1996; Healy and Hurly 2004). For example, the hummingbird *Phaethornis superciliosus* does not show any colour preference in field-studies, but rather visit red and yellow colour morphs of *Heliconia irrasa* equally frequent (McDade 1983). In *Fuchsia excorticata* the flowers pass through a colour change from green in the nectar-producing phase of anthesis towards red colour in the post-reproductive and nectar-less phase (Delph and Lively 1989). Here, honeyeaters discriminate against the red morph and prefer green, nectar-producing flowers (Delph and Lively 1989). Grant (1966) hypothesized that the prevalence of red colouration among the Californian flora is due to the fact that red might be the best colour for quick detection of suitable flowers. He argued that this region is perambulated by migrating hummingbirds, which need to locate food sources in permanently varying habitats along their migration route (Grant 1966). Other explanations for the strong association between red bird-pollinated flowers and birds as flower-visitors take into account, that the red-blindness in bees might exert selective pressure on floral colouration (Porsch 1931; Grant 1966; Grant und Grant 1968; Raven 1972; Stiles 1976). Evolution towards reflectance of red wavelengths negatively affect interactions between these flowers and bees, leading to a reduced visitation frequency (Porsch 1931). As an example he refers to Hawaii as study system where bees are absent and bird-pollinated flowers are commonly blue (Porsch 1931). In fact, bird-pollinated flowers suffer from being visited by bees through direct as well as indirect competition between bees and birds as flower-visitors (Roubik 1982; Roubik 1985; Waser 1979; Irwin and Brody 1998, 1999, 2000; Irwin *et al*. 2001; Navarro 2001; Irwin 2003, 2006; Pohl *et al*. 2006; Botes *et al*. 2009). This has developed to the extent that hummingbirds use bite-marks left by nectar-robbing bees as visual cue that communicates the amount of nectar reward and avoid visitation of flowers with insufficient energy supply (Irwin 2000). However, bees are able to detect red flowers, as the sensitivity of the green photoreceptor-type in the bees' eye is comparably lesser, but to some extend still sensitive to red lights (Chittka and Waser 1997; Reisenman und Giurfa 2008). Rodríguez-Gironés and Santamaría (2004) made a further attempt to explain the mystery of red bird-pollinated flowers using the optimal foraging strategy hypothesis. Assuming that two equally abundant flower types differ exclusively in their colour but neither in their nutrition, morphology nor other floral traits, the authors suggest that there is complete resource partitioning, with bees foraging exclusively at blue flowers and birds exclusively at red flowers (Rodríguez-Gironés and Santamaría 2004). This is because bees more easily detect blue flowers as compared to red ones and hummingbirds prefer flowers with more nectar reward left (Rodríguez-Gironés and Santamaría 2004). Thus, the red colouration of bird-pollinated flowers creates a private communication channel in which birds are free from competition for nectar (Rodríguez-Gironés and Santamaría 2004). In fact, the foraging ability of bees at red flowers is comparably less effective as detecting red targets needs comparably more time as compared to blue or yellow due to adjustment of flight speed (Spaethe *et al*. 2001). Moreover, with increasing complexity of the background, red stimuli became more difficult to detect by bees as compared to blue ones (Forrest and Thomson 2009). Lunau (1990) hypothesized that beside red, other colours like ultraviolet (UV)-reflecting white likewise might lead to reduced visitation frequencies by bees due to comparably lesser performance of bees to detect such coloured stimuli. Compared to a mechanical exclusion, the sensory exclusion of bees by bird-pollinated flowers might be of advantage for the flowers' reproductive success if visitation frequency by bees and concomitant nectar robbing by bees is reduced. Thus, flower colour might influence plant-pollinator interactions by creating communication channels between the flowers and their pollinators. However, the reason for the strong association between red floral colouration and bird-pollinated flowers is still unknown.

Regarding classical pollination syndromes, the correlation between flower colour and pollinator finds, by times, strong evidence (McCall and Primack 1992; Chittka *et al*. 2001; Danieli-Silva *et al.* 2012). In the genus *Mimulus*, evolutionary shifts from bee-pollinated ancestors towards bird-pollinated derived plants are well studied (Rausher 2008; Hopkins and Rausher 2012). Here, the floral colouration leads to reproductive isolation of closely related plant species, as it affects the visitation behaviour by different flower-visitors (Rausher 2008; Hopkins and Rausher 2012). In this genus, floral colouration undergoes an evolutionary shift from the blue-coloured bee-pollinated flowers of *M. lewisii* to pink-coloured bird-pollinated *M. cardinalis* flowers, which is a result of a mutation leading to the lack of pigments (Bradshaw *et al*. 1998; Bradshaw and Schemske 2003). When bees and bird forage in a common garden setup with both species and their hybrids present, bees visit blue flowers more frequently than pink flowers, whereas birds choose more often red anthocyanin-rich flowers (Schemske and Bradshaw 1999; Bradshaw and Schemske 2003). Due to this opposing colour choice behaviour of flower-visitors, reproductive isolation between the two colour types within a population takes place, as pollen flow between the different types is reduced (Bradshaw *et al*. 1995; Schemske and Bradshaw 1999; Ramsey *et al*. 2003). That flower colour is an important cue determining the flower-visitor composition of bees and birds and that this influences reproductive isolation between plant species, could been assessed for other study systems, too (Wilson *et al*. 2004; Zufall and Rausher 2004; Gegear and Burns 2007; Rausher 2008; Thomson and Wilson 2008). However, shifts in flower colour between species pollinated by either bees or birds arising simultaneously with changes in other floral traits like morphological parameters (Castellanos *et al*. 2003, 2004; Wilson *et al*. 2004).

Moreover, correlations between flower colour and flower-visitors are lacking in several other study systems (Waser *et al*. 1996; Kingston and McQuillan 2000; Chittka *et al*. 2001). The following paragraph gives an overview over principles in floral colouration, regarding pigment chemistry, physical properties of flowers influencing their colouration, and the further insight into the ecology of interactions between flower colours and flower-visitors.

Flower Colours

Flower colours result primarily from pigments located in flower petals (for review, see Mol *et al.* 1998; Grotewold 2006; Davies 2004). Flower pigments are complex and selectively absorb a specific range of wavelengths from the ambient light environment. The reflected light is not monochromatic, i.e. the reflectance of a single wavelength, but rather a step function of weak slope with reflectance over one or more wavelength ranges (Chittka and Waser 1997). Several different pigment classes from biosynthetic pathways are known and occur in different concentrations and combinations among petals (Scogin 1983; for review, see Mol *et al.* 1998; Grotewold 2006). Main pigment classes in plants are flavonoids, carotenoids, betalains, and chlorophylls (Scogin 1983; Grotewold 2006; Davies 2004). The former three ones are common in flower tissues (Scogin 1983; Grotewold 2006; Davies 2004). Flavonoids are phenylpropanoid compounds and are the most common pigments in flowers as well as other showy plant organs (Scogin 1983; Davies 2004). Among the 7000 flavonoids known, anthocyanins are the most abundant and widespread pigments in flowers (Scogin 1983; Davies 2004). Flavonoids absorb light at the longest wavelengths, and appear thus pink, red, magenta, purple, blue and blue-black for the human observer (Scogin 1983; Brouillard and Dangles 1993; Davies 2004). Carotenoids are terpenoid structures (Davies 2004), and 600 different carotenoids are known, including xanthophylls and carotenes, which are present in all photosynthetically active plants (Goodwin 1984; Davies 2004). In flowers, carotenoids evoke bright yellow, orange or red colourations for the human observer (Davies 2004). The less common betalains (including betaxanthins and betacyanins) generate a yellow and violet flower colouration and replace anthocyanins in most plant families of the order Caryophyllales (Clement and Mabry 1996; Davies 2004), as the biosynthetic pathways of both pigments cannot operate at the same time (Wyler and Dreiding 1961; Kimler *et al*. 1971). Additional co-pigments, the prevalent pH in the vacuole, metal ions, pigment packaging, and tertiary structures arising from self-association and inter- and intramolecular interactions change the appearance of flower colours (Scogin 1983; Gottsberger and Gottlieb 1981; for review, see Mol *et al.* 1998; Davies 2004). Moreover, the cellular and subcellular localisation of the different pigment groups within the flowers' tissue is also generally distinct and influences the visual appearance of the flower colour (Kay *et al*. 1981). Flavonoids occur in several subcellular and extra-cellular locations, but are most frequently located within vacuoles in epidermal cells (Kay *et al*. 1981; Bohm 1998; Brouillard and Dangles 1993). Carotenoids are, in general, lipid-soluble and located within plastids. However, some carotenoids are water-soluble and located in the vacuole (Bouvier *et al*. 2003). The same is true for betalains (Davies 2004). Beside floral colouration, pigments adopt several other functions within plants. Among them, photo-protection, protecting against pathogens, acting as antioxidants, handling of biotic and abiotic stress, influencing hormone transport, and enabling plant fertility are noteworthy (Gronquist *et al*. 2001; Davies 2004). Former research has focused on correlations between specific flower-visitor groups and flower pigments (Scogin *et al*. 1977; Scogin 1980, 1983, 1988; Harborne and Grayer 1994). Scogin (1988) described a "bird-visitation pigment syndrome" and claimed that anthocyanidins, especially pelargonidins, are the most frequent flower pigments, at least among Neotropical flowers visited by hummingbirds.

Several recent studies focus on the impact of epidermal cell structure on floral colouration. Conical cells, which are common in epidermal cells among angiosperms, can act as lenses and light traps, directing the incident light into specific cell parts (Exner and Exner 1910; Kay *et al.* 1981; Gorton and Vogelmann 1996). In the snapdragon *Antirrhinum majus*, conical epidermal cells direct incident light into basal parts of the epidermal cells (Gorton and Vogelmann 1996). The formation of conical epidermal cells is due to a single gene, called *mixta* (Noda *et al.* 1994). In contrast, flat epidermal cells in a *mixta*-mutant of the same species, focusing light beneath the epidermal cells into the mesophyll (Gorton and Vogelmann 1996). Depending on whether the pigments are located within the flower tissue the shape of epidermal cells affect the amount of incident light directed towards the pigments, and thus affect the floral colouration (Exner and Exner 1910; Noda *et al.* 1994). Several researchers have used snapdragon wild-types and mutants to study the interactions between flowers and pollinators in regard to epidermal cell structure. Bumblebees, which are the pollinators of *Antirrhinum majus*, are not able to visually detect differences in the floral colouration between wild-type and mutant flowers (Dyer *et al.* 2007), but prefer to visit wild-type flowers (Glover and Martin 1998; Comba *et al.* 2000). Beside floral colouration, the epidermal cell shape additionally affect the floral temperature (Comba *et al.* 2000), floral shape (Baumann *et al.* 2007), floral wettability (Whitney *et al.* 2011b), presence of tactile nectar guides (Kevan and Lane 1985), and floral grip (Whitney *et al.* 2009a, 2009b; Rands *et al.* 2011; Alcorn *et al.* 2012). Moreover, the shape of epidermal cells affects the amount of gloss, which is reflected from the flower surface and might influence plant-pollinator interactions (Vignolini *et al.* 2012a, b). Gloss increases if the surface becomes flat, and thus flat epidermal cells in flowers might increase the amount of gloss of flowers (Parkin 1928; Galsterer *et al.* 1999; Vignolini *et al.* 2012a, b; Whitney *et al.* 2011c, 2012). In turn, with increasing amounts of gloss a smaller amount of the incident light enters the flowers' tissue and is directed towards the pigments. However, the behaviour of pollinators in respect of gloss is not well studied (Whitney *et al.* 2011c, 2012). In addition, surface structures on epidermal petal cells affect the flower colour impression and the amount of gloss (Bradshaw *et al.* 2010; Kourounioti *et al.* 2013). Besides evoked by pigment chemistry, floral colouration can also be generated by means of structural colours produced by coherent and incoherent scattering, but their role in floral colouration was investigated only recently (Vogelmann 1993; Glover and Whitney 2010; Whitney *et al.* 2011a; Vignolini *et al.* 2013). Colours generated by pigments result from a diffuse reflection of light, and thus do not change with the angle of view of the beholder. In contrast, structural colours might be angle-dependent and are of higher intensity due to reflective structures that are comparably more restricted in the wavelength regions that they reflect (Glover and Whitney 2010; Vignolini *et al.* 2013). At times, patterns of varying epidermal cell structures have the same contours than pigment-based coloured flower patterns, and induce iridescence of restricted coloured patterns

(Whitney *et al*. 2009c). Several plant species are known to exhibit iridescent flowers produced by multilayers or diffraction gratings (for review, see Glover and Whitney 2010; van der Kooi *et al*. 2014a; Vignolini *et al*. 2012a, b, 2014a). However, there is an ongoing debate about the floral iridescence' impact on interactions between flowers and their visitors (Morehouse and Rutowski 2009; Vignolini *et al*. 2014b). In fact, bumblebees are able to distinguish between optical signals arising from iridescent and non-iridescent petals, and moreover, bumblebees can be trained to use these signals as a cue to identify rewarding flowers (Whitney *et al*. 2009c). However, other studies suggest that pigments rather than structural coloration determine the optical appearance of flowers for flower-visitors (van der Kooi *et al*. 2014b). Further research is needed to understand the complex relationships between pigment- and structure-based colours. As a whole, flower colours appear in a remarkable diversity among angiosperms, and this diversity is even more remarkable when the diversity of visual systems among animals is considered.

Flower-visiting species, including bees and flower-visiting birds (Peitsch *et al*. 1992; Herrera *et al*. 2008; Ödeen and Håstad 2010), can detect UV-light, which is commonly reflected by flowers (Kühn 1924; Silberglied 1979; Chittka *et al*. 1994; Kevan *et al*. 2001; Bennett and Cuthill 1994). Chalcone- and flavonol-type flavonoids absorb UV-light, and, together with UV-reflecting carotenoids, often form colour patterns within flowers, which are invisible for the human observer (Harborne and Grayer 1994; Bohm 1998). This is especially the case in yellow flowers, which often show distinct intra-floral colour patterns in the UV (Horovitz and Cohen 1972; Guldberg and Atsatt 1975; Primack 1982), but floral colour patterns are also common in other wavelength ranges and are thus, at times, also visible for the human observer (Sprengel 1793; Lunau 2006). Nectar guides are most noteworthy, as interactions between them and flower-visitors, especially bees, are well studied, suggesting that nectar guides increase the attractiveness of the flower (Free 1970a; Jones and Buchmann 1974; Waser and Price 1985; Lunau 1993; Lehrer *et al*. 1995; Lunau *et al*. 1996; Heuschen *et al*. 2005; Owen and Bradshaw 2011; Orbán and Plowright 2013).

Among flower colours, blue is assumed to be more common in the temperate ecosystems, in which bees are the most important pollinators (Weevers 1952; Ostler and Harper 1978; Gottsberger and Gottlieb 1981; Menzel and Shmida 1993). In contrast, a red flower colour is assumed to be more common in tropical or mediterranean ecosystems, in which beetles or birds are more important (Grant 1966; Dafni *et al*. 1990). Floral colouration, whether it is pigment- or structure-based, is not a static flower trait, but rather might changes during floral anthesis. Flowers or flower parts of at least 456 species in 253 genera in 78 plant families undergo ontogenetic colour changes, which are assumed to play important roles in flower-animal interactions, especially between flowers and their pollinators (Weiss and Lamont

1997). In the majority of cases, floral colour change take place after the flowers became pollinated (Delph and Lively 1989; Harborne and Grayer 1994; Weiss 1995; Weiss and Lamont 1997; Bohm 1998; Oberrath and Bohning-Gaese 1999). The retention of older, already pollinated flowers by plants is assumed to increase the attractiveness from a distance, and direct flower-visitors to rewarding and sexually viable flowers while near-orientation (Delph and Lively 1989; Weiss and Lamont 1997).

Research on evolutionary tuning between the colour vision system (or innate colour preferences in bees) on the one hand and flower colours on the other hand has been carried out by a number of researchers (Goldsmith 1991; Menzel and Backhaus 1991; Chittka and Briscoe 2001; Chittka *et al*. 2001; for review, see Briscoe and Chittka 2001). Chittka and Menzel (1992) investigated the colouration of a large variety of flowers and found sharp steps in spectral reflectance at those wavelengths where bees are most sensitive to spectral differences. The set of photoreceptor-types found in hymenopteran species corresponds well with a calculated optimal set of photoreceptors crucial for the detection of flower colours (Chittka and Menzel 1992; Chittka 1996). However, the trichromatic set of photoreceptor-types known from hymenoptera was already present in ancestors, which did not visit flowers as a source of food, and thus these results suggest that the evolutionary tuning of the hymenopteran colour vision system is phylogenetically constrained (Chittka 1996). Moreover, the evolution of flower colours might be phylogenetically constrained due to restrictions of the plant species' pigment-biosynthesis, or due to pleiotropic effects (Chittka *et al*. 2001). For example, Osche (1979) claimed that UV-absorbing yellow colouration produced by flavonoids in pollen was already present in primarily wind-pollinated ancestors. This yellow colouration might have shaped innate preferences of bees since it is assumed to represent the very first colour signal of flowers (Osche 1979). Thus, the yellow colouration of nectar guides, which has been assumed to evolve in order to replace the attractive function of anthers, when hidden in the flowers' corolla, was probably predetermined (Osche 1979; Lunau 2000; Chittka *et al*. 2001; Heuschen *et al*. 2005). Other studies focus on the shaping of innate preferences of bees due to floral colouration. Studies including subspecies of the bumblebees *Bombus terrestris* reveal remarkably differences in innate preferences for specific hues among the subspecies (Briscoe and Chittka 2001; Chittka *et al*. 2004). Most of the tested subspecies exhibit strong preferences for different shades of blue and violet, but some island populations show an additional preference for red (Briscoe and Chittka 2001; Chittka *et al*. 2004). This is true for the island population of the subspecies *B. t. sassaricus* and the investigation of spectral sensitivity function in this subspecies reveal that the green photoreceptor-type is comparably shifted towards longer wavelengths (Skorupski *et al.* 2007). Moreover, the preference for red in the bumblebee *Bombus occidentalis* might facilitate the foraging efficiency of this species, as workers of this species often rob the nectar of hummingbird-pollinated flowers exhibiting a red colouration (Chittka and Waser 1997; Briscoe and Chittka 2001; Chittka and Wells 2004). However, colour parameters other than hue were not considered in these studies and colours were, at times, colours were termed from the point of human observers. In fact, bees with preferences for violet over blue flowers gather comparably more nectar than bees possessing a preference for blue over violet in a specific field-site (Raine and Chittka 2007). The authors argued that local variation in floral colouration could drive the evolution for innate preferences in bees (Raine and Chittka 2007). In addition, Giurfa *et al.* (1995) found a good fit between colour preferences in bees and flowers with comparably higher rewards in a study site in Germany. The same is true for a study site in Israel (Menzel and Shmida 1993).

Among different floral characteristics, flower colour is one of the most striking attributes of flowers for humans. As early as 1793, Christian Konrad Sprengel noticed the relationship between flower colours and flower-visitors, and Charles Darwin followed in 1876. However, the first evidence about colour vision in flower-visitors was verified much later. John Lubbock (1888) was the first to demonstrate colour vision in animals. Beside a positive phototaxis in the Crustaceaen genus *Daphnia*, he further investigated the preference for yellow and white light environments (Lubbock 1888). Kevan *et al.* (2001) cited Wallace with his words, "*The primary necessity which led to the development of the sense of colour was probably the need of distinguishing objects much alike in form and size, but differing in important properties, such as ripe and unripe, or eatable and poisonous fruits, flowers with honey or without, the sexes of the same or closely allied species. In most cases the strongest contrast would be the most useful, especially as the colours of objects to be distinguished would form but minute spots or points when compared with the broad masses of tint of sky, earth, or foliage against which they would be set.*" (Wallace 1878, p. 243), revealing the importance of flower-visitors to detect proper food sources via colours. In fact, floral colouration is a crucial parameter for the detection of food resources for different flower-visitors including bees (von Frisch 1914; Chittka and Menzel 1992; Menzel and Shmida 1993) as well as birds (Stiles 1976). As the visitation by pollinating flower-visitors is in turn also necessary for the plants' reproductive success, mutually adaptations are conceivable. However, colour vision systems vary remarkably among flower-visitors and thus, flower colours selectively attract different flower-visitors.

Colour Perception

Colour is an attribute that results from the composition of reflected light by an object. If objects strongly reflect or transmit all wavelengths of incident light to an equal degree, then the beholder perceives the colour sensation "white". In contrast, if objects absorb all wavelengths of light, then it is perceived as black, and intermediate but equal reflectance of all wavelengths results in a grey colouration (Gegenfurtner and Kiper 2003). However, if an object absorbs all light except a specific set of wavelengths, then the object is chromatic (Gegenfurtner and Kiper 2003). Colour is, however, not a property of the object, but emerges as a colour sensation in the eye of the beholder, and includes physical, physiological and psychological dimensions (Wyszecki and Stiles 1982; Vorobyev *et al.* 2001; for review, see Kelber and Osorio 2010). Thus, the beholder-subjective sensation depends on the visual system and further neural processing in the brain (Gegenfurtner and Kiper 2003). For example, an object that absorbs all light except blue wavelengths remains black, if the beholder does not exhibit a photoreceptor-type, sensitive for these blue wavelengths. Several definitions of colour vision for different coherences exist. A general definition applicable for all animals of variable colour vision systems is, "*Colour is that aspect of visual perception by which an observer may distinguish differences between two structure-free fields of view of the same size and shape, such as may be caused by differences in the spectral composition of the radiant energy concerned in the observations*." (Wyszecki and Stiles 1982). Thus, colour vision is the ability to recognise objects of different spectral properties irrespective of their intensity (Wyszecki and Stiles 1982). The requirement for colour vision is the possession of at least two different photoreceptor-types of different sensitivity functions, and subsequent neural processing of receptor excitations (Gegenfurtner and Kiper 2003). Photoreceptor-types contain different visual photo-pigments, which are excitable by a restricted set of wavelengths, each. Then, retina-located, light-sensitive carotenoid chromophores absorb photons and consequently isomerizes (Aidley 1998). This, in turn, leads to a changing conformation of opsin proteins that are likewise located within the photopigments, and activates photo-transduction (Aidley 1998). Both, opsin proteins and chromophores, are associated with different amino acids, affecting the visual pigments' sensitivity for specific wavelengths. The human colour vision system implies two different types of photoreceptors that are subsequently active, i.e. rods and cones. Rods are uniformly constituted and are maximally sensitive for wavelengths of 498nm (Bowmaker and Dartnall 1980). Rods are active under dim light-conditions and serve for the detection of brightness, only. In contrast, three different types of cones with sensitivity peaks at 420nm, 534nm, or 563nm exist and accomplish colour vision tasks (Bowmaker and Dartnall 1980). Specific cone-types occur in different quantities and with an irregular distribution across the retina (Bowmaker and Dartnall 1980). Depending on the beholders' brain capacities, the quantum catch in the different photoreceptor-types will be neural processed, evoke a colour sensation in the beholders' brain and might affect specific reactions and behaviours.

A concept of colour vision is that a specific colour can be defined by three distinct colour parameters, i.e. dominant wavelength, spectral purity, and intensity (Wyszecki and Stiles 1982;

Kelber *et al*. 2003). This concept derived from investigations of human-subjective perception and the physical properties of dominant wavelength, spectral purity, and intensity, correspond to colour qualities of hue, saturation, and brightness in humans, respectively and determine a specific colour (Wyszecki and Stiles 1982; Kelber *et al*. 2003). The dominant wavelength can be described by the wavelength of maximal reflectance within the visible spectrum of the beholder (Wyszecki and Stiles 1982; Kelber *et al*. 2003). Spectral purity increases when only a few wavelengths are reflected or, in other words, decreases when objects additionally reflect higher amounts of white light (Wyszecki and Stiles 1982; Kelber *et al*. 2003). Thus, monochromatic lights that only reflect a single wavelength are of highest spectral purity (Wyszecki and Stiles 1982; Kelber *et al*. 2003). Intensity describes the strength of reflected light over the visible spectrum (Wyszecki and Stiles 1982; Kelber *et al*. 2003). The higher the cumulative reflectance over the visible spectrum of the beholder, the higher is the intensity of an object and is thus in contrast to dominant wavelength and spectral purity, an achromatic rather than a chromatic aspect of colour (Wyszecki and Stiles 1982; Kelber *et al*. 2003). However, whether and how animals respond to underlying properties of colour is not well studied. Bees and flower-visiting birds are well-known groups of visually oriented pollinators that differ in their capabilities of colour perception as well as in their foraging abilities. In the following, principles of colour vision and colour perception by these two flower-visitor groups are summarized.

Colour Vision in Bees and their Implication for Foraging Strategies

Foraging bees rely, among others, on visual cues including shape, pattern, size and colour to detect flowers (Menzel and Shmida 1993; Giurfa *et al*. 1994). Beside three dorsal ocelli (Goldsmith and Ruck 1958), bees possess two apposition compound eyes consisting of several hexagonal optical modules, called facets or ommatidia (Lehrer 1998; Jander and Jander 2002). Each ommatidium consists of optical systems including a laminar, chitinous, transparent cuticular lens, a crystalline cone, and light receptive pigment cells, which built the rhabdom (Varela and Wiitanen 1970; Land and Nilsson 2012). Thus, except ommatidia in the dorsal rim which contribute to polarized light detection only (Wehner and Bernard 1993), ommatidia serve as basis for colour vision in bees (Varela and Porter 1969; Varela and Wiitanen 1970; Gribakin 1975; Wakakuwa *et al*. 2005). Already Lubbock (1888) investigated the ability of bees to possess colour vision, but his experiments did not exclude that bees associate rewards with colour solely on differences in their brightness. Karl von Frisch (1914) was the first to demonstrate the ability of colour vision in bees by training honeybees to discriminate specific colours from different shades of grey. By using specific sets of colours, von Frisch (1914) concluded that the visual spectrum in bees covers the wavelength ranges from about 300 to 650 nm. Years later, intracellular electrophysiological recordings approved his results (Autrum and von Zwehl 1964; Menzel and Blakers 1976; Peitsch *et al*. 1992), and the colour vision in bees is well studied nowadays (for review, see Menzel and Backhaus 1991, and Hempel de Ibarra *et al.* 2014; Vorobyev and Brandt 1997; Dyer *et al*. 2010).

Bees, as all other hymenopteran insects except ants, have a trichromatic colour vision system with three different photoreceptor-types possessing sensitivity peaks in the UV, blue, and green wavelength range, respectively (von Helversen 1972; Menzel and Blakers 1976; Briscoe and Chittka 2001). The sensitivity of UV-receptors peak around 350 nm, of blue-receptors around 440 nm, and of green-receptors around 530 nm, and thus fell well within the range of most insect species yet investigated (Briscoe and Chittka 2001). Trichromaticity appears to date back at least to the Devonian ancestor of all winged insects (Pterygota), but was more probably already present in the common ancestor of crustaceans and insects (Chittka 1996; Briscoe and Chittka 2001). Differences in peak sensitivities among different species of bees are known (Peitsch *et al*. 1992), and even small changes of a few nanometres can lead to varying discrimination tasks by these species. However, the shift of UV-receptor peak sensitivities to longer wavelengths in stingless bees as proposed by Peitsch *et al*. (1992) due to inhabitation of forests in these bees, could not been verified in further studies (Briscoe and Chittka 2001). In fact, the possession of three photoreceptor-types with more or less similar peak sensitivities the UV, blue, and green is the basic set-up in bees, although different bee genera and even different bee species within a single genus differ in their lifestyle (Briscoe and Chittka 2001). In contrast, the possession of an additional photoreceptor-type which is sensitive to red wavelengths is a derived trait in bees (Briscoe and Chittka 2001), and has been exclusively found in the andrenid species *Callonychium petunia* (Wittmann *et al*. 1990; Briscoe and Chittka 2001). This solitary bee is a specialist solely foraging at red-flowering *Petunia* species (Wittmann *et al*. 1990). However, all other bee species are also more or less able to detect red colours due to the fact that the sensitivity of the green photoreceptor-type extends slightly into the red wavelength range (Chittka and Waser 1997; Reisenman und Giurfa 2008). Moreover, UV-receptors seem to be involved in detecting of polarized light, besides being involved in colour-vision (von Frisch 1960; von Helversen and Edrich 1974; Labhart and Meyer 1999). Green-receptors promote motion-dependent behaviour and thus, the detection of shapes and position (Srinivasan and Lehrer 1988). The honeybees' eyes contain more than 5000 ommatidia (Lehrer 1998; Jander and Jander 2002) and possess three different types of ommatidia, differing in the presence and number of photoreceptor-types (Spaethe and Briscoe 2005; Wakakuwa *et al*. 2005). All three types of ommatidia contain six green photoreceptor-types, but differ in their presence and number of blue and green photorecptor-types: Type I ommatidia contain one ultraviolet and one blue, type II two ultraviolet, and type III two blue photoreceptor-types (Wakakuwa *et al*. 2005). In addition, the ratio of ommatidia-types across the bees' eye is not balanced, and their distribution depends on the part of the eye (Spaethe and Briscoe 2005; Wakakuwa *et al*. 2005). Colour discrimination takes place in the ventral, frontal and lateral eye region, whereas the dorsal eye region, however, was found to be incapable of colour discrimination (Giger and Srinivasan 1997).

Beyond the receptor-level, receptor signals in bees are analysed by two visual pathways, i.e. a colour-blind pathway system (also referred to as achromatic vision system) and a colour vision system (also referred to as chromatic vision system; Lehrer and Bischof 1995; Giurfa *et al*. 1996; Giurfa *et al*. 1997; Spaethe *et al*. 2001). Processing in the achromatic pathway is comparably faster than in the chromatic system (Skorupski and Chittka 2012). If the colour-blind system is active, receptor-specific signals exclusively from those photoreceptor-types which are maximally sensitive in the green wavelength range are processed (also referred to as "green contrast"; Menzel 1974; Giurfa *et al*. 1996; Giurfa *et al*. 1997; Giurfa and Vorobyev 1998; Spaethe *et al*. 2001). Thus, discrimination of objects will be made solely by means of differences in their brightness (Menzel 1974; Giurfa *et al*. 1996; Giurfa *et al*. 1997; Giurfa and Vorobyev 1998; Spaethe *et al*. 2001). The same is true for the human eye when rods instead of cones are active (Brown and Wald 1964). In contrast to humans, who use their colour-blind system under very dim light conditions, the colour-blind system in bees is active if objects are seen under small visual angles (Giurfa *et al*. 1996; Giurfa *et al*. 1997; Giurfa and Vorobyev 1998; Spaethe *et al*. 2001). For honeybees, visual angles of less than 15° are crucial for the perception of achromatic contrasts (Giurfa *et al*. 1996; Giurfa *et al*. 1997; Giurfa and Vorobyev 1998; Spaethe *et al*. 2001). The higher the achromatic contrast between two objects the more reliable bees can distinguish these objects from each other (Giurfa *et al*. 1996; Giurfa *et al*. 1997; Giurfa and Vorobyev 1998; Spaethe *et al*. 2001). Consequently, when looking at flowers from large distances, flowers need to display achromatic contrasts to their backgrounds to be detected by bees. However, if colour contrast is lacking, bees have difficulty to detect objects, even if achromatic contrast is present (Lehrer and Bischof 1995; Spaethe *et al*. 2001). Only under large visual angles (>15°) and thus, during near-orientation objects become coloured for bees (Giurfa *et al*. 1996; Spaethe *et al*. 2001). In contrast to the colour-blind vision system, differences in intensities are not analysed during colour vision, but rather differences in the spectral composition (Backhaus *et al*. 1987; Backhaus 1992; Vorobyev and Brandt 1997). Experimental evidence shows that the higher the chromatic contrast between two objects the better bees can distinguish them from each other

(Chittka 1992; Lunau *et al.* 1996; Vorobyev and Brandt 1997). High colour contrasts between flowers and their backgrounds are crucial parameters in the detection of flowers by bees.

The colour choice behaviour of newly emerged bees is assumed to be influenced by innate preferences for specific colour parameters, aiding the bees to find their first flowers. Several authors claim that bees have an innate preference for specific bee-subjective hues, i.e. for blue and violet colours (Menzel and Shmida 1993; Giurfa *et al.* 1995; Chittka *et al.* 2001). The statements that bees learn blue colours comparably faster and choose it more accurately supported this hypothesis (Menzel 1967, 1985). Other studies assess preferences in bees for colours of high spectral purity (Lunau 1990, 1992). However, several behavioural studies with bees regarding their choice behaviour towards specific colour parameters were conducted, but analysed in different ways, and focusing on different colour parameters. Moreover, the independent variation of a specific colour parameter is complex and the alteration of one colour parameter is most often concomitant with changes in the other parameters, too. Thus, preferences in bees for specific colour parameters are still unknown or ambiguous. In addition, inter-individual variations in colour choice behaviours within colonies or populations are also known (Briscoe and Chittka 2001). Beyond that, within-flower colour patterns determine the choice behaviour of bees, as well as their behaviour during near-orientation at flowers (Lunau 1990, 1991, 1992; Horridge and Zhang 1995; Lehrer *et al.* 1995; Lunau *et al.* 1996; Hempel de Ibarra *et al.* 2001, 2002; Simonds and Plowright 2004; Heuschen *et al.* 2005; Lunau *et al.* 2006; Pohl *et al.* 2008). More precisely, bees use the coloured patterns as functional orientation cues, which aid bees to locate the floral reward more rapidly as compared to uni-coloured flowers (Waser and Price 1985; Leonard and Papaj 2011; Jones and Buchmann 1974). However, colour-naïve bees prefer those colours that experienced bees learn faster (Giurfa *et al.* 1995). For colour vision in bees, several models are established and applied (for review, see Hempel de Ibarra *et al.* 2014). The first attempt was the Maxwell triangle, where colour information in form of quantum catches reaching the bees' eye are calculated and plotted in the colour space (Daumer 1956). Other authors postulate other models, which imply assumptions about further neural processing (Backhaus 1991; Chittka 1992; Vorobyev and Osorio 1998). Several results indicate that colour in bees is coded by opponent (subtractive) mechanisms (Menzel and Backhaus 1991; Backhaus 1991; Chittka 1992; Vorobyev *et al.* 2001). The COC-model (colour opponent coding model) bases on physiological data obtained from colour-coding neurons in the bees' brain, whereas the colour hexagon model bases on generic opponent processes (Chittka 1992) and the receptor-noise limited model on receptor noise considerations (Vorobyev and Osorio 1998; Vorobyev *et al.* 2001). By means of colour vision models bee-subjective colour parameters can be calculated and predictions about the choice behaviour of bees due to colour similarity are possible.

Beside innate colour preferences, colour can be associated with food and effectively learnt by bees (Menzel 1967, 1985; Giurfa 1991; Greggers and Mauelshagen 1997; for review, see Avarguès-Weber and Giurfa 2014). After learning, generalisation in bees takes place, i.e. bees choose novel colours in accordance to their similarity to the learnt ones (Gumbert 2000). Temporarily, individual bees selectively visit flowers of a specific colour, rather than visit all flowers in their environment. This behaviour is called flower-constancy (Grant 1950; Free 1963, 1970b; Heinrich 1976; Waser 1986; Hill *et al*. 1997; for review, see Chittka *et al*. 1999). At times, benefits for bees as well as plants arise through flower-constant foraging behaviour, but the adaptive value is still disputed (for review, see Chittka *et al*. 1999). For example, plants might benefit from enhanced pollen transfer between conspecifics, but this depends on the plants' reproductive strategy (Waser 1986; for review, see Chittka *et al*. 1999). At times, plants mimicking the flower colour of another plant benefit from visits by flower-constant bees, although no costly rewards are produced (Dafni 1983; Peter and Johnson 2008). Flower-constant bees might forage more efficiently when handling of complex flowers was already learnt, but might forage less efficiently when not visiting flowers which contain comparably higher rewards (for review, see Chittka *et al*. 1999). Flower-constant foraging behaviour due to flower colour is facilitated by imperfect colour-constancy possessed by bees (Neumeyer 1981; Dyer 1999). Colour-constancy is the ability of the beholder that a perceived colour remain constant even if the surrounding illumination changes (Hurvich 1981; for review, see Foster 2012). The bees' flight speed additionally affects the choices of colours by bees (Chittka *et al*. 2003; Bogacz *et al*. 2010). Several studies, which deal with the flowers' choice behaviour by bees, imply handling time as relevant parameter determining the bees' foraging efficiency. For example, flowers with complex morphologies need to be longer handled by bees as compared to flowers in which rewards are easy to reach (Harder 1986; Laverty 1994). The same is true for floral colouration, as the acquisition of colours determine their detectability by bees and thus, the bees' handling time, which furthermore determines the foraging efficiency in dependence on the reward. A low flight speed facilitates the detection of flowers with less well detectable colour (Chittka *et al*. 2003; Carrasco *et al*. 2006). Flowers with less well detectable colours might compensate the resulting increase handling time by bees caused by low flight speed through larger floral rewards. Nevertheless, under certain circumstances, high flight speed can be more efficiently, even if inaccuracy increases (Burns 2005). Speed-accuracy trade-offs are thus expected for individual bees (Skorupski *et al.* 2006; for review, see Chittka *et al*. 2009). Both foraging strategies concerning the foraging speed, i.e. fast-flying and inaccurate, and slow-flying and accurate, can be found in different individual bees within a single colony (Chittka *et al*. 2003; Dyer and Chittka 2004). Depending on the heterogeneity of the distribution of floral rewards in association to flower colour, the within-colony variance might increases the fitness of the whole colony (Burns and Dyer 2008).

Flower-visiting birds possess in general a tetrachromatic colour vision system covering the visible spectrum from the UV over blue and green to red range of wavelengths (Goldsmith 1980; Cuthill *et al*. 2000; Hart 2001; Endler and Mielke 2005; Hart and Vorobyev 2005; Hart and Hunt 2007; Ödeen and Håstad 2010). Regardless of differing lifestyles, this is most probably true for all birds due to retention of four photoreceptor-types already present in the ancestor, teleost fishes (Hisatomi *et al*. 1994). However, there are at least two major groups of birds, which differ in respect of their colour vision systems: Ultraviolet-sensitive (UVS-) type birds and violet-sensitive (VS-) type birds (Ödeen and Håstad 2010).

The three major groups of flower-visiting birds are hummingbirds (Trochilidae), honeyeaters (Meliphagidae), and sunbirds (Nectariniidae), although several other groups of birds are generalist flower-visitors (Cronk and Ojeda 2008). Hummingbirds and honeyeaters belong to the VS-type birds, whereas sunbirds and other generalist foraging flower-visiting birds belong to the UVS-type birds (Ödeen and Håstad 2010). In VS-type birds, the sensitivity peak of the photoreceptor-type sensitive for short wavelengths is comparably shifted towards longer wavelengths (Ödeen and Håstad 2010). The VS-type eye is the ancestral state in birds from which UVS-type eyes evolved independently several times (Ödeen and Håstad 2010). The four different photoreceptor-types of both eye-types contain oil droplets (Bowmaker 1980; Vorobyev 2003), whereby each visual pigment of a specific photoreceptor-type is associated with a specific coloured oil droplet-type (Vorobyev 2003). The oil droplets act as long-pass filters and thus, reduce overlapping sensitivities of different photoreceptor-types in the range of shorter wavelengths (Maier and Bowmaker 1993; Bowmaker *et al*. 1997; Vorobyev 2003; Hart and Vorobyev 2005). Ultimately, oil droplets enhance the birds' ability to discriminate colours (Vorobyev 2003). Beside four types of single cones involved in colour vision, birds additionally possess double cones, whose function is thought to be in brightness discrimination, but also in motion detection, polarized light detection and magnetic field orientation (Osorio *et al*. 1999). Modelling of the tetrachromatic colour vision system requires a tetrahedron colour space (Goldsmith 1990; Neumeyer 1991, 1992; Vorobyev *et al*. 1998). The preferred colour vision model for birds is the receptor-noise limited model (Vorobyev *et al*. 1998).

Birds in general are known to rely on visual cues during food search (Schaefer *et al*. 2006), migratory path finding (Beason 1987), mate recognition (Bennett *et al*. 1994) or communication (Butcher and Rohwer 1989), but in experimental approaches, no innate preferences for distinct colours or colour parameters in respect of flower choice have been discovered yet. This is true for the flower colour choice of hummingbirds (Bené 1941; Collias and Collias 1968; Miller and Miller 1971; Stiles 1976; Goldsmith and Goldsmith 1979; McDade 1983; Delph and Lively 1989) as well as for other flower-visiting birds (Kaczorowski *et al*. 2014). However, some bird species, which forage occasionally on nectar, show preferences for specific colours, which were termed for the human visual perception (Winkel 1969). Several bird species are known to possess superior sensitivity in red wavelength range (Kühn 1929), especially hummingbirds (Herrera *et al*. 2006). The enhanced sensitivity in the red wavelength range leads to higher chromatic contrasts to the background for red flowers (Herrera *et al*. 2006). However, innate preferences for red colours in flower-visiting birds are absent (Collias and Collias 1968; Miller and Miller 1971; Stiles 1976; Goldsmith and Goldsmith 1979; McDade 1983). Nevertheless, the main driver of colour choices in flower-visiting birds are learnt associations between colour and food rewards (Bené 1941; Miller and Miller 1971; Stiles 1976; Goldsmith and Goldsmith 1979; Goldsmith *et al*. 1981; Melendez-Ackerman *et al*. 1997; Altshuler 2003). For example, territorial hummingbirds do not rely on floral colour cues, but exploit all flowers within their territory irrespective of their colouration (Brown and Kodric-Brown 1979). Thus, flower-constancy in hummingbirds is lacking. Other visual cues than colours are used by nectarivorous birds to estimate adequate floral rewards before probing the flowers (Smith *et al*. 1996; Temeles 1996; Irwin 2000). Moreover, spatial location is rather more important than colour or colour patterns for the detection of flowers, as could been verified in hummingbirds (Bené 1941; Miller and Miller 1971; Stiles 1976; Goldsmith and Goldsmith 1979; Hurly and Healy 1996; Healy and Hurly 2001, 2004) as well as in sunbirds (Kaczorowski *et al*. 2014).

Outlook of my Dissertation

This dissertation bases on four articles, published in international peer-reviewed journals, and one manuscript under review, and investigates the impact of floral colouration on the structure of competitive pollination networks. More precisely, behavioural and ecological dynamics in pollination networks comprising bird-pollinated flowers and flower-visiting birds as pollinators are investigated, verifying the role of bees as antagonistic non-pollinating and competitive visitors regarding floral colouration as filtering mechanism.

First, the spontaneous choice behaviour regarding distinct colours by bees is investigated in order to verify if specific colour parameters are necessary to attract bees as flower-visitors (Rohde *et al*. 2013). As there is an ongoing debate about which colour parameters are crucial in the colour choice behaviour in bees, different experimental set-ups are assigned, with a single colour parameter varying at time (Rohde *et al*. 2013). Bee-subjective achromatic contrasts of stimuli to the background, chromatic contrasts of stimuli to the background as well as between different stimuli, and spectral purity as colour parameters are under investigation (Rohde *et al*. 2013). The results are assigned to the natural red colouration of flowers pollinated by either bees or birds (Lunau *et al*. 2011). Differences in several colour parameters between bee- and bird-pollinated red flowers are determined and assigned to further choice experiments in bees and hummingbirds towards the colours, found in nature (Lunau *et al*. 2011). This is done in order to estimate the role of pollinators and non-pollinating visitors on the selection of flower colouration in these two pollination systems and in order to gain insight into the common, but still non-clarified association between a red colouration and flowers pollinated by birds (Lunau *et al*. 2011). Moreover, it is verified whether other colours of bird-pollinated flowers create private communication channels in which birds are free from competition (Lunau *et al*. 2011, Chapter 4).

Chapter 5 and 6 cover the floral pigment chemistry and the impact of the structure of epidermal cells on the colouration of flower colours and consequences for animal-plant interactions. Chapter 5 deals with the impact of varying pigment concentration on the colour choice by bees (Papiorek *et al*. 2013). The results gain insight into evolutionary selection on flower colours by bees and help to understand behavioural dynamics in competitive pollination networks (Papiorek *et al*. 2013). Moreover, the results are discussed concerning natural variability of floral colouration and the evolution of floral colouration in respect of the colour vision system of bees as flower-visitors (Papiorek *et al*. 2013). Finally, chapter 6 deals with the contribution of the surface structure to floral colouration by determining the epidermal cell shape of bee- and bird-pollinated flowers and studying their impact on floral colouration and on gloss properties (Papiorek *et al.* 2014). This is done in order to study developmental possibilities of flowers to adapt their colouration to specific pollinators and non-pollinating antagonists on the one hand and in order to consider the impact of mechanical floral traits on the pollination-network structure on the other hand (Papiorek *et al.* 2014). Moreover, studying the contribution of the surface structure to floral colouration might gain insight into the previously encountered low reflectance in the visual range of less than 30% measured in white flowers, which would be adequate for a dark grey colour. More precisely, white flowers do not reflect 100 percent of incident light, although this is theoretically predicted (Lunau *et al.* 2011). Thus, gaining insight into surface-dependent scattering of incident light in flowers might explain former results and help to understand how flower colours appear for distinct flower-visitors depending on their point of view (Papiorek *et al.* 2014).

CHAPTER 2

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ORIGINAL PAPER

Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours

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Abstract Differences in the concentration of pigments as well as their composition and spatial arrangement cause intraspecific variation in the spectral signature of flowers. Known colour preferences and requirements for flower-constant foraging bees predict different responses to colour variability. In experimental settings, we simulated small variations of unicoloured petals and variations in the spatial arrangement of colours within tricoloured petals using artificial flowers and studied their impact on the colour choices of bumblebees and honeybees. Workers were trained to artificial flowers of a given colour and then given the simultaneous choice between three test colours: either the training colour, one colour of lower and one of higher spectral purity, or the training colour, one colour of lower and one of higher dominant wavelength; in all cases the perceptual contrast between the training colour and the additional test colours was similarly small. Bees preferred artificial test flowers which resembled the training colour with the exception that they preferred test colours with higher spectral purity over trained colours. Testing the behaviour of bees at artificial flowers displaying a centripetal or centrifugal arrangement of three equally sized colours with small differences in spectral purity, bees did not prefer any type of artificial flowers, but preferentially choose the most spectrally pure area for the first antenna contact at both types of artificial flowers. Our results indicate that innate preferences for flower colours of high spectral purity in pollinators might exert selective pressure on the evolution of flower colours.

Keywords *Bombus terrestris* · *Apis mellifera* · Colour preference · Flower colour · Spectral purity

Introduction

Entomophilous flowers display visual, olfactory, gustatory and tactile stimuli to attract pollinating insects (Kugler 1935; von Frisch 1967; Kevan and Lane 1985; Dafni et al. 1997; Raine and Chittka 2006; de Brito Sanchez et al. 2007). The colour of flowers is used for detection and recognition of food plants in various taxa of pollinators such as hummingbirds (Trochilidae), hoverflies (Syrphidae), butterflies (Lepidoptera) and bees (Apoidea) (Ilse 1949; Stiles 1976; Lunau and Maier 1995; Weiss 1997); even nocturnal pollinators such as hawkmoths are known to orient by means of floral colours (Kelber et al. 2002).

Bees are known to use flower colour for the detection of flowers by evaluating the green contrast of the target object against the background (Lehrer and Bischof 1995). For flower-constant foraging bees, the flower colour is an important cue to identify flowers of the current food plant species (Chittka et al. 1999). Flower colours are, however,

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subject to variation, because the concentration as well as the composition of floral pigments varies due to genetical or environmental influences. Many studies about variation in flower colours deal with discontinuous variation, e.g. distinct colour morphs occurring without intermediate coloured individuals (Meléndez-Ackerman et al. 1997; Matsumura et al. 2006; Whibley et al. 2006; Shipunov et al. 2011). Less well studied but probably more common is continuous colour variation of a single morph caused by more subtle differences in the composition and concentration of floral pigments (Tourjee et al. 1993; Tastard et al. 2008).

There are probably major selective pressures on flower colours exerted by pollinators (Chittka and Menzel 1992; Dyer et al. 2012). Since some pollinators learn the flower colour of their concurrent food plant, flower-constant foraging pollinators might overlook flowers exhibiting a strong deviance from the prevalent flower colour and thus less effectively pollinate flowers of deviant colour. On the other hand, individual plants displaying more attractive flowers than other conspecifics might benefit in terms of increased pollination success. It is plausible that bees which forage flower constant at flowers of a given wild type would probably not visit highly distinct colour morphs rather than subtle variations in flower colours fitting better to their spontaneous preferences. The ability (and limitations) (Dyer and Chittka 2004a; Goulson et al. 2007) for within-species and between-species preferences in bees has been demonstrated in cases in which the flowers change colour (Asmussen 1993) or odour (Dobson et al. 1999) during anthesis.

In this study, we use artificial flowers with small (via the colour hexagon) and defined differences in selected colour parameters to test how trained bees respond to test colours offering small shifts towards shorter and longer dominant wavelength or towards lower and higher spectral purity. Shifts of the dominant wavelength affect the perceived hue of a colour, whereas changes of the spectral purity affect the saturation of a colour with less spectrally pure colours appearing less saturated or more greyish. Although the colour vision system of bees and humans differ, it is likely that these changes are similar for both (Lunau et al. 1996). The differences in dominant wavelength and spectral purity used here provide small and similar differences in colour contrast. The shifts of the dominant wavelength are denominated in relation to the reference colour due to the direction of the shift, e.g. more bluish, more greenish, whereas the changes of the spectral purity are denominated in relation to the reference colour more (+) or less (−) spectrally pure. The experimental design allows for testing the hypotheses (1) whether trained bees prefer any test colour over the trained colour, (2) which colour parameter, i.e. dominant wavelength or spectral purity, predicts the choice behaviour, and (3) which direction of these colour parameters, i.e. to shorter or to longer dominant wavelength, resp. higher or lower spectral purity, predicts choice behaviour. Offering a triple choice that included the trained stimulus, a stimulus falling below the trained stimulus in a distinct colour parameter, and a stimulus surpassing the trained stimulus in this parameter, we aim at testing which, if any, colour stimulus trained bees will prefer over the trained one. By contrast with differential conditioning (training method using rewarded stimulus and unrewarding distractor stimuli), the absolute condition (training method using only rewarding stimuli) applied in this study, facilitates choices of novel colours in the tests. Moreover, absolute conditioning hampers peak shift (see below) and fine colour discrimination in the test phase (Dyer and Chittka 2004a).

The assortment of training and test colours takes into account that the colour choice behaviour of naïve bumblebees (Lunau 1990) has been interpreted as preference of spectrally pure colours (Lunau et al. 1996) and, alternatively, as preference of a distinct dominant wavelength, i.e. 410 nm (Gumbert 2000). Moreover, Gumbert (2000) showed that trained bees generalised flower colours; among well-distinguished test colours they chose the test colour which was most similar to the trained one and only chose flower colours according to their innate preferences, if test colours were too different from the training stimuli. To reflect the natural variability of flower colours, the test colours used in this study are quite similar and not well distinguished from the trained colour at least due to estimates from spectral reflection properties and colour differences in the colour hexagon (Figs. 1, 2). The maximal colour contrast between the training colour and one of the test colours (training colour excluded) amounts to 0.068 hexagon units for bumblebees and to 0.075 hexagon units for honeybees.

A further cause of intraspecific variation among flowers arises from the uneven distribution of floral pigments within the visual signalling apparatus of flowers, causing petal venation and colour patterns (Heuschen et al. 2005; Lunau 2006; Shang et al. 2011). The role of small contrastingly coloured areas, often termed as floral guides, for

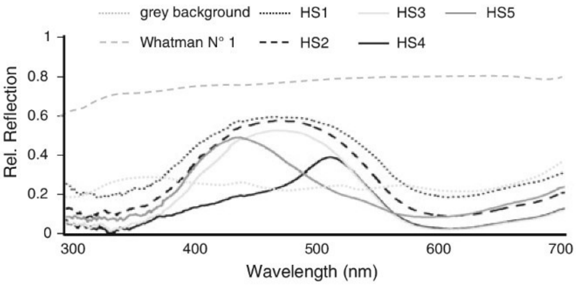

Fig. 1 Spectral reflection of the training and test stimuli due to the HSB system and spectral reflection of uncoloured Whatman filter paper No. 1 and of grey background; stimuli of Experiments 1, 2 and 3

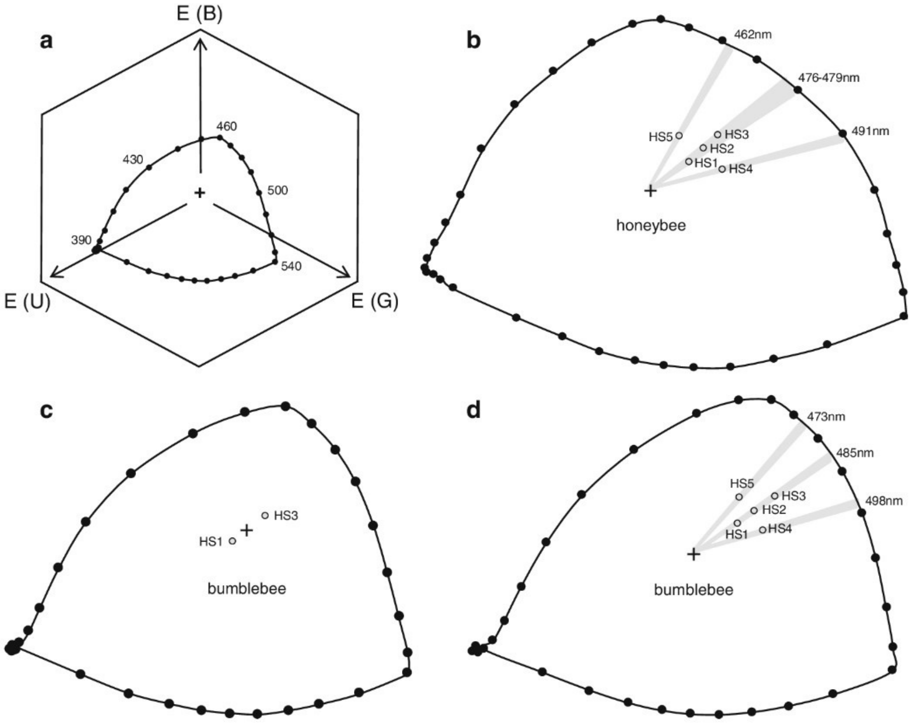

Fig. 2 Colour loci (*open circles*) of training and test stimuli in the colour hexagon (Chittka 1992) showing the calculated excitation of ultraviolet [E(UV)], blue [E(BL)] and green [E(GR)] photoreceptor types, indicated by *arrows*. The + in the centre of the colour hexagon indicates the locus of the background colour to which the photoreceptors are assumed to be completely adapted. **a** Overview of the total colour hexagon for the bumblebee *Bombus terrestris*. The spectral locus is indicated by *filled black circles* connected by a *line*. Some loci of the spectral line are labelled with the dominant wavelength. **b** Colour loci of stimuli used in Experiment 1 inside the spectrum locus for the honeybee *Apis mellifera* in natural daylight condition and grey background. The hues of stimuli are indicated and highlighted in grey. **c** Colour loci of stimuli used in Experiment 2 inside the spectrum locus for the bumblebee *Bombus terrestris* in fight cage light conditions and background HS2. **d** Colour loci of stimuli used in Experiments 1 and 3 inside the spectrum locus for the bumblebee *Bombus terrestris* in fight cage light conditions and grey background. The hues of stimuli are indicated and highlighted in grey

receiving attention from approaching bees and guiding them towards landing sites, is well known (Lunau et al. 1996, 2006, 2009; Hempel de Ibarra et al. 2001; Pohl et al. 2008). The function of colour patterns caused by small variations of a single colour, however, is not fully understood. By analysing the bees' behaviour at an artificial flower displaying a subtle colour gradient as compared to an artificial flower displaying an inverse colour gradient, we test the hypotheses (1) whether the colour contrast of the peripheral area of the artificial flower against the background colour is important for the preferential choice behaviour, and (2) if not, whether the spatial arrangement of colours impacts the choice of the landing site on the artificial flower. The former hypothesis is related to the assumed importance of the contrast between the target and the background colour for the detectability. The latter hypothesis is based on the observation that honeybees as well as bumblebees use colour cues to choose the site of the first antennal contact with flowers (Lunau et al. 1996, 2006; Hempel de Ibarra et al. 2001; Pohl et al. 2008). The trained bumblebees' approach was tested at two artificial flowers which offered a three-stepped gradient of coextensive circular equal-sized areas, in which the middle ring offered the training colour and the outer and the inner ring offered a colour of slightly lower bee-subjective spectral purity or slightly higher spectral purity, respectively; in addition the area of the first antennal contact was tested.

Bumblebees and honeybees are well suited for testing the attractiveness of non-trained colours in choice tests, because many aspects of their colour vision and their use of colour cues have been studied. Both genera are best known to possess innate colour preferences (Lunau 1990; Giurfa et al. 1995; Lunau et al. 1996) and to use colour signals for distant attraction towards flowers (Simonds and Plowright 2004; Dyer et al. 2008) and for targeting landing sites on flowers (Lunau 1991, 1992; Lunau et al. 2006; Pohl et al. 2008). Moreover, bumblebees learn to orient by means of floral colour cues, although innate preferences may still affect choice behaviour (Gumbert 2000; Pohl et al. 2008). A distinctive feature in the visual detection of flowers by honeybees and bumblebees is that they evaluate the green contrast when the flower is viewed under a small visual angle and evaluate colour signals of flowers when the visual angle surpasses a certain level (Giurfa et al. 1997; Spaethe et al. 2001; Dyer 2006; Dyer et al. 2008).

Many aspects of colour vision in bumblebees and honeybees have been thoroughly studied, such as learning of spectral colours (Menzel 1967), wavelength discrimination (von Helversen 1972) and simultaneous and successive colour contrast (Neumeyer 1980, 1981), and modelling of colour vision for bees (Backhaus 1991; Chittka 1992; Brandt and Vorobyev 1997; Vorobyev and Osorio 1998; Chittka 1999; Vorobyev 1999; Vorobyev et al. 1999). It is less well known whether and how innate colour preferences affect learning, since previous studies of colour preferences in trained bees had different outcomes: Guirfa and Núñez (1989) found no asymmetry for colour choice in tests with reciprocal reward, whereas Smithson and McNair (1996) found a bias towards the more conspicuous artificial flowers.

We explicitly test whether there are more attractive colours than the trained colours for distant attraction and for targeting at landing sites, which colour parameter is responsible for an increased attractiveness beyond learned values, and how pigment concentration is related to the attractiveness of colours.

Materials and methods

Training experiments

The experiments with bumblebees indoors and honeybees outdoors used an absolute conditioning paradigm, in which only rewarding stimuli, but no distractor stimuli providing either no reward or a repellent reward, were used during the training (Dyer and Chittka 2004a).

Experiment 1: variation in spectral purity versus dominant wavelength

In this experiment, we intended to study the colour choice of trained bees, bumblebees and honeybees, for colours of a slightly deviant dominant wavelength or a slightly deviant spectral purity as compared to the training colour. For this purpose, we used the Corel Draw 12 software (HSB system, i.e. Hue, Saturation and Brightness) to produce five different colours which were printed with a Canon Pixma MP630 onto Whatman No. 1 filter paper. Three of these colours (H180S30B100/H180S50B100/H180S70B100; HS1/HS2/HS3 in the text) provide bee-subjective differences in spectral purity, whereas three others (H170S70B100/H180S50B100/H250S60B100; HS4/HS2/HS5 in the text) provide differences in the bee-subjective dominant wavelength (Fig. 1; Table 1). One colour (HS2) offers a very similar hexagon distance to the other four colours (Fig. 2b, d).

Each bee was trained to artificial flowers offering the training colour HS2 and presented on a grey background. Three artificial training flowers were presented simultaneously side by side with 7 cm interspace and were filled with a small droplet (5 µl) of 100 % Apiinvert as a reward. Using a transparent tube, the bee was placed on the artificial flower until it started to take up the reward. Having emptied the reward, the bee flew to the next artificial flower. During the visit to this artificial flower, the bee was caught with the transparent tube. From this tube the bee was then released at a distance of 60 cm from the artificial flowers and caught again after each visit. After ten visits and rewards on the artificial training flower the training was stopped. The test started immediately after the training. In the test, three different artificial flowers were presented including the artificial training flower. The artificial test flowers were presented in the same location as the artificial training flowers. During the test, the artificial flowers offered a droplet of pure water which was of the same size as the droplet of Apiinvert reward on artificial training flowers, ensuring that the bees were not able to visually discriminate between training and artificial test flowers. Unrewarded test trials and rewarded training trials alternated. Again, each trial started from the transparent tube at a distance of 60 cm from the artificial flowers. This was iterated until ten visits towards the test situation had been monitored. The artificial training flowers were refilled after a bee had visited them. Altogether, 20 individual bumblebee workers were tested. Although the bees were captured up to 15 times, they cooperated well in this training and test procedure.

This experiment was performed with bumblebee workers in a flight cage with artificial light, and also with workers of *Apis mellifera* under natural daylight conditions to check whether the findings have more general validity. The comparative experiments with freely flying honeybees were done on sunny days in the Botanical Garden of the Heinrich-Heine University Düsseldorf.

Experiment 2: impact of sign of spectral purity contrast

This experiment was planned as an experiment to study the impact of the sign of the spectral purity contrast on the colour choice of bumblebees. Since spectral purity is a signed measure of perceptual distance, not only the value of spectral purity of the artificial flower colour, but also the fact that the artificial flower colour is of higher or of lower spectral purity than the background colour might affect the colour choice. For this purpose, we modified experiment 1, in which the difference in spectral purity between HS1 and

Table 1 Quantum fluxes, receptor-specific contrasts (including green contrast), colour contrasts and spectral purity of training and test colours

Bombus terrestris in flight cage illumination

Stimulus	Exp., Fig.	Quantum flux			Receptor-specific contrast to the background			Colour contrast		
		UV	BL	GR	UV	BL	GR	ΔS	H	SP
Grey background	Exp. 1–2	0.61	4.02	4.32	1.00	1.00	1.00	0.00	0.00	0.00
HS1	Exp. 1, Fig. 3	0.70	8.57	8.61	1.13	2.13	2.00	0.42	0.14	+0.31
HS2	Exp. 1, Fig. 3	0.51	7.92	7.58	0.84	1.97	1.76	0.56	0.20	+0.44
HS3	Exp. 1, Fig. 3	0.33	6.93	6.32	0.54	1.72	1.46	0.75	0.26	+0.59
HS4	Exp. 1, Fig. 3	0.26	3.43	4.39	0.42	0.85	1.02	0.56	0.19	+0.42
HS5	Exp. 1, Fig. 3	0.42	6.89	5.15	0.68	1.71	1.19	0.59	0.20	+0.42
HS1 versus HS2	Exp. 2, Fig. 4	0.70	8.57	8.61	1.35	1.08	1.14	0.14	0.05	−0.24
HS3 versus HS2	Exp. 2, Fig. 4	0.33	6.93	6.32	0.65	0.88	0.83	0.19	0.07	+0.30

Apis mellifera in natural daylight

Stimulus	Fig.	Quantum flux			Receptor-specific contrast to the background			Colour contrast		
		UV	BL	GR	UV	BL	GR	ΔS	H	SP
Grey background		4.91	16.87	28.34	1.00	1.00	1.00	0.00	0.00	0.00
H1	Fig. 3	5.54	34.30	51.12	1.13	2.03	1.80	4.12	0.13	+0.29
H2	Fig. 3	4.04	31.29	43.42	0.82	1.85	1.53	5.69	0.18	+0.40
H3	Fig. 3	2.74	27.06	34.94	0.56	1.60	1.23	7.42	0.23	+0.51
H4	Fig. 3	1.92	14.01	27.66	0.39	0.83	0.98	5.72	0.20	+0.39
H5	Fig. 3	3.43	26.51	27.61	0.70	1.57	0.97	6.22	0.17	+0.39

The receptor-specific contrasts were calculated as quotient of quantum fluxes of stimulus and background. The chromatic contrast to the background Δ*S* is given in just noticeable difference units (JNDs) and calculated using Receptor noise limited model (Vorobyev and Osorio 1998), whereas H is given in Hexagon units and calculated using Colour Hexagon model (Chittka 1992)

UV ultraviolet-receptor type, *BL* blue-receptor type, *GR* green-receptor type, *SP* spectral purity given in relative units

HS3 as well as HS2 and HS3 was similar for bumblebees and honeybees (Table 1). During training, two identical artificial flowers (HS2) were presented against a grey background, whereas in the tests (without intermittent training) two rewarding artificial flowers (HS1, HS3) were offered against a background formerly used as training colour of the artificial flowers (HS2), which was identical to the colour of the trained artificial flowers in Experiment 1 (Figs. 1, 2c). The artificial flowers were the same as in Experiment 1 and presented with 3 cm interspace. As in Experiment 1, the bumblebees were caught in a transparent tube after each visit and then released at the opposite end of the flight box at a distance of 136 cm from the artificial flowers. Following ten training trails, the bumblebees accomplished ten test trials with rewarded artificial flowers. Altogether ten individual bumblebee workers were tested.

Experiment 3: reciprocity of within-flower spectral purity gradient

This experiment was done to evaluate the impact of spatial arrangement of colours on overall colour choice and on the bumblebees' choice of a landing site. In this experiment, the conditions were similar to those in Experiment 2. In addition, a digital camera (Casio Exlim Ex-F1) placed above the artificial flowers was used to document the site of the first antennal contact made by the tested bumblebees at the artificial flowers (see supplementary material, Fig. S1). The artificial test flowers consisted of three coextensive concentric rings, made up by the colours HS1, HS2, HS3 (Corel Draw 12, HSB system) printed with a Canon Pixma MP630 onto Whatman No. 1 filter paper (Figs. 1, 2d). These colours were identical to those tested in Experiment 1. The inner, middle, and outer rings of the artificial flowers had an equal surface area. The bumblebees were trained with two single-coloured artificial flowers (HS2). In the test, two artificial flowers were offered simultaneously in which the inner/middle/outer ring consisted of HS1/HS2/HS3 and HS3/HS2/HS1, respectively. All artificial flowers were presented against a grey background.

Bee keeping and training

The flower naïve, individually marked workers of a bumblebee colony (*Bombus terrestris*; Koppert, NL) were kept in a nesting box which was connected via a transparent

Perspex tunnel to a feeding box made of transparent, UV-permeable Perspex. The bees were fed with 50 % Apiinvert solution (72 % sugar syrup of sucrose [30 %], glucose [31 %] and fructose [39 %]) through plastic syringes suspended in the feeding box. Pollen was provided in the nesting box.

The Experiment 1 was conducted in a flight cage (2 m × 2 m × 2 m) illuminated by eight UV- and VIS-emitting fluorescent tubes (Osram L58W/865). The light intensity in the flight cage at the artificial flowers was about 2000 lx. The artificial flowers were composed of a coloured circular corolla of "Whatman" filter paper No. 1 and were connected to a transparent plastic stick offering a droplet of sugar syrup in a small drilled hole during training as a reward and a droplet of water during the tests. Individual bees were trained using absolute conditioning and in the test given the choice between the three test stimuli, including the training stimulus; during the test phase test trials and training trials alternated. This experimental design allows comparison of the impact of innate and trained preferences upon the colour choice behaviour as well as observation of modifications of colour choice under continued training. The colour differences between the tested artificial flower colours were large enough that the bees respond noticeable different towards the stimuli but at the same time were small enough that the bees did not solely respond to the trained colour which in all experiments was one of the test colours (Gumbert 2000). According to Dyer (2006), bumblebees cannot discriminate between colours, in which the colour contrast is smaller than 0.06 hexagon units, and poorly discriminate between colours (63 % correct choices), in which the colour difference is smaller than 0.1 hexagon units. The colour contrast between the training colour and the test colours (training colour excluded) averages to 0.59 hexagon units for bumblebees and to 0.63 hexagon units for honeybees.

For training one individually marked bumblebee worker was caught when walking from the nesting box through the tunnel to the feeding box, using a transparent tube. The transparent tube was used to carry the worker to the flight cage, which was in the same room but separated from the colony setup. The worker was released at one of the training stimuli in a manner such that the worker could detect the sugar-syrup reward. Up to three attempts were sufficient for the workers to approach the training stimuli by themselves when released at a starting point situated 60 cm in front of the stimuli. Only one worker was allowed to fly in the flight cage each time. For the subsequent training and test with individual workers, each worker was tested only once in a given experiment, but some workers were tested under different conditions. Training stimuli were not used as test stimuli and visited test stimuli were replaced by new or cleaned test stimuli to avoid choices based upon chemical cues such as olfactory footprints (Witjes and Eltz 2007). When a worker visited a test stimulus, it was caught in the transparent tube; after the visited stimulus was exchanged the worker was released from the starting point. The short distance of 60 cm between the starting point and the test stimuli enabled the experimenter to release the bumblebee at the starting point and to catch it at the test stimulus without disturbing the bumblebee by his movements. When the worker did not approach the stimuli any longer, it was caught and released in the tunnel. After the worker had emptied the honey stomach, it was caught again and the training or test was continued. The results of the choice tests were assorted in groups of five subsequent trials to check whether the bumblebees retain or change their spontaneous preferences.

The experiments 2 and 3 were conducted in a flight tunnel which offered two rewarding artificial flowers at one end of a grey, plastic-walled flight box (136 cm × 27 cm × 19 cm) which was covered with UV-transmittent Perspex plates. In this experiment, individual bumblebees can be tested without training. The flight box was illuminated by UV- and VIS-emitting fluorescent tubes (Osram L58W/865) providing a light intensity in the flight box of 2000 lx. The reason for working with this experimental setup was its suitability for experiments with reduced training, because the bumblebees usually approached the artificial flowers in the first test trial. Moreover, in Experiment 2 the flight tunnel and its length better ensured that all bumblebees viewed the artificial flowers for some time against the background before landing. In experiment 3, the flight tunnel facilitated the use of a video camera. Each bumblebee worker was used only once for the experiments.

Experiment 1 was also conducted with honeybees in an outdoor setting. A small number of experienced workers of the Western honeybee were ad libitum rewarded with sugar water at an outdoor feeding place without colour cues. The concentration was varied between 4 and 26 % such that between 10 and 20 workers were visiting the feeding place. The honeybee workers were individually marked when feeding using water-soluble colours. Individually marked workers were brought in a plastic tube to the experimental setup which was housed in an outdoor flight cage to avoid visits of other bees. This flight cage was of similar size to that used for the indoor experiments with bumblebees, made of a mosquito net, and was placed in half shade under a tree.

Spectral reflection properties of colour stimuli

The spectral reflection of the artificial flowers (Fig. 1) was measured with the spectrophotometer USB 2000 (Ocean Optics, Inc., Ostfildern, Germany). Illumination was provided by a deuterium–halogen light source (D_2H; World Precision Instruments, Sarasota, FL, USA). Measurements were taken at an angle of 45° to the surface of the measuring spot (Chittka and Kevan 2005) and calibrated against a white standard of a barium sulphate pellet and against a black standard of black film can.

In many recent publications about colour vision, colour discrimination and colour learning of bees, the green contrast (Dyer 2006), dominant wavelength (Giurfa et al. 1995; Gumbert 2000) and the colour distance between target and background (Giurfa et al. 1997) have been considered as relevant parameters. We use the terms intensity, dominant wavelength and spectral purity as physical-physiological analogues to the perceptual attributes brightness, hue and colour saturation. We calculated these and other parameters as follows.

The quantum flux (Q_i) is given by:

$$Q_i = \int_{300}^{700} S_i(\lambda) I(\lambda) D(\lambda)\, \mathrm{d}\lambda \tag{1}$$

where $S_i(\lambda)$ denotes the spectral sensitivity function of the photoreceptor type i (UV, Blue and Green) of the bumblebees' (Peitsch et al. 1992) and of the honeybees' photoreceptors (Menzel and Backhaus 1991), $I(\lambda)$ denotes the spectral reflectance function of the stimulus, $D(\lambda)$ denotes the spectral distribution of the illuminant and $\mathrm{d}\lambda$ denotes the wavelength step size.

The receptor-specific contrast between stimulus and background (q_i) is given by

$$q_i = \frac{Q_i(\text{stimulus})}{Q_i(\text{background})}. \tag{2}$$

The green contrast between target and background defines the achromatic contrast perceived by the green type of photoreceptors. To calculate the chromatic contrast in the hexagon colour space (Chittka 1992) the relative amount of light absorbed by each photoreceptor type (P) is given by:

$$P = Q_i \times R \tag{3}$$

where the sensitivity factor R is given by:

$$R = 1 \Big/ \int_{300}^{700} S_i(\lambda) I_B(\lambda) D(\lambda)\, \mathrm{d}\lambda \tag{4}$$

simulating the adaptation of the photoreceptor types to the background function (I_B).

The transduction of photoreceptor absorption (P) into receptor excitation (E) is given by:

$$E = \frac{P}{P+1}. \tag{5}$$

The colour contrast results from the perceptual distance of the colour of the artificial flower and the background and is given in hexagon units (Chittka 1992). The spectral purity results from the perceptual distance between a colour and the centre of the hexagon in relation to the perceptual distance between the colour of the spectral line of corresponding dominant wavelength and the centre of the hexagon (Lunau et al. 1996):

$$SP = \frac{H_{i(\text{target}-\text{background})}}{H_{i(\text{spectral locus}-\text{background})}} \tag{6}$$

where i is the corresponding dominant wavelength. The colours of the spectral locus thus all possess a spectral purity of 1. Note that in the colour hexagon model the colours of the spectral loci have different perceptual distances to the background.

Chromatic contrasts were also calculated for the receptor noise limited model (Vorobyev and Osorio 1998). Chromatic contrasts (ΔS), in just-noticeable difference (JND) units, are given by:

$$\Delta S = \sqrt{\frac{\omega_{\mathrm{UV}}^2(\Delta f_G - \Delta f_B)^2 + \omega_B^2(\Delta f_G - \Delta f_{\mathrm{UV}})^2 + \omega_G^2(\Delta f_{\mathrm{UV}} - \Delta f_B)^2}{(\omega_S\omega_M)^2 + (\omega_S\omega_L)^2 + (\omega_M\omega_L)^2}} \tag{7}$$

where ω_i denotes the standard deviation of noise [$\omega_{\mathrm{UV}} = 0.13$, $\omega_B = 0.06$ and $\omega_G = 0.12$ for the honeybee (Vorobyev et al. 2001); $\omega_{\mathrm{UV}} = 1.3$, $\omega_B = 0.9$ and $\omega_G = 0.9$ for the bumblebee (Skorupski and Chittka 2010)] and Δf_i the difference in receptor signal (f_i) between stimulus and background:

$$f_i = \ln(q_i). \tag{8}$$

Colours are indiscriminable, if $\Delta S < 2.3$ (Vorobyev et al. 2001). It should be noted that Skorupski and Chittka (2010) found different receptor noise in bumblebees and honeybees which has been considered here. Moreover, Dyer and Neumeyer (2005) reported different ΔS values depending on the conditioning method.

The dominant wavelength assigns a wavelength to colour according to a given colour vision model. The colour distance between target and background describes the perceptual colour distance according to a given colour vision model (for all calculated data see Table 1).

Statistical analysis

Preference for test stimuli: The difference between the observed distributions for the two or three treatments for each trial was compared with a random distribution using a Wilcoxon matched-pairs signed-rank test. To test for an effect of the number of training events during the tests, when unrewarded choice test trials alternated with rewarded training trials, the observed distributions of choices of all bumblebees for the trained colour and for one test colour between the first five trials and the last five trials were compared using a Chi-square test.

Results

Stimuli

The colour hexagon (Chittka 1992) as a standard colour vision model (see handbook of pollination biology by Dafni et al. 2005) was used to depict the perceptual distances between the tested colours as perceived by the bumblebee *B. terrestris* and the Western honeybee *A. mellifera* (Fig 2). From the centre to the spectral locus the spectral purity increases, whereas the dominant wavelength varies along a circular around the centre. The colour loci of the artificial flower colours of Experiment 1 are arranged crosswise in the colour hexagon in a manner that three of the loci represent a variation in dominant wavelength and three others represent a variation in spectral purity holding for bumblebees and honeybees (Fig. 2b, d; Table 1). The quantum flux values for each receptor type, the values of receptor-specific contrasts including green contrast, the chromatic contrast calculated with the colour hexagon model and with the receptor-noise limited model as well as the spectral purity of all training and test colours are listed for both tested species in Table 1.

Experiment 1: variation in purity versus dominant wavelength

Following training to artificial flowers of a medium spectral purity, the bumblebees significantly preferred artificial flowers of higher spectral purity and significantly discriminated against artificial flowers of lower spectral purity (Fig. 3a; Table 1, Supplemental material Table S1). An effect of the number of training events during the sequence of test trials was not found (Fig. 3a, Supplemental material Table S1). Following training to artificial flowers of a specific colour, bumblebees approached artificial flowers of the training colour significantly more often than artificial flowers of equal spectral purity, but of different dominant wavelength (Fig. 3c; Table 1, Supplemental material Table S1). An effect of the number of training events during the test stage was not found (Fig. 3c, Supplemental material Table S1).

Also honeybees, following training to artificial flowers of a medium spectral purity, significantly preferred artificial flowers of higher spectral purity over artificial flowers with the trained colour and significantly discriminated against artificial flowers of lower spectral purity (Fig. 3b; Table 1). Following training to artificial flowers of the same colour, the honeybees approached artificial flowers of the training colour significantly more often than artificial flowers of equal spectral purity, but of different dominant wavelength (Fig. 3d; Table 1).

Experiment 2: impact of sign of spectral purity contrast

After being trained to artificial flowers of a distinct colour presented against a grey background, bumblebees significantly preferred an artificial flower of higher spectral purity over an artificial flower of lower spectral purity presented against a background of the training colour of a middle spectral purity. The green contrast between target colour and background did not predict the bumblebees' choices (Fig. 4; Table 1). An effect of the number of training events during the test stage was not found (Fig. 4, Supplemental material Table S1).

Experiment 3: reciprocity of within-flower spectral purity gradient

When trained to single-coloured artificial flowers and tested by artificial flowers displaying a pattern of three coextensive circular rings, the bumblebees exhibited no preference when approaching the artificial flowers; 136 approaches were directed towards the artificial flower offering the spectrally purest colour in the centre and 164 approaches were directed towards the artificial flower offering the spectrally purest colour in the periphery (Fig. 5a). At close range, the bumblebees exhibited significant preferences for targeting at the site of and making antennal contact with the area of highest spectral purity irrespective of its position in the innermost or outermost ring (Fig. 5b); at artificial flowers with a centripetal spectral purity pattern 77.2 % of the approaches were directed towards the innermost ring and ended up with an antennal contact at this area of highest spectral purity, whereas at artificial flowers with a centrifugal spectral purity pattern only 28.0 % of the approaches were directed towards the innermost ring and ended up with an antennal contact at this area of lowest spectral purity (Fig. 5b). During the test stage, the bumblebees learned to make antennal contact with the area closer to the centre, even if the spectrally purest colour was at the outermost ring of the artificial flowers (Supplemental material Table S1). Photographs of antennating bumblebees are shown in the supplementary material (Fig. S1).

Discussion

This study was intended to survey the relevant colour parameter determining decisions about flower visits of colour-experienced bees when choosing among already acquired colours and similar, but slightly deviant colours. The dominant wavelength and spectral purity of the target stimuli, as well as the green contrast and colour contrast between colour stimuli and the background have been

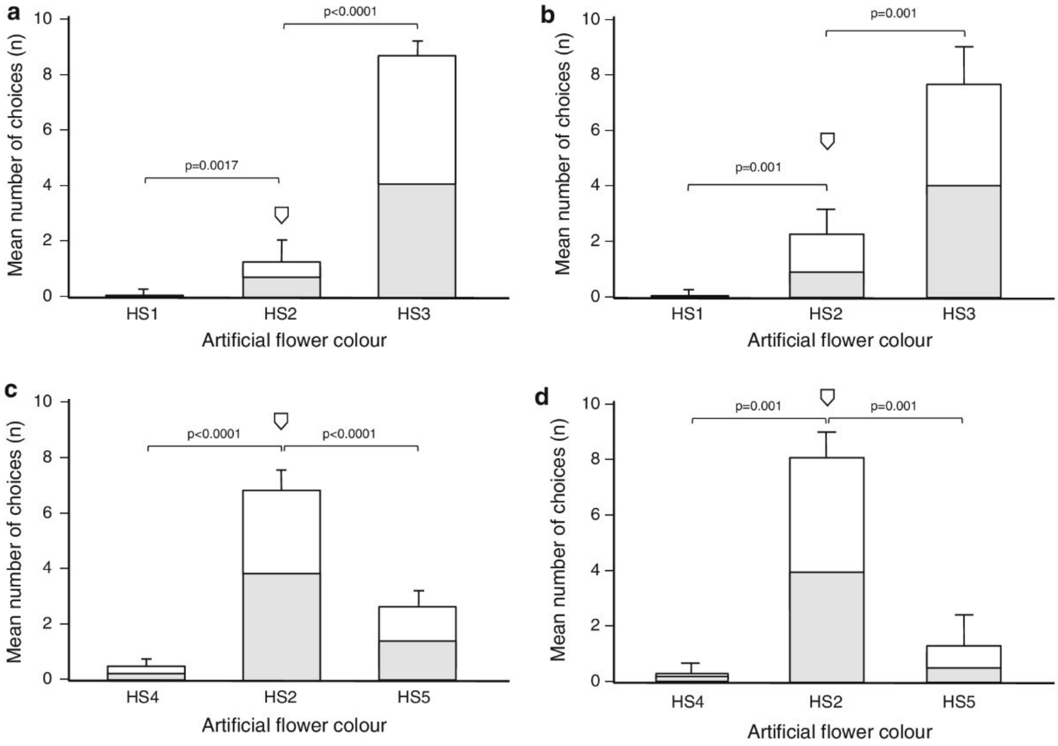

Fig. 3 Mean number of approaches by flower-naïve bumblebees (*Bombus terrestris*) ($n = 20$) and honeybees (*Apis mellifera*) ($n = 10$) during a sequence of ten approaches that were directed to three artificial flowers of different colours (Experiment 1) which varied **a** in spectral purity (HS1; HS2; HS3) for *B. terrestris*; and **b** for *A. mellifera*; **c** in dominant wavelength (HS4; HS2; HS5) for *B. terrestris*; and **d** for *A. mellifera*. The mean number of approaches is indicated with the upper 95 % confidence interval. The training stimulus is indicated by a *white arrow*. Statistical analysis: Wilcoxon matched-pairs signed-rank test. *Grey part of columns* first five approaches of each bee, *white part of columns* last five approaches of each bee

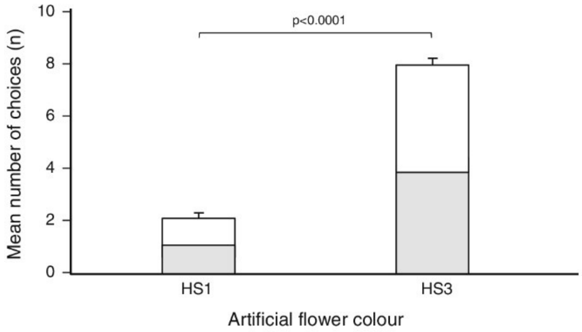

Fig. 4 Mean number of approaches by flower-naïve bumblebees (*Bombus terrestris*) ($n = 20$) with training on HS2 during a sequence of ten approaches that were directed to two artificial flowers of different sign of colour purity (HS1; HS3) presented against a background of HS2 (Experiment 2). The mean number of approaches is indicated with the upper 95 % confidence interval. Statistical analysis: Wilcoxon matched-pairs signed-rank test. *Grey part of columns* first five approaches of each bumblebee, *white part of columns* last five approaches of each bumblebee

considered as colour parameters determining their spontaneous colour preferences (Lunau 1990; Lunau et al. 1996; Giurfa et al. 1995, 1996, 1997; Gumbert 2000; Dyer et al. 2008). The results indicate that, if the differences between training and test colours are attributed to their dominant wavelength, honeybees as well as bumblebees exhibit strong fidelity for the trained colour. In contrast, if the differences between training and test colours are attributed to their colour purity, honeybees as well as bumblebees exhibit preferences for colours of higher colour purity and discriminated against colours of lower colour purity. Besides the amount of spectral purity, also the sign of spectral purity contrast between target colour and background influences the bumblebees' choice behaviour.

In the experiments presented in this paper, there was no shift towards shorter dominant wavelengths as might be predicted by studies stating one major peaks of wavelength-dependent colour choice at 410 nm (Gumbert 2000); however, it remains to be tested whether dominant wavelength effects occur with colour stimuli that have a dominant wavelength more close to 410 nm. A shift

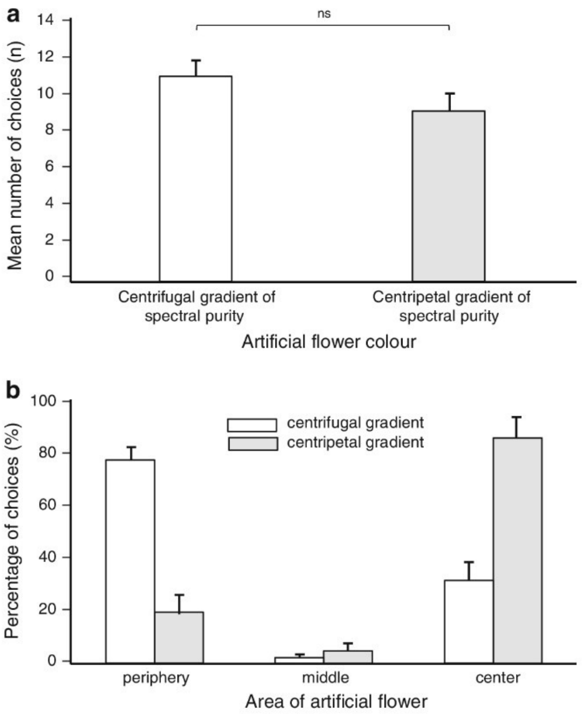

Fig. 5 **a** Mean number of approaches by flower-naïve bumblebees (*Bombus terrestris*) ($n = 10$) towards artificial flowers, which were arranged in a spectral purity gradient and as an inverse spectral purity gradient. **b** Mean percentage of approaches by flower-naïve bumblebees ending up with antennal contact with one of three differently coloured areas of the artificial flowers. All means are indicated with the upper 95 % confidence interval. Statistical analysis: Wilcoxon matched-pairs signed-rank test

towards colours of higher spectral purity was found indicating that spontaneous choices of experienced bees rely more on spectral purity than on dominant wavelength.

Which is the best predictor of the spontaneous colour preference in experienced honeybees and bumblebees? All three measurements of chromatic contrast used here, i.e. JND (ΔS) according to the receptor noise limited model, hexagon distance (H) according the hexagon model and spectral purity (SP) calculated with the hexagon model, predict the choices of the bees quite well. Are ΔS, H and SP just three different methods to calculate colour contrast or is there any evidence that one of these three measurements, ΔS, H and SP is more relevant to bees? Remarkably, there is an important difference between these measurements of colour contrast: JNDs and hexagon distances measure the magnitude of perceptible contrast between any two colours. In contrast, spectral purity is an attribute of the colour. That means, if any two colours are compared with respect to their spectral purity, the spectral purity difference provides—besides the magnitude of this difference—also a signed measure of the perceptual distance, i.e. its direction, meaning that one colour has a higher spectral purity than the other (Lunau et al. 1996). This aspect is not important, if only the flower colour against its background is considered, but becomes relevant for floral guides colours contrasting against the overall flower colour as in our experiment 3. Previous studies have also shown that the bumblebees' choice of a site for antennal contact with artificial flowers is dependent on the colour pattern (Manning 1956; Lunau et al. 1996. 2006; Pohl et al. 2008), but these studies have investigated the antennation behaviour at artificial flowers with small floral guides, assuming that the floral guides mimic stamens in colour and size (Lunau 2000). The interpretation given here that the bumblebees target at the colour of highest spectral purity is supported by Lunau et al. (1996) who have shown that the magnitude and the sign of colour contrast between artificial flower and floral guide predicted antennation behaviour of bumblebees. This means, that a distinct magnitude of the colour contrast is necessary, but not sufficient to predict the area of the bumblebees' antenna reaction, because the spatial arrangement of the contrasting colours was also important.

The spontaneous colour choice during the antennation behaviour may help the bumblebees to locate the reward of natural flowers, which is often hidden in the floral tube, and hence reduce handling time of flowers (Pernal and Currie 2002) by means of a subtle flower colour gradient. The detected preferences of the bumblebees might therefore be best explained by spectral purity, although it is unknown whether bumblebees use spectral colours as a reference as we did in our definition of spectral purity.

How does green contrast impact the choice behaviour? The green contrast of flowers against their background is a critical parameter in the search of foraging bees for flowers. Visual angle of target objects and amount of green contrast are decisive parameters for target detection (Giurfa et al. 1996; Dyer et al. 2008). Flowers offering high green contrast against the background are detected from a larger distance than flowers which exhibit only colour contrast (Giurfa et al. 1996; Hempel de Ibarra et al. 2001; Dyer et al. 2008). Since colour contrast and green contrast may change concordantly in some colours, the active sensorial colour parameter had to be tested separately. While it is possible that bees chose HS2 over HS4 and over HS5 because of its higher intensity contrast to background, not because it was the correct training colour, the results of experiment with HS1, HS2 and HS3 as test colours and that with HS1 and HS3 as test colours against HS2 background rule out the possibility of green contrast being the decisive parameter. When trained to a distinct colour and tested with two colours offering the same green contrast against the background provided by the training colour, the preferences observed in Experiment 2 clearly indicate that green contrast is not the decisive parameter. However, also in the other experiments, no indication was found that the green contrast between the target and the background influenced the choice of honeybees or bumblebees in our experiments (see Table 1). The green contrast between target and background colour thus seemingly did not affect colour choice in the experimental setup.

The phenomenon of bees preferring a novel colour over a trained one has been observed in some previous studies (Lehrer 1999; Lynn et al. 2005; Dyer and Murphy 2009). What makes a bee prefer a novel colour over a trained one? Detecting a small flower may be a difficult task for a bee, particularly if the flower does not stand out against the background. It has been demonstrated that the colour of flowers increases its detectability, if it contrasts strongly against the background (Giurfa et al. 1996; Spaethe et al. 2001). Hempel de Ibarra et al. (2000) found that honeybees detected bright stimuli on dim backgrounds better than dim stimuli on bright backgrounds. Bees may possess fixed innate preferences for a distinct colour that cannot be fully overridden by acquired preferences. As shown by Gumbert (2000) bumblebees trained to a distinct colour choose novel colours according to their similarity to the trained one, if the trained colour was similar to the test colours, but choose novel colours according to their innate preferences, if the trained colour was largely different from the test colours. It is noteworthy to mention that, different from the experiments in this study, the training colour was not among the test colours in the experiments of Gumbert (2000). Recently Leonard et al. (2011) have described other experimental conditions in which bumblebees prefer novel colours over trained ones. They showed that learning of slightly different hues in bumblebees is different in the presence of scent as compared to learning the same task in the absence of scent. Bumblebees trained with unscented artificial flowers, i.e. differential training with one rewarded and one non-rewarded stimulus, preferred the trained colour, whereas bumblebees trained with scented artificial flowers show a peak shift and preferred a test colour (hue) that was different from the rewarded training stimulus and even more different from the unrewarded training stimulus. In our experiments—with absolute rather than differential conditioning—a preference shift was observed towards spectrally more pure colours. Based on the findings that bumblebees exhibit peak shift and preferentially visit novel flower colours over previously rewarded ones, Lynn et al. (2005) discuss the peak shift phenomenon as a possible strategy to reduce the risk of flower-type misidentification. In general, the way how bees store and retrieve memories may have fundamental implications for how they choose between flowers (Chittka and Raine 2006).

Colour preferences are not necessarily attributed to a distinct hue due to the multidimensionality of colour vision (Kelber and Osorio 2010). Besides hue, saturation, and brightness of colours as well as the contrast between the target colour and another colour, e.g. the background colour, a simultaneously offered additional target colour, or a trained colour, provide further dimensions of colour potentially suited to trigger colour choices of experienced bees. In addition, combinations of some of these parameters might be relevant for choice decisions. Moreover, some flower-visiting animals that have colour vision orient colour-blind for opto-motor reactions (Srinivasan and Guy 1990). It is particularly interesting that the orientation of bees by means of green contrast or other colour parameters is dependant of the size of the floral targets, respectively, the distance of the bee towards the floral targets (Spaethe et al. 2001).

It is well known that despite repeated trainings, innate preferences of bumblebees are still detectable in learning experiments (Smithson and McNair 1996; Gumbert 2000). It has also been shown that bumblebees following absolute conditioning cannot discriminate colours with a colour contrast of <0.06 hexagon units, that discrimination was 63 % for colour contrast above 0.1 hexagon units, and that discrimination was 90 % for colour contrasts above 0.14 hexagon units (Dyer 2006). In our Experiment 1 with bumblebees, the perceptual distances between the test colours were smaller than 0.08 hexagon units, but the bumblebees discriminated between test colours only if the differences between the test colours were mostly attributed to their dominant wavelength. If the perceptual colour contrast between tested artificial flowers is small, bees show only 70 % of correct choices after numerous trainings (Dyer and Chittka 2004b). Our results indicate that not the perceptual distance between training and test colours, but a distinct property of the test colour affects choice behaviour. Though the test colours were perceptually similar, the bumblebees clearly preferred distinct test colours even over the training colour. When the test colours differed in their spectral purity, the bumblebees preferred another than the training colour, but when the test colours differed in their dominant wavelength the bees preferred the training colour over the test colours.

Interestingly, almost no effect of the number of training events was observed in bumblebees if the differences were attributed to their spectral purity. It should be stated that the estimation of the effect of training length on choice behaviour follows a very simple procedural method and does not exclude learning with larger number of training events. There are at least two different interpretations of this result: Bees possess a strong innate preference, which—with a limited amount of training—is dominant over learning, or bees are unable to learn (small) differences in spectral purity. It is known that for bees spectrally pure colours have a different salience from colours with low spectral purity: Daumer (1956), Menzel (1967), and

Giurfa et al. (1995) found that honeybees can be more easily trained to monochromatic lights than to white light or mixtures of white and monochromatic lights. Since differential training with rewarded and unrewarded artificial flowers in the training phase is known to facilitate discrimination learning (Dyer and Chittka 2004a), our training procedure, in which three rewarding identical artificial flowers were offered, may make colour discrimination learning difficult for the bumblebees and thus may have favoured the persistence of innate preferences. Differential training, in which three differently coloured artificial flowers similar to the artificial test flowers were simultaneously offered, but only one of them bore a reward, would more likely improve colour discrimination. In general, the absence of an effect, the number of training events could have been caused by the relatively short training experience of the bumblebees (10 trials during pre-training and 10 or 15 training trials alternating with test trials). The outcome of Experiment 1 indicates that the bumblebees learned to discriminate test stimuli differing in respect to the dominant wavelength from training stimuli within the series of test trials. We can, however, not exclude that the learning speed is different for colours of different dominant wavelengths as compared to colours of different spectral purity.

Natural light conditions are hard to simulate in laboratory settings, due to lower light intensity and lower UV-content in flight rooms illuminated with fluorescent tubes. However, comparative outdoor experiments with freely flying honeybees achieved similar results as given by bumblebees in the laboratory setting. Also honeybees preferred colours of higher spectral purity over trained colours but not colours of deviant dominant wavelength when identical artificial flowers were used as in the indoor experiments with bumblebees. This is an important outcome showing that the basic findings with flower-naïve bumblebees are valid for another species as well with experienced worker bees and also in natural light environment.

Can floral pigmentation honestly indicate the amount of reward? Models of co-optimisation of floral display and nectar reward predict a mosaic of cheating and honesty in plant-pollinator coevolution, with some plants having a variable proportion of empty or cheater flowers and others in which the display reflects the reward with some degree of honesty (Belsare et al. 2009). It is still open whether flower pigment concentration and composition potentially serve as honest flower signals indicating the amount of reward as has been shown for the floral display size (Armbruster et al. 2005). Principally colours of plant organs are suited for honest signalling of reward via the amount of pigment concentration (Schäfer et al. 2008). The results of this study show that bees forage and choose flowers by means of spectral purity, which likely is suitable as a signal indicating floral reward. It remains to be demonstrated that plants possess variability in flower colour caused by pigment concentration, and that pigment concentration is positively correlated both with a parameter of floral reward and with the size of a bee-subjective colour parameter. Besides pigment concentration also the surface, here the epidermal cell shape, might contribute to saturation of flower colours, since conical epidermal cells act as light traps and intensify colours (Whitney et al. 2011). Tests with *Antirrhinum majus* mutants have shown that bumblebees do not have innate preferences for flowers with conical epidermal cells as compared to those with flat epidermal cells, but are able to distinguish both surface types on flowers (Dyer et al. 2007). Our study shows that the visual capabilities and colour preferences in bees are strong enough to select among the intraspecific variability among floral colours.

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References

Armbruster WS, Antonsen L, Pélabon C (2005) Phenotypic selection on *Dalechampia*-blossoms: honest signaling affects pollination success. Ecology 86:3323–3333

Asmussen CB (1993) Pollination biology of the Sea Pea, *Lathyrus japonicus*: floral characters and activity and flight patterns of bumblebees. Flora 188:227–237

Backhaus W (1991) Color opponent coding in the visual system of the honeybee. Vis Res 31:1381–1397

Belsare PV, Sriram B, Watve MG (2009) The co-optimization of floral display and nectar reward. J Biosci 34:963–967

Brandt R, Vorobyev M (1997) Metric analysis of threshold spectral sensitivity in the honeybee. Vis Res 37:425–439

Chittka L (1992) The colour hexagon: a chromacity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J Comp Physiol A 170:533–545

Chittka L (1999) Bees, white flowers, and the color hexagon—a reassessment? No, not yet. Naturwissenschaften 86:595–597

Chittka L, Kevan PG (2005) Flower colour as advertisement. In: Dafni A, Kevan PG, Husband BC (eds) Practical pollination biology. Enviroquest Ltd., Cambridge

Chittka L, Menzel R (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. J Comp Physiol A 171:171–181

Chittka L, Raine NE (2006) Recognition of flowers by pollinators. Curr Opin Plant Biol 9:428–435

Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86:361–377

Dafni A, Lehrer M, Kevan PG (1997) Spatial flower parameters and insects spatial vision. Biol Rev 72:239–382

Dafni A, Kevan PG, Husband BC (2005) Practical pollination biology. Enviroquest Ltd, Cambridge

Daumer K (1956) Reizmetrische Untersuchungen des Farbensehens der Bienen. Z Vergl Physiol 38:413–478 de Brito Sanchez MG, Ortigao-Farias J, Gauthier M, Liu F, Giurfa M (2007) Taste perception in honeybees: just a taste of honey. Arthropod Plant Interact 1:69–76

Dobson HEM, Danielson EM, Wesep IDV (1999) Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). Plant Spec Biol 14:153–166

Dyer AG (2006) Bee discrimination of flower colours in natural settings. Entomol Gener 28:257–268

Dyer AG, Chittka L (2004a) Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften 91:224–227

Dyer AG, Chittka L (2004b) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. J Comp Physiol A 190:105–114

Dyer AG, Murphy AH (2009) Honeybees choose "incorrect" colors that are similar to target flowers in preference to novel colors. Isr J Plant Sci 57:203–210

Dyer AG, Neumeyer C (2005) Simultaneous and successive colour discrimination in the honeybee (*Apis mellifera*). J Comp Physiol A 191:547–557

Dyer AG, Whitney HM, Arnold SEJ, Glover BJ, Chittka L (2007) Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of *Antirrhinum majus* flower colour. Isr J Plant Sci 57:203–210

Dyer AG, Spaethe J, Prack S (2008) Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. J Comp Physiol A 194:617–627

Dyer AG, Boyd-Gerny S, McLoughlin S, Rosa MGP, Simonov V, Wong BBM (2012) Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision. Proc R Soc B 279:3606–3615

Giurfa M, Núñez J (1989) Colour signals and choice behavior of the honeybee (*Apis mellifera ligustica*). J Insect Physiol 35:907–910

Giurfa M, Núñez J, Chittka L, Menzel R (1995) Colour preferences of flower-naive honeybees. J Comp Physiol A 177:247–259

Giurfa M, Vorobyev M, Kevan P, Menzel R (1996) Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrast. J Comp Physiol A 178:699–709

Giurfa M, Vorobyev M, Brandt R, Posner B, Menzel R (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. J Comp Physiol A 180: 235–243

Goulson D, Cruise JL, Sparrow KR, Harris AJ, Park KJ, Tinsley MC, Gilburn AS (2007) Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees. Behav Ecol Sociobiol 61:1523–1529

Gumbert A (2000) Color choice by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. Behav Ecol Sociobiol 48:36–43

Hempel de Ibarra N, Vorobyev M, Brandt R, Giurfa M (2000) Detection of bright and dim colours by honeybees. J Exp Biol 203:3289–3298

Hempel de Ibarra N, Giurfa M, Vorobyev M (2001) Detection of coloured patterns by honeybees through chromatic and achromatic cues. J Comp Physiol A 187:215–224

Heuschen B, Gumbert A, Lunau K (2005) A generalised mimicry system involving angiosperm flower colour, pollen and bumblebees' innate colour preferences. Plant Syst Evol 252:121–137

Ilse D (1949) Colour discrimination in the dronefly, *Eristalis tenax*. Nature 163:255–256

Kelber A, Osorio D (2010) From spectral information to animal colour vision: experiments and concepts. Proc R Soc Lond B 277:1617–1625

Kelber A, Balkenius A, Warrant EJ (2002) Scotopic colour vision in nocturnal hawkmoths. Nature 419:922–925

Kevan PG, Lane MA (1985) Flower petal microtexture is a tactile cue for bees. Proc Natl Acad Sci USA 82:4750–4752

Kugler H (1935) Blütenökologische Untersuchungen mit Hummeln. VII. Die Anlockung von „Neulingen" durch Blüten. Planta 23:692–714

Lehrer M (1999) Dorsoventral asymmetry of colour discrimination in bees. J Comp Physiol A 184:195–206

Lehrer M, Bischof S (1995) Detection of model flowers by honeybees: the role of chromatic and achromatic contrast. Naturwissenschaften 82:145–147

Leonard AS, Dornhaus A, Papaj DR (2011) Flowers help bees cope with uncertainty: signal detection and the function of floral complexity. J Exp Biol 214:113–121

Lunau K (1990) Color saturation triggers innate reactions to flower signals—flower dummy experiments with bumblebees. J Comp Physiol A 166:827–834

Lunau K (1991) Innate flower recognition in bumblebees (*Bombus terrestris, B. lucorum*; Apidae)—optical signals from stamens as landing reaction releasers. Ethology 88:203–214

Lunau K (1992) A new interpretation of flower guide coloration: absorption of ultraviolet light enhances colour saturation. Plant Syst Evol 183:51–65

Lunau K (2000) The ecology and evolution of visual pollen signals. Plant Syst Evol 222:89–111

Lunau K (2006) Stamens and mimic stamens as components of floral colour patterns. Bot Jahrb Syst 127:13–41

Lunau K, Maier EJ (1995) Innate colour preferences of flower visitors. J Comp Physiol A 177:1–19

Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. J Comp Physiol A 178:477–489

Lunau K, Fieselmann G, Heuschen B, van de Loo A (2006) Visual targeting of components of floral colour patterns in flower naïve bumblebees (*Bombus terrestris*; Apidae). Naturwissenschaften 93:325–328

Lunau K, Unseld K, Wolter F (2009) Visual detection of diminutive floral guides in the bumblebee *Bombus terrestris* and in the honeybee *Apis mellifera*. J Comp Physiol A 195:1121–1130

Lynn SK, Cnaani J, Papaj DR (2005) Peak shift discrimination learning as a mechanism of signal evolution. Evolution 59:1300–1305

Manning A (1956) The effect of honey-guides. Behaviour 9:114–139

Matsumura S-I, Yokoyama J, Tateishi Y, Maki M (2006) Intraspecific variation of flower colour and its distribution within a sea lavender, *Limonium wrightii* (Plumbaginaceae), in the north-western Pacific Islands. J Plant Res 119119:625–632

Meléndez-Ackerman E, Campbell DR, Waser NM (1997) Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. Ecology 78:2532–2541

Menzel R (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). Z Vergl Physiol 56:22–62

Menzel R, Backhaus W (1991) Colour vision in insects. In: Gouras P (ed) Vision and visual dysfunction: the perception of colour. MacMillan Press, London, pp 262–288

Neumeyer C (1980) Simultaneous colour contrast in the honey bee. J Comp Physiol 139:165–176

Neumeyer C (1981) Chromatic adaptation in the honey bee: successive color contrast and color constancy. J Comp Physiol 144:543–553

Peitsch D, Fietz A, Hertel H, de Souza J, Ventura DF, Menzel R (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol A 170:23–40

Pernal SF, Currie RW (2002) Discrimination and preferences for pollen-based cues by foraging honeybees, *Apis mellifera* L. Anim Behav 63:369–390

Pohl M, Watolla T, Lunau K (2008) Anther-mimicking floral guides exploit a conflict between innate preference and learning in bumblebees (*Bombus terrestris*). Behav Ecol Sociobiol 63:295–302

Raine NE, Chittka L (2006) Flower constancy and memory dynamics in bumblebees (Hymenoptera: Apidae: *Bombus*). Entomol Gener 29:179–199

Schäfer HM, McGraw K, Catoni C (2008) Birds use fruit color as honest signal of dietary antioxidant rewards. Funct Ecol 22:303–310

Shang Y, Venail J, Mackay S, Bailey P, Schwinn K, Jameson P, Martin C, Davies K (2011) The molecular basis for venation patterning of pigmentation and its effect on pollinator attraction in flowers of Antirrhinum. New Phytol 189:602–615

Shipunov A, Kosenko Y, Volkova P (2011) Floral polymorphism in common primrose (*Primula vulgaris* Huds., Primulaceae) of the Northeastern Black Sea coast. Plant Syst Evol 296:167–178

Simonds V, Plowright CMS (2004) How do bumbles first find flowers? Unlearned approach responses and habituation. Anim Behav 67:379–386

Skorupski P, Chittka L (2010) Differences in photoreceptor processing speed for chromatic and achromatic vision in the bumblebee *Bombus terrestris*. J Neurosci 30:3896–3903

Smithson A, McNair MR (1996) Frequency-dependent selection by pollinators: mechanisms and consequences with regard to behaviour of bumblebees *Bombus terrestris* (L.) (Hymenoptera: Apidae). J Evol Biol 9:571–588

Spaethe J, Tautz J, Chittka L (2001) Visual constraints in foraging bumble bees: flower size and colour affect search time and flight behavior. Proc Natl Acad Sci USA 98:3898–3903

Srinivasan MV, Guy RG (1990) Spectral properties of movement perception in the dronefly *Eristalis*. J Comp Physiol A 166:287–295

Stiles FG (1976) Taste preferences, color preferences, and flower choice in hummingbirds. Condor 78:10–26

Tastard E, Andalo C, Giurfa M, Burrus M, Thébaud C (2008) Flower colour variation across a hybrid zone in *Antirrhinum* as perceived by bumblebee pollinators. Arthropod Plant Interact 2:237–246

Tourjee KR, Harding J, Byrne TG (1993) Calorimetric analysis of *Gerbera* flowers. Hortic Sci 28:735–737

von Frisch K (1967) The dance language and orientation of bees. Harvard University Press, Cambridge

von Helversen O (1972) Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. J Comp Physiol A 80:439–472

Vorobyev M (1999) Evolution of flower colours—a model against experiments: reply to comments by Chittka. Naturwissenschaften 86:598–600

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc Lond B 265:351–358

Vorobyev M, Hempel de Ibarra N, Brandt R, Giurfa M (1999) Do "white" and "green" look the same to a bee? Naturwissenschaften 86:592–594

Vorobyev M, Brandt R, Peitsch D, Laughlin SB, Menzel R (2001) Colour threshold and receptor noise: behaviour and physiology compared. Vis Res 41:639–653

Weiss MR (1997) Innate colour preferences and flexible colour learning in the pipevine swallowtail. Anim Behav 53:1043–1052

Whibley AC, Langlade NB, Andalo C, Hanna AI, Bangham A, Thébaud C, Coen E (2006) Evolutionary paths underlying flower color variation in *Antirrhinum*. Science 313:963–966

Whitney HM, Bennett KMV, Dorling M, Sandbach L, Prince D, Chittka L, Glover BJ (2011) Why do so many petals have conical epidermal cells? Ann Bot 108:609–616

Witjes S, Eltz T (2007) Influence of scent deposits on flower choice: experiments in an artificial flower array with bumblebees. Apidologie 38:12–18

CHAPTER 3

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RESEARCH ARTICLE

Avoidance of achromatic colours by bees provides a private niche for hummingbirds

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SUMMARY

That hummingbird-pollinated plants predominantly have red flowers has been known for decades, but well-investigated research studies are still rare. Preference tests have shown that hummingbirds do not have an innate preference for red colours. In addition, hummingbirds do not depend solely upon red flowers, because white-flowered hummingbird-pollinated plants are also common and temporarily abundant. Here we show that both white and red hummingbird-pollinated flowers differ from bee-pollinated flowers in their reflection properties for ultraviolet (UV) light. Hummingbird-pollinated red flowers are on average less UV reflective, and white hummingbird-pollinated flowers are more UV reflective than the same coloured bee-pollinated ones. In preference tests with artificial flowers, neotropical orchid bees prefer red UV-reflecting artificial flowers and white UV-nonreflecting flowers over red and white flowers with the opposite UV properties. By contrast, hummingbirds showed no preference for any colour in the same tests. Plotting floral colours and test stimuli into the honeybees' perceptual colour space suggests that the less attractive colours are achromatic for bees and therefore more difficult to detect against the background. This underlying colour preference in bees might provide hummingbirds with a private niche that is not attractive to bees.

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Key words: flower colour, hummingbirds, orchid bees, colour preference, sensory exclusion, private niche.

INTRODUCTION

Red flower colours has long been thought to determine the specific interrelationships between hummingbirds and the trochilophilous (hummingbird-pollinated) plants that they visit (Porsch, 1931; Grant, 1966; Raven, 1972; Bradshaw et al., 1995; Lunau and Maier, 1995; Cronk and Ojeda, 2008). Only a few flower-visiting insects, such as some butterflies and beetles, are attracted to red flowers (Dafni et al., 1990; Kinoshita et al., 1999). The absence of an expected innate preference for red colours in hummingbirds (Bené, 1941; Miller and Miller, 1971; Stiles, 1976; Goldsmith and Goldsmith, 1979; McDade, 1983; Delph and Lively, 1989) has fostered the idea that floral colours of bird-pollinated plants evolved mainly to discourage nectar-robbing insects, rather than to attract birds (Raven, 1972; Bradshaw et al., 1995; Lunau and Maier, 1995; Cronk and Ojeda, 2008).

Bees have trichromatic colour vision, based on three photoreceptor types maximally sensitive in the ultraviolet (UV), blue and green waveband. Flower-visiting birds, such as hummingbirds, have an advanced tetrachromatic colour vision with photoreceptor sensitivities peaking in the UV, blue, green and red waveband (Autrum and von Zwehl, 1964; Herrera et al., 2008), suggesting that bees are less able to detect red colours (Grant, 1966; Raven, 1972). Bees are very abundant visitors to flowers in the subtropical and tropical regions, and are the main competitors of hummingbirds for nectar (Castellanos et al., 2004; Rodríguez-Gironés and Santamaría, 2004; Freitas et al., 2006), but bees are often far less effective pollinators than hummingbirds (Roubik, 1982; Irwin and Brody, 2000). Floral traits have a twofold role in pollinator attraction as well as in plant defence. Long-tongued bees are able to extract the nectar from the deep floral tubes of hummingbird-pollinated plants, but rarely pollinate the flowers (Castellanos et al., 2004; Rodríguez-Gironés and Santamaría, 2004). Some bees are even able to pierce the floral tubes and to take up nectar through the perforations without touching anthers and stigma. As a consequence, trochilophilous plants are pollinated more efficiently if their flowers attract hummingbirds, but at the same time exclude bees (Irwin, 2006). Resource partitioning in nectar sources between hummingbirds and bees is known to be mediated by differences in flower morphology (Raven, 1972; Castellanos et al., 2003; Rodríguez-Gironés and Santamaría, 2004). However, direct evidence for a role of sensory exclusion through floral colours is lacking. One prediction of the sensory exclusion through floral colour hypothesis is the occurrence of differences in the spectral reflection properties between trochilophilous and melittophilous (bee-pollinated) flowers. To test this prediction we recorded the spectral reflection in the ultraviolet and visual range of wavelengths of neotropical bee- and hummingbird-pollinated flowers. We considered red flowers as well as white flowers, as the latter is the second most frequent flower colour of hummingbird-pollinated plants (Porsch, 1924; Burr and Barthlott, 1993; Dziedzioch et al., 2003). Another prediction of the hypothesis is the difference in response to the colours by bees compared with hummingbirds. To test this prediction we performed colour preference tests with orchid bees and hummingbirds, using a set of test colours matching the differences in the spectral reflection properties in trochilophilous and melittophilous flowers.

MATERIALS AND METHODS

Spectral reflection measurements

The spectral reflection of the test stimuli and of natural flowers was measured with a USB2000 spectrophotometer (Ocean Optics, Inc., Ostfildern, Germany) calibrated between 300 nm and 700 nm. Illumination was provided with a deuterium–halogen light source (D_2H; World Precision Instruments, Sarasota, FL, USA) emitting light between 215 nm and 1700 nm. Measurements were taken at an angle of 45 deg to the surface of the spot being measured (Chittka and Kevan, 2005). A pellet of barium sulphate was used as a white standard and a black film can was used as a black standard for recordings of the spectral reflection. Flowers were collected in botanical gardens of North Rhine Westphalia, Germany, and in the Atlantic rainforest of Sao Paulo, Brazil. The flowers were preserved in storage boxes with wet paper until measurement on the same day. Flowers were categorized as bee- or hummingbird-pollinated from literature information (see supplementary material Table S1). For this purpose we included only plant species in our analysis for which information about visitation and/or pollination by bees or hummingbirds was available. Plant species for which the literature stated only potential pollinators based on morphological conditions (pollination syndrome) were excluded. Also plant species for which the literature reported bees as well as birds as visitors or pollinators were excluded form this study (supplementary material Table S2). The sample size of these flowers is too small to include them in our study. In order to approximate the phylogenetic independence of the data the following procedure was applied: the means and standard errors of the spectral reflection curves were calculated for species belonging to the same genus and for genera belonging to the same family. Only the latter curves were used to calculate the mean spectral reflection curves. For statistical analysis we compared the spectral reflection of trochilophilous and melittophilous flowers in four distinct wavebands, i.e. UV (301–400 nm), blue (401–500 nm), green (501–600 nm) and red (601–700 nm), using an unpaired, two-tailed *t*-test (Table 1).

Choice experiments

Hummingbirds as well as orchid bees were offered the same set of artificial flowers in a multiple choice test. We used white UV-reflecting and red UV-reflecting colour stimuli [non-iridescent red feathers of the grey parrot, *Psittacus erithacus* (Linnaeus 1758) and white feathers of the mute swan, *Cygnus olor* (Gmelin 1789)] and combined both with either a UV-transmitting or a UV-absorbing transparent foil to assemble the four test colours (Fig. 1A,B).

Humans were unable to see any difference between the two colour stimuli in each of the pairs of red and white artificial flowers, which differed only in the UV spectrum. In the preference tests we presented eight artificial flowers (diameter 30 mm) to which Eppendorf tubes carrying a reward were appended in a manner that each of the four colour types was presented twice. The reward was 400 μl of the customary 7% nectar surrogate for hummingbirds (Avian Bird Food Products, Oosterend, Texel, Netherlands) and 10 μl of 50% honey water for orchid bees. The hummingbirds were not able to deplete the reward during the test intervals, whereas the reward in tests with euglossine bees was refilled after visits by bees. To prevent inaccuracies through position preferences the individuals were tested in eight trials with a pseudorandom placement of artificial flowers such that each of the eight artificial flowers was presented once in every position. For hummingbirds each trial lasted 10 min. For euglossine bees the trials lasted 90 min. Amounts of reward and duration of trials took into account the different foraging activity of the orchid bees compared with the hummingbirds. Seven individual hummingbirds [*Amazilia amazilia* (Lesson 1827) 1 male; *Eugenes fulgens* (Swainson 1827) 1 female, 1 male; *Thaumastura cora* (Lesson and Garnot 1827) 1 male; *Hylocharis cyanus* (Vieillot 1818) 2 female, 1 male] belonging to the subfamily Trochilinae were tested singly. The hummingbirds were bred in Germany and fed exclusively out of glassy colourless nectar tubes before and between the experiments. The hummingbirds were thus completely flower-naïve and not rewarded in association with colour cues before testing. Sixteen individuals of marked *Euglossa viridissima* (Friese 1899) (three females and 13 males) were tested simultaneously in a flight cage measuring 3×2×2 m. All choices of orchid bees of which the markers could not be ascertained after each feeding event were treated as choices of a single individual bee. The preference of the few bees caught in the wild did not differ from those bred in the flight cage, so the data from both were pooled. For statistical analysis of colour preferences a one-tailed Mann–Whitney *U*-test was performed according to our hypothesis that bees should prefer red UV-reflecting and white UV-absorbing artificial flowers as revealed from our spectral reflection measurements.

Perceptual bee colour space

To obtain a bee-subjective view of the natural and artificial flower colours we used the colour hexagon, a widely accepted model system for bee colour vision (Chittka, 1992) (Figs 1, 2).

For the calculation of colour loci in the colour hexagon a standard background of green leaves and a standard daylight illumination (D65) was used. The centre point of the colour hexagon represents the colour locus of the background to which the photoreceptor types are assumed

Table 1. Mean reflection of 63 neotropical red (r) and white (w) trochilophilous and melittophilous flowers in the ultraviolet, blue, green and red wavebands

Waveband	Flower colour	Mean reflection of melittophilous flowers	Mean reflection of trochilophilous flowers	*P*	*t*	d.f.
Ultraviolet	w	0.036	0.087	0.0067	2.957	25
	r	0.102	0.033	0.0001	5.164	19
Blue	w	0.258	0.220	0.3548	0.943	25
	r	0.118	0.032	0.0001	5.882	19
Green	w	0.329	0.242	0.0615	1.958	25
	r	0.195	0.041	0.0001	5.916	19
Red	w	0.336	0.277	0.2539	1.168	25
	r	0.488	0.255	0.0006	4.077	19

An unpaired two-tailed *t*-test was used for comparison of mean reflection values of trochilophilous and melittophilous flowers of the same human-visible floral colour. The mean spectral reflection was calculated for species belonging to the same genus and for genera belonging to the same family; only the latter reflection data were used to calculate the mean spectral reflection of each waveband.

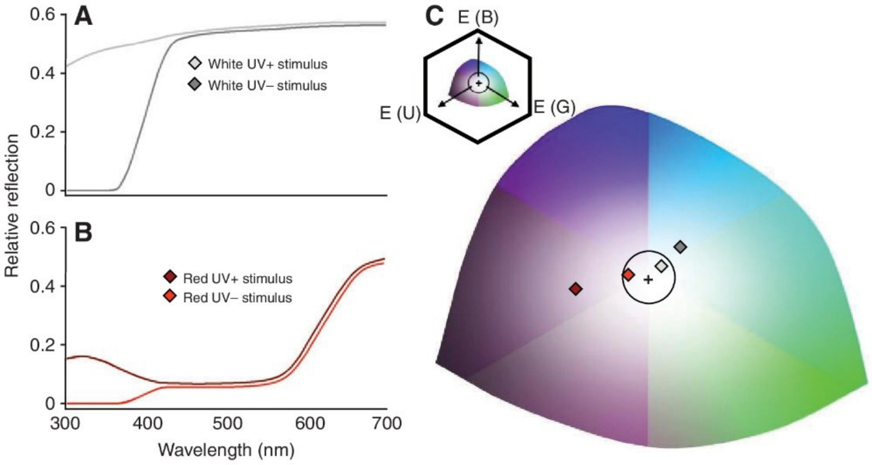

Fig. 1. Spectral reflection curves of the test stimuli and the representation of the respective colour loci in the honeybees' perceptual colour space. (A) Spectral reflection of white test stimuli. (B) Spectral reflection of red test stimuli. (C) Colour loci of red and white ultraviolet (UV)-reflecting and UV-non-reflecting test stimuli in the colour hexagon (Chittka, 1992). The centre point is indicated by +. The spectrum locus borders the coloured area within the colour hexagon. Achromatic colours with fewer than 0.1 hexagon units between the respective colour locus and the centre point are located in the circled area around the centre point. Inset: overview of the total colour hexagon. The excitations of the ultraviolet [E (U)], blue [E (B)] and green [E (G)] receptor types are indicated by arrows.

to be adapted (Chittka, 1992). The colour locus of a coloured object in the colour hexagon is generated by the calculated excitation of the UV, blue and green photoreceptors. The spectrum locus represents the colour loci of monochromatic colours and mixtures between the most extreme short- and long-wavelength ('bee-purple') colours. The spectrum locus borders the bee-visible colour space. Bees navigate and evaluate visual information of target objects by means of input exclusively to the green photoreceptor when an object is viewed at an angle of <15 deg; if the visual angle exceeds 15 deg bees use input from all three photoreceptor types and exhibit colour vision (Spaethe et al., 2001). The green contrast was calculated as the excitation difference of the green photoreceptor produced by a given visual target stimulus and its background, i.e. the artificial flowers and the background used in the choice experiments or the natural flowers and a standard background of green leaves (Chittka et al., 1994), because green foliage is the prevalent background for most flowers. Most studies so far have shown that the absolute value rather than the direction of green contrast is relevant for orientation in bees (Giurfa et al., 1996; Spaethe et al., 2001). Searching time for flowers negatively correlates with the amount of green contrast between flower and background (Spaethe et al., 2001). Colour loci of achromatic colours are located close to the centre point, whereas colour loci of high colour purity are located close to the spectrum locus. Behavioural tests with honeybees and bumblebees – the standard systems for the study of colour perception and preference in bees – show that a minimum colour distance is needed to discriminate colour stimuli from the background (Chittka et al., 1994; Spaethe et al., 2001).

RESULTS

Reflection properties of red and white neotropical flowers

The mean spectral reflection curves show that white melittophilous flowers (*N*=20 species from 19 genera in 17 families) reflect less in the UV than trochilophilous flowers (*N*=15 species from 13 genera in 10 families) of the same colour group (Fig. 2A), and red melittophilous flowers (*N*=5 species from five genera in five families) reflect more in the UV than the respective trochilophilous flowers (*N*=32 species from 25 genera in 16 families) that seem to be the same colour to the human eye (Fig. 2B). The individual species are listed in the supplementary information (supplementary material Table S1). In white flowers the differences in the spectral reflection between melittophilous and trochilophilous flowers are confined to the ultraviolet waveband, whereas in red flowers the spectral reflection of melittophilous flowers surpasses that of trochilophilous flowers in the UV, blue, green and red wavebands (Table 1).

Colour preferences of hummingbirds and neotropical bees

Hummingbirds did not show a preference for any of the four colour types of artificial flowers tested (white/UV absorbing *vs* white/UV reflecting; red/UV absorbing *vs* red UV reflecting; *P*>0.05; one-tailed Mann–Whitney *U*-test; Fig. 3A), whereas euglossine bees significantly preferred UV-reflecting red artificial flowers over UV-absorbing red flowers (*P*=0.0036, *U*=66; one-tailed Mann–Whitney *U*-test), and UV-absorbing white flowers over UV-reflecting white flowers (*P*=0.0044, *U*=68; one-tailed Mann–Whitney *U*-test; Fig. 3B). The data from the preference tests with euglossine bees and those with hummingbirds were pooled following a statistical test that showed that the individuals did not exhibit significant differences in their colour preferences.

Bee perceptual colour space

The colour loci of the artificial flowers less preferred by euglossine bees in the choice experiments were bee-achromatic as revealed in the perceptual colour space of the honeybee (Chittka, 1992) (Fig. 1C). The hexagon distance from the centre point of the UV-absorbing red test stimulus is 0.070 hexagon units and is smaller than that of the UV-reflecting red test stimulus, which was 0.279 hexagon units (Fig. 1C). The hexagon distance of the UV-reflecting white test stimulus was 0.078 hexagon units and is smaller than the UV-absorbing white test stimulus, which was 0.187 hexagon units (Fig. 1C).

The flowers of many trochilophilous plant families (31.3% of red, 40.0% of white) but none of the white-flowered melittophilous

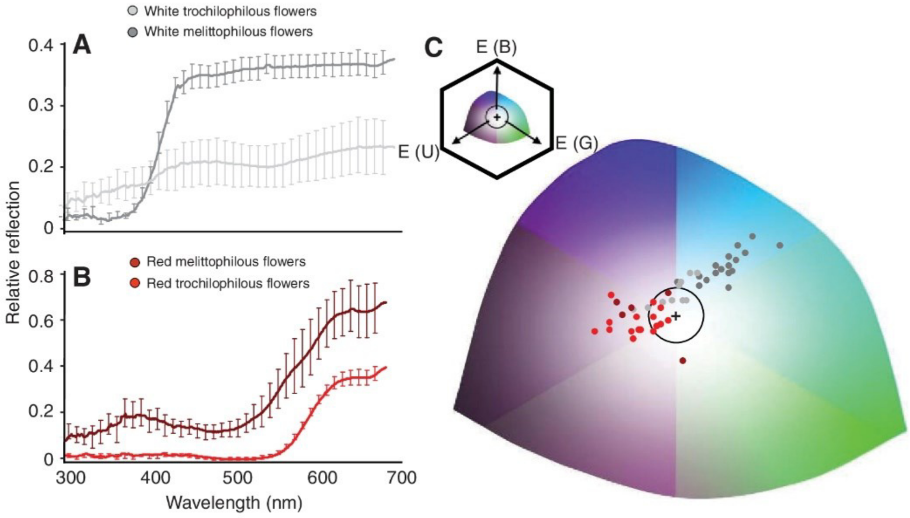

Fig. 2. Mean spectral reflection curves of hummingbird-pollinated and bee-pollinated neotropical flowers and the representation of the respective colour loci in the honeybees' perceptual colour space. (A) Mean spectral reflection of white trochilophilous and melittophilous flowers. (B) Mean spectral reflection of red trochilophilous and melittophilous flowers. The mean relative spectral reflection is given with the standard error. For calculation of the mean spectral reflection curves see Material and methods. (C) Colour loci of red and white melittophilous and trochilophilous flowers in the colour hexagon (Chittka, 1992). The centre point is indicated by +. Achromatic colours with fewer than 0.1 hexagon units between the colour locus and the centre point are located in the circled area around the centre point. Inset: overview of the total colour hexagon. The excitations of the ultraviolet [E (U)], blue [E (B)] and green [E (G)] receptors are indicated by arrows. Each plant family is represented by one colour locus.

plant families are achromatic for bees and only one of the red-flowered melittophilous plant species (Fig. 2C). The mean hexagon distance between the colour loci of white melittophilous flowers and the centre point was 0.199±0.047 hexagon units and was greater that that of trochilophilous flowers of the same colour, which was 0.108±0.032 hexagon units (Fig. 2C). The respective value of red melittophilous flowers was 0.135±0.04 hexagon units and was greater that that of trochilophilous flowers of the same colour, which was 0.127±0.054 hexagon units (Fig. 2C). For bees, the green contrast between the test colours and the background of the test stimuli was similar for the UV-reflecting and the UV-absorbing test stimuli (Table 2). The green contrast between white melittophilous flowers and the background of (standard) green leaves was greater than that of trochilophilous flowers, whereas the green contrast between red melittophilous flowers and the background of (standard) green leaves was less than that of trochilophilous flowers (Table 2).

DISCUSSION

It has been largely overlooked that red is not the exclusive floral colour of hummingbird-pollinated plants. Most non-red hummingbird-pollinated flowers, particularly among the Monocotyledonae, are white or pale pink (Porsch, 1924; Burr and Barthlott, 1993; Dziedzioch et al., 2003), or even undergo a colour change from green nectar-producing flowers to red non-rewarding flowers (Delph and Lively, 1989). In this study we demonstrate not only that neotropical red and white bee-pollinated and hummingbird-pollinated flowers differ in their spectral reflection properties, but also that orchid bees are less attracted to artificial flowers simulating the colour of trochilophilous flowers than to those of melittophilous flowers that appear identical in colour to human observers. Because hummingbirds do not exhibit a colour preference in tests with the same set of artificial flowers, the colour preference of orchid bees may contribute to them not visiting trochilophilous flowers.

Reflection properties of red and white neotropical flowers

Previous studies on floral colour preferences in bees suggested that bees, despite their low sensitivity in the red range of wavelengths, visit red flowers even if they have no additional reflection in the ultraviolet waveband (Chittka and Waser, 1997). Our results do not conflict with these observations, but suggest that the attractiveness of red colours is enhanced by additional ultraviolet reflection. Forrest and Thomson showed that red floral colours must be interpreted together with the background against which they are presented (Forrest and Thomson, 2009). Bees take longer to detect UV-absorbing red coloured flowers if presented against a complex background such as natural green leaves (Forrest and Thomson, 2009).

Spectral reflection properties of flowers in temperate regions demonstrated that white melittophilous flowers regularly absorb UV light (Kevan et al., 1996), whereas red melittophilous flower reflect UV light (Chittka and Waser, 1997). These findings are in accordance with our findings in neotropical flowers. The few UV-reflective white flowers might use alternative strategies to become conspicuous to bees, such as strong green contrast against the background or dissected shape (Chittka et al., 1994; Kevan et al., 1996). The spectral reflection of white trochilophilous flowers has not been systematically studied so far, but semi-quantitative UV photography revealed a high proportion of UV-reflective white trochilophilous flowers (Burr and Barthlott, 1993) and thus confirms our findings.

Colour preferences of hummingbirds and neotropical bees

Behavioural tests with hummingbirds have demonstrated the lack of innate colour preferences (Bené, 1941; Miller and Miller, 1971; Stiles, 1976; Goldsmith and Goldsmith, 1979; McDade, 1983; Delph and Lively, 1989). Bumblebees as well as honeybees exhibit innate

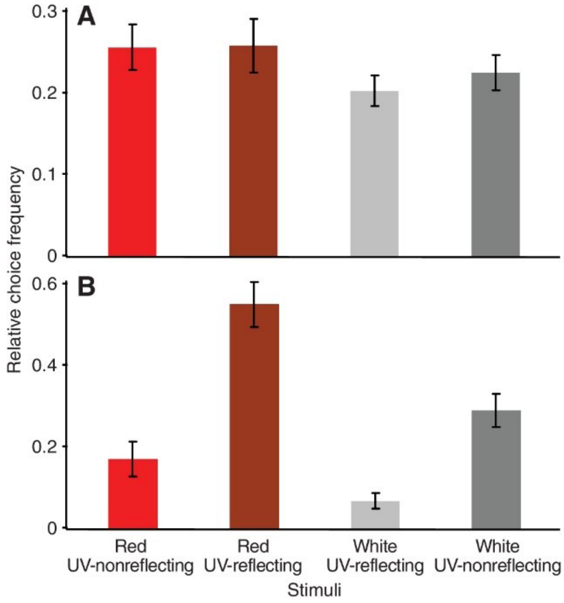

Fig. 3. Colour preferences of hummingbirds and euglossine bees for red and white artificial flowers with and without ultraviolet reflection. (A) Mean choice frequency of hummingbirds (N=7) for four types of simultaneously presented permanently rewarding artificial flowers, and (B) mean choice frequency of euglossine bees (N=17) for the same set of artificial flowers. The mean percentage of choice is given with the standard error.

colour preferences encompassing preferences for colours dominated by blue wavelengths (Giurfa et al., 1995; Gumbert, 2000), preferences for colours that contrast with the background (Giurfa et al., 1996; Lunau et al., 1996; Vorobyev and Brandt, 1997; Spaethe et al., 2001; Dyer and Chittka, 2004) as well as preferences for colours of high colour purity (Lunau et al., 1996). In addition, learning speed and learning capacity in bees are also dependent on colour (Menzel, 1967). Our colour-preference tests with neotropical orchid bees differed from those in the above-mentioned studies in that we used artificial flowers with only those colours that simulated the colour differences in the UV waveband of red and white trochilophilous and melittophilous flowers. Under these conditions, experienced orchid bees maintain preferences for distinct colours even in the absence of differentiating rewards. The maintenance of colour preferences in experiments with two different colour stimuli associated with the same amount of reward has previously been demonstrated with bumblebees (Smithson and Macnair, 1996; Smithson and Macnair, 1997). The results of the comparative preference tests with orchid bees and hummingbirds closely correspond to those expected from the spectral reflection measurements. However, it is noteworthy that hummingbirds do not show reciprocal colour preferences to orchid bees; instead, they showed no colour preference at all, as in previous studies (Bené, 1941; Miller and Miller, 1971; Stiles, 1976; Goldsmith and Goldsmith, 1979; McDade, 1983; Delph and Lively, 1989). Given that hummingbirds do not exhibit any colour preferences, the colour preferences of orchid bees may contribute to them not visiting the trochilophilous flowers. From studies of honeybees and bumblebees it is known that workers take longer to detect achromatic colours, which prolongs their searching time and reduces foraging efficiency (Spaethe et al., 2001; Reisenman and Giurfa, 2008) and may result in a preference for easily detectable colours. In addition, the colour loci of many red, as well as white, hummingbird-pollinated flowers appear achromatic to bees and thus would provide only limited colour contrast against a background of green leaves (Chittka et al., 1994; Spaethe et al., 2001; Dyer and Chittka, 2004).

Bee perceptual colour space

The bees' perceptual colour space developed by Chittka has been successfully applied to quantify and visualize the perceptual colour contrast (Chittka, 1992). The colour hexagon is an equidistant colour space in which the distance between the colour loci of flower and background, as well as between two flowers, represents the perceptual colour contrast between the pairs of colour loci (Chittka, 1992; Chittka et al., 1994; Spaethe et al., 2001; Dyer and Chittka, 2004). Experimental studies have shown that bees can detect a coloured target against a background with a minimum perceptual distance exceeding 0.1 hexagon units; otherwise the colours appear achromatic to bees (Chittka et al., 1994; Spaethe et al., 2001). Our study shows that, using this criterion, many floral colours of red and white trochilophilous flowers appear achromatic to bees, whereas melittophilous flowers do not. Because the amount of green contrast between UV-reflecting and UV-absorbing target stimuli and background was similar in the choice tests, it was concluded that the observed preference of the orchid bees was independent of green contrast; this holds for red as well as for white test stimuli. Moreover, because white melittophilous flowers offer more green contrast to the background than white trochilophilous flowers, whereas red melittophilous flowers do not, green contrast does not seem to be an overall key parameter, which could explain the flowers' attractiveness to bees.

Hummingbird-pollinated plants benefit more from the exclusion of flower-visiting bees than *vice versa* (Castellanos et al., 2003), thereby favouring strategies of trochilophilous flowers to exclude

Table 2. Excitation values of ultraviolet (UV), blue and green photoreceptor types and values for green contrast for 63 neotropical red (r) and white (w) trochilophilous and melittophilous flowers and UV-reflecting and UV-absorbing artificial flowers used in the choice experiments

			Photoreceptor excitation values			
Flower type	Flower colour	Flowering syndrome/colour type	UV	Blue	Green	Green contrast values
Natural	r	Melittophilous	0.709	0.639	0.581	–0.081
		Trochilophilous	0.383	0.306	0.234	0.266
	w	Melittophilous	0.535	0.751	0.706	–0.206
		Trochilophilous	0.627	0.626	0.513	–0.013
Artificial	r	UV-reflecting	0.749	0.547	0.430	0.070
		UV-absorbing	0.370	0.449	0.426	0.074
	w	UV-reflecting	0.904	0.878	0.816	–0.316
		UV-absorbing	0.645	0.849	0.809	–0.309

bees. Besides colour, some other floral adaptations to visitation and pollination by hummingbirds are also known to exclude bees: absence of a landing platform, inappropriate size of the corolla tube, and low-concentration, dilute nectar (Raven, 1972; Irwin et al., 2004; Rodríguez-Gironés and Santamaría, 2004). In addition, hummingbird-pollinated flowers typically do not emit odours and thus lack chemical attractants typically associated with bee-pollinated flowers (Raguso, 2008). Our results show that the floral colours of hummingbird-pollinated plants are effective sensorial barriers that contribute to exclude bees from visiting these flowers. The colours of hummingbird-pollinated flowers thus have a dual function: the attraction of hummingbirds and the repellence of bees. Sensory exclusion of bees is not necessarily restricted to red and white colours, but may also be the case for pink, orange, green and blue flowers and for bird-pollinated flowers from the paleotropics.

Applying Possingham's model (Possingham, 1992) Rodríguez-Gironés and Santamaría predicted that resource-partitioning among bees and hummingbirds will develop solely based on the fact that bees require more time to find flowers of one species with a specific colour as compared with differently coloured flowers of another species (Rodríguez-Gironés and Santamaría, 2004). This prediction completely fits our results. In their study Rodríguez-Gironés and Santamaría assumed, that if bee visits were costly for the ancestral yellow and blue bird flowers, the yellow flowers would experience a selective pressure to become red (Rodríguez-Gironés and Santamaría, 2004). In this example they considered that, at any given time, this bird flower occurred in only two shades of colour, one of them with a slightly longer wavelength (an orange morph). By contrast our results indicate that the predicted main shift in the spectral reflection may have occurred in the UV waveband, and that pure red colours may be only one of several solutions to distract bees. Owing to the bees' preference for colours of high spectral purity (Lunau et al., 1996), the predicted colour shift is not restricted to longer wavelengths, but may also result in less spectrally pure colours (a pale morph or a dull morph). Moreover, hummingbirds learn to associate floral colours with expected rewards very well, whereas even experienced bees retain their innate colour preference to some degree (Smithon and Macnair, 1996; Pohl et al., 2008).

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REFERENCES

Autrum, H. and von Zwehl, V. (1964). Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. *J. Comp. Physiol. A* **48**, 357-384.

Bené, F. (1941). Experiments on the color preference of black-chinned hummingbirds. *Condor* **43**, 237-323.

Bradshaw, H. D., Jr, Wilbert, S. M., Otto, K. G. and Schemske, D. W. (1995). Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature* **376**, 762-765.

Burr, B. and Barthlott, W. (1993). Untersuchungen zur Ultraviolettreflexion von Angiospermenblüten I. Monocotyledoneae. *Trop. Subtrop. Pflanzenwelt* **87**, 1-193.

Castellanos, M. C., Wilson, P. and Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* **57**, 2742-2752.

Castellanos, M. C., Wilson, P. and Thomson, J. D. (2004). Anti-bee and pro-bird changes during the evolution of hummingbird pollination in *Penstemon* flowers. *J. Evol. Biol.* **17**, 876-885.

Chittka, L. (1992). The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *J. Comp. Physiol. A* **170**, 533-543.

Chittka, L. and Kevan, P. G. (2005). Flower colours as advertisement. In *Practical Pollination Biology* (ed. A. Dafni, P. G. Kevan and B. C. Husband), pp. 157-230. Cambridge, ON, Canada: Enviroquest Ltd.

Chittka, L. and Waser, N. M. (1997). Why red flowers are not invisible to bees. *Isr. J. Plant Sci.* **45**, 169-183.

Chittka, L., Shmida, A., Troje, N. and Menzel, R. (1994). Ultraviolet as a component of flower reflections, and the colour perception of hymenoptera. *Vision Res.* **34**, 489-1508.

Cronk, Q. C. B. and Ojeda, I. (2008). Bird-pollinated flowers in an evolutionary and molecular context. *J. Exp. Bot.* **59**, 15-27.

Dafni, A., Bernhardt, P., Shmida, A., Ivri, Y., Greenbaum, S., O'Toole, C. and Losito, L. (1990). Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. *Isr. J. Bot.* **39**, 81-92.

Delph, L. F. and Lively, C. M. (1989). The evolution of floral color change: pollinator attraction versus physiological constraints in *Fuchsia excorticata*. *Evolution* **43**, 1252-1261.

Dyer, A. G. and Chittka, L. (2004). Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *J. Comp. Physiol. A* **190**, 105-114.

Dziedzioch, C., Stevens, A. D. and Gottsberger, G. (2003). The hummingbird plant community of a tropical montane rain forest in southern Ecuador. *Plant Biol.* **5**, 331-337.

Forrest, J. and Thomson, J. D. (2009). Background complexity affects colour preference in bumblebees. *Naturwissenschaften* **96**, 921-925.

Freitas, L., Galetto, L. and Sazima, M. (2006). Pollination by hummingbirds and bees in eight syntopic species and a putative hybrid of Ericaceae in southeastern Brazil. *Plant Syst. Evol.* **258**, 49-61.

Giurfa, M., Núñez, J., Chittka, L. and Menzel, R. (1995). Colour preferences of flower-naive honeybees. *J. Comp. Physiol. A* **177**, 247-259.

Giurfa, M., Vorobyev, M., Kevan, P. and Menzel, R. (1996). Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J. Comp. Physiol. A* **178**, 699-709.

Goldsmith, T. H. and Goldsmith, K. M. (1979). Discrimination of colors by the black-chinned hummingbird, *Archilochus alexandri*. *J. Comp. Physiol. A* **130**, 209-220.

Grant, K. A. (1966). A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. *Am. Nat.* **100**, 85-97.

Gumbert, A. (2000). Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behav. Ecol. Sociobiol.* **48**, 36-43.

Herrera, G., Zagal, J. C., Diaz, M., Fernández, M. J., Vielma, A., Cure, M., Martinez, J., Bozinovic, F. and Palacios, A. G. (2008). Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). *J. Comp. Physiol. A* **194**, 785-794.

Irwin, R. E. (2006). The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *Am. Nat.* **167**, 315-328.

Irwin, R. E. and Brody, A. K. (2000). Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* **81**, 2637-2643.

Irwin, R. E., Adler, L. S. and Brody, A. K. (2004). The dual role of floral traits: pollinator attraction and plant defense. *Ecology* **85**, 1503-1511.

Kevan, P. G., Giurfa, M. and Chittka, L. (1996). Why are there so many and so few white flowers? *Trends Plant Sci.* **1**, 280-284.

Kinoshita, M., Shimada, N. and Arikawa, K. (1999). Colour vision of the foraging swallowtail butterfly *Papilio xuthus*. *J. Exp. Biol.* **202**, 95-102.

Lunau, K. and Maier, E. J. (1995). Innate colour preferences of flower visitors. *J. Comp. Physiol. A* **177**, 1-19.

Lunau, K., Wacht, S. and Chittka, L. (1996). Colour choices of naive bumble bees and their implications for colour perception. *J. Comp. Physiol. A* **178**, 477-489.

McDade, L. A. (1983). Long-tailed hermit hummingbird visits to inflorescence color morphs of *Heliconia irrasa*. *Condor* **85**, 360-364.

Menzel, R. (1967). Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). *Z. Vgl. Physiol.* **56**, 22-62.

Miller, R. S. and Miller, R. E. (1971). Feeding activity and color preference of ruby-throated hummingbirds. *Condor* **73**, 309-313.

Pohl, M., Watolla, T. and Lunau, K. (2008). Anther-mimicking floral guides exploit a conflict between innate preference and learning in bumblebees (*Bombus terrestris*). *Behav. Ecol. Sociobiol.* **63**, 295-302.

Porsch, O. (1924). Vogelblumen-Studien I. *Naturwissenschaften* **63**, 553-605.

Porsch, O. (1931). Grellrot als Vogelblumenfarbe. *Biol. Gen.* **2**, 647-674.

Possingham, H. P. (1992). Habitat selection by two species of nectarivore: habitat quality isolines. *Ecology* **73**, 1903-1912.

Raguso, R. A. (2008). Wake up and smell the roses: the ecology and evolution of floral scent. *Annu. Rev. Ecol. Evol. Syst.* **39**, 549-569.

Raven, P. H. (1972). Why are bird-visited flowers predominantly red? *Evolution* **26**, 674.

Reisenman, C. E. and Giurfa, M. (2008). Chromatic and achromatic stimulus discrimination of long wavelength (red) visual stimuli by the honeybee *Apis mellifera*. *Arthropod Plant Interact.* **2**, 137-146.

Rodríguez-Gironés, M. A. and Santamaría, L. (2004). Why are so many bird flowers red? *PLoS Biol.* **2**, 1515-1519.

Roubik, D. W. (1982). The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. *Ecology* **63**,354-360.

Smithson, A. and Macnair, M. R. (1996). Frequency-dependent selection by pollinators: mechanisms and consequences with regard to the behaviour of bumblebees *Bombus terrestris* (L.) (Hymenoptera, Apidae). *J. Evol. Biol.* **9**, 571-588.

Smithson, A. and Macnair, M. R. (1997). Density-dependent and frequency-dependent selection by bumblebees *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Biol. J. Linn. Soc.* **60**, 401-417.

Spaethe, J., Tautz, J. and Chittka, L. (2001). Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. *Proc. Natl. Acad. Sci. USA* **98**, 3898-3903.

Stiles, F. G. (1976). Taste preferences, color preferences, and flower choice in hummingbirds. *Condor* **78**, 10-26.

Vorobyev, M. and Brandt, R. (1997). How do insect pollinators discriminate colours? *Isr. J. Plant Sci.* **45**, 103-113.

CHAPTER 4

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Bees, birds and yellow flowers: Pollinator-dependent convergent evolution of UV-patterns

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Abstract

Colour is one of the most obvious advertisements of flowers and occurs in a huge diversity among the angiosperms. Flower colour is responsible for the attraction from a distance, whereas contrasting colour patterns within flowers aid orientation of flower-visitors after approaching the flowers. Due to the striking differences in colour vision systems and neural processing across animal taxa, flower colours evoke specific behavioural responses by different flower-visitors. We tested whether and how yellow flowers differ in their spectral reflectance depending on the main pollinator. We focused on bees and birds and examined whether the presence or absence of the widespread UV-reflectance pattern of yellow flowers predicts the main pollinator.

Most bee-pollinated flowers displayed a pattern with UV-absorbing centres and UV-reflecting peripheries, whereas the majority of bird-pollinated flowers are entirely UV-absorbing. In choice experiments we found that bees did not show consistent preferences for any colour- or pattern-types. However, all tested bee species made their first antennal contact preferably at the UV-absorbing area of the artificial flower irrespective of its spatial position within the flower. The appearance of UV-patterns within flowers is the main difference in spectral reflectance between yellow bee- and bird-pollinated flowers, and affects the foraging behaviour of flower-visitors. The results support the hypothesis that flower colours and the visual capabilities of their efficient pollinators are adapted to each other.

Introduction

Several flower-visitors are highly dependent on flower resources for their own or their offspring. Likewise, flowers are highly dependent on efficient pollinators and thus ensuring their reproductive success. Flower colours play an important role in the attraction of flower-visitors, but due to the striking differences in colour vision and colour preferences among different animals, specific flower colours selectively attract flower-visitors (Grant 1949; Melendez-Ackerman & Campbell 1998; Campbell *et al.* 2010; Junker *et al.* 2013). For example, bees have trichromatic colour vision with three different photoreceptor classes maximally sensitive in the UV, blue and green range of wavelengths (Peitsch *et al.* 1992), whereas birds are tetrachromatic and have further receptors sensitive to red lights (Ödeen & Håstad 2003). Beside physiological properties, neural processing and therefore the behaviours of bees and birds towards colours are different: For chicks it is known that chromatic and achromatic colour signals are used during food search, with high contrasts crucial for the detection of objects (Osorio *et al.* 1999). The preferred flower colour choice by flower-visiting birds is mainly due to individual experience as birds associate colours with rewards, but innate preferences for specific colour parameters are not known (Stiles 1976; Kaczorowski *et al.* 2014). In contrast, foraging bees rely more on distinct colour parameters (for review, see Dyer *et al.* 2010). Under small visual angles, bees evaluate information solely in the green receptor channel, i.e. they analyse only achromatic contrasts (Giurfa *et al.* 1997; Spaethe *et al.* 2001). If the visual angle of an object exceeds a specific value, bees switch to colour vision (Giurfa *et al.* 1997; Spaethe *et al.* 2001). Then, a high chromatic contrast between two colours facilitates discrimination in bees (Lunau *et al.* 1996). Thus, high colour contrast between flower and background colour is important for the detection of flowers by bees (Giurfa *et al.* 1996). Moreover, bees are known to prefer colours of high spectral purity, a parameter that increases if stimuli reflect only one or two of the three specific ranges of wavelengths, i.e. if they selectively excite one or two of the three colour photoreceptors (Lunau 1990; Rohde *et al.* 2013).

Bees and birds have in common that they possess colour vision extending into the UV range of wavelength and are able to discriminate between UV-absorbing and UV-reflecting colours (Peitsch *et al.* 1992; Ödeen & Håstad 2003). Entirely yellow flowers are common among bee- as well as bird-pollinated plants and potentially contain UV-patterns, which may influence the attractiveness for bees and birds. Many flowers display those colour patterns (Lunau 2007; Davies *et al.* 2012), which have been described as early as 1793 by Christian K. Sprengel as “Saftmale”, i.e. nectar guides. Irrespective of the overall flower colour, nectar guides in general absorb ultraviolet (UV)-light (Kugler 1963; Silberglied 1979; Lunau 1993, 1995), most notably in yellow flowers (Horovitz & Cohen 1972; Guldberg & Atsatt 1975; Primack 1982). Within those yellow flowers, the apical parts contain pervasive UV-reflecting yellow carotenoids, whereas the central parts of the signalling apparatus additionally contain UV-absorbing flavonoids (Thompson *et al.* 1972; Harborne & Smith 1978). Due to their shape and uniformity central elements of floral colour patterns were named “bull’s eye” known in many radially-symmetric flowers, especially in species belonging to the plant family Asteraceae (Silberglied 1979).

The role of this intra-floral colour pattern for the visual orientation of pollinators has been revealed by studies focusing on the behaviour of bees towards nectar guides (Free 1970; Lehrer *et al.* 1995; Lunau 1993; Lunau *et al.* 1996; Heuschen *et al.* 2005; Plowright *et al.* 2006; Owen & Bradshaw 2011; Orbán & Plowright 2013). In contrast, birds seem to rely less on floral colour patterns. Previous studies on a few flowering plants that are frequently visited by birds found that nectar guides are absent or have been replaced by structural floral features (Grant & Grant 1968; Smith *et al.* 1996; Schemske & Bradshaw 1999; Temeles & Rankin 2000).

In this study we tested if yellow flowers from the Neo- and Paleotropics and -subtropics consistently differ in their spectral reflectance properties depending on the pollination system, bees or birds. We compared the spectral reflectance properties of bee- and bird-pollinated human all-yellow flowers focusing on differences in UV reflectance. Specifically, we tested if the overall flower colour differs in UV reflectance, and whether colour parameters are affected. In addition, we tested if nectar guides in the UV range of wavelengths that are invisible to humans, are presented. Moreover, we performed choice experiments with bees using yellow artificial flowers, which either reflect or absorb UV-light or artificial flowers either displaying the natural (central UV-absorbance) or the inverse pattern of UV-reflectance (central UV-reflectance). The combined results of quantitative flower colour analysis and preference tests for three eusocial bee species provide a basis for the discussion of differences between bee- and bird-pollinated yellow flowers and their impact on the foraging behaviour of different flower-visitors.

Materials and methods

Yellow flowers

Yellow flowers were collected in botanical gardens in Germany and Brazil. The flowers were stored in moist boxes until measurement on the same day. In order to evaluate pollinator-mediated selection on flower colouration, we categorize the flowers into bee- or bird-pollination through literature analysis. We included in our data set only plant species, for which literature identified either bees or birds as “effective pollinators”. Effective pollinators are those, which remove pollen from stamens and deposit pollen to stigmas with additional information about their visitation frequency (Mayfield *et al.* 2001, and references within). If no data about pollination of specific plant species were available, we use the ones from other species within the same genus with corresponding morphological traits (after Rosas-Guerrero *et al.* 2014). Thus, we excluded from our analysis those plant species, for which pollinators were classified solely according to classical pollination syndromes by visual floral traits (after Faegri & van der Pijl 1979). Flowers that can be pollinated by both bees and birds are rare and were excluded from our analysis. Then, plant species were categorized into New World and Old World according to their native habitats, as bee as well as bird species from different habitats differ in their visual capabilities. There are three major families of flower-visiting birds: Hummingbirds (Trochilidae) from the New World, sunbirds (Nectariniidae) and honeyeaters (Meliphagidae) from the Old World. Sunbirds and other generalist foraging birds belong to the UV sensitive-(UVS-) type, whereas hummingbirds and honeyeaters belong to the violet sensitive-(VS-) type with a sensitivity peak of the short-wavelength cones shifted towards longer wavelengths as compared to sunbirds and other generalists (Ödeen & Håstad 2010; Endler & Mielke 2005). Although there are some flower-visiting perching birds in the New World, this analysis focused mainly on hummingbird-pollinated flowers from the New World and perching bird-pollinated flowers from the Old World (Supplemental Material 1). Even though differences in the colour vision of bees from the New as compared to bees from the Old World are known (Peitsch *et al.* 1992), these differences are statistically not significant (Briscoe & Chittka 2001). However, we tested for differences in the spectral reflectance between flowers from the New and Old World for both pollination systems before pooling the data.

To evaluate the presence of UV-nectar guides each flower was separated into two parts: the inner centred part (hereinafter referred to as “centre”) includes ray florets, corolla orifices, basal parts of petals or flags, and reproductive organs (i.e. those parts of the flowers where nectar guides are common). The outer apices (hereinafter referred to as “periphery”) include disc florets, lips, adaxial parts of corollas and peripheral parts of petals or flags. However, before categorizing the flower parts, we control for variation of size, shape and position of nectar guides, by scanning the entire signalling apparatus of the flower for differences in spectral reflectance. This means that we relocate the probe of the spectrophotometer on different parts of the flower and check for any differences in spectral reflectance. Thus, any UV-nectar guide was recorded. A list of tested plant species with reference to their habitat, literature with pollinator reference and measured flower parts for centre and periphery is given in Supplemental Material 1.

Spectral reflectance of flowers was measured with a spectrophotometer (USB 4000, Ocean Optics, Inc., Dunedin, FL, USA) relative to a white (pressed pellet of barium sulphate) and a black standard (black film can) in an angle of 45° to the measuring spot. The spectrophotometer was connected with a coaxial fibre cable (QR400-7-UV-VIS, Ocean Optics, Inc., Dunedin, FL, USA) to a deuterium-halogen light source (DH-2000-BAL, Ocean Optics, Inc., Dunedin, FL, USA). Spectral reflectance was recorded from 300 nm to 700 nm.

To approximate the phylogenetic independence of the analysis of plant species, the means and standard errors of spectral reflectance data were calculated for species belonging to the same genus (this was the case for five species within a genus with bee-pollinated flowers in the family Fabaceae and two species within a genus with bee-pollinated flowers in the family Xanthorrhoaceae) and for genera belonging to the same family within bee- and bird-pollinated flowers, respectively (this was the case for four plant families with bee-pollinated flowers and for two plant families with bird-pollinated flowers). Only the latter data were used to calculate the mean spectral reflectance curves and each plant family was regarded as a single data point for further analyses.

Yellow test stimuli

For choice experiments with bees, we prepare yellow stimuli resembling the spectral reflectance of natural yellow flowers with or without UV-reflectance (Supplemental Material 2). For this purpose, discs of 3 cm in diameter of Whatman filter paper No. 1 was immersed for three seconds in a solution of 1.82 ml of the flower pigment carotene (oily solution from Carl Roth GmbH and Co. KG, Karlsruhe, Germany) dissolved in 50 ml hexane. After sufficient evaporation of the solvent the coloured filter paper was covered with foils of different UV-transmitting properties and connected with centrally located transparent Eppendorf tubes, containing the reward. The foils were either UV-absorbing (LEE 226, LEE Filter, Hampshire, UK) or UV-transmitting (NOWOFOL® ET 6235, NOWOFOL® Kunststoffprodukte GmbH & Co. KG, Siegsdorf, Germany) and combined variously in order to produce four artificial flower types (afterwards referred as “test flowers”): One test flower was entirely UV-reflecting, one was entirely UV-absorbing, and two possessed a pattern of UV-reflectance, with one test flower possessing a UV-absorbing centre and a UV-reflecting periphery, and the other possessing a reciprocal pattern. The centre of the patterned test flowers was 1.5 cm in diameter each. The prepared test flowers were stored in the dark until being used in the choice experiments to prevent changes in light absorbing properties of the pigments. After about 30min of exposure to light, newly fabricated ones replaced the artificial flowers in order to prevent effects of bleaching of the colour stimuli for choice behaviour of the bees. Spectral reflectance of test stimuli was measured using the same method as with natural flowers and are given in Supplemental Material 2A. In order to illustrate the negligible effect of bleaching Supplemental Material 2B shows the spectral reflectance of fresh artificial flowers as well as of artificial flowers that had been exposed to light for 30min.

Choice experiments and bee keeping

Choice experiments were performed with three different social species of the subfamily Apinae, i.e. honeybees (*Apis mellifera carnica* Pollmann), bumblebees (*Bombus terrestris dalmatinus* Dalla Torre), and stingless bees (*Melipona quadrifasciata* Lepeletier). All three bee species are known to use colour cues to detect flowers as food sources (Giurfa *et al.* 1994; Spaethe *et al.* 2001; Spaethe *et al.* 2014). We have chosen these bee species to include flower-naïve (*B. t. dalmatinus*) as well as flower-experienced workers (*A. m. carnica* and *M. quadrifasciata*) in our analysis and also to include different experimental conditions, i.e. laboratory environment with artificial lights as well as natural daylight environment. Moreover we wanted to include bee species from the New as well as from the Old World; but flower-naïve bees from the New World were not at hand.

The decisions of bees were examined with dual choice tests by offering four test flowers (i.e. two of each type) at a time. The four test flowers were presented in a rectangular arrangement in a distance of 10 cm each on a green cardboard. The green cardboard was 30 cm x 30 cm in size. The cardboard and the test flowers were presented vertically. We offered two different test set-ups to the bees: One set-up of test flowers comprised two entirely UV-reflecting and two entirely UV-absorbing yellow stimuli, the other set-up comprised two of both types of patterned test flowers each. We tested 10 workers of each bee species and recorded approaches towards the test flower types and additionally antenna reactions towards the centre or periphery for the patterned test flower types.

For training, individually marked workers were released directly on one of the four training artificial flowers (afterwards referred to “training flowers”) presented in the same arrangement than the test set-ups. The training flowers were of the same size and shape as the test flowers and were made of the green cardboard background used in the tests.

Training and test flowers were permanently rewarded, as the reward was supplied in an amount of 200 µl, such that the bees were not able to deplete a single artificial flower without being saturated. The rewards were adjusted for each bee species according to their regular nectar sources, i.e. 50% honey solution for stingless bees, 50% sugar water for honeybees and 50% Biogluc® (re-natur GmbH, Ruhwinkel, Germany) solution for bumblebees. The training set-up was replaced by a test set-up when workers had approached the training stimuli by themselves. If a bee landed on one of the four test flowers and took up the reward, the choice was counted as approach. Antenna reactions were counted when the bees’ antennae contacted any area of the test flowers while approaching before landing and drinking.

Each individual bee was tested in both test set-ups in a pseudo-randomly changed order. To prevent position preferences of the individual bees, test and training flowers were changed pseudo-randomly after each approach that any artificial flower was once at each position. All bees were tested individually to prevent competition and potentially resulting altered choice behaviour among individuals.

Choice experiments with honeybees (*A. m. carnica*) were performed in the Botanical Garden of the Heinrich-Heine University of Düsseldorf, Germany, in June 2013 under natural daylight conditions. Freely foraging and therefore flower-experienced honeybees of two colonies were attracted to a feeder in a distance of 30 m from the hives. From this feeder, individual bees were transported into a flight cage of 2 m x 4 m x 2 m in size in half-shade environment, holding the training and test area in a distance of 10 m from the feeder. The flight cage was necessary to prevent competition with other recruited honeybees as well as other hymenopteran visitors at the training and test set-up. Ten approaches and/or antenna reactions were recorded for honeybees.

Bumblebee colonies were purchased from re-natur GmbH (*B. t. dalmatinus*; Ruhwinkel, Germany) and kept in flight cages in the laboratory of the University of Düsseldorf. Individuals were trapped in plastic tubes directly from the hive entrance and brought to an indoor flight cage of 2m x 2m x 2 m for choice experiments. Both flight cages were illuminated by L58 W/865 fluorescent tubes (Osram, Munich, Germany) providing an intensity of about 2,000 lux of 6,500 K colour temperature and moderate emission of UV-light. Twenty approaches and/or antenna reactions were recorded for bumblebees.

Experiments with stingless bees (*M. quadrifasciata*) were performed on the Campus of the State University of Campinas, Campinas, Brazil, in February 2012 and on the Campus of the Federal University of Paraná, Curitiba, Brazil, in March 2013 under natural daylight conditions. Also freely foraging and therefore flower-experienced workers from two hives were trapped in plastic tubes directly from the hive entrance when they intended to fly out and were brought to the training and test area at a distance of approximately 30 m from the hives under natural daylight conditions. For these experiments no flight cage was necessary and rare visitors other than the evaluated individuals were directly trapped. Twenty approaches towards entirely coloured test flowers and 10 approaches and antenna reactions towards patterned test flowers were recorded for stingless bees.

As the training and test flowers were permanently rewarded, each individual bee of all three species chose one artificial flower, drank the reward till she was sated and flew back (honeybees and stingless bees) or was alternatively brought back (bumblebees) to the hive. Bumble- and honeybees were released in a distance of 60 cm to the test area and stingless bees approach the test area by themselves when leaving their hives. Hence, the visual angles under which they detect the test flowers in first instance were so small, that the bees' achromatic vision system was active, and subsequently switched to chromatic vision when approaching the flowers (Giurfa *et al.* 1997; Spaethe *et al.* 2001). This procedure was chosen in order to simulate natural conditions under which bees usually detect flowers.

Colour vision models and calculation of colour parameter

To gain insight into natural flower colouration and the choice behaviour of bees, we calculated several colour parameters known to influence bees' foraging behaviour. Colour parameters include achromatic contrasts and were calculated between flower peripheries and their backgrounds, as those flower parts capture the main part of the whole flower and are crucial for the detection of flowers by bees when the colour-blind vision is active, i.e. under small visual angles (Giurfa *et al.* 1997; Spaethe *et al.* 2001). Further on, we evaluate chromatic contrasts between flower peripheries and the background, as well as between flower peripheries and flower centres, as those contrasts are analysed by bees with their colour-active systems (Lunau *et al.* 1996). The same is true for bee-subjective spectral purity as crucial parameter in the foraging behaviour of bees (Lunau 1990; Rohde *et al.* 2013).

The colour-blind system analyse information in the green photoreceptors only, and therefore we calculated achromatic contrast as the quotient of the relative quantum flux of stimulus and background to the green receptor types. The quantum flux is calculated as the sum of the product of spectral sensitivity of a photoreceptor type, the spectral distribution of the illuminant and the spectral reflectance of the stimulus. The quantum flux is also multiplied with a sensitivity factor for each photoreceptor type assuming that the bee's eye is adapted to the background (Laughlin 1981; calculated as 1 divided by the sum of the product of spectral sensitivity of a photoreceptor type, the spectral distribution of the illuminant and the spectral reflectance of the background). This procedure was done for the three tested bee species, but not for flower-visiting birds. Here, double cones are active to analyse achromatic contrasts (see Receptor-noise limited model). As photoreceptor sensitivities for bees we used functions from Menzel and Backhaus (1991) for the honeybee *A. mellifera*, from Skorupski *et al.* (2007) for the bumblebee *B. t. dalmatinus*, and from Menzel *et al.* (1989) for the stingless bee *M. quadrifasciata*. As illumination we used the daylight function D65 (Wyszecki & Stiles 1982), and as background, to which the bees´ or birds´ eyes were assumed to be adapted, we used a standard function of green leaves.

For further colour parameters we used two different colour vision models: The colour hexagon (Chittka 1992) and the receptor-noise limited model (Vorobyev & Osorio 1998). The former one includes specific assumptions about neural processing in the bees' eye, whereas the latter one assumptions tracked in several animal species and can be applied for tri- as well as tetrachromatic colour vision systems and hence for bees as well as birds.

By using the colour hexagon model (Chittka 1992) bee-subjective spectral purity and bee-subjective chromatic contrast to the background as well as chromatic contrast between parts within a flower can be calculated. Chromatic contrast results from the perceptual distance of the colour loci and was given in hexagon units (HU; Chittka 1992). Spectral purity according to the colour hexagon model (Chittka 1992) was calculated as the perceptual distance between target and background divided by the perceptual distance between the corresponding spectral locus, i.e. the locus of the corresponding monochromatic light, and background (Lunau *et al.* 1996). The same functions for spectral sensitivities, background and illumination as before were used. By using the receptor-noise limited model chromatic contrast and achromatic contrast to the background, as well as chromatic contrast between parts within a flower can be calculated (Vorobyev & Osorio 1998). Chromatic contrast between stimulus and background is given in JND units (just noticeable differences; Vorobyev & Osorio 1998). Achromatic contrasts in the receptor-noise limited model results from dividing the contrast between stimulus and background in the green receptor for bee species and in the double cone for birds by the noise values of the corresponding receptor and is also given in JND units (Vorobyev & Osorio 1998). To apply this model, noise values for photoreceptor types were required, but were not available for stingless bees. Noise values in JND units for trichromatic colour vision systems for the UV-, blue- and green-photoreceptor type of 1.3, 0.9 and 0.9 for bumblebees (Skorupski & Chittka 2010), and of 0.13, 0.06 and 0.12 for honeybees (Vorobyev & Osorio

1998) were used. Noise values for tetrachromatic colour vision systems for the SWS1-, SWS2-, MWS- and LWS-photoreceptor type of 0.1, 0.07, 0.07 and 0.05 for birds possessing UVS-type eyes and of 0.1, 0.1, 0.1 and 0.07 for birds possessing VS-type eyes were used. These noise values are in accordance to published ratios of relative numbers of cone-types of 1:2:2:4 for UVS-type birds (Maier & Bowmaker 1993) and 1:1:1:2 for VS-type birds (Bowmaker *et al.* 1997) of SWS1-, SWS2-, MWS- and LWS-photoreceptor type, respectively, and also in accordance to the sole known weber fraction values for 0.1 for the LWS-photoreceptor of a UVS-type bird species (Maier 1992). As photoreceptor sensitivities for bees we used the same functions as for the colour hexagon model. Spectral sensitivity functions of the four single cones of pollinating bird species were adopted from Ödeen and Håstad (2010) for UVS-type and UV-type bird eyes. The spectral sensitivity function of double cones was adopted from Osorio *et al.* (1999) with an affiliated Weber fraction value of 0.05 (Siddiqi *et al.* 2004).

Statistical analysis

The machine learning algorithm random forest (Breiman 2001) is a preferred method to analyse ecological data (Cutler *et al*. 2007). To apply this method, we classified specific wavelength ranges each corresponding to the main sensitivity range of a photoreceptor type. This was done by calculating the mean spectral reflectance in the UV (301-400 nm), blue (401-500 nm), green (501-600 nm) and red (601-700 nm) wavelength range for each plant family (see yellow flowers section). By means of this classification method we evaluated whether a specific wavelength range contributes to structuring multiple datasets, i.e. natural yellow flowers, into classes. In this case we determine four classes, i.e. central parts of bee-pollinated flowers, peripheral parts of bee-pollinated flowers, and both parts of bird-pollinated flowers. The outputs of random forest analyses are confusion matrices revealing the classification and variable importance (*E*) values for the underlying factors, i.e. the four wavelength ranges. The higher the *E*-value of a wavelength range the more important is this factor for the class separation and correct assignment to a class. Each analysis based on 100,000 decision trees with 2 variables each, which are randomly selected from the four ranges of wavelengths, and which were not included into the analysis. To evaluate the results of random forest, further analyses were done using one-way analysis of variance (ANOVA) with Tukey HSD as *post-hoc* test, evaluating significant differences in the mean spectral reflectance between the four classes for each wavelength range (Junker *et al.* 2011).

To evaluate if bees and birds can detect differences in spectral reflectance between bee- and bird-pollinated flowers we compare achromatic as well as chromatic contrasts between flower peripheries and background as well as chromatic contrast between flower centres and peripheries using unpaired two-tailed *t*-tests for each flower-visitor. Spectral purity of centres and peripheries of bee- and bird-pollinated flowers each were compared using ANOVA with Tukey HSD as *post-hoc* tests.

To analyse the choice behaviour of the bees, we performed a paired two-tailed *t*-test comparing the number of approaches towards the two stimuli in each set-up between individual bees for each bee species. Likewise a paired two-tailed *t*-test was used to compare the number of antenna contacts towards centre and periphery or towards UV-reflective and UV-absorbing colours in the patterned set-up. The numbers of approaches as well as antenna contacts were logarithm transformed to meet the assumptions of normal distribution and variance homogeneity. All statistical analyses were performed with R 2.14.0 (R Development Core Team 2009), using the R packages "randomForest", "party", and "MASS" for random forest analyses and the package “stats” for other statistical analyses.

Results

Yellow flower colours

In total, we measured the spectral reflectance of 38 species (out of 32 genera in 19 families) with human all-yellow coloured flowers including bee-pollinated flowers in 13 species (out of 12 genera in 8 families) of the New World, and 14 species (11 genera in 8 families) of the Old World, as well as bird-pollinated flowers in 8 species (8 genera in 5 families) of the New World and 3 species (3 genera in 3 families) of the Old World. We pooled the data of flowers from the Old and New World, because we did not find differences in the mean spectral reflectance between peripheries and between centres within the two bee-pollinated or the two bird-pollinated groups in any range of wavelength with random forest analysis. The pooled data comprises 27 bee-pollinated species of 22 genera in 14 plant families and 11 bird-pollinated species of 11 genera in 7 plant families (Fig. 1; Supplemental Material 1).

Random forest analysis revealed that centres as well as peripheries of bee-pollinated flowers were more often correctly assigned to their specific groups, whereas centres and peripheries of bird-pollinated flowers were more often incorrectly assigned to other groups, but never to the group of peripheries of bee-pollinated flowers (Table 1). The most important range of wavelength for group separation was UV, followed by blue and green and the least contributing wavelength range was red (Table 1). We found significant differences in spectral reflectance properties only in the UV wavelengths range (ANOVA; $F=16.54$, $df=3$, $p<0.001$), but not in the blue, green or red range (ANOVA; $F=1.48$, $df=3$, $p=0.24$ for blue, $F= 2.25$, $df=3$, $p=0.10$ for green, and $F= 2.31$, $df=3$, $p=0.09$ for red). Peripheries of bee-pollinated flowers reflected significantly more UV-light than all other flower parts with their specific pollinators (Tukey HSD: $p<0.001$), but all other comparisons were not significant (Tukey HSD: $p>0.05$ respectively; Fig.

1). The colour hexagon revealed that peripheries of bee-pollinated flowers are most often bee-UV-green coloured (Fig. 2; after nomenclature from Chittka *et al.* 1994). In contrast, centres of bee-pollinated flowers as well as both parts of bird-pollinated flowers were more often bee-green coloured (Fig. 2; after nomenclature from Chittka *et al.* 1994).

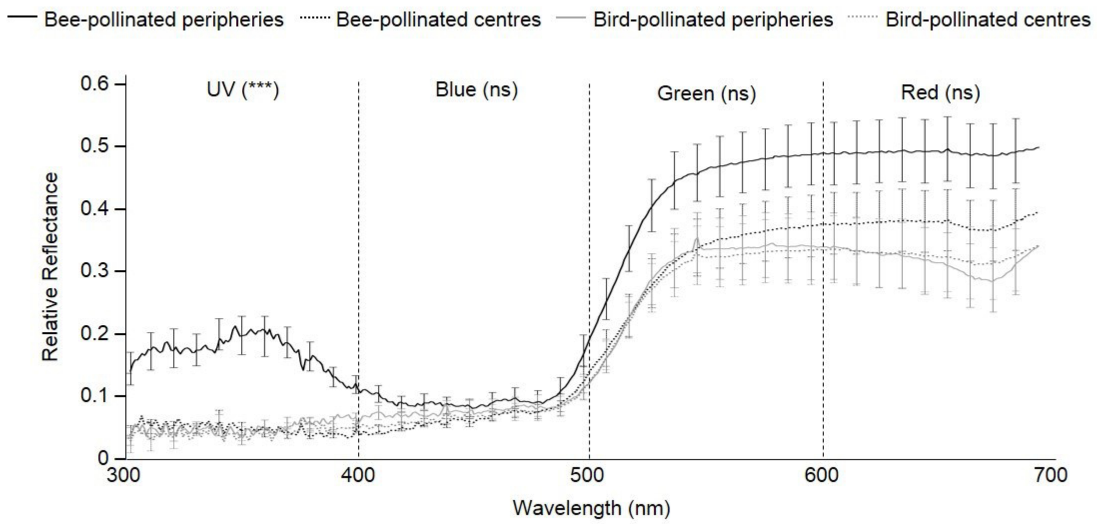

Fig. 1. Mean spectral reflectance with standard errors of the mean in 10 nm steps of central and peripheral parts of yellow coloured flowers pollinated by either bees or birds. For calculation of means see method section. Statistical analyses: Mean spectral reflectance was calculated in four wavelength ranges, i.e. UV, blue, green, and red, for each plant family and compared between bee-pollinated centres, bee-pollinated peripheries, bird-pollinated centres and bird-pollinated peripheries using one-way analysis of variance (ANOVA) with Tukey HSD as post-hoc test. ns=not significant; *** $p<0.001$.

(A) Confusion matrix

	bee_centre	bee_periphery	bird_centre	bird_periphery	class error
bee_centre	8	1	2	3	0.43
bee_periphery	2	11	0	1	0.21
bird_centre	6	0	0	1	1.00
bird_periphery	4	0	1	2	0.71

(B) Variable importance (C) ANOVA

	E	df	F	p
UV	45.67	3	16.54	<0.001
B	27.69	3	1.48	0.24
G	-24.25	3	2.25	0.10
R	-44.20	3	2.31	0.09

Table 1. Random forest analyses and statistical interference of spectral reflectance properties of yellow coloured flowers divided into centred and peripheral parts and pollinated by either bees or birds. (A) Confusion matrices showing the number of correctly assigned groups and the proportional class error for the mean spectral reflectance values in four wavelength ranges (UV=Ultraviolet, B=blue, G=green, R=red) with (B) variable importance (E) values. (C) Results of one-way analysis of variance (ANOVA).

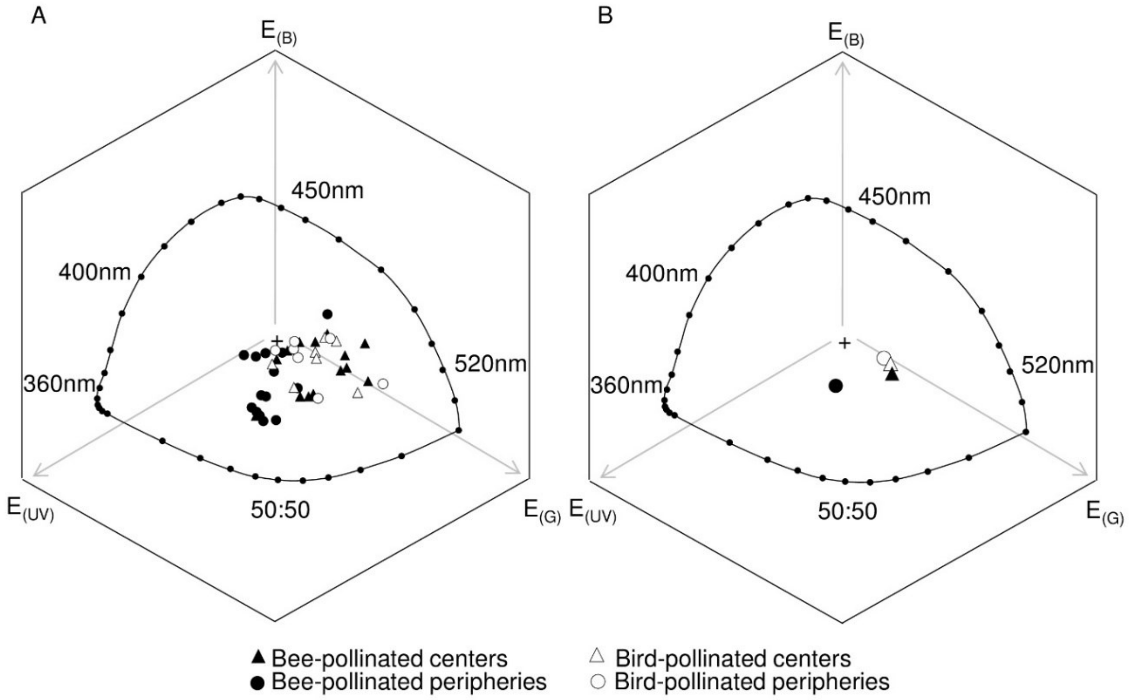

Fig. 2. Colour hexagon (Chittka 1992) of yellow coloured flower parts pollinated by either bees or birds for *Apis mellifera*. Each colour locus represents (A): flower parts of single plant families or (B): the mean of bee- and bird-pollinated centres and peripheries. For calculations of the means see method section. The excitation (E) of the ultraviolet (UV), blue (B) and green (G) photoreceptor types are indicated by arrows. + represents the centre of the hexagon. Colour loci of monochromatic lights are connected by a black line and labelled with selected hues or mixtures of UV- and Green-light.

The comparison between flower periphery and background between bee- and bird-pollinated flowers revealed significant differences only in achromatic contrasts for two out of three tested bee species, as well as in chromatic contrasts in the receptor-noise limited model for UVS-type birds (Fig. 3). Among that, peripheries of bird-pollinated flowers significantly displayed less achromatic and chromatic contrasts to the background as compared to peripheries of bee-pollinated flowers (Fig. 3).

More clearly were the results for intra-floral contrasts; chromatic contrasts between centre and periphery within the same flower significantly differed from each other for all flower-visitors and all calculation methods, with bee-pollinated flowers displaying higher colour contrasts within flowers than bird-pollinated ones did (Fig. 3). This was true for the visual capacities of all tested flower-visitors as well as for both tested colour vision models (Fig. 3).

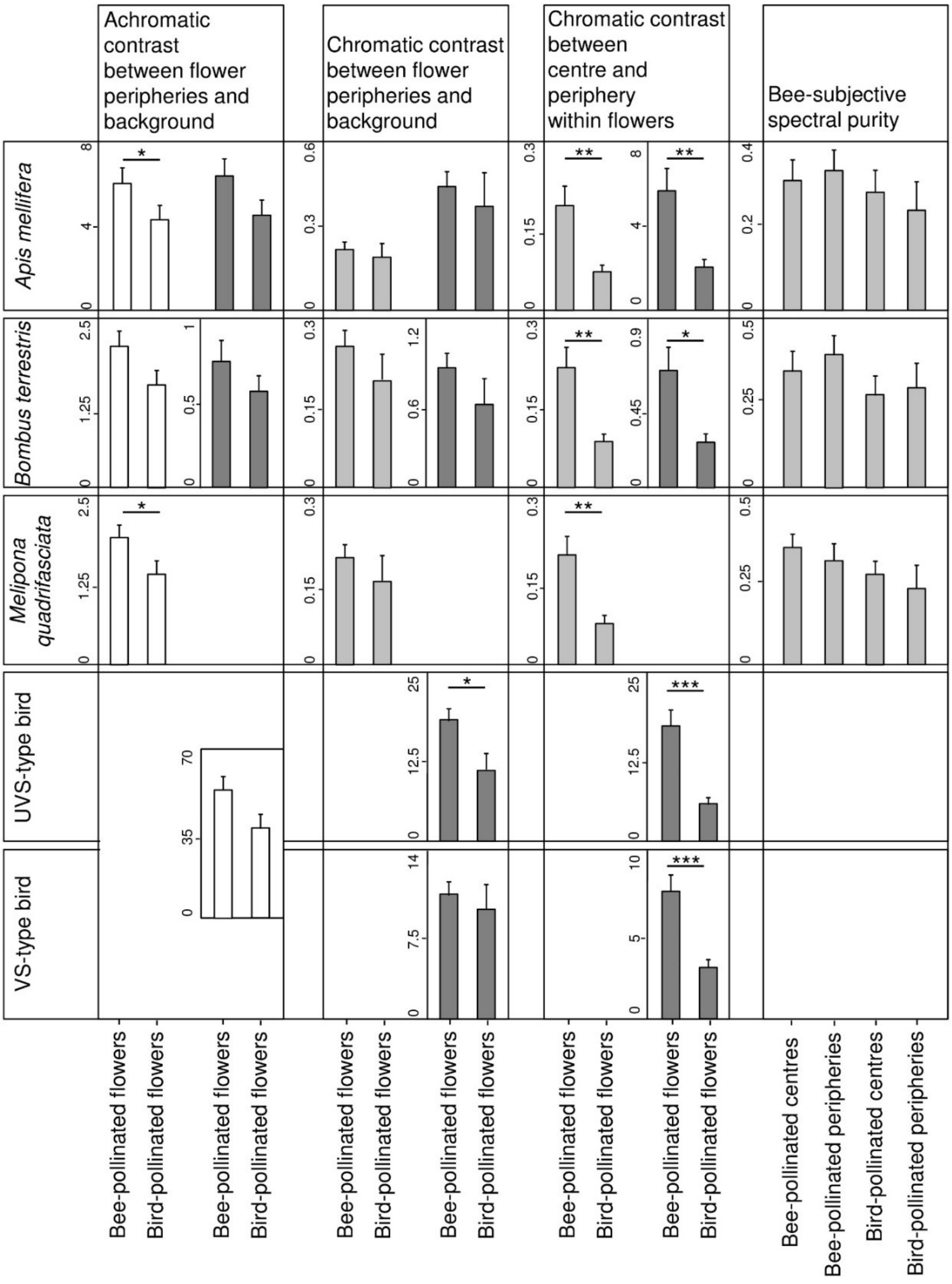

Fig. 3. Colour parameters as perceived by different species of flower visitors. White bars represent calculations based on physical properties in relative units. Light-grey bars represent calculations based on the colour hexagon model (Chittka 1992) in hexagon units. Dark grey bars represent calculations based on the receptor-noise limited model (Vorobyev and Osorio 1998) in JND units. Statistical analyses: Colour parameters between flower peripheries and background as well as between centre and periphery within flowers were compared between bee- and bird-pollinated flowers paired two-tailed t-tests. Spectral purities of centers and peripheries of bee- and bird-pollinated flowers were compared using one-way analysis of variance (ANOVA). * p<0.05; ** p<0.01; *** p<0.001; all other comparisons were not significant.

Choice experiments

Giving the choice between entirely UV-reflecting and entirely UV-absorbing test flowers, none tested bee species showed a significant preference for a test flower (Fig. 4A). The same was true when giving the choice between patterned test flowers, except for *A. m. carnica*, preferring test flowers with UV-absorbing centres and UV-reflecting peripheries over the reciprocal pattern (Fig. 4B).

While approaching the patterned flowers, *A. m. carnica* as well as *B. t. dalmatinus* made their first antennal contact significantly more often at the centre of the artificial flowers compared to the periphery, whereas *M. quadrifasciata* did not show any preference (Fig. 4C).

All three tested bee species made their first antenna contact significantly more often towards UV-absorbing areas within the patterned artificial flowers (Fig. 4D).

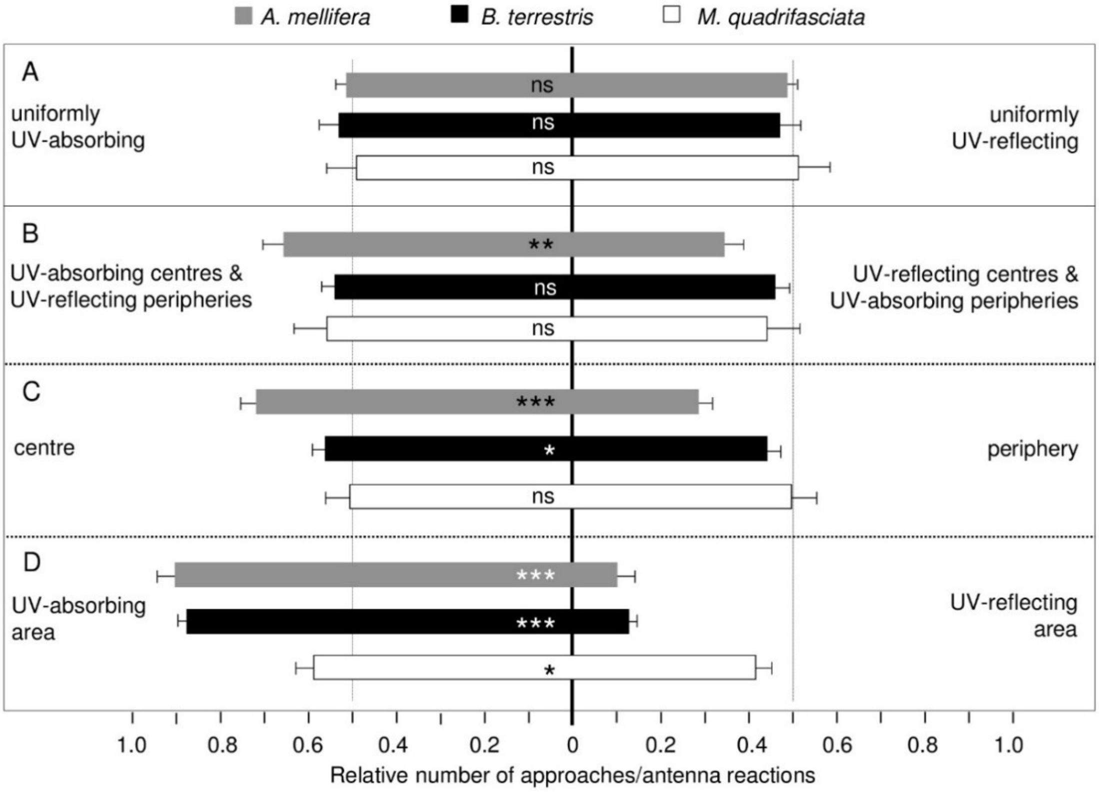

Fig. 4. Dual choice tests with three species of bees. Choices were represented in grey bars for the honeybee *Apis mellifera carnica*, in black bars for the bumblebee *Bombus terrestris dalmatinus*, and in white bars for the stingless bee *Melipona quadrifasciata*. (A, B) Mean relative number of approaches with standard errors of the mean towards yellow test flowers (A): with entirely UV-reflectance and entirely UV-absorbance or (B): with UV-patterns each. (C, D) Mean relative number of antenna contacts with standard errors of the mean towards (C) the centre or the periphery or towards (D) the UV-absorbing or UV-reflecting area of yellow test flowers, both within yellow flowers with an UV pattern. Statistical analyses: Paired, two-tailed t-test. ns=not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Discussion

In the current study we demonstrate that bee- and bird-pollinated yellow flowers differ in their spectral reflectance properties. Yellow bee-pollinated flowers show a pattern of spectral reflectance with UV-absorbing centre and UV-reflecting periphery, whereas yellow bird-pollinated flowers are mostly uniformly UV-absorbing. Bees do not prefer any test flowers that resemble flower colours found in nature, and yellow bee- and bird-pollinated flowers do not differ in colour parameter, except for the achromatic contrast between peripheries and background of bee-pollinated flowers. For bee-pollinated flowers the achromatic contrast is higher than for bird-pollinated ones indicating that yellow bee-pollinated flowers are easier to detect for bees. However, this is only true for two tested bee species, which did not significantly prefer any of the artificial flowers. Recently, it was shown that red and white flowers pollinated by bees are of higher spectral purity as compared to bird-pollinated ones and are therefore easier to detect for bees. In red and in white flowers, spectral purity for bees depends on the amount of UV-reflectance, with red flowers being more attractive for bees if they reflect UV-light and white flowers being more attractive for bees if they absorb UV-light (Lunau *et al.* 2011). This has been interpreted as a sensorial floral filter discouraging potential nectar robbing bees (Lunau *et al.* 2011). However, bees did not discriminate between yellow flowers with differing UV-reflectance. More precisely, spectral purity of yellow flowers cannot be altered simply by additional reflectance or absorption of UV-light, as in red or white colours, although bees as pollen-collecting flower-visitors respond innately to yellow UV-absorbing colours (Jones & Buchman 1974; Heuschen *et al.* 2005). Both experienced and naïve bees did not show significant preference for any of the artificial flowers, indicating that former experience of bees with flowers does not affect the choice behaviour of bees for the offered colour stimuli.

Despite the lack of a preference for yellow either with or without UV-reflectance, the tested bees showed striking behaviours: Workers of all tested bee species preferred to make their first contact by means of their antennal tips at the UV-absorbing area at the patterned yellow artificial flowers, irrespective of its spatial position within the test flower. Chromatic contrasts in the hexagon model has to be higher than 0.1 HU in the hexagon model (Spaethe *et al.* 2001) and higher than 1 JND in the receptor-noise limited model (Vorobyev *et al.* 2001), in order that bees can perceive them as differently coloured and can distinguish them from each other. This was the case in bee-pollinated flowers, but not in bird-pollinated ones (except for bumblebees in the receptor-noise limited model, which were not able to distinguish centres and peripheries of either bee- or bird-pollinated flowers). Thus, bird-pollinated flowers appeared uniformly coloured for bees, whereas bee-pollinated flowers showed a pattern of differently coloured areas for the bees. Previous studies have shown that bees usually make contact and land on the edges of flowers if nectar guides are lacking (Manning 1956; Free 1970). It is thus assumed that the presence of nectar guides aids in-flight-orientation and directs the bees to the floral reward more quickly, decreasing bees' handling time (Waser & Price 1985; Leonard & Papaj 2011). Thus, the occurrence of UV-patterns in yellow bee-pollinated flowers might be an adaptation of flowers to the pollinators' visual system. This is further suggested by the fact that nectar guides orient the flower-visitor in a way that contact between the visitors' body and the flowers' reproductive organs is ensured and thus improve the effectiveness of pollination (Owen & Bradshaw 2011). If bees enter the corolla tube of guideless mutants of *Mimulus lewisii* which lack yellow nectar guides present in wild-type flowers, their orientation towards the flower is upside-down, and therefore improper to promote pollination (Owen & Bradshaw 2011). The authors argued that the lack of nectar guides and the resulting decreased visitation frequency of bees in this species could act as an adaptive trait to other pollinators, i.e. hummingbirds or moths (Owen & Bradshaw 2011). Moreover, several other studies reveal that bees prefer to visit flowers displaying a pattern over those ones which are uni-coloured (Free 1970; Waser & Price 1985; Heuschen *et al.* 2005).

However, for hummingbirds other floral traits than colour promote effective foraging behaviour: in hummingbird-pollinated flowers which lack nectar guides other flower traits like lower lips or trumpet-shaped orifices can incur a tactile function guiding the flower-visiting bird (Smith *et al.* 1996; Temeles & Rankin 2000). Ornithophilous flowers from the New and from the Old World share characteristic floral traits including the lack of scent and high amounts of dilute nectar due to convergent evolution (Faegri & van der Pijl 1979). Nonetheless there are differences in the floral traits within bird-pollinated flowers mainly due to differences in the foraging behaviour and the visual systems of the various bird families (Ödeen & Håstad 2010; Rocca & Sazima 2010). Floral colouration seems to be a shared trait within bird-pollinated yellow flowers, as we did not find differences among flowers pollinated by either New or Old World bird species.

In conclusion, the yellow colouration of bee- and bird-pollinated flowers relies on convergent coevolution between flowers and flower-visitor species from the New as well as from the Old World. Yellow flowers pollinated by bees and birds differ in respect of the occurrence of UV-patterns but not in specific colour parameters. The occurrence of UV-reflectance patterns exclusively in bee-pollinated flowers represents an adaptation to bees in accordance to published behavioural data. The results support the hypothesis that flower colours and the visual systems of pollinators are well adapted to each other.

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References

Bowmaker J.K., Heath L.A., Wilkie S.E., Hunt D.M. (1997) Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research*, **37**, 2183-2194.

Breiman L. (2001) Random forests. *Machine Learning*, **45**, 5-32.

Briscoe A.D., Chittka L. (2001) The evolution of color vision in insects. *Annual Review of Entomology*, **46**, 471-510.

Campbell D.R., Bischoff M., Lord J.M., Robertson A.W. (2010) Flower color influences insect visitation in alpine New Zealand. *Ecology*, **91**, 2638-2649.

Chittka L. (1992) The colour hexagon: A chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A*, **170**, 533-543.

Cutler D.R., Edwards Jr T.C., Beard K.H., Cutler A., Hess K.T., Gibson J., Lawler J.J. (2007) Random forests for classification in ecology. *Ecology*, **88**, 2783-2792.

Davies K.M., Albert N.W., Schwinn K.E. (2012) From landing lights to mimicry: the molecular regulation of flower colouration and mechanisms for pigmentation patterning. *Functional Plant Biology*, **39**, 619-638.

Endler J.A., Mielke P.W. (2005) Comparing entire colour patterns as birds see them. *Botanical Journal of the Linnean Society*, **86**, 405-431.

Faegri K., Pijl L. van der (1979) *The Principles of Pollination Ecology*. Pergamon Press, Oxford, UK.

Free J.B. (1970) Effect of flower shapes and nectar guides on the behaviour of foraging honeybees. *Behaviour*, **37**, 269-285.

Giurfa M., Núñez J., Backhaus W. (1994) Odour and colour information in the foraging choice behaviour of the honeybee. *Journal of Comparative Physiology A*, **175**, 773-779.

Giurfa M., Vorobyev M., Kevan P., Menzel R. (1996) Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *Journal of Comparative Physiology A*, **178**, 699-709.

Giurfa M., Vorobyev M., Brandt R., Posner B., Menzel R. (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology A*, **180**, 235-243.

Grant V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution*, **3**, 82-97.

Grant K.A., Grant V. (1968) *Hummingbirds and their flowers*. Columbia University Press, New York, USA.

Guldberg L.D., Atsatt P.R. (1975) Frequency of reflection and absorption of ultraviolet light in flowering plants. *American Midland Naturalist*, **93**, 35-43.

Harborne J.B., Smith D.M. (1978) Anthochlors and other flavonoids as honey guides in the Compositae. *Biochemical Systematics and Ecology*, **6**, 287-291.

Heuschen B., Gumbert A., Lunau K. (2005) A generalised mimicry system involving angiosperm flower colour, pollen and bumblebees' innate colour preferences. *Plant Systematics and Evolution*, **252**, 121-137.

Horovitz A., Cohen Y. (1972) Ultraviolet reflectance characteristics in flowers of crucifers. *American Journal of Botany*, **59**, 706-713.

Jones C.E., Buchmann S.L. (1974) Ultraviolet floral patterns as functional orientation cues in hymenopterous pollination systems. *Animal Behaviour*, **22**, 481-485.

Junker R.R., Loewel C., Gross R., Dötterl S., Keller A., Blüthgen N. (2011) Composition of epiphytic bacterial communities differs on petals and leaves. *Plant Biology*, **13**, 918-924.

Junker R.R., Blüthgen N., Brehm T., Binkenstein J., Paulus J., Schaefer H.M., Stang M. (2013) Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. *Functional Ecology*, **27**, 329-341.

Kaczorowski R.L., Blumenfeld G., Koplovich A., Markman S. (2014) Color and side preferences in Palestine sunbirds (*Nectarinia osea*). *Israel Journal of Ecology & Evolution*, **60**, 35-40.

Kugler H. (1963) UV-Musterungen auf Blüten und ihr Zustandekommen. *Planta*, **59**, 296-329.

Lehrer M., Horridge G.A., Zhang S.W., Gadagkar R. (1995) Shape vision in bees: innate preference for flower-like patterns. *Philosophical Transactions of the Royal Society of London B*, **347**, 123-137.

Leonard A.S., Papaj D.R. (2011) 'X'marks the spot: The possible benefits of nectar guides to bees and plants. *Functional Ecology*, **25**, 1293-1301.

Lunau K. (1990) Colour saturation triggers innate reactions to flower signals: flower dummy experiments with bumblebees. *Journal of Comparative Physiology A*, **166**, 827-834.

Lunau K. (1993) Interspecific diversity and uniformity of flower colour patterns as cues for learned discrimination and innate detection of flowers. *Experientia*, **49**, 1002-1010.

Lunau K. (1995) Notes on the colour of pollen. *Plant Systematics and Evolution*, **198**, 235-252.

Lunau K., Wacht S., Chittka L. (1996) Colour choices of naive bumble bees and their implications for colour perception. *Journal of Comparative Physiology A*, **178**, 477-489.

Lunau K. (2007) Stamens and mimic stamens as components of floral colour patterns. *Botanische Jahrbücher*, **127**, 13-41.

Lunau K., Papiorek S., Eltz T., Sazima M. (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology*, **214**, 1607-1612.

Maier E.J. (1992) Spectral sensitivities including the ultraviolet of the passeriform bird *Leiothrix lutea*. *Journal of Comparative Physiology A*, **170**, 709-714.

Maier E.J., Bowmaker J.K. (1993) Colour vision in the passeriform bird, *Leiothrix lutea*: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. *Journal of Comparative Physiology A*, **172**, 295-301.

Manning A. (1956) Some aspects of the foraging behaviour of bumble-bees. *Behaviour*, **9**, 164-201.

Mayfield M.M., Waser N.M., Price M.V. (2001) Exploring the ‘most effective pollinator principle’with complex flowers: bumblebees and *Ipomopsis aggregata*. *Annals of Botany*, **88**, 591-596.

Melendez-Ackerman E., Campbell D.R. (1998) Adaptive significance of flower color and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution*, **52**, 1293-1303.

Menzel R., Ventura D.F., Werner A., Joaquim L.C.M., Backhaus W. (1989) Spectral sensitivity of single photoreceptors and color vision in the stingless bee, *Melipona quadrifasciata*. *Journal of Comparative Physiology A*, **166**, 151-164.

Menzel R., Backhaus W. (1991) Color vision in insects. In: Gouras P. ed. *Vision and visual dysfunction. VI. Perception of color.* Macmillan, London, UK: pp 262–293.

Ödeen A., Håstad O. (2003) Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Molecular Biology and Evolution*, **20**, 855-861.

Ödeen A., Håstad O. (2010) Pollinating birds differ in spectral sensitivity. *Journal of Comparative Physiology A*, **196**, 91-96.

Orbán L.L., Plowright C.M.S. (2013) The effect of flower-like and non-flower-like visual properties on choice of unrewarding patterns by bumblebees. *Naturwissenschaften*, **100**, 621-631.

Osorio D., Vorobyev M., Jones C.D. (1999). Colour vision of domestic chicks. *Journal of Experimental Biology*, **202**, 2951-2959.

Owen C.R., Bradshaw H.D. (2011) Induced mutations affecting pollinator choice in *Mimulus lewisii* (Phrymaceae). *Arthropod-Plant Interactions*, **5**, 235-244.

Peitsch D., Fietz A., Hertel H., de Souza J., Ventura D.F., Menzel R. (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology A*, **170**, 23-40.

Plowright C.M.S, Simonds V.M., Butler M.A. (2006) How bumblebees first find flowers: habituation of visual pattern preferences, spontaneous recovery, and dishabituation. *Learning and Motivation*, **37,** 66-78.

Primack R.B. (1982) Ultraviolet patterns in flowers, or flowers as viewed by insects. *Arnoldia*, **42**, 139-146.

R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rocca M.A., Sazima M. (2010) Beyond hummingbird-flowers: The other side of ornithophily in the Neotropics. *Oecologia Australis*, **14**, 67-99.

Rohde K., Papiorek S., Lunau K. (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. *Journal of Comparative Physiology A*, **199**, 197-210.

Rosas-Guerrero V., Aguilar R., Martén-Rodríguez S., Ashworth L., Lopezaraiza-Mikel M., Bastida J. M., Quesada M. (2014) A quantitative review of pollination syndromes: do floral traits predict effective pollinators?. *Ecology letters*, **17**, 388-400.

Schemske D.W., Bradshaw H.D. (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences*, **96**, 11910-11915.

Siddiqi A., Cronin T.W., Loew E.R., Vorobyev M., Summers K. (2004) Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *Journal of Experimental Biology*, **207**, 2471-2485.

Silberglied R.E. (1979) Communication in the ultraviolet. *Annual Review of Ecology and Systematics*, **10**, 373-398.

Skorupski P., Döring T.F., Chittka L. (2007) Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. *Journal of Comparative Physiology A*, **193**, 485-494.

Skorupski P., Chittka L. (2010) Differences in photoreceptor processing speed for chromatic and achromatic vision in the bumblebee, *Bombus terrestris*. *The Journal of Neuroscience*, **30**, 3896-3903.

Smith C.E., Stevens J.T., Temeles E.J., Ewald P.W., Hebert R.J., Bonkovsky R.L. (1996) Effect of floral orifice width and shape on hummingbird-flower interactions. *Oecologia*, **106**, 482-492.

Spaethe J., Tautz J., Chittka L. (2001) Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences USA*, **98**, 3898-3903.

Spaethe J., Streinzer M., Eckert J., May S., Dyer A.G. (2014) Behavioural evidence of colour vision in free flying stingless bees. *Journal of Comparative Physiology A*, **200**, 485-496.

Sprengel C.K. (1793) *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen.* Vieweg, Berlin, Germany.

Stiles F.G. (1976) Taste preferences, color preferences, and flower choice in hummingbirds. *Condor*, **78**, 10-26.

Temeles E.J., Rankin A.G. (2000) Effect of the lower lip of *Monarda didyma* on pollen removal by hummingbirds. *Canadian Journal of Botany*, **78**, 1164-1168.

Thompson W.R., Meinwald J., Aneshansley D., Eisner T. (1972) Pigments responsible for ultraviolet absorption in nectar guide of flower. *Science*, **177**, 528-530.

Vorobyev M., Osorio D. (1998) Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London, Series B*, **265**, 351-358.

Vorobyev M., Brandt R., Peitsch D., Laughlin S.B., Menzel R. (2001) Colour thresholds and receptor noise: behaviour and physiology compared. *Vision Research*, **41**, 639-653.

Waser N.M., Price M.V. (1985) The effect of nectar guides on pollinator preference: experimental studies with a montane herb. *Oecologia*, **67**, 121-126.

Wyszecki G., Stiles W.S. (1982) *Color science*. Wiley, New York, USA.

CHAPTER 5

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Bees` subtle colour preferences: How bees respond to small changes in pigment concentration

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Bees' subtle colour preferences: how bees respond to small changes in pigment concentration

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Abstract Variability in flower colour of animal-pollinated plants is common and caused, inter alia, by inter-individual differences in pigment concentrations. If and how pollinators, especially bees, respond to these small differences in pigment concentration is not known, but it is likely that flower colour variability impacts the choice behaviour of all flower visitors that exhibit innate and learned colour preferences. In behavioural experiments, we simulated varying pigment concentrations and studied its impact on the colour choices of bumblebees and honeybees. Individual bees were trained to artificial flowers having a specific concentration of a pigment, i.e. Acridine Orange or Aniline Blue, and then given the simultaneous choice between three test colours including the training colour, one colour of lower and one colour of higher pigment concentration. For each pigment, two set-ups were provided, covering the range of low to middle and the range of middle to high pigment concentrations. Despite the small bee-subjective perceptual contrasts between the tested stimuli and regardless of training towards medium concentrations, bees preferred neither the training stimuli nor the stimuli offering the highest pigment concentration but more often chose those stimuli offering the highest spectral purity and the highest chromatic contrast against the background. Overall, this study suggests that bees choose an intermediate pigment concentration due to its optimal conspicuousness. It is concluded that the spontaneous preferences of bees for flower colours of high spectral purity might exert selective pressure on the evolution of floral colours and of flower pigmentation.

Keywords *Apis mellifera* · *Bombus terrestris* · Colour preference · Pigment concentration · Spectral purity · Floral colour variation

Introduction

The impressive diversity of floral colours is mainly caused by a large diversity of flower pigments occurring in different concentrations and combinations (Kay et al. 1981). In addition, epidermal cell shape, position and thickness of the pigment layer, and total internal light reflection might contribute to flower colour (Kay et al. 1981; Noda et al. 1994). Intraspecific small differences in flower pigment concentration are doubtless perceptible to the human eye and cause natural within-species variability of flower colours or continuous flower colour variation. Many studies about variation in flower colours deal with discontinuous variation, e.g. distinct colour morphs occurring without intermediately coloured individuals (Meléndez-Ackerman et al. 1997; Matsumura et al. 2006; Whibley et al. 2006; Shipunov et al. 2011). Less well studied but probably more common is continuous colour variation of a single morph caused by more subtle differences in the composition and concentration of floral pigments (Tourjee et al. 1993; Tastard et al. 2008). This variability of pigment concentration and composition likely affects differences in various colour parameters, i.e. spectral purity (saturation), dominant wavelength (hue) and/or intensity (brightness) influencing the foraging behaviour of flower-visiting insects, especially bees. Beside these three parameters of colours, pigment composition

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and/or concentration can affect the contrast between one flower colour and another colour like that of green leaves or the background, which also is important for foraging bees.

For the detection of flowers from some distance, bumblebees as well as honeybees use green contrast: a colour-blind visual orientation system mediated by the green receptor contrast (Giurfa et al. 1996, 1997; Giurfa and Vorobyev 1998; Hempel de Ibarra et al. 2001, 2002). Flower-constant foraging bees are known to rely on the flower colour as an important cue to identify conspecific flowers of a current food plant species (Neumeyer 1981) and even discriminate against conspecific flowers of a differently coloured floral colour morph (Hill et al. 1997). Since the flowers' attractiveness for naïve bees can be attributed to floral colour cues, flower visitors may exhibit innate preferential flower choice according solely to flower colour (Giurfa et al. 1995; Lunau et al. 1996). In tests in which either the spectral purity or the dominant wavelength of colours was varied, it has been shown that trained honeybees and bumblebees respond to subtle differences in the spectral purity and dominant wavelength of artificial flowers in a different way (Rohde et al. 2013). Whereas both bumblebees and honeybees preferred trained colours of a distinct dominant wavelength over colours of slightly different dominant wavelength, they preferred colours of slightly higher spectral purity over the trained colour (Rohde et al. 2013). It has, however, not been shown how experienced bees respond to colours of slightly deviant pigment concentrations, in which several colour parameters, i.e. spectral purity, dominant wavelength and colour intensity, vary. Moreover, the study of Rohde et al. (2013) tested only colours in the blue waveband part; therefore, it is an open question if the findings hold for other colours such as yellow.

There are probably major selective pressures on flower colours exerted by pollinators (Menzel and Shmida 1993 and references within). Since some pollinators learn the flower colour of their current food plant, flower-constant foraging pollinators might overlook flowers exhibiting strongly deviant colours and thus less effectively pollinate those flowers (Hill et al. 1997). On the other hand, individual plants displaying more attractive flowers than conspecifics might benefit in terms of increased pollination success (Chittka et al. 1999). It is plausible that bees foraging flower-constantly at flowers of a given wild type would probably not visit highly deviant colour morphs, but instead prefer subtle variations in flower colours fitting better to their spontaneous preferences over the average flower colour. The ability (Raine et al. 2006; and limitations, see Dyer and Chittka 2004a; Goulson et al. 2007) for within-species and between-species preferences in bees has been demonstrated in cases in which flowers undergo a change of colour (Asmussen 1993) or odour (Dobson et al. 1999) during anthesis.

Floral colours affect flower-visiting behaviour of bees in various respects. Flower colours are, however, subject to variation because the concentration as well as the composition of floral pigments varies due to genetic or environmental influences. However, flower colours are a main attractant for bee pollinators, which might respond to intraspecific variability of flower colours. The conspicuousness of flowers is attributed to commonly high concentrations of floral pigments (Kay et al. 1981) and hence the resulting highly saturated flower colours, probably not only for human eyes. The brothers Franz and Sigmund Exner noticed as early as 1910 that “we can discern manifold designs in flower petals which generate relatively high colour saturation. Thus we must…conclude that the more saturated colours are more conspicuous than unsaturated ones for insects…as is the case for us. Only the most brilliantly coloured jewels surpass certain flower colours in colour saturation” (translated from German by the authors from Exner and Exner 1910). However, the relationship between pigment concentration and colour saturation is neither simple nor well studied. To a human observer, colour saturation of a pigment solution appears to increase with increasing pigment concentration, but only up to an intermediate concentration, beyond which saturation appears to decrease. Thus, with increasing concentration, colour saturation of a pigment solution undergoes an optimum.

How bees respond to small differences in visual signals is already known to some extent (Gumbert 2000; Dyer and Chittka 2004a; Giurfa 2004; Dyer and Neumeyer 2005; Goulson et al. 2007; Benard and Giurfa 2008; Avarguès-Weber et al. 2010), but which differences in visual signals are caused by variability in floral pigment concentration and pigment composition among individual flowers of the same species and how bees respond to that have not been well investigated. In this study, we use artificial flowers with small and defined differences in the concentration of a pigment to test if and how trained bumblebees as well as honeybees respond to test colours offering small shifts of colour cues caused by pigment concentration. The tests provided a triple choice including one trained stimulus of a given pigment concentration, one stimulus of lower and one of higher pigment concentration as compared to the trained stimulus. Thus, we simulate the effect of slightly varying floral pigment concentrations and test the impacts of colour variability on the foraging behaviour of important bee species. We explicitly test if spontaneous preferences shape the colour choice behaviour of trained bees with respect to variation in pigment concentration.

Materials and methods

Stimuli and test set-ups

Aniline Blue and Acridine Orange were used as floral pigment analogues. For the construction of the artificial flowers, 10 cm^2 of UV-reflecting filter paper (Whatman No. 1, GE Healthcare, USA) was soaked for 1 min in 20-ml pigment solution of different concentrations of Aniline Blue and Acridine Orange in water. After 1 min, even when the pigment solution was not fully absorbed, the filter paper was removed from the solution and was dried by hanging for 24 h to achieve a uniform distribution of colour over the whole surface. Circular pieces 30 mm in diameter were punched out of the filter paper in order to construct the corolla of the artificial flowers with a central circular cutout 5 mm in diameter, which was stuck to a plastic stick offering the reward in a small-bore hole. Three artificial flowers in each training situation and each experimental set-up were presented simultaneously on a grey cardboard (50×50 cm) with 7 cm interspace.

The stimuli were offered to the bees in two test set-ups for Aniline Blue and two for Acridine Orange. For each pigment, one test set-up with three test stimuli having pigment concentrations in the range from low to middle and one test set-up with three stimuli in the range from middle to high were prepared. In each set-up, three artificial flowers were offered: one of an intermediate concentration also used for training, one of lower concentration and one of higher concentration as compared to the training concentration. Aniline Blue was offered in concentrations of 0.5, 1.5 and 2.5 g/l or 1, 6 and 10 g/l, and Acridine Orange in concentrations of 0.325, 0.625 and 1.0 g/l or 1, 5 and 10 g/l.

Spectral reflection properties of colour stimuli and calculation of colour parameters

Relative spectral reflections of the stimuli were measured with the spectrophotometer USB 2000 (Ocean Optics, Inc., Dunedin, FL, USA) in an angle of 45° to the measuring spot, illuminated with a deuterium-halogen light source D_2H (World Precision Instruments, Inc., Sarasota, FL, USA) and connected with a bifurcated fibre-optic cable (UV–VIS 400 μm; World Precision Instruments, Inc., Sarasota, FL, USA; Fig. 1). A pressed pellet of barium sulphate powder was used as white standard and a black film can as black standard. According to the spectral reflection measurements, we calculate colour parameters (i.e. spectral purity and intensity) and physiological properties of photoreceptors in the bee's eye under daylight conditions (i.e. green contrast and colour contrast to the background) to analyse which colour parameters affect the choice of bees for visiting distinct artificial flowers.

Green contrast, G, is the specific contrast between stimulus and background in the green photoreceptor type and is calculated as the quotient of the relative quantum flux of stimulus and background to the green receptor. The quantum flux is the sum of the product of spectral sensitivity of a photoreceptor type [UV, blue or green; for bumblebees acquired according to Skorupski et al. (2007) and for honeybees acquired according to Menzel and Backhaus (1991)], the spectral distribution of the illuminant [for bumblebees the spectral distribution of the fluorescent tubes and for honeybees the daylight function D65 (Wyszecki and Stiles 1982)] and the spectral reflection of the stimulus over a wavelength interval from 300 to 700 nm, multiplied with a sensitivity factor, for each photoreceptor type. The sensitivity factor assumes that the bee's eye is adapted to the background and is calculated as 1 divided by the sum of the product of spectral sensitivity of a photoreceptor type, the spectral distribution of the illuminant and the spectral reflection of the background (grey cardboard) over a wavelength interval from 300 to 700 nm (Laughlin 1981).

Colour contrast was calculated according to the colour hexagon model (Chittka 1992), i.e. H, and also according to the receptor noise-limited model (Vorobyev and Osorio 1998), i.e. ΔS. The colour contrast in the colour hexagon results from the perceptual distance of the colour loci of the stimulus and the background and is given in hexagon units (HU; Chittka 1992; Fig. 2). Chromatic contrasts according to the receptor noise-limited model (Vorobyev and Osorio 1998) were calculated with standard deviations of 1.3, 0.9 and 0.9 for the UV-, blue- and green-photoreceptor type, respectively, for the bumblebee (Skorupski and Chittka 2010) and 0.13, 0.06 and 0.12 for the UV-, blue- and green-photoreceptor type, respectively, for the honeybee (Vorobyev et al. 2001), and are given in JND units (just noticeable differences).

Spectral purity according to the colour hexagon model (Chittka 1992), SP_H, was calculated as the perceptual distance between target and background divided by the perceptual distance between the corresponding spectral locus, i.e. the locus of the corresponding monochromatic light, and background (Lunau et al. 1996; Fig. 2). The closer the colour loci of stimuli are located to the spectrum locus, the more spectrally pure they are. Since there is an on-going debate about colour vision modelling in bees (Menzel and Backhaus 1991; Chittka 1992, 1999; Brandt and Vorobyev 1997; Vorobyev and Osorio 1998), we also calculate spectral purity independent of a colour vision model, SP, by dividing the difference of maximal and minimal spectral reflection by the average spectral reflection over the wavelength from 300 to 700 nm (Endler 1990).

Intensity, I, was calculated as the cumulative sum of relative spectral reflection of stimuli over the wavelength range from 300 to 700 nm (Endler 1990). For a better representation of all colour parameters in one graphic (Fig. 3), I was divided by 100.

The values of relative quantum flux, green contrast G, colour contrasts to the background H and ΔS, spectral purity SP_H and SP and intensity I of all training and test colours are listed in Table 1.

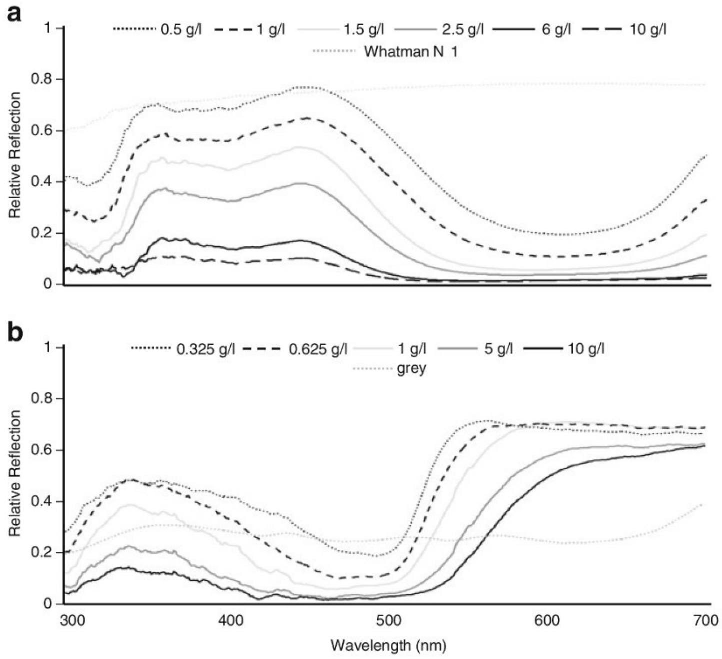

Fig. 1 Spectral reflection of the training and test stimuli: (**a**) stimuli with Aniline Blue concentrations on Whatman filter paper No. 1 and uncoloured Whatman filter paper No. 1; (**b**) stimuli comprising Acridine Orange concentrations on Whatman filter paper No. 1 and the *grey background*

Bee keeping, training and choice experiments

We tested ten flower-naïve workers of a bumblebee colony (*Bombus terrestris dalmatinus*; Koppert Biological Systems, Berkel en Rodenrijs, Netherlands) in an indoor flight cage illuminated by L58 W/865 fluorescent tubes (Osram, Munich, Germany) providing an intensity of about 2,000 lux of a 6,500 K colour temperature and moderate emission of UV-light. We also tested 25 experienced workers of the Western honeybee (*Apis mellifera carnica*) in an outdoor flight cage

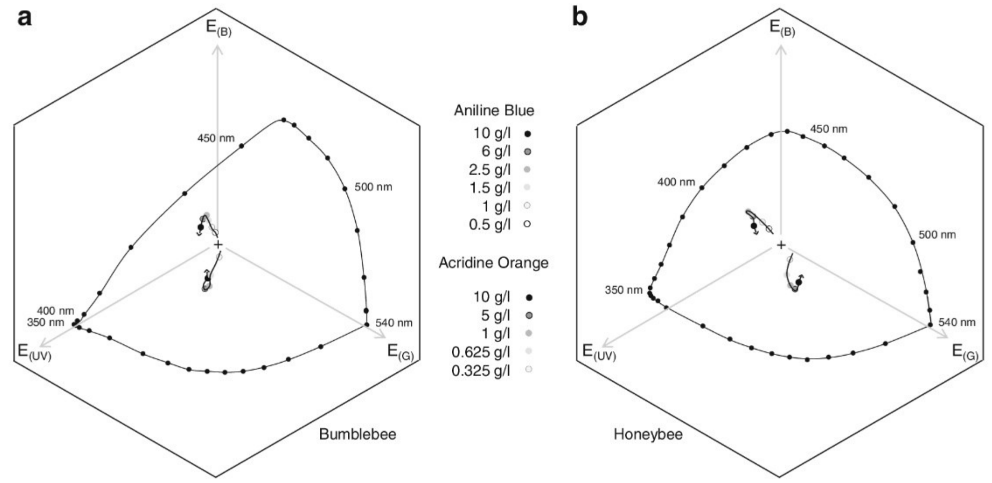

Fig. 2 Colour loci of training and test stimuli in the colour hexagon (Chittka 1992) showing the calculated excitation of ultraviolet [$E_{(UV)}$], blue [$E_{(BL)}$] and green [$E_{(GR)}$] photoreceptor types indicated by *grey arrows*. *Circles* denote the loci of the colours made by different concentrations of Aniline Blue and Acridine Orange for the colour vision system (**a**) of the bumblebee *Bombus terrestris dalmatinus* and (**b**) of the honeybee *Apis mellifera carnica*. The *cross in the centre of the colour hexagon* indicates the locus of the background colour to which the photoreceptors are assumed to be completely adapted. The spectral locus is indicated by *small black circles connected by a line* (selected loci of spectral line are labelled). *Black arrows* follow the increasing Aniline Blue or Acridine Orange concentrations

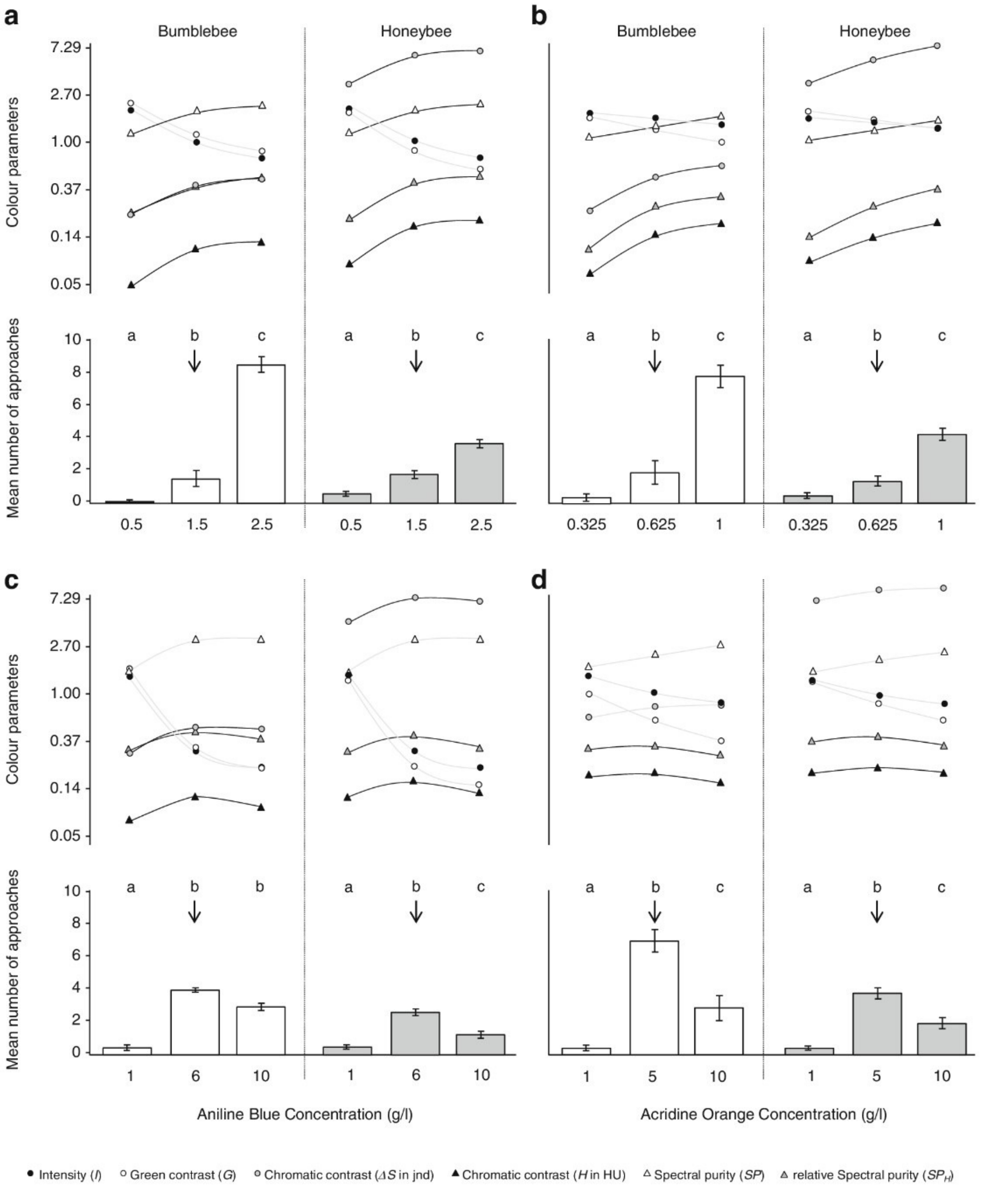

Fig. 3 Mean number of approaches with standard errors of the mean during a sequence of ten approaches by flower-naïve bumblebees (*Bombus terrestris dalmatinus*; $n=10$) or during a sequence of six approaches by experienced honeybees (*Apis mellifera carnica*; $n=25$) that were directed to artificial flowers with three different pigment concentrations and colour parameters of stimuli. (**a**, **b**) Set-ups in the low-to-middle concentration range and (**c**, **d**) in the middle-to-high concentration range of (**a**, **c**) Aniline Blue and (**b**, **d**) Acridine Orange. The training of bees has always been performed using the intermediate concentration of each test trial (indicated by *arrows above the bars*). Colour parameters are represented using the same scale following natural logarithm transformation. *Black lines between values* indicate correspondence of the respective colour parameter with choice frequencies; *grey lines between values* indicate missing correspondence of the respective colour parameter with choice frequencies. Statistical analysis: repeated measures analysis of variance (RM-ANOVA). *Different letters* denote differences according to pairwise comparison with *t* test as post-hoc test

under natural illumination. All tested workers of both species were individually marked and tested successively. Each bee was tested in both set-ups. Workers of each species were trained to three identical artificial flowers of intermediate

Table 1 Colour parameters of training and test stimuli

	Rel. quantum flux (receptor types)				Chromatic contrast to the background				
Stimulus	UV	Blue	Green	*G*	Δ*S* [JND]	*H* [HU]	SP_H	*SP*	*I*/100
Grey background	0.06; 0.10	0.40; 0.34	0.54; 0.57	1.00; 1.00	0.00; 0.00	0.00; 0.00	0.00; 0.00	0.77	1.01
AB 0.5 g/l	0.05; 0.10	0.47; 0.43	0.48; 0.47	2.22; 1.84	0.22; 3.33	0.05; 0.08	0.23; 0.21	1.21	1.96
AB 1 g/l	0.05; 0.11	0.49; 0.46	0.46; 0.43	1.68; 1.31	0.30; 4.42	0.07; 0.12	0.32; 0.31	1.54	1.46
AB 1.5 g/l	0.05; 0.12	0.52; 0.51	0.42; 0.38	1.17; 0.83	0.42; 6.10	0.11; 0.18	0.40; 0.44	1.94	1.03
AB 2.5 g/l	0.05; 0.12	0.54; 0.52	0.41; 0.36	0.82; 0.57	0.45; 6.61	0.12; 0.19	0.48; 0.49	2.17	0.73
AB 6 g/l	0.06; 0.12	0.55; 0.53	0.39; 0.34	0.33; 0.22	0.51; 7.23	0.12; 0.17	0.46; 0.42	3.07	0.31
AB 10 g/l	0.07; 0.14	0.54; 0.51	0.39; 0.35	0.21; 0.15	0.49; 6.64	0.10; 0.13	0.40; 0.33	3.09	0.22
AO 0.325 g/l	0.06; 0.09	0.34; 0.25	0.60; 0.65	1.68; 1.87	0.24; 3.32	0.06; 0.09	0.11; 0.14	1.11	1.84
AO 0.625 g/l	0.08; 0.10	0.29; 0.22	0.64; 0.68	1.32; 1.60	0.48; 5.45	0.15; 0.14	0.26; 0.27	1.40	1.66
AO 1 g/l	0.08; 0.10	0.25; 0.18	0.66; 0.72	1.02; 1.32	0.62; 7.32	0.18; 0.19	0.32; 0.37	1.74	1.44
AO 5 g/l	0.09; 0.10	0.22; 0.15	0.69; 0.76	0.58; 0.82	0.77; 9.32	0.19; 0.21	0.34; 0.41	2.22	1.04
AO 10 g/l	0.09; 0.09	0.22; 0.14	0.69; 0.77	0.38; 0.59	0.79; 9.76	0.16; 0.19	0.28; 0.34	2.68	0.86

Calculations of colour parameters of grey background and of colour stimuli of different Aniline Blue (*AB*) and Acridine Orange (*AO*) concentrations for training and tests given to bumblebees (left from semicolon) and honeybees (right from semicolon). Relative quantum flux; relative green contrast *G*; colour contrast to the background measured with the receptor-noise limited model (Vorobyev and Osorio 1998), *ΔS* (in just noticeable differences); colour contrast to the background measured with the colour hexagon (Chittka 1992), *H* (in hexagon units); relative spectral purity in the colour hexagon model, SP_H; spectral purity independent of a colour vision model, *SP*; and intensity, *I*

pigment concentrations in the test set-up by releasing them from a plastic tube directly on the artificial flower; they were rewarded with sugar water. One to three of those training attempts were sufficient for the workers to start approaching the stimuli by themselves after release at a distance of 60 cm from the stimuli. Afterwards, the test with new unrewarded artificial flowers took place with an equidistant starting point and alternated with a rewarded training situation. The positions of training and test stimuli were changed after each trial to prevent position preferences of bees. The first ten approaches of bumblebees and the first six approaches of honeybees towards the three artificial flowers of a setup were recorded. The divergence of the number of approaches and of individual bees tested between the two species is due to the facts that honeybees needed much more time to unload the collected sugar water in the hive than bumblebees and that the tests with honeybees were conducted under less stable field conditions, limiting the daily time span for training and testing individual bees. For more detailed information about bee keeping, materials and methods, see Rohde et al. (2013).

Statistical analysis

All statistical analyses were performed using R (V 2.14.0; R Development Core Team 2008). Data were arc sin transformed to meet the assumption of normal distribution. The differences in approaches towards the simultaneously presented three stimuli of different concentrations in a test set-up (in the range of low pigment concentration or in the range of high pigment concentration, respectively, for each pigment) were compared using repeated measures analysis of variance (RM-ANOVA) with a nested design. A paired *t* test as post-hoc test was applied to assessing where the divergences are constituted. *P* values were adjusted by false discovery rate (FDR; Benjamini and Hochberg 1995).

Results

Stimuli and colour parameters

The increase of Aniline Blue and Acridine Orange pigment concentrations presented on dried filter paper results in a stronger absorption throughout the range of visible and UV wavelengths (Fig. 1). Increasing pigment concentration is associated with a decrease of intensity and with an increase of spectral purity if spectral purity is calculated independent of a colour vision model for bees (Table 1 and Fig. 3). The bumblebee-subjective spectral purity calculated according to the colour hexagon model (Chittka 1992), green contrast and colour contrast of the tested colours are less simply dependent on pigment concentration, i.e. there is no linear relationship between pigment concentration and those colour parameters (Table 1). Plotted into the colour hexagon (Chittka 1992), which is one of the standard colour vision models commonly employed for bees (Cittka and Kevan 2005), the position of the colour loci of the tested Aniline Blue and Acridine Orange concentrations indicates the perception of these colour shades in the bees' perceptual colour space (Fig. 2). With increasing pigment concentration, the respective colour loci are positioned closer to the spectral locus, except for the highest tested pigment concentrations. Likewise, spectral purity increases in parallel with increasing concentration of Aniline Blue as well as Acridine Orange initially, except for the highest pigment concentrations (Table 1). This is apparent by a regressive shift of colour loci in the colour hexagon due to increasing pigment concentrations, indicated by black arrows (Fig. 2). Likewise, chromatic contrast to the background calculated with the colour hexagon model (H; Chittka 1992) is regressive if pigment concentrations exceed a distinct value, which is a pigment concentration of 2.5 g/l for Aniline Blue and 5 g/l for Acridine Orange (Table 1 and Fig. 2). If chromatic contrasts are calculated with the receptor noise-limited model (ΔS; Vorobyev and Osorio 1998), this regression applies only for concentration series of Aniline Blue, but not for Acridine Orange, where chromatic contrasts to the background increase with increasing amount of pigment up to the highest concentration (Table 1). As with spectral purity and chromatic contrasts to the background calculated with the colour hexagon model (Chittka 1992), a small shift of the colours' hue occurs if pigment concentrations exceed distinct values. An increase of Aniline Blue concentration leads to a shift from bee-blue colours to more bee-UV-blue colours [according to the bee colour nomenclature from Menzel and Shmida (1993)], and this is true for bumblebees as well as honeybees (Fig. 2). However, the shift of hue with increasing concentration of Acridine Orange follows different directions for bumblebees and honeybees: for bumblebees, an increase of Acridine Orange concentration leads to a shift from bee-purple to bee-UV colours, whereas for honeybees, the same increase leads to a shift to bee-green colours (Fig. 2).

For the choice experiments, we selected three-stimulus groups in the range of low pigment concentrations of Aniline Blue and Acridine Orange, respectively, and also three-stimulus groups in the range of high concentrations of both pigments. The rationale of this selection of groups of pigment concentrations was to include sets in which the pigment concentration of artificial flowers increases in parallel with their SP_H and SP for both bee species (low concentrations), and other sets in which this is not the case for SP_H, but for SP (high concentrations; Table 1 and Figs. 1 and 2). The colour contrasts between the stimuli of different concentrations used in one set-up are in maximum 0.13 HU for bumblebees and 0.12 HU for honeybees, close to the threshold for bees to recognize the stimuli as distinct (Dyer and Chittka 2004b).

Choice experiments

Following training to artificial flowers having an intermediate pigment concentration of Aniline Blue (1.5 g/l) and Acridine Orange (0.625 g/l) within the range from low to middle concentrations, both bumblebees and honeybees altered their choice behaviour, producing significant differences among the test stimuli with respect to the number of approaches towards them (RM-ANOVA, $P<0.001$ for both bee species and both pigments; Table 2). Bumblebees as well as honeybees significantly preferred artificial flowers with a pigment concentration (2.5 g/l Aniline Blue and 1 g/l Acridine Orange) higher than that of the intermediate-range training stimulus (t test, $P<0.001$ for both bee species and both pigments; Table 2 and Fig. 3) and significantly discriminated against those artificial flowers with the lower pigment concentration (0.5 g/l Aniline Blue and 0.325 g/l Acridine Orange, respectively, t test, $P<0.05$ for bumblebees and Aniline Blue, $P<0.01$ for honeybees and Aniline Blue and $P<0.001$ for both bee species and Acridine Orange; Table 2 and Fig. 3).

In contrast to that are the results of the choice experiments in the range from middle to high pigment concentrations: The bees were trained likewise to artificial flowers of intermediate pigment concentrations (6 g/l Aniline Blue and 5 g/l Acridine Orange), and there were also significant differences among the test stimuli with respect to the number of approaches towards them for bumblebees as well as honeybees (RM-ANOVA, $P<0.001$ for both bee species and both pigments; Table 2). Instead of preferring the highest pigment concentrations (10 g/l Aniline Blue and 10 g/l Acridine Orange) as they did in the experiments covering the range from low to middle pigment concentrations, both bee species most often approached those artificial flowers with intermediate pigment concentration (Fig. 3). This is statistically significant for honeybees (t test, $P<0.01$; Table 2) but not for bumblebees (t test, $P=0.33$; Table 2) and Aniline Blue, and statistically significant for both bee species and Acridine Orange (t test, $P<0.05$ for bumblebees and for honeybees; Table 2). The bees also significantly discriminated against those artificial flowers with the lowest pigment concentrations in the tested range (1 g/l Aniline Blue and 1 g/l Acridine Orange; t test, $P<0.001$ for both bee species and both pigments; Table 2 and Fig. 3).

Discussion

The colour preferences of bees are thought to be determined by the dominant wavelength (Giurfa et al. 1995; Gumbert 2000) and spectral purity (Lunau 1990) of the target stimuli, as well as by the green contrast (Giurfa et al. 1996, 1997; Giurfa and Vorobyev 1998; Hempel de Ibarra et al. 2001, 2002; Dyer et al. 2008) and colour contrast between colour stimulus and background (Giurfa et al. 1996; Dyer and Chittka 2004b). In this study, we show that varying pigment concentrations affect all mentioned colour parameters (Table 1), but which of those colour parameters affects the choice behaviour of bees between stimuli differing in the amount of pigment concentration? With increasing pigment concentration, spectral purity has an optimum at medium

Table 2 Choice preferences of bees

Pigment; concentration set-up	Test	Comparison of pigment concentrations	Bumblebees		Honeybees	
			F value	*P* value	*F* value	*P* value
AB; low	RM-ANOVA	All	56.98	1.63E-08	31.03	2.24E-09
	t test	0.5 vs. 1.5 g/l		0.03		1.15E-03
		0.5 vs. 2.5 g/l		5.70E-05		9.20E-04
		1.5 vs. 2.5 g/l		5.40E-08		5.00E-09
AB; high	RM-ANOVA	All	16.24	9.31E-05	19.39	6.73E-07
	t test	1 vs. 6 g/l		5.30E-04		3.60E-07
		1 vs. 10 g/l		1.84E-03		0.01
		6 vs. 10 g/l		0.33		3.60E-03
AO; low	RM-ANOVA	All	99.21	1.90E-10	26.87	1.48E-08
	t test	0.325 vs. 0.625 g/l		3.40E-04		6.65E-03
		0.325 vs. 1 g/l		4.00E-07		2.40E-07
		0.625 vs. 1 g/l		5.40E-07		4.20E-04
AO; high	RM-ANOVA	All	21.7	1.60E-05	23.55	7.47E-08
	t test	1 vs. 5 g/l		1.00E-05		1.00E-08
		1 vs. 10 g/l		0.02		1.70E-03
		5 vs. 10 g/l		0.02		0.01

Choice preferences of naïve bumblebees (*Bombus terrestris dalmatinus*; ten approaches of ten individuals each) and experienced honeybees (*Apis mellifera carnica*; six approaches of 25 individuals each). Statistical evaluation of the approaches by bees towards artificial flowers with different pigment concentrations of Aniline Blue (*AB*) and Acridine Orange (*AO*) in set-ups covering the range from low to middle concentrations (*low*) and covering the range from middle to high concentrations (*high*) for both pigments, using a repeated measures analysis of variance (RM-ANOVA) and a paired *t* test as post-hoc test with FDR adjusted *P* values

concentrations of both pigments if it is calculated as SP_H, but leads to an increasing spectral purity if it is calculated as SP (see formulas in the "Materials and methods" section). Both bee species more often visit those stimuli offering a high spectral purity SP in the experiments covering the low range of pigment concentrations but prefer intermediate values of SP in the experiments covering the high range of pigment concentrations. Assuming that bees prefer stimuli of high spectral purity as is hypothesized by some authors (Lunau et al. 1996, 2011), SP as colour parameter does explain the choice behaviour of both bee species for the artificial flowers only in the set-ups of range from low to middle concentrations, but not in the set-ups of the range from middle to high concentrations (Table 1 and Fig. 3). SP as a measure of spectral purity is obviously not relevant for bees. By contrast, measurements of spectral purity in accordance with the colour hexagon model (SP_H, Chittka 1992) fit very well to the behavioural data and predict and best explain the choices of bees: both bee species most often approach those stimuli offering the highest values for SP_H, and this is true for both pigments and both concentration ranges (Fig. 3).

Intensity I decreases with increasing pigment concentration of Aniline Blue as well as of Acridine Orange (Table 1 and Fig. 3). In the experiments covering the range from low to middle concentrations of both pigments, both bee species prefer those stimuli offering the lowest colour intensities, whereas in the experiments covering the range from middle to high concentrations of both pigments, both bee species more often approach those stimuli offering intermediate colour intensity (Table 1 and Fig. 3). Therefore, the colour parameter intensity does not explain the choice behaviour. These results are consistent with other experiments on bees, stating that intensity (i. e. brightness) of colours is a cue which is not evaluated by bees (Backhaus 1992).

Similarly, green contrast G decreases with increasing pigment concentration of Aniline Blue as well as of Acridine Orange (Table 1 and Fig. 3). In our experimental set-ups, bees start their approaches towards the artificial flowers 3 cm in diameter from a distance of 60 cm; from that distance, single artificial flowers were viewed under an angle of 2.9° each and thus were probably detected via the green contrast system and not the chromatic system. Other studies found a preference of bees for flowers offering a high green contrast against the background (Giurfa et al. 1996; Hempel de Ibarra et al. 2001; Dyer et al. 2008), as those flowers will be detected by bees from a larger distance if flower size is comparable (Giurfa et al. 1996). This is in contrast to the results of the current study: if a high green contrast G is a crucial colour parameter to detect and approach stimuli, bees should prefer stimuli with the lowest concentrations in both set-ups for both pigments, but they did not (Fig. 3).

Chromatic contrast ΔS to the background increases with increasing concentration of Acridine Orange, whereas it has an optimum at intermediate concentrations of Aniline Blue, i.e. a concentration of 6 g/l Aniline Blue (Table 1 and Fig. 3). Therefore, both bee species prefer those stimuli offering the highest values for ΔS in the experiments covering the range of low to middle concentrations of both pigments, but in the experiments covering the range from middle to high pigment concentrations, bees prefer these stimuli only for Aniline Blue, and bumblebees as well as honeybees prefer stimuli with intermediate values of ΔS of Acridine Orange (Table 1 and Fig. 3).

Chromatic contrast H to the background has an optimum at intermediate pigment concentrations, i.e. 2.5 g/l Aniline Blue and 5 g/l Acridine Orange (Table 1 and Fig. 3). For both pigments and in both set-ups, bees prefer those stimuli offering the highest chromatic contrasts H against the background (Table 1 and Fig. 3). Chromatic contrast H to the background appears to be, beside spectral purity SP_H, an important colour parameter for bees to approach flowers (Table 1 and Fig. 3). Since colour contrast and spectral purity change in parallel for our test stimuli (Table 1 and Figs. 2 and 3), the active sensorial colour parameter has to be tested separately. Rohde et al. (2013) did so by changing the experimental design and offered artificial flower colours which differed in their spectral purity, but displayed the same values for H. In that case, bees preferred those stimuli which offered the higher spectral purity, and thus, H was eliminated as explanatory colour parameter (Rohde et al. 2013; see Experiment 2).

Our experiments therefore support the conclusion that spectral purity is an important cue of colours for bees to detect and approach flowers and that spectral purity determines choice behaviour of bees (Lunau et al. 1996, 2011). The examined bee species preferred artificial flowers of superior spectral purity and significantly discriminated against artificial flowers of lesser spectral purity if calculated with the bee-subjective colour vision model from Chittka (1992). Beyond that, we could show that minor differences in pigment concentrations can affect floral colouration to a degree that is perceived by bees and affects choice behaviour of experienced bees (Figs. 2 and 3). In the tests, the bees preferred a spectrally more pure colour over the trained colour irrespective of whether colour purity was correlated with pigment concentration (Table 1 and Fig. 3).

One might argue that the differences in the dominant wavelength between the training stimulus and the test stimuli might have affected the choice behaviour. Indeed, the colour stimuli produced by varying pigment concentrations do not only vary in their intensity (Table 1 and Fig. 1) and spectral purity (Table 1 and Fig. 2), but also in their dominant wavelength (Fig. 2). Particularly, the colour produced by the lowest concentration of Acridine Orange possesses a dominant wavelength that differs from all colours produced by higher concentrations of Acridine Orange, particularly for bumblebees, whereas the colours produced by high concentrations of Acridine Orange possess a very similar dominant wavelength for bumblebees (Fig. 2). In contrast, the colour produced by the highest concentration of Aniline Blue possesses a dominant wavelength for honeybees as well as bumblebees that differs from all colours produced by lower concentrations of Aniline Blue (Fig. 2). For bumblebees, different concentrations of Aniline Blue are more diverse in their dominant wavelengths, i.e. hues, especially for the highest concentration, than they are for honeybees (Fig. 2). Nevertheless, the results show that the ability of bumblebees to discriminate against the stimuli, especially the stimuli 6 and 10 g/l Aniline Blue, is worse than that of honeybees (Table 2 and Fig. 3). In the case of Acridine Orange, honeybees should more easily discriminate the stimuli by means of their hue than can bumblebees, for which the stimuli are more similar in their hues (Fig. 2). However, both bee species show a similar behaviour in their choice for specific stimuli (Table 2 and Fig. 3). This indicates clearly that the choice behaviour was probably not markedly affected by differences in dominant wavelength and this holds for both bee species, known to differ in their spectral sensitivities (Menzel and Backhaus 1991; Skorupski et al. 2007), and to differ in their ability of fine chromatic discrimination between two colours of different dominant wavelengths (Giurfa 2004) when tested under different light conditions.

These interpretations of the experimental results have to be considered carefully because some argumentations comprise the implicit assumption that the bees' preference is monotonically increasing with an increasing value of a given parameter. However, bees might also prefer intermediate values of a given parameter over lower and higher values. Nevertheless, we have purposely chosen an overall range of pigment concentration in order to ensure that colour parameters are not monotonically increasing with pigment concentration. Moreover, it cannot be excluded that bees might possess preferences consisting of combinations of more than one colour parameter; for example, bees might prefer more saturated colours only if bee-blue or bee-green, but prefer brighter colours if bee-blue-green. Taking such complex preferential choices into account, rigorous statistical testing is only possible if more numerous series of stimuli are tested as has been done in this study.

Why should bumblebees exhibit innate preferences for floral colours caused by high pigment concentrations? The detectability of flowers may increase with pigment concentration because colours produced by higher floral pigment concentration are perceptually more distinct and distant from background colours (Kevan et al. 1996). Floral pigment concentration may be correlated with the flowers' detectability for flower visitors, especially if pigment concentration is associated with spectral purity, which is a key parameter for foraging bees to detect flowers (Lunau et al. 2011, Rohde et al. 2013). The ultimate cause for the preference of spectrally pure colours may be related to the yet hypothetical assumption that flowers may honestly signal the availability of nectar reward via colour signals. Floral pigment concentration bears the potential to serve as an honest signal (Armbruster et al. 2005) indicating the quality or quantity of expectable reward because both the increase of floral pigment concentration and the production of floral reward is costly for plants. Parallel investment in floral advertisement and floral reward may benefit the plant's pollination success. It is, however, unknown whether the pigment concentration of flowers in nature is beneath the level that will result in an optimal spectral purity for bees; only then could bees exert positive directional selection for higher flower pigment concentrations. For fruits, it was demonstrated that pigmentation may serve as an honest indicator of nutritional value to birds (Schaefer et al. 2008). It is still unknown if pigment concentration can also act as honest flower signal indicating the amount of reward (Armbruster et al. 2005) and if natural variability of flower colours of given plant species or flowering morph affects the behaviour of foraging bees. It is known that plant species displaying a floral colour change of the corolla or of the floral guide (Sprengel 1793; Kugler 1936; Vogel 1949; Weiss 1991) communicate the nectar availability (Schaefer et al. 2004) by means of spectrally pure colours (Lunau 1996). The attractiveness of *Mimulus* flowers for insect pollinators increased for example with the nectar sugar concentration as well as the flower pigment (Schemske and Bradshaw 1999). Our results show that the increase of pigment concentration indeed causes an increase of the attractiveness of colours due to a parallel increase of bees' subjective spectral purity up to excessive concentrations. Models of co-optimization of floral display and nectar reward predict a mosaic of cheating and honesty in plant–pollinator coevolution with plants having a variable proportion of empty or cheater flowers and plants in which the display reflects the reward with some degree of honesty (Belsare et al. 2009). So far, floral pigment concentration has not been reported as an honest flower signal. Future studies will show how this preferential behaviour in bees might have shaped the evolution of floral pigment concentration.

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References

Armbruster WS, Antonsen L, Pélabon C (2005) Phenotypic selection on *Dalechampia* blossoms: honest signaling affects pollination success. Ecology 86:3323–3333. doi:10.1890/04-1873

Asmussen CB (1993) Pollination biology of the Sea Pea, *Lathyrus japonicus*: floral characters and activity and flight patterns of bumblebees. Flora 188:227–237

Avarguès-Weber A, de Brito Sanchez MG, Giurfa M, Dyer AG (2010) Aversive reinforcement improves visual discrimination learning in free-flying honeybees. PLoS One 5:e15370. doi:10.1371/journal.pone.0015370

Backhaus W (1992) The Bezold–Brücke effect in the color vision system of the honeybee. Vision Res 32:1425–1431

Belsare PV, Sriram B, Watve MG (2009) The co-optimization of floral display and nectar reward. J Biosci 34:963–967. doi:10.1007/s12038-009-0110-7

Benard J, Giurfa M (2008) The cognitive implications of asymmetric color generalization in honeybees. Anim Cogn 11:283–293

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B Met 57:289–300

Brandt R, Vorobyev M (1997) Metric analysis of threshold spectral sensitivity in the honeybee. Vision Res 37:425–439

Schemske DW, Bradshaw HD Jr (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc Natl Acad Sci USA 96:11910–11915. doi:10.1073/pnas.96.21.11910

Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J Comp Physiol A 170:533–545

Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86:361–377

Cittka L, Kevan PG (2005) Advertisement in flowers. In: Dafni A, Kevan PG, Husband BC (eds) Practical pollination biology. Enviroquest Ltd., Cambridge, pp 147–230

Dobson HEM, Danielson EM, Wesep IDV (1999) Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). Plant Spec Biol 14:153–166

Dyer AG, Chittka L (2004a) Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften 91:224–227

Dyer AG, Chittka L (2004b) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. J Comp Physiol A 190:105–114

Dyer AG, Neumeyer C (2005) Simultaneous and successive colour discrimination in the honeybee (*Apis mellifera*). J Comp Physiol A 191:547–557

Dyer AG, Spaethe J, Prack S (2008) Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. J Comp Physiol A 194:617–627

Endler JA (1990) On the measurement and classification of colour in studies of animal colour patterns. Biol J Linn Soc 41:315–352. doi:10.1111/j.1095-8312.1990.tb00839.x

Exner F, Exner S (1910) Die physikalischen Grundlagen der Blütenfärbungen. Sitz der Akad Wiss Wien math naturw. Klasse 119(Abt 1):1–55

Giurfa M, Núñez J, Chittka L, Menzel R (1995) Colour preferences of flower-naive honeybees. J Comp Physiol A 177:247–259

Giurfa M, Vorobyev M, Kevan P, Menzel R (1996) Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrast. J Comp Physiol A 178:699–709

Giurfa M, Vorobyev M, Brandt R, Posner B, Menzel R (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. J Comp Physiol A 180:235–243

Giurfa M, Vorobyev M (1998) The angular range of achromatic target detection by honey bees. J Comp Physiol A 183:101–110

Giurfa M (2004) Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. Naturwissenschaften 91:228–231

Goulson D, Cruise JL, Sparrow KR, Harris AJ, Park KJ, Tinsley MC, Gilburn AS (2007) Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees. Behav Ecol Sociobiol 61:1523–1529

Gumbert A (2000) Color choices by bumble bees (*Bombus terrestris*) innate preferences and generalization after learning. Behav Ecol Sociobiol 48:36–43

Hempel de Ibarra N, Giurfa M, Vorobyev M (2001) Detection of coloured patterns by honeybees through chromatic and achromatic cues. J Comp Physiol A 187:215–224

Hempel de Ibarra N, Giurfa M, Vorobyev M (2002) Discrimination of coloured patterns by honeybees through chromatic and achromatic cues. J Comp Physiol A 188:503–512

Hill PSM, Wells PH, Wells H (1997) Spontaneous flower constancy and learning in honey bees as a function of colour. Anim Behav 54:615–627

Kay QON, Daoud HS, Stirton CH (1981) Pigment distribution, light reflection and cell structure in petals. Bot J Linn Soc 83:57–83. doi:10.1111/j.1095-8339.1981.tb00129.x

Kevan PG, Giurfa M, Chittka L (1996) Why are there so many and so few white flowers? Trends Plant Sci 1:280–284

Kugler H (1936) Blütenökologische Untersuchungen mit Hummeln VIII. Planta 25:346–363

Laughlin SB (1981) Neural principles in the peripheral visual systems of invertebrates. In: Autrum H (ed) Vision in invertebrates (Handbook of sensory physiology). Springer, Berlin, pp 133–280

Lunau K (1990) Color saturation triggers innate reactions to flower signals—flower dummy experiments with bumblebees. J Comp Physiol A 166:827–834

Lunau K (1996) Unidirectionality of floral colour change. Plant Syst Evol 200:125–140

Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. J Comp Physiol A 178:477–489

Lunau K, Papiorek S, Eltz T, Sazima M (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. J Exp Biol 214:1607–1612. doi:10.1242/jeb.052688

Matsumura S, Yokoyama J, Tateishi Y, Maki M (2006) Intraspecific variation of flower colour and its distribution within a sea lavender, *Limonium wrightii* (Plumbaginaceae), in the northwestern Pacific Islands. J Plant Res 119:625–632

Meléndez-Ackerman E, Campbell DR, Waser NM (1997) Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. Ecology 78:2532–2541. doi:10.1890/0012-9658(1997) 078

Menzel R, Backhaus W (1991) Color vision in insects. In: Gouras P (ed) Vision and visual dysfunction. VI. Perception of color. Macmillan, Houndmills, pp 262–293

Menzel R, Shmida A (1993) The ecology of flower colours and the natural colour vision of insect pollinators: the Israeli flora as a study case. Biol Rev 68:81–120. doi:10.1111/j.1469-185X.1993.tb00732.x

Neumeyer C (1981) Chromatic adaptation in the honeybee: successive color contrast and color constancy. J Comp Physiol 144:543–553

Noda K-I, Glover BJ, Linstead P, Martin C (1994) Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. Nature 369:661–664. doi:10.1038/369661a0

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, http://www.R-project.org. ISBN 3-900051-07-0

Raine NE, Ings TC, Dornhaus A, Saleh N, Chittka L (2006) Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. Adv Stud Behav 36:305–354

Rohde K, Papiorek S, Lunau K (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. J Comp Physiol A 199:197–210. doi:10.1007/s00359-012-0783-5

Schaefer HM, Schaefer V, Levey DJ (2004) How plant-animal interactions signal new insights in communication. Trends Ecol Evol 19:577–584

Schaefer HM, Spitzer K, Bairlein F (2008) Long-term effects of previous experience determine nutrient discrimination abilities in birds. Front Zool 5:4. doi:10.1186/1742-9994-5-4

Shipunov A, Kosenko Y, Volkova P (2011) Floral polymorphism in common primrose (*Primula vulgaris* Huds., Primulaceae) of the Northeastern Black Sea coast. Plant Syst Evol 296:167–178

Skorupski P, Döring TF, Chittka L (2007) Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. J Comp Physiol A 193:485–494

Skorupski P, Chittka L (2010) Photoreceptor spectral sensitivity in the bumblebee, *Bombus impatiens* (Hymenoptera: Apidae). PLoS One 5:e12049. doi:10.1371/journal.pone.0012049

Sprengel CK (1793) Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen. Fascimile-Druck. Mayer & Müller, Berlin

Tastard E, Andalo C, Giurfa M, Burrus M, Thébaud C (2008) Flower colour variation across a hybrid zone in *Antirrhinum* as perceived by bumblebee pollinators. Arthropod-Plant Inter 2:237–246. doi:10.1007/s11829-008-9046-3

Tourjee KR, Harding J, Byrne TG (1993) Calorimetric analysis of *Gerbera* flowers. HortSci 28:735–737

Vogel S (1949) Farbwechsel und Zeichnungsmuster bei Blüten. Plant Syst Evol 97:44–100

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc Lond B 265:351–358. doi:10.1098/rspb.1998.0302

Vorobyev M, Brandt R, Peitsch D, Laughlin SB, Menzel R (2001) Colour thresholds and receptor noise: behaviour and physiology compared. Vision Res 41:639–653

Weiss MR (1991) Floral color changes as cues for pollinators. Acta Hort 288:294–298

Whibley AC, Langlade NB, Andalo C, Hanna AI, Bangham A, Thebaud C, Coen E (2006) Evolutionary paths underlying flower color variation in *Antirrhinum*. Science 313:963–966

Wyszecki G, Stiles WS (1982) Color science: concepts and methods, quantitative data and formulae. Wiley, New York

CHAPTER 6

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Gloss, colour and grip: Multifunctional epidermal cell shapes in bee- and bird-pollinated flowers

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Gloss, Colour and Grip: Multifunctional Epidermal Cell Shapes in Bee- and Bird-Pollinated Flowers

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Abstract

Flowers bear the function of filters supporting the attraction of pollinators as well as the deterrence of floral antagonists. The effect of epidermal cell shape on the visual display and tactile properties of flowers has been evaluated only recently. In this study we quantitatively measured epidermal cell shape, gloss and spectral reflectance of flowers pollinated by either bees or birds testing three hypotheses: The first two hypotheses imply that bee-pollinated flowers might benefit from rough surfaces on visually-active parts produced by conical epidermal cells, as they may enhance the colour signal of flowers as well as the grip on flowers for bees. In contrast, bird-pollinated flowers might benefit from flat surfaces produced by flat epidermal cells, by avoiding frequent visitation from non-pollinating bees due to a reduced colour signal, as birds do not rely on specific colour parameters while foraging. Moreover, flat petal surfaces in bird-pollinated flowers may hamper grip for bees that do not touch anthers and stigmas while consuming nectar and thus, are considered as nectar thieves. Beside this, the third hypothesis implies that those flower parts which are vulnerable to nectar robbing of bee- as well as bird-pollinated flowers benefit from flat epidermal cells, hampering grip for nectar robbing bees. Our comparative data show in fact that conical epidermal cells are restricted to visually-active parts of bee-pollinated flowers, whereas robbing-sensitive parts of bee-pollinated as well as the entire floral surface of bird-pollinated flowers possess on average flat epidermal cells. However, direct correlations between epidermal cell shape and colour parameters have not been found. Our results together with published experimental studies show that epidermal cell shape as a largely neglected flower trait might act as an important feature in pollinator attraction and avoidance of antagonists, and thus may contribute to the partitioning of flower-visitors.

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Introduction

Plant-animal interactions include mutualistic as well as antagonistic relationships. Animal pollination was traditionally regarded as mutualism including reciprocal benefits for both interaction partners. Nowadays the view that the flowers' signalling apparatus single task is the attraction of flower visitors has changed. Flowers are interpreted as sensorial and/or morphological filters supporting the attraction of pollinators as well as the deterrence of floral antagonists such as herbivores, pollen and nectar robbers or thieves (reviewed in [1]). These interactions bear on different communication tasks, with colour as one of the most important floral features that structures the flower-visitor composition [2], [3]. The diversity of flower colours in angiosperms is mainly attributed to pigments deriving from different biochemical pathways, their combinations, variable concentrations as well as additional co-pigments, the prevalent pH in the vacuole, metal ions, pigment packaging and location within the tissue layers [4]. Next to these factors, also the petals' epidermal cell structure affects the visual appearance of flowers [5], [6], [7], [8], [9]. Particularly, conical epidermal cells can act as lenses and light traps, changing optical properties by refracting and focusing light into the pigment containing tissue layer of petals [5]. Gorton & Vogelman [5] investigated this function in the Snapdragon *Antirrhinum majus*, whose wild type flowers have conical epidermal cells and a comparably enhanced colour signal. By contrast, mutants with flat instead of conical epidermal cells are focusing incident light into the mesophyll beneath the pigment-containing epidermal cell layers, thereby reducing the colour signal as the pigments absorb comparably less light [5]. Thus, the presence of conical epidermal cells in contrast to flat ones might alter colour impression for flower-visitors by enhancing light absorption by pigments in a yet unexplored manner [10].

Moreover, the structure of epidermal cells affects the amount of gloss at the petals' surface. Gloss is defined by the total reflectance of incident light at a surface in the identical angle to that of the incident light. Both, in theory and as shown in experimental studies on flowers [8], [9], [11], gloss is strongest if the surface is flat, in this case, if the epidermal cells are flat. Thus, at smooth surfaces a smaller portion of the incident light enters the plant tissues and passes the pigment containing cells as compared to rough surfaces. With decreasing reflectance at mirror geometry (i.e. gloss), the colour signal increases, as a higher amount of light enters the tissue and might be absorbed by pigments. Gloss is a phenomenon appearing in fruits and petals of some plant species [8], [9], [12], [13], [14], [15], but the vast majority of angiosperm flowers exhibits some form of conical epidermal cells [11] and possesses only slightly glossy surfaces. Absorption of light by flower pigments causes a spectral signal restricted to specific ranges of wavelengths, whereas gloss phenomena cover the whole range of visible light, including the ultraviolet range of wavelengths, and are strictly angle-dependent [14].

Beside visual appearance, the epidermal cell structure also determines floral temperature [16], floral shape [6], wettability [17], microsculptural patterns forming nectar guides [18], and floral grip [19], [20], [21]. The latter involves that conical epidermal cells provide contact between bee and flower petal, making flowers easier to handle as they are less slippery [19].

In the current study we investigate the shape of epidermal cells on flowers pollinated by either bees or birds. Due to differences in the visual capabilities as well as differences in the foraging behaviour between these two flower-visitor groups, differences in respect to epidermal cell shapes and their consequent functions are conceivable. The colour vision system of bees and flower-visiting birds differ in respect to the number of photoreceptor types, with superior colour discrimination abilities in birds as compared to bees [22], [23], [24]. Colour is an important trait for foraging bees to detect flowers evoked by innate colour preferences and learned responses ([25], and references within), whereas flower-visiting birds do not show preferences for specific colour properties, but nevertheless associate colours with floral rewards [26], [27]. Thus, we investigate three different hypotheses concerning the effects of epidermal cell shape on 1) the colour sensation for bees as well as birds, 2) the importance of floral grip for bees and birds, and 3) on floral grip for nectar robbers.

The first hypothesis implies that bee-pollinated flowers might benefit from enhancing the flower's colour signal for bees, whereas bird-pollinated flowers benefit from avoiding frequent visits by bees due to flower colours which are comparatively less attractive to bees [2]. As bird-pollinated flowers become commonly thieved by bees representing competitors for pollinators, negative effects on the plants' fitness arise [28], [29], and avoidance of frequent visitation by bees should be beneficial for these plant species [2]. Unlike conical epidermal cells, flat ones may bear that function by producing less attractive colours for bees with similar pigment concentrations.

The second hypothesis based on the knowledge that bees need grip to effectively forage on flowers [19], [20]. Again, bee-pollinated flowers might benefit from conical cells promoting floral grip. In contrast, bird-pollinated flowers might benefit from flat epidermal cells, hampering bees from effective foraging and thus, avoiding the loss of rewards for their pollinators.

The third hypothesis implies that those flower parts vulnerable to nectar robbing benefit from flat epidermal surfaces, hampering grip and handling for bees while robbing the flower [19], [20]. Since this parameter seems relevant for bee- as well as bird-flowers we investigate whether robbing-sensitive parts possess mechanical properties, i.e. flat epidermal cell surfaces, which may help to avoid nectar robbing.

Testing these three hypotheses allows us to demonstrate that epidermal cell shape is a multifunctional flower trait which may act as an important feature in pollinator attraction.

Materials and Methods

Plant material

In total we studied the epidermal cell shape, spectral reflectance and gloss of the flowers of 58 plant species from 48 genera in 26 families (29 species from 28 genera in 16 families which are adapted to the pollination by bees, and 29 species from 23 genera in 15 families adapted to the pollination by birds, Text S1). Flowers were collected in the Botanical Garden of the Heinrich-Heine University, Düsseldorf, Germany. The permission for collecting three to five flowers was obtained by the academic advisor Dr. Sabine Etges. Flowers were stored in moist storage boxes until measurements as soon as possible after picking the flowers at the same day.

Plant species were categorized into bee- and bird-pollination, with effective pollinators assigned from literature (Text S1). For this purpose, plant species were only included, if literature reports seed or fruit set caused by specific flower-visitors or if literature explicitly supports morphological fit between frequent visitors and the flowers' reproductive organs, as evidence for effective pollination. To circumvent incorrectly assigned pollinators from our data set, we excluded those plant species from our analysis for which only assumptions of probable pollinators were made from morphological floral traits according to pollination syndromes [30]. For example, the red flower colour is often assigned with bird-pollination, but bees can contribute to effective pollen transfer, performing selection pressure towards flower traits promoting visitation by bees [31], [32].

Flower parts were categorized into those sites which belong to the visually signalling apparatus of the flower and provide a landing platform for bees (afterwards referred to as 'visually-active parts') and those that are averted from the visitor and are vulnerable to nectar robbing by bees (afterwards referred to as 'robbing-sensitive parts') (Text S1). In tubular flowers the former parts were adaxial parts of lips and the latter ones abaxial basal parts of the corolla; in open flowers the former parts were adaxial parts of petals and the latter ones abaxial basal parts of the petals (Text S1).

Shape measurements

Several types of epidermal cells can be found in flower petals [11], with six main types comprised in our data set (Figure 1A, C). Light that already entered the epidermal cell tissue of petals will be reflected from the underlying mesophyll towards the outside and thereby passing the epidermal cells again [11]. If the basal epidermal cell parts are convex or conical, and the refractive indices differ between the epidermal cell and the mesophyll cell, light might be focused again into the pigment containing area. Thus, we assumed that the apical as well as basal part of the epidermal cells contribute to light refraction. Therefore we defined a shape index S from three angles, i.e. α, β, and γ, describing the degree of bending of the surface of the apical, lateral as well as the basal cell part:

$$S=\frac{\alpha_1+\alpha_2}{180}*\frac{\gamma_1+\gamma_2}{180}*\frac{\beta_{min}}{\beta_{\max}} \quad (1)$$

Cell shape was characterized by means of transverse sections of petals using light microscopy. Slices were made at the same parts and in the same direction, where spectral reflection and floral gloss measurements were taken, as the arrangement of epidermal cells may vary with respect to their position on the petal and as microstructural pattern can differ in various parts of petals [18]. Slices had a thickness of three to four cell-rows and we focused on the inner cell-row in order to assess the cell morphology without any underestimation of cell parameters, especially cone steepness. Those slices where cells were only cut were not considered. LM-photographs were analysed using AxioVision Rel. 4.8 Software (ZEISS, Oberkochen, Germany) by evaluating the maximal cell height (h) and the cell width orthogonal to half the maximal cell height (w; Figure 1A).

Each angle is located between a length parameter, i.e. h or w, and the adjacent cell wall in a distance of 5 µm from the intercept of cell wall and length parameter. α_1 and α_2 describe the surface structure in the apical part of the epidermal cell (Figure 1A). The smaller α_1 and α_2, the more conical, the larger α_1 and α_2 the more flat is the cell towards the petal outside (Figure 1B). The same is true for γ_1 and γ_1, describing the bending of the cells' basal part (Figure 1A, B). The ratio between β_{min} and β_{max} describe the degree of bending of the lateral cell part towards the adjacent epidermal cells (Figure 1A). This term was added in order to include those cell forms, which consist of a flat part with attached papillae, forming a cell form with flat as well as conical parts and therefor intermediate *S*-values (Figure 1B).

For each flower part of each flower we evaluated the mean values of each shape parameter of five haphazardly chosen cells and afterwards calculated *S*. In summary, the smaller *S*, the more conical is the cell towards the apical and basal part and the higher *S*, the more flat is the cell form towards each side (Figure 1B). Reverse-conical cells for example have intermediate values for *S* (Figure 1B). *S* values for all investigated flower parts of all plant species are given in Text S1.

Gloss measurements

Gloss measurements were made using a ZGM 1120 Glossmeter (Zehnter Testing Instruments, Sissach, Switzerland), measuring the amount of scattered light in the mirror angle of the incident light, under an angle of 60° and recorded with *GlossTools* 1.7 software. Gloss was measured relative to a standard and given in gloss units (GU). All measurements were compared to a standard of black cardboard (HKS97N; standardized colour paper of the HKS-N-series; Hostmann-Steinberg K+E Druckfarben, H. Schmincke & Co., Germany) instead of the accompanied calibration standard of black polished glass. Since the normal use of this glossmeter is for highly glossy materials like car coatings, this procedure was done to achieve gloss data more widely distributed over the range of values provided by the glossmeter and therefore to examine differences in gloss between flowers more accurately. The black cardboard showed gloss of 1.74±0.28GU (n = 10) if measured with the manufactory standard. The particular flower parts were removed from flowers and positioned as flat as possible on black cardboard. For a more detailed insight into the technique of measurements with glossmeter see [15]. For each flower part of all investigated plant species, floral gloss values are included in Text S1. Gloss measurements were taken in the same direction of the flower petals as was used for transverse sections.

Reflectance measurements and calculation of colour parameters

Reflectance measurements were performed with an USB4000 spectrophotometer (Ocean Optics, Inc., Ostfildern, Germany) and illumination was provided by a DH-2000-BAL light-source (Ocean Optics, Inc., Ostfildern, Germany), both connected via a coaxial fibre cable (QR400-7-UV-VIS, Ocean Optics, Inc., Dunedin, FL, USA). Since the values for photoreceptor excitation are not angle-dependent [14], all measurements were taken in an angle of 45° to the measuring spot with a pellet of barium sulphate used as white standard and a black film can used as black standard. Reflectance measurements were taken in the same direction towards flower petals than transverse sections.

As there is only little known about colour preferences of flower-visiting birds and even evidence that birds do not have spontaneous preferences for any colour or specific colour

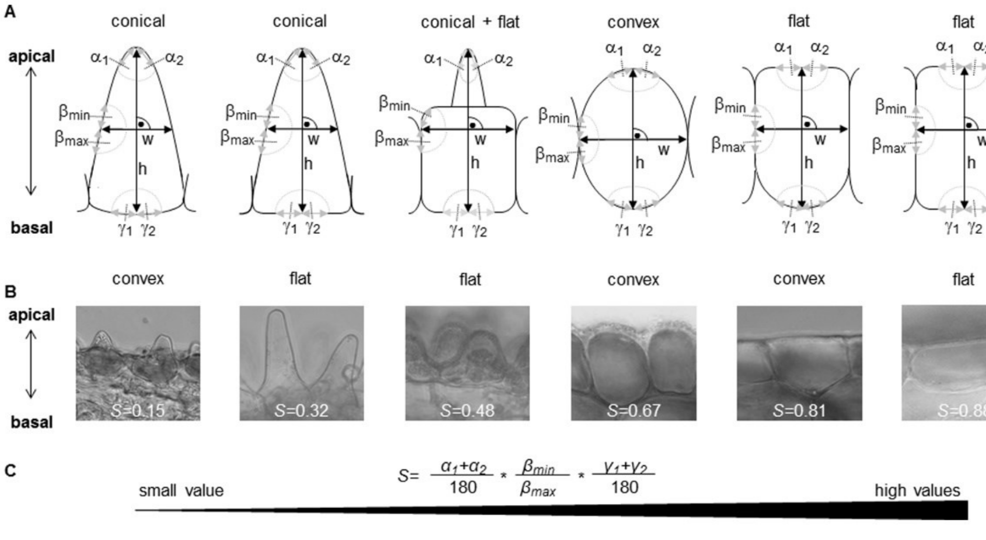

Figure 1. Cell shapes and explanation of the shape index *S*. A) Drawings and B) photographs of epidermal cell shapes with *S*-values for the represented shape types (from left to right: *Chritia gueilinensis*, *Proboscidea fragrans*, *Tecomaria capensis*, *Columnea gloriosa*, *Aloe vogtsii*, *Polygala myrtifolia*), found in the epidermal surfaces of investigated flowers. h = maximal cell height. w = cell width at half height. Dashed grey curves indicate angles. Grey arrows indicate a length of 5 µm. C) Formula of shape index *S*.
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parameters, we evaluated the impact of epidermal cell shape on floral colouration for bees only. For this purpose, we calculated several colour parameters relevant for honeybees' response to colour cues [33], i.e. colour contrast to the background (we used an average spectrum of several green leaves), bee-subjective spectral purity, green contrast, and intensity. These colour parameters are thought to affect the foraging behaviour of bees and determine preferences [25]. The honeybee serves as an example for several trichromatic hymenopteran species with similar photoreceptor sensitivities in the ultraviolet, blue and green wavelength parts [22]. Colour contrast to the background was calculated using two colour vision models, i.e. the colour hexagon [34] and the receptor-noise limited model [35], both aligned to the colour vision system of hymenopterans. Bee-subjective spectral purity was calculated according to the colour hexagon model [34], [36], [37]. Green contrast, chroma and intensity were calculated independent of colour vision models [37], [38].

For exact calculations of colour parameters see [36]. All colour parameters of the investigated flower parts of all plant species are summarized in the Text S1.

Statistical analysis

Correlations between epidermal cell shape, floral gloss, and colour parameters were analysed using Spearman rho correlations. To compare epidermal cell shape, floral gloss and all investigated colour parameters between each two flower parts of different pollinators we used one-way analysis of variance (ANOVA) with Tukey HSD as post-hoc test. Data were logarithm transformed to meet the assumption of variance homogeneity for the ANOVA. All statistical analyses were performed with the statistical computing software R 3.0.2 [39].

Results

Differences between cell shape, floral gloss, and colour parameters in relation to pollinators were found; main pollinator (i.e. bee or bird) as well as flower part (i.e. visually-active or robbing-sensitive parts) had a significant effect on shape, floral gloss as well as all investigated colour parameters (Figure 2).

Visually-active parts of bee-pollinated flowers had more often conical epidermal cells (corresponding to lower *S*-values), whereas robbing-sensitive parts of bee- as well as both parts of bird-pollinated flowers had more often flat epidermal cells (larger *S*-values) (Figs. 1, 2A). Visually-active parts of bird-pollinated flowers had more often intermediate values for S, and therefore convex or intermediate formed epidermal cells (Figures 1, 2A). Floral gloss was minimal in visually-active parts of bee-pollinated flowers, but did not significantly differ from both parts of bird-pollinated flowers (Figure 2B). Colour contrast to the background in the colour hexagon model, spectral purity in the hexagon model, green contrast, and intensity were larger for bee-pollinated flowers as compared to bird-pollinated ones, independent of the flower part (Figure 2C, E–F, H); however pairwise post-hoc comparisons for colour contrasts to the background in the colour hexagon model were not significant (Figure 2C). In contrast, colour contrast to the background in the receptor-noise limited model as well as chroma tended to be larger for both parts of bird-pollinated as compared to bee-pollinated flowers, but pairwise comparisons were not all significant (Figure 2D, G).

In the flowers studied there was a positive correlation between epidermal cell shape *S* and floral gloss, but all other correlations between *S* and the investigated colour parameters, as well as between floral gloss and the latter ones were not significant (Figure S1). As an exception, there was a significant positive correlation between floral gloss and bee-subjective spectral purity, (Figure S1). Moreover, we found significant correlations between specific colour parameters (Figure S1). However, due to the mathematical background and physiological conditions co-linearity between some colour parameter are given. For example, bee-subjective spectral purity in the hexagon model and colour contrast to the background in the colour hexagon model result both from the perceptual distance to the background in a rather similar manner.

Discussion

A first survey of epidermal cell structure in angiosperms revealed that 79% of the investigated plant species show some form of papillate or conical epidermal cells [11]. The current study provides more differentiated results and demonstrates that the distribution of epidermal cell shape is largely explained by the effective pollinator as well as by their position on petals. Considering visually-active flower-parts among the 58 species studied, conical epidermal cells are more common in bee-pollinated flowers, whereas bird-pollinated flowers have more often flat epidermal cells. In contrast, flower parts which are vulnerable to nectar robbing are more often flat in bee- as well as in bird-pollinated flowers.

Both, the correlation between epidermal cell shape and pollinator guild as well as the within-flower patterns suggest that epidermal cell structure may play a significant role in determining flower-visitor choices.

Effects of epidermal cell shape on colour sensation for bees and birds

Other than expected, the investigated colour parameters did not correlate with epidermal cell shape. Bee-pollinated flowers entirely appear of higher investigated colour parameters (i.e. bee-subjective spectral purity, chromatic contrast to the background, green contrast, chroma and intensity) as compared to bird-pollinated ones, irrespective of their epidermal cell shape. Thus, the tested colour parameters are not determined by means of cell shape only, provided that flower pigment concentration is similar in visually-active and robbing-sensitive flower parts. That the colouration of bird-pollinated flowers act as sensorial filter has been demonstrated previously, with comparably lower spectral purities and lower chromatic contrasts to the background as compared to bee-pollinated flowers of the same colour [2]. However, the light focusing effect of conical epidermal cells may enhance colour parameters like spectral purity only, if pigments are located properly within the epidermal cells [5].

These results are consistent with the work from Dyer *et al.* [40], who found no significant differences in the detectability for naïve bumblebees between wild-type and mutant flowers of *Anthirrinum majus*. However, Glover & Martin [41] as well as Comba *et al.* [16] showed that wild type flowers with conical epidermal cells received a higher frequency of approaches by bumblebees and yielded also a higher reproductive success as compared to flat-celled mutants.

Furthermore, epidermal cell shape does correlate with the amount of floral gloss. On epidermal surfaces with conically shaped cells, floral gloss appears only at the small apical tips of epidermal cells, producing a pattern of regularly arranged angle-dependent highlights on the petal. This is the case in bee-pollinated flowers, and the bright flashes arising from floral gloss at mirror geometry can act as an attractant for insects [14].

Other than expected, epidermal cell shape in bird-pollinated flowers does not predict floral gloss in a way that it fits to

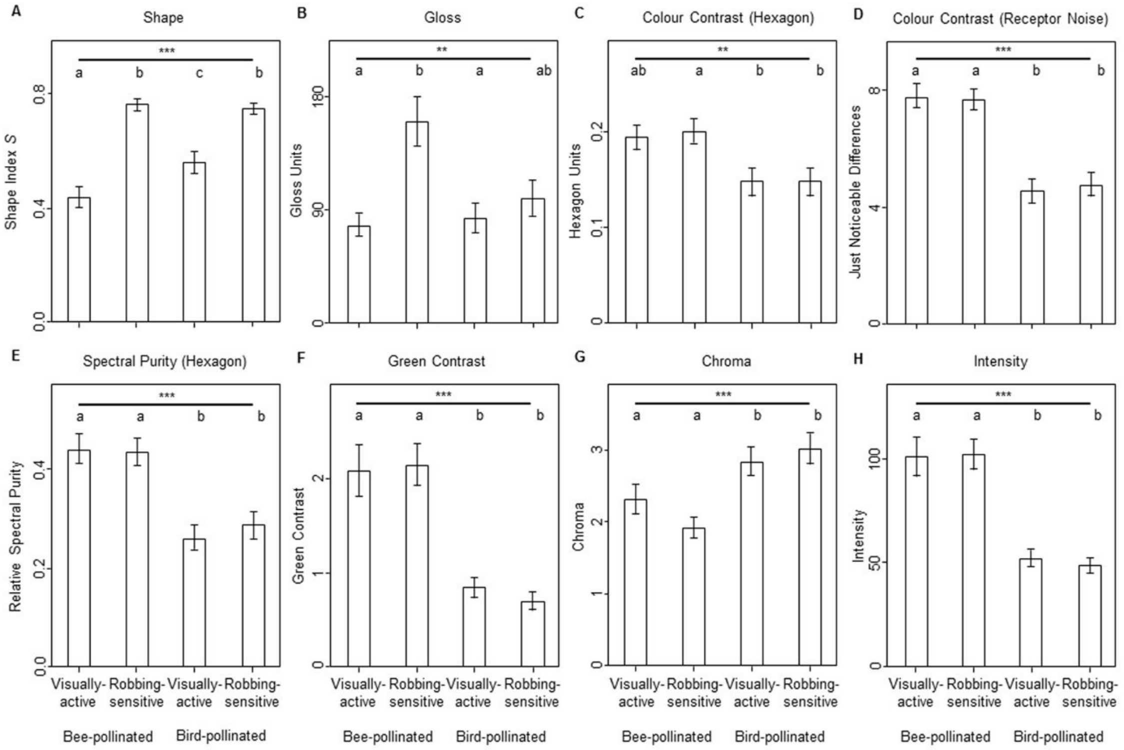

Figure 2. Epidermal cell shape, gloss and colour parameters of bee- and bird-pollinated flower parts. Means and standard errors of A) epidermal cell shape, B) floral gloss, colour contrast to the background in C) the colour hexagon model and D) in the receptor-noise limited model,(E) bee-subjective spectral purity according to the colour hexagon model, F) green contrast, G) chroma, and H) intensity for visually-active and for robbing-sensitive flower parts of bee- and bird-pollinated flowers. Asterisks above the bold line indicate differences according to one-way analysis of variance (ANOVA) with significance levels of ** for $p<0.01$ and *** for $p<0.001$. Different letters below the bold line denote significant differences according to pairwise comparisons using Tukey HSD.
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theoretical predictions. In fact, epidermal cell shape is only one factor among others determining gloss, and low glossiness might be caused by additional devices on (flat) epidermal cells like trichomes, surface-active residues, or surface micro-textures: these devices have been reported to decrease the amount of gloss on otherwise flat surfaces [13], [42].

But why do bird-pollinated flowers have flat epidermal cells at all, when it takes additional effort to reduce their intrinsic glossiness? Birds are fast flying flower visitors in habitats with adverse or alternating light conditions. Thus, on the one hand, dynamic visual displays in terms of floral gloss might improve the attention of birds, as plants might additionally exploit a pre-existing sensory bias for sparkling objects in birds [43]. However, flower-visiting birds, especially hummingbirds, need reliable floral cues operating on sunny as well as on cloudy and rainy days, because they need to feed on nectar more regular than bees. Thus the lacking invariability of gloss as visual cue in different ambient light conditions might foster a reduced glossiness of bird-pollinated flowers with flat surfaces.

The investigation of colour parameters and floral gloss in dependence on the epidermal cell shape suggest that the sensorial-floral-filter hypothesis does not apply. In fact, in our data set conical epidermal cells are restricted to visually-active parts of bee-pollinated flowers, but do not enhance the colour signal for bees.

Effects of epidermal cell shape on floral grip for bees and birds

In contrast to hovering hummingbirds and perching flower-visiting birds, bees need to land on flowers while consuming rewards and thus need micro-textural surface structures for floral grip [19]. Thus, the results suggest that our second hypothesis applies, as we found the predicted distribution of conical epidermal cells in bee and flat epidermal cells in bird-pollinated flowers. Bumblebees are able to tactile discriminate between conical-celled flowers and flat-celled flowers, and prefer those flowers with rough surfaces, especially if the flowers are difficult to handle [19], [21] (but see [44]). Moreover, colour produced by differing surface properties can be used by bees as a cue to visually discriminate against flowers which lack grip [19]. Thus, even though bees do not show innate preferences for the colours produced by conical epidermal cells, bees might use slightly differences in their colouration for discrimination before landing. However, the results indicate that bees are not able to distinguish the colours produced by conical and flat epidermal cells.

Effects of epidermal cell shape on floral grip for nectar robbers

Considering our dataset of flower parts which are vulnerable to nectar robbers, bee- as well as bird-pollinated flowers have more often flat surfaces consisting of flat epidermal cells. Therefore the third hypothesis applies, and flat surfaces might constitute a mechanical filter, hampering floral grip for nectar-robbing bees.

Surfaces consisting of flat epidermal cell surfaces make flowers difficult to handle for bees due to their slipperiness, and hamper bees to rest while consuming nectar [19]. The limitation of nectar robbing through a mechanical filter by flat surfaces might enhance the plants' reproductive success [28], [29].

Beside floral colouration and grip, several other flower traits are affected by epidermal cell shape, possessing bi-functionality for bees and birds as pollinators: Conical epidermal cells as compared to flat ones are suited to increase floral temperature used as floral reward by some insect visitors [16], (but see also [45]), but less important for homoiothermic birds. Beside this, conical as compared to flat epidermal cells improve overall flower size by influencing corolla reflexing abilities [6], and thereby promoting the detectability of flowers for bees [46]. Flower texture in form of conical epidermal cells acts additionally as tactile cue after landing and guide the bee towards the reward [18]. All these flower traits have different meanings for bees and birds, with conical epidermal cells more suitable in bee-pollinated flowers and flat epidermal cells more suitable for bird-pollinated ones. Bird-pollinated plants evolve mainly from bee-pollinated ancestors ([47], and references within). Therefore an evolutionary shift from conical epidermal cells in bee-pollinated ancestors towards bird-pollinated plants with a derived flat epidermal surface structure is conceivable. The evolutionary shift includes adaptations towards birds but at the same time maladaptations for bees, as was shown already for several other flower traits [48], [49].

The current study shows for the first time that epidermal cell shape is pollinator and flower part dependent. This ambiguity provides, together with experimental studies, evidence that epidermal cell shape is a multi-functional adaptive floral trait affecting grip required for bees as well as the floral colour signal as one of the most selective floral attractants. Petals' surface structure might be an important, but hitherto neglected, flower trait structuring the visitor composition of flowers and should be considered in plant-pollinator and plant-antagonist networks.

Supporting Information

Figure S1 Heatmap visualizing the results of Spearman's rho correlation between epidermal cell shape S, floral gloss, and all investigated colour parameters. The significance level of correlation is colour-coded with black for $p<0.001$, dark grey for $p<0.05$, light grey for $p>0.05$, and white for $p>0.1$. In the upper triangle of the symmetric matrix, rho-values are given. In the lower triangle, corresponding scatter plots are presented.
(TIF)

Text S1 Excel file including a list of studied plant species with literature reference about pollination mode, measured flower parts, floral colour, cell shape parameters and shape index S, floral gloss values and colour parameters.
(XLSX)

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Author Contributions

Conceived and designed the experiments: SP KL. Performed the experiments: SP. Analyzed the data: SP RRJ. Contributed reagents/materials/analysis tools: KL. Wrote the paper: SP KL RRJ.

References

1. Schiestl FP, Johnson SD (2013) Pollinator-mediated evolution of floral signals. Trends Ecol Evol 28: 307–315.
2. Lunau K, Papiorek S, Eltz T, Sazima M (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. J Exp Biol 214: 1607–1612.
3. Junker RR, Blüthgen N, Brehm T, Binkenstein J, Paulus J, et al. (2013) Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. Funct Ecol 27: 329–341.
4. Grotewold E (2006) The genetics and biochemistry of floral pigments. Annu Rev Plant Biol 57: 761–780.
5. Gorton HL, Vogelmann TC (1996) Effects of epidermal cell shape and pigmentation on optical properties of *Antirrhinum* petals at visible and ultraviolet wavelengths. Plant Physiol 112: 879–888.
6. Baumann K, Perez-Rodriguez M, Bradley D, Venail J, Bailey P, et al. (2007) Control of cell and petal morphogenesis by R2R3 MYB transcription factors. Development 134: 1691–1701.
7. Whitney HM, Glover BJ, Walker R, Ellis AG (2011) The contribution of epidermal structure to flower colour in the South African flora. Curtis's Bot Mag 28: 349–371.
8. Vignolini S, Davey MP, Bateman RM, Rudall PJ, Moyroud E, et al. (2012) The mirror crack'd: both pigment and structure contribute to the glossy blue appearance of the mirror orchid, *Ophrys speculum*. New Phytol 196: 1038–1047.
9. Vignolini S, Thomas MM, Kolle M, Wenzel T, Rowland A, et al. (2012) Directional scattering from the glossy flower of *Ranunculus*: how the buttercup lights up your chin. J R Soc Interface 9: 1295–1301.
10. Noda K, Glover BJ, Linstead P, Martin C (1994) Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. Nature 369: 661–664.
11. Kay QON, Daoud HS, Stirton CH (1981) Pigment distribution, light reflection and cell structure in petals. Bot J Linn Soc 83: 57–84.
12. Parkin J (1928) The glossy petal of *Ranunculus*. Ann Bot 42: 739–755.
13. Nussinovitch A, Ward G, Mey-Tal E (1996) Gloss of fruits and vegetables. LWT-Food Sci Technol 29: 184–186.
14. Galsterer S, Musso M, Asenbaum A, Fürnkranz D (1999) Reflectance measurements of glossy petals of *Ranunculus lingua* (Ranunculaceae) and of non-glossy petals of *Heliopsis helianthoides* (Asteraceae). Plant Biol 1: 670–678.
15. Whitney HM, Rands SA, Elton NJ, Ellis AG (2012) A technique for measuring petal gloss, with examples from the Namaqualand flora. PloS One 7: e29476.
16. Comba L, Corbet SA, Hunt H, Outram S, Parker JS, et al. (2000) The role of genes influencing the corolla in pollination of *Antirrhinum majus*. Plant Cell Environ 23: 639–647.
17. Whitney HM, Poetes R, Steiner U, Chittka L, Glover BJ (2011) Determining the contribution of epidermal cell shape to petal wettability using isogenic *Antirrhinum* lines. PLoS One 6: e17576.
18. Kevan PG, Lane MA (1985) Flower petal microtexture is a tactile cue for bees. Proc Natl Acad Sci USA 82: 4750–4752.
19. Whitney HM, Chittka L, Bruce TJA, Glover BJ (2009) Conical epidermal cells allow bees to grip flowers and increase foraging efficiency. Curr Biol 19: 948–953.
20. Whitney HM, Federle W, Glover BJ (2009) Grip and slip. Commun Integr Biol 2: 505–508.
21. Alcorn K, Whitney H, Glover B (2012) Flower movement increases pollinator preference for flowers with better grip. Funct Ecol 26: 941–947.
22. Peitsch D, Fietz A, Hertel H, de Souza J, Ventura DF, et al. (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol A 170: 23–40.
23. Herrera G, Zagal JC, Diaz M, Fernández MJ, Vielma A, et al. (2008) Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). J Comp Physiol A 194: 785–794.
24. Ödeen A, Håstad O (2010) Pollinating birds differ in spectral sensitivity. J Comp Physiol A 196: 91–96.
25. Lunau K, Maier EJ (1995) Innate colour preferences of flower visitors. J Comp Physiol A 177: 1–19.
26. Collias NE, Collias EC (1968) Anna's hummingbirds trained to select different colors in feeding. Condor 70: 273–274.
27. Stiles FG (1976) Taste preferences, color preferences, and flower choice in hummingbirds. Condor 78: 10–26.
28. Irwin RE, Brody AK (1998) Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. Oecologia 116: 519–527.
29. Irwin RE, Brody AK (1999) Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). Ecology 80: 1703–1712.
30. Faegri K, Van der Pijl L (1966) The principles of pollination ecology. Oxford: Pergamon Press.
31. Botes C, Johnson SD, Cowling RM (2009) The birds and the bees: using selective exclusion to identify effective pollinators of African tree aloes. Int J Plant Sci 170: 151–156.

32. Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, et al. (2009) A global test of the pollination syndrome hypothesis. Ann Bot 103: 1471–1480.
33. Menzel R, Backhaus W (1991) Colour vision in insects. Vision and Visual Dysfunction 6: 262–293.
34. Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J Comp Physiol A 170: 533–543.
35. Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc London B 265: 351–358.
36. Papiorek S, Rohde K, Lunau K (2013) Bees' subtle colour preferences: how bees respond to small changes in pigment concentration. Naturwissenschaften 100: 633–643.
37. Rohde K, Papiorek S, Lunau K (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. J Comp Physiol A 199: 197–210.
38. Valido A, Schaefer HM, Jordano P (2011) Colour, design and reward: phenotypic integration of fleshy fruit displays. J Evol Biol 24: 751–760.
39. R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
40. Dyer AG, Whitney HM, Arnold SE, Glover BJ, Chittka L (2007) Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of *Antirrhinum majus* flower colour. Arthropod-Plant Interact 1: 45–55.
41. Glover BJ, Martin C (1998) The role of petal cell shape and pigmentation in pollination success in *Antirrhinum majus*. Heredity 80: 778–784.
42. Kooi CJ, Wilts BD, Leertouwer HL, Staal M, Elzenga JTM, et al. (2014) Iridescent flowers? Contribution of surface structures to optical signaling. New Phytol 203: 667–673.
43. Fitzpatrick S (1998) Colour schemes for birds: structural coloration and signals of quality in feathers. Ann Zool Fennici 35: 67–77.
44. Rands SA, Glover BJ, Whitney HM (2011) Floral epidermal structure and flower orientation: getting to grips with awkward flowers. Arthropod-Plant Interact 5: 279–285.
45. Whitney HM, Bennett KV, Dorling M, Sandbach L, Prince D, et al. (2011) Why do so many petals have conical epidermal cells? Ann Bot 108: 609–616.
46. Spaethe J, Tautz J, Chittka L (2001) Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. Proc Natl Acad Sci USA 98: 3898–3903.
47. Thomson JD, Wilson P (2008) Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. Int J Plant Sci 169: 23–38.
48. Castellanos MC, Wilson P, Thomson JD (2004) 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. J Evol Biol 17: 876–885.
49. Wilson P, Castellanos MC, Wolfe AD, Thomson JD (2006) Shifts between bee and bird pollination among Penstemons. In: Waser NM, Ollerton J, editors. Specialization and Generalization in Pollination Systems. Chicago: University of Chicago Press. pp. 47–68.

CHAPTER 7

SYNTHESIS

Animal-pollinated plants occupy a large number of habitats with distinct conditions and possess a large number of different lifestyles and reproductive strategies (Barrett 1998). Thus, the effectiveness to act as pollen-vector for flowers of specific plants varies across different flower-visitor species (Schemske and Horvitz 1984). Flowers need to attract pollinators on the one hand, but also need to restrict interactions with antagonistically operating, non-pollinating visitors, which might reduce the plants' reproductive success, on the other hand (Strauss and Whittall 2006). The interactions between flowers and their visitors are influenced by specific chemical and morphological flower traits (Faegri and van der Pijl 1979). As different flower-visitors impose different requirements towards flowers and their specific trait formation, the identity of specific flower traits structure the flower-visitor composition. Studying the impact on the evolution of flower traits by pollinators as well as by antagonistically operating, non-pollinating visitors implicates great value, as the traditional view in respect of associations between the flowers' trait identity and a specific guild of flower-visitors is assumed to be caused by adaptations exclusively of flowers to their pollinators (Baker 1963; Stebbins 1970; Faegri and van der Pijl 1979; Brown 2002; Fenster *et al*. 2004).

Flower-visitors face a heterogeneous environment, in which flowers need to be easily detectable and easily to handle, in order to facilitate effective foraging, as the sufficient exploitation of floral resources mostly need many flower visits, often more than 1000 per day (Seeley 1985; Heinrich 2004). Especially bees play a significant role in crop pollination (Delaplane *et al.* 2000). The investigation of foraging behaviours in bees might aid conservation of plants and pollinators (Matheson *et al*. 1996; Kearns *et al*. 1998). My results are the first to demonstrate that the bees´ preference for colours of high bee-subjective spectral purity causes the exclusion from flowers adapted to the pollination by birds (Chapter 2 and 3). The innate preferences for spectrally pure colours in bees are even persisting when bees are trained to distinct colours (Chapter 2), which is in disagreement with Gumbert (2000) claiming that irrespective of innate preferences, bees choose novel colours in accordance to their similarity to the trained ones. Furthermore, these results are in disagreement with other studies assessing preferences in bees for other colour parameters like hue (Menzel and Shmida 1993; Giurfa *et al*. 1995; Chittka *et al*. 2001).

Due to different colour vision systems across flower-visitors, colouration is assumed to act as a flower trait structuring the flower-visitor composition. Several aspects of my results are in agreement with this assumption. Other than previously suggested, the colouration of flowers does not always match colour preferences of the main pollinators, but, at times, rather causes colours which are obviously difficult to detect for antagonistically operating flower-visitors due to their visual capabilities (Fenster *et al*. 2004; Chapter 3). This was shown for flowers of red and white colour, which are pollinated by hummingbirds (Chapter 3). In fact, flower colour acts as a sensorial floral filter, selectively attracting flower-visitors and additionally diminishing the frequency of visitation by non-pollinating visitors (Chapter 3). Furthermore, my results gain insight into the strong association between red flowers and bird-pollination as commonly stated in the literature (Porsch 1931; Grant 1966; Grant und Grant 1968; Raven 1972; Stiles 1976), and reveal that not reflectance of red wavelengths alone, but the additional absorbance of UV-light is crucial to avoid frequent visitation from bees (Chapter 3). Only then bird-pollinated flowers create a private communication channel for birds as their pollinators, in which flower-visiting birds suffer from less competition for nectar. Moreover, my results support the hypothesis from Lunau (1990) that the same mechanism is valid for white flowers. In fact, the colouration of white bird-pollinated flowers also selectively filter birds as flower-visitors, likewise not due to innate colour preferences in birds, but due to reducing frequent visitation by non-pollinating bees; again the UV-reflectance properties are crucial for this effect, but in this case UV-reflecting flowers are less attractive for bees (Chapter 3). Other than previously suggested, the results show that not the red-blindness by bees leads to a prevalence in red colouration of bird-pollinated flowers, but rather the evolution towards colours with low bee-subjective spectral purity (Porsch 1931; Grant 1966; Grant und Grant 1968; Raven 1972; Stiles 1976; Chapter 3). In fact, this is most parsimonious in red and white flowers, as here the amount of UV-light determines bee-subjective spectral purity. In contrast, bee- and bird-pollinated flowers with a yellow floral colouration do not differ in any colour parameter, and thus, bird-pollinated yellow flowers do not avoid frequent visitation by bees due to a comparably less attractive flower colours (Chapter 4). Different from red and white colours, the amount of bee-subjective spectral purity of yellow colours is not as simply determined by the amount of UV-reflectance as in red and white flowers. Some of my results corroborate the view that flower colours are well adapted to their effective pollinator (Fenster *et al*. 2004; Chapter 3 and 4). This is the case for the colouration of red, white, and yellow flowers which are pollinated by bees, as these colours are well detectable and of high spectral purity for bees (Chapter 3 and 4). Moreover, intra-floral colour patterns are displayed exclusively in bee-, but not in bird-pollinated yellow flowers, indicating once more the importance of nectar guides for bees, but not for birds (Free 1970a; Chapter 4). In sum, the colouration of flowers might represent a floral trait under selective pressure exerted by both, pollinators as well as antagonists.

Several aspects of my results support the understanding of flower-colour evolution. Tuning of floral pigment concentration in plants might improve the interaction-strength between flowers and several flower-visitors, which differ in their impact on the plants' reproductive success (Chapter 5). Moreover, the gain of insight into foraging behaviour of bees towards colours arising from slightly varying pigment concentrations, improve the understanding of evolutionary adaptations of flower colours towards bees (Chapter 5). Thus, even flower-constant bees exert selective pressure on the possession of spectrally pure colours in flowers (Chittka *et al*. 1999). Other aspects of my results indicate that, other than previously stated, the petals' epidermal cell shape does not affect floral colouration (Chapter 6). Thus, the detectability of flowers by bees is not affected by means of petals' epidermal cell shape alone, as it was previously shown for a single plant species only (Dyer *et al*. 2007; Gorton and Vogelmann 1969). The results show that epidermal cell shape in flowers might also shape competitive pollination networks due to mechanical properties (Whitney *et al*. 2009a; Chapter 6).

CHAPTER 8

DETAILED SUMMARY

Flowers serve as reproductive organs of angiosperms and interact with several different flower-visitors. The relation between the interacting partners can be of advantage, but also of disadvantage for one or both of the partners. The current study focuses on the role of floral colouration as a floral trait that structures the pollination network of plants and animals. Flower colours serve as floral filters, attracting pollinators of the plant species on the one hand, but on the other hand are non-enticing for those flower-visitors, which do not promote effective pollination service. This bi-functionality of one and the same flower colour is caused by different colour vision systems and colour preferences across the guilds of flower-visitors. Bees in general possess a trichromatic colour vision system with three different photoreceptor-types with peak-sensitivities in the UV, blue and green wavelength range. In contrast, all flower-visiting bird species studied yet are tetrachromatic, and possess photoreceptor-types maximally sensitive in the UV, blue and green wavelength range as well as a photoreceptor-type with a peak-sensitivity in the red wavelength range. In addition, the neural processing of visual input also differs between both flower-visitor groups. Flower-inexperienced bees detect flowers by means of innate preferences for distinct colour parameters, which can be altered by experience. However, innate preferences in experienced bees persist to some degree. In contrast, no innate preferences for distinct colour parameters are known for inexperienced flower-visiting birds. However, birds learn to associate flower colours with the quantity and quality of rewards and possess colour preferences only after experience. The divergent colour perception across flower-visitors and their implications for the colouration of natural flowers are under investigation. The focus will be on bees and birds as flower-visitors and flowers pollinated by either of them.

Chapter 2 - Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours.

To investigate which colour parameters are used by bees during foraging, colour choice experiments with workers of different bee species and uni-coloured artificial flowers, which vary only in one specific colour parameter were conducted. When giving the choice between colours varying only in their dominant wavelength, bees prefer those colours they were trained to. In contrast, when giving the choice between colours varying only in bee-subjective spectral purity, bees prefer colours of higher spectral purity in the tests, even over the training colour. As natural flowers often bear intra-floral colour patterns, further experiments were conducted offering artificial flowers with three concentric colours of equally sized spatial proportions, differing only in the arrangement of areas with colours of different spectral purity. Irrespective of their spatial position within the artificial flower bees always choose the most spectrally pure colour area for the first contact with the artificial flower. The results show that far- as well as near-orientation by bees towards flowers is determined by means of spectral purity as important key parameter.

Chapter 3 - Avoidance of achromatic colours by bees provides a private niche for hummingbirds.

This publication includes the comparative analysis of flower colours and preference tests with bees and hummingbirds with regard to artificial colour stimuli, resembling natural flower colours. The focus is on flowers, which appear red or white for humans, and are exclusively pollinated by either bees or hummingbirds. The studied flowers differ in their reflectance of ultraviolet (UV) light in dependence on the flower colour as well as on the pollinator group. Red as well as white flowers exclusively pollinated by bees are of higher bee-subjective spectral purity compared to those ones, which are pollinated by birds, and are therefore easier to detect by bees. Moreover, choice experiments using artificial flowers with Neotropical bees of the genus *Euglossa* reveal that bees exhibit preferences for those colours, which are of higher bee-subjective spectral purity. In contrast, hummingbirds do not show colour preferences for any of the offered colours. The results support the hypothesis that flower colours act as floral filters. The colouration of red and white flowers pollinated exclusively by bees represents adaptations to their pollinators. In contrast, the red and white colouration of bird-pollinated flowers is not directly adapted to the visual system of birds as pollinators, but rather represents an adaptation to antagonistic flower-visitors, which exploit the floral rewards without performing pollination, i.e. bees. In doing so, the visitation frequency of pollinators, which prefer to visit non-depleted, nectar-rich flowers, will be enhanced and the fitness of the plant will be ensured.

Chapter 4 - Bees, birds and yellow flowers: Pollinator-dependent convergent evolution of UV-patterns.

This manuscript bases on chapter 2 and extends the comparison between bee- and bird-pollinated flowers, which appear yellow for the human observer. The yellow colouration of bee- and bird-pollinated flowers rely on convergent coevolution between flowers and flower-visitor species in the New and the Old World, respectively. However, yellow colours of bee- and bird-pollinated flowers do not evoke a selective attractiveness for either bees or birds, as bee-subjective spectral purity cannot be altered simply by additional reflectance or absorption of UV-light in yellow flowers, as it is the case in red and white colours. Nevertheless, UV-patterns are restricted to yellow bee-pollinated flowers with UV-absorbing central parts within the flowers and UV-reflecting peripheral parts. In contrast, yellow bird-pollinated flowers entirely absorb UV-light. The pollinator-dependent occurrence of UV-patterns within flowers affects the foraging behaviour of flower-visitors, and the presence of UV-reflectance patterns exclusively in bee-pollinated flowers represents an adaptation between flowers and the visual capacities of bees as their pollinators.

Chapter 5 - Bees` subtle colour preferences: How bees respond to small changes in pigment concentration.

It was already shown that the foraging behaviour of bees is determined by specific colour parameters. To assess how flowers might utilize these innate preferences by means of their colouration, the impact of pigment concentration on specific colour parameters is investigated. For this purpose, artificial flowers are constructed by slightly varying pigment concentration, their bee-subjective colour parameters assigned, and the choice behaviour towards these modifications investigated. Up to excessive concentrations, increasing pigment concentration leads to increasing bee-subjective spectral purity as well as chromatic contrast to the background. A further increase of pigment concentration exceeding these profuse concentrations leads to a decrease in both colour parameters. Choice experiments in two different set-ups, offering three artificial flowers each comprising colours, which covering the range from low to intermediate or the range of intermediate to high pigment concentrations, reveal that pigment concentration affects the choice behaviour of workers in different species of bees. Bees do not prefer colours of highest pigment concentration, but colours of highest bee-subjective spectral purity and highest chromatic contrast to the background. These results show that pigment concentration in petals affects the detectability of flowers by bees.

Moreover, innate preferences for colours of high spectral purity in bees might exert selective pressure on the evolution of floral colouration.

Chapter 6 - Gloss, colour and grip: Multifunctional epidermal cell shapes in bee- and bird-pollinated flowers.

The epidermal cell structure of flower petals has several functions, which, among others, influence plant-animal interactions. Epidermal cells are often conically formed and affect the refraction of light on petal surfaces. Conical cells focusing incident light into those petal layers which commonly contain the pigments and thus, increasing the strength of the flowers' colour signal. Hence, this publication investigates the impact of diverse surface structures in petals on floral colouration. Flowers pollinated by bees have conical epidermal cells on those flower parts, which contribute to the advertisement for flower-visitors. In contrast, bird-pollinated flowers have flat epidermal cells at the same flower parts. However, a correlation between epidermal cell form and specific bee-subjective colour parameters has not been demonstrated and thus, epidermal cell form alone does not induce filtering effects in flowers through specific colouration. Nevertheless, epidermal cell structure affects the grip by bees handling the flowers, and this work indicates a mechanical filter by means of cell form of petals. Flat floral surfaces appear on those flower parts on which bees usually rob the nectar of flowers. Those flat surfaces hamper effective nectar robbing by bees due to reduced grip.

CHAPTER 9

DETAILLIERTE ZUSAMMENFASSUNG

Blüten dienen als Reproduktionsorgane von Angiospermen und stehen in direkten Wechselbeziehungen mit einer Vielzahl verschiedener Blütenbesucher. Die Wechselbeziehungen können dabei von Vorteil, aber auch von Nachteil für beide oder einen der beiden interagierenden Partner sein. Die Farbe von Blüten dient dabei als floraler Filter und lockt selektiv Bestäuber der jeweiligen Pflanzen an, erscheint gleichzeitig jedoch nicht attraktiv für solche Blütenbesucher, die keinen Bestäubungsdienst leisten. Das gleichzeitige Auftreten unterschiedlicher Funktionen ein und derselben Blütenfarbe beruht auf der unterschiedlichen Farbwahrnehmung verschiedener Blütenbesucher. Im Allgemeinen haben Bienen ein trichromatisches Farbsehsystem mit drei Rezeptortypen die jeweils im ultravioletten, blauen und grünen Wellenlängenbereich maximal sensitiv sind. Alle bisher untersuchten blütenbesuchenden Vogelarten dagegen sind Tetrachromaten und haben neben Rezeptoren, die im ultravioletten, blauen und grünen Wellenlängenbereich maximal sensitiv sind, einen weiteren Rezeptortyp, der für rotes Licht sensitiv ist. Neben diesen physiologischen Grundlagen kommen weitere neuronale Verarbeitungsprozesse hinzu, die sich ebenfalls bei beiden Blütenbesucher-Gruppen unterscheiden. Bei blütennaiven Bienen bestimmen angeborene Präferenzen für bestimmte Farbparameter das Auffinden von Blüten, die jedoch durch Erfahrungen modifiziert werden können. Für blütenbesuchende Vögel konnten dagegen bis heute keine angeborenen Präferenzen für bestimmte Farbparameter nachgewiesen werden. Vielmehr assoziieren blütenbesuchende Vögel Blütenfarben mit Belohnungsparametern und entwickeln erst nach einiger Erfahrung Präferenzen. Die in dieser Arbeit zusammengefassten Publikationen und Manuskripte befassen sich mit dem unterschiedlichen Wahrnehmungsvermögen unterschiedlicher Blütenbesucher und deren Konsequenzen für die Farbgebung natürlicher Blütenfarben. Im Fokus stehen dabei Bienen und Vögel und die durch diese Blütenbesucher bestäubten Pflanzenarten.

Kapitel 2 - Hummeln (*Bombus terrestris*) und Honigbienen (*Apis mellifera*) bevorzugen Farben von höherer spektraler Reinheit gegenüber solchen Farben, auf die sie trainiert wurden und die sich ähneln.

Um zu untersuchen welche Farbparameter Bienen bei der Nahrungssuche nutzen, werden Farbwahlexperimente mit Arbeiterinnen verschiedener Bienenarten und einfarbigen

Kunstblüten durchgeführt, die sich jeweils in nur einem Farbparameter unterscheiden. Wenn Farben angeboten werden, die sich jeweils nur in ihrer dominanten Wellenlänge unterscheiden, präferieren Biene diejenigen Farben, auf die sie trainiert werden. Werden Farben angeboten, die sich jeweils nur in ihrer spektralen Reinheit unterscheiden, präferieren Bienen im Testversuch dagegen nicht diejenigen Farben auf die sie trainiert wurden, sondern diejenigen, die von höchster spektraler Reinheit sind. Da natürliche Blüten oftmals intra-florale Farbmuster aufweisen, werden weitere Experimente mit Kunstblüten durchgeführt, die jeweils drei verschiedene konzentrisch angeordnete Farben von gleichem Flächenanteil zeigen, die sich jeweils nur in der Anordnung der farbigen Flächen unterschiedlicher spektraler Reinheit unterscheiden. Unabhängig von der räumlichen Position innerhalb der Kunstblüte, wählen Bienen diejenige Farbe von höchster spektraler Reinheit für den ersten Kontakt mit der Kunstblüte. Die Ergebnisse zeigen, dass sowohl die Fern- als auch die Nahorientierung von Bienen gegenüber Blüten durch spektrale Reinheit als Schlüsselparameter bestimmt sind. Weiterhin legen die Ergebnisse den Schluss nahe, dass die angeborene Präferenz für Farben von hoher spektraler Reinheit bei Bienen einen Selektionsdruck auf die Farbgebung von Blüten ausübt.

Kapitel 3 - Vermeidung achromatischer Farben von Bienen führt zu einer privaten Nischenbildung für Kolibris.

Inhalt dieser Publikation ist die vergleichende Analyse von Blütenfarben und Präferenztests mit Bienen auf Farbstimuli die den in der Natur gefundenen Blütenfarben entsprechen. Der Fokus liegt hierbei auf für den Menschen rot und weiß erscheinenden Blüten, die ausschließlich von Bienen oder ausschließlich von Kolibris bestäubt werden. Die Blütenfarben unterscheiden sich in Bezug auf die Reflexion von ultraviolettem (UV) Licht sowohl in Abhängigkeit von der Blütenfarbe, als auch in Abhängigkeit von der Bestäubergruppe. Solche Blüten, die ausschließlich von Bienen bestäubt werden, sind von höherer bienensubjektiver spektraler Reinheit als solche, die von Kolibris bestäubt werden. Wahlexperimente mit Bienen der neotropischen Gattung *Euglossa* zeigen weiterhin, dass Bienen solche Farben bevorzugen, die von höherer spektraler Reinheit sind und somit Blüten bevorzugen, die von Bienen bestäubt werden. Kolibris dagegen zeigen keinerlei Präferenz für eine der dargebotenen Farben. Die Ergebnisse unterstützen die Hypothese, dass Blütenfarbe als floraler Filter dienen kann. Die Farbe von roten und weißen Blüten die ausschließlich von Bienen bestäubt werden, stellt eine direkte Anpassung an den Bestäuber dar. Die rote und weiße Farbe von vogelbestäubten Blüten dagegen stellen keine direkte Anpassung an den Bestäuber dar, sondern eine Anpassung an solche Blütenbesucher, die das Nahrungsangebot der Blüten aufbrauchen, aber keine Bestäubung gewährleisten. Dies verbessert wiederum das Besuchsverhalten der eigentlichen Bestäuber und gewährleistet somit die biologische Fitness der Pflanze.

Kapitel 4 - Bienen, Vögel und gelbe Blüten: Bestäuber-abhängige konvergente Evolution von UV-Mustern.

Diese Publikation basiert auf Kapitel 2 und erweitert den Vergleich von bienen- und vogelbestäubten Blüten um Blütenfarben, die für das menschliche Auge gelb erscheinen. Die gelbe Färbung bienen- und vogelbestäubter Blüten ist auf koevolutive Zusammenhänge zwischen Blüten und Blütenbesuchern zurückzuführen. Eine gelbe Blütenfärbung führt jedoch nicht zu selektiver Anlockung von Bienen, da die bienensubjektive spektrale Reinheit bei gelben Farben nicht wie bei roten und weißen durch die Menge an UV-Reflexion bestimmt wird. Das Auftreten von UV-Mustern ist jedoch auf solche gelben Blüten beschränkt, die von Bienen bestäubt werden. Hier weisen zentrale Bereiche innerhalb der Blüten eine UV-Absorption auf und periphere Bereiche eine UV-Reflexion. Vogelbestäubte gelbe Blüten absorbieren dagegen einheitlich UV-Licht. Das vom Bestäuber abhängige Auftreten von UV-Mustern beeinflusst das Verhalten von Blütenbesuchern und das Vorhandensein dieser Muster ausschließlich in von Bienen bestäubten Blüten, repräsentiert eine Anpassung zwischen Blüten und dem visuellen Wahrnehmungsvermögen ihrer Bestäuber, den Bienen.

Kapitel 5 - Feinsinnige Farbpräferenzen bei Bienen: Wie sich Bienen gegenüber kleinen Änderungen in der Pigment-Konzentration verhalten.

Es konnte bereits gezeigt werden, dass die Nahrungssuche von Bienen durch spezifische Farbparameter von Blüten bestimmt wird. Um zu untersuchen, inwiefern Blüten diese angeborenen Präferenzen durch Färbung nutzen können, wird der Einfluss der Pigmentkonzentration auf bienensubjektive Farbparameter untersucht. Daher werden Kunstblüten durch jeweils gering variierende Pigmentkonzentrationen hergestellt, deren bienensubjektive Farbparameter bestimmt und das Wahlverhalten von Bienen gegenüber solchen Änderungen getestet. Bis zu einer bestimmt hohen Konzentration, steigen mit zunehmender Pigmentkonzentration ebenfalls die bienensubjektive spektrale Reinheit und der chromatische Kontrast zum Hintergrund für die jeweilige Farbe. Eine weitere Zunahme der Pigmentkonzentration führt dagegen wieder zu einer Abnahme beider Farbparameter. Wahlexperimenten in zwei Versuchsreihen, in denen jeweils drei Kunstblüten angeboten werden, deren Farben zum einen den Bereich von geringen bis mittleren Konzentrationen und zum anderen den Bereich von mittleren bis hohen Pigmentkonzentrationen umfassen, zeigen, dass die Pigmentkonzentration die Wahl der Arbeiterinnen verschiedener Bienenarten beeinflusst. Bienen präferieren nicht immer diejenigen Farben von höchster Pigmentkonzentration, jedoch immer diejenigen von höchster bienensubjektiver spektraler Reinheit und chromatischem Kontrast zum Hintergrund. Die Ergebnisse zeigen, dass die Pigmentkonzentration in Blütenblättern die Erkennung von Blüten für Bienen beeinflusst und dass die angeborene Präferenz von Bienen für Farben von hoher spektraler Reinheit einen eventuellen Selektionsdruck auf die Blütenfärbung ausübt.

Kapitel 6 - Glanz, Farbe und Grip: Multifunktionale epidermale Zellformen bei bienen- und vogelbestäubten Blüten.

Der epidermalen Zellstruktur von Blütenblättern werden mehrere verschiedene Funktionen angerechnet, die unter anderem auch Auswirkungen auf Interaktionen zwischen Pflanze und Tier haben. Häufig sind diese epidermalen Zellformen von konischer Natur, die Auswirkungen auf die Lichtbrechung an der Blütenoberfläche haben. Konische Zellen fokussieren dabei auftreffendes Licht in eine bestimmte Schicht des Blütenblattes, die oftmals die Pigmente enthält und somit das Farbsignal der Blüte verbessert. In dieser Veröffentlichung wird daher der Einfluss von diversen Oberflächenstrukturen auf die Farbgebung von Blütenblättern untersucht. Von Bienen bestäubte Blüten weisen konische Epidermiszellen an solchen Blütenbereiche auf, die eine Lockfunktion für Blütenbesucher darstellen. Von Vögeln bestäubte Blüten dagegen besitzen an gleicher Stelle flach ausgeprägte Epidermiszellen. Eine Korrelation zwischen der Zellform und spezifischen bienensubjektiven Farbparametern kann jedoch nicht gefunden werden und somit bedingt die epidermale Zellstruktur allein nicht die sensorische Filterwirkung von Blüten durch gewisse Färbung. Da die epidermale Zellform jedoch ebenfalls Einfluss auf die Griffigkeit der Blüte durch Bienen hat, zeigt diese Arbeit eine mechanische Filterfunktion durch Zellform bei Blütenblättern. Glatte Blütenoberflächen treten sowohl bei von Bienen als auch bei von Vögeln bestäubten Pflanzen an denjenigen Bereichen auf, an denen Nektarräuber üblicherweise ansetzten. Diese flachen Oberflächen hindern Bienen an effektivem Nektarraub durch das Fehlen von Griffigkeit.

CHAPTER 10

REFERENCES

(Excluding references of papers and manuscripts)

Aidley, D. J. (1998). *The Physiology of Excitable Cells. 4th edn*. Cambridge University Press, Cambridge, UK.

Alcorn, K., Whitney, H. and Glover, B. (2012). Flower movement increases pollinator preference for flowers with better grip. *Functional Ecology*, 26, 941-947.

Altshuler, D. L. (2003). Flower Color, Hummingbird pollination, and habitat irradiance in four Neotropical forests. *Biotropica*, *35*, 344-355.

Armbruster, W. S. (1984). The role of resin in angiosperm pollination: ecological and chemical considerations. *American Journal of Botany*, 71, 1149-1160.

Autrum, H. and Zwehl, V. von (1964). Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. *Zeitschrift für vergleichende Physiologie*, 48, 357-384.

Avarguès-Weber, A. and Giurfa, M. (2014). Cognitive components of color vision in honey bees: how conditioning variables modulate color learning and discrimination. *Journal of Comparative Physiology A*, 200, 449-461.

Backhaus, W., Werner, A. and Menzel, R. (1987). Color vision in honeybees: Metric, dimensions, constancy, and ecological aspects. In: Menzel, R. and Mercer, A. (eds.) *Neurobiology and behavior of honeybees*. Springer, Berlin, Heidelberg, Germany, pp 172-190.

Backhaus, W. (1991). Color opponent coding in the visual system of the honeybee. *Vision Research*, 31, 1381-1397.

Backhaus, W. (1992). Color vision in honeybees. *Neuroscience and Biobehavioral Reviews*, 16, 1-12.

Baker, H. G. (1963). Evolutionary mechanisms in pollination biology. *Science*, 139, 877-883.

Baker, H. G. (1975). Sugar concentrations in nectars from hummingbird flowers. *Biotropica*, 7, 37-41.

Barrett, S. C. H. (1998). The evolution of mating strategies in flowering plants. *Trends in Plant Science*, 3, 335-341.

Bascompte, J. and Jordano, P. (2006). The structure of plant-animal mutualistic networks. In: Pascual, M., and Dunne, J. A. (eds.). *Ecological networks: linking structure to dynamics in food webs.* Oxford University Press, Oxford, UK, pp 143-159.

Bascompte, J. and Jordano, P. (2007). Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 38, 567-593.

Bastolla, U., Fortuna, M. A., Pascual-García, A., Ferrera, A., Luque, B. and Bascompte, J. (2009). The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature*, 458, 1018-1020.

Baumann, K., Perez-Rodriguez, M., Bradley, D., Venail, J., Bailey, P., Jin, H., Koes, R., Roberts, K. and Martin, C. (2007). Control of cell and petal morphogenesis by R2R3 MYB transcription factors. *Development*, 134, 1691-1701.

Bawa, K. S. and Beach, J. H. (1981). Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden*, 68, 254-274.

Beason, R. C. (1987). Interaction of visual and non-visual cues during migratory orientation by the Bobolink (*Dolichonyx oryzivorus*). *Journal für Ornithologie*, 128, 317-324.

Bené, F. (1941). Experiments on the color preference of black-chinned hummingbirds. *Condor*, 43, 237-242.

Bennett, A. T. D. and Cuthill, I. C. (1994). Ultraviolet vision in birds: what is its function?. *Vision Research*, 34, 1471-1478.

Bennett, A. T. D., Cuthill, I. C. and Norris, K. J. (1994). Sexual selection and the mismeasure of color. *American Naturalist*, 144, 848-860.

Bogacz, R., Wagenmakers, E. J., Forstmann, B. U. and Nieuwenhuis, S. (2010). The neural basis of the speed-accuracy tradeoff. *Trends in Neurosciences*, 33, 10-16.

Bohm, B. A. (1998). *Introduction to flavonoids. Chemistry and Biochemistry of Organic Natural Products Vol 2*. Harwood Academic Publishers, Amsterdam, Netherlands.

Bolten, A. B. and Feinsinger, P. (1978). Why do hummingbird flowers secrete dilute nectar?. *Biotropica*, 10, 307-309.

Botes, C., Johnson, S. D. and Cowling, R. M. (2009). The birds and the bees: using selective exclusion to identify effective pollinators of African tree aloes. *International Journal of Plant Sciences*, 170, 151-156.

Bouvier, F., Suire, C., Mutterer, J. and Camara, B. (2003). Oxidative remodeling of chromoplast carotenoids: identification of the carotenoid dioxygenase CsCCD and CsZCD genes involved in crocus secondary metabolite biogenesis. *Plant Cell*, 15, 47-62.

Bowmaker, J. K. (1980). Colour vision in birds and the role of oil droplets. *Trends in Neurosciences*, 3, 196-199.

Bowmaker, J. K. and Dartnall, H. (1980). Visual pigments of rods and cones in a human retina. *The Journal of Physiology*, 298, 501-511.

Bowmaker, J. K., Heath, L. A., Wilkie, S. E. and Hunt, D. M. (1997). Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research*, 37, 2183-2194.

Bradshaw Jr, H. D., WIlbert, M. and Otto, K. G. (1995). Genetic mapping of floral traits associated with reproductive isolation in. *Nature*, *376*, 31.

Bradshaw, H. D., Otto, K. G., Frewen, B. E., McKay, J. K. and Schemske, D. W. (1998). Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). *Genetics*, 149, 367-382.

Bradshaw, H. D. and Schemske, D. W. (2003). Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature*, 426, 176-178.

Bradshaw, E., Rudall, P. J., Devey, D. S., Thomas, M. M., Glover, B. J. and Bateman, R. M. (2010). Comparative labellum micromorphology of the sexually deceptive temperate orchid genus *Ophrys*: diverse epidermal cell types and multiple origins of structural colour. *Botanical Journal of the Linnean Society*, 162, 504-540.

Briscoe, A. D. and Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology*, 46, 471-510.

Brouillard, R. and Dangles, O. (1993). Flavonoids and flower colour. In: Harborne, J. B. (ed). *The Flavonoids: Advances in Research Since 1986.* Chapman and Hall, London, UK, pp 565-587.

Brown, P. K. and Wald, G. (1964). Visual pigments in single rods and cones of the human retina. *Science*, 144, 45-52.

Brown, J. H. and Kodric-Brown, A. (1979). Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology*, 60, 1022-1035.

Brown, K. (2002). A compromise on floral traits. *Science*, 298, 45-46.

Buchmann, S. L. (1987). The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics*, 18, 343-369.

Buchmann, S. L. and Nabhan, G. P. (1997). *The forgotten pollinators*. Island Press, Washington, USA.

Burns, J. G. (2005). Impulsive bees forage better: the advantage of quick, sometimes inaccurate foraging decisions. *Animal Behaviour*, 70, e1-e5.

Burns, J. G. and Dyer, A. G. (2008). Diversity of speed-accuracy strategies benefits social insects. *Current Biology*, 18, R953-R954.

Butcher, G. S. and Rohwer, S. (1989). The evolution of conspicuous and distinctive coloration for communication in birds. *Current Ornithology,* 6, 51-108

Carrasco, M., Giordano, A. M. and McElree, B. (2006). Attention speeds processing across eccentricity: Feature and conjunction searches. *Vision Research*, 46, 2028-2040.

Castellanos, M. C., Wilson, P. and Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution*, 57, 2742-2752.

Castellanos, M. C., Wilson, P. and Thomson, J. D. (2004). ‘Anti-bee’and ‘pro-bird’changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, 17, 876-885.

Chittka, L. (1992). The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A*, 170, 533-543.

Chittka, L. and Menzel, R. (1992). The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A*, 171, 171-181.

Chittka, L., Shmida, A., Troje, N. and Menzel, R. (1994). Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Research*, 34, 1489-1508.

Chittka, L. (1996). Does bee color vision predate the evolution of flower color?. *Naturwissenschaften*, 83, 136-138.

Chittka, L. and Waser, N. M. (1997). Why red flowers are not invisible to bees. *Israel Journal of Plant Sciences*, 45, 169-183.

Chittka, L., Thomson, J. D. and Waser, N. M. (1999). Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften*, 86, 361-377.

Chittka, L. and Briscoe, A. (2001). Why sensory ecology needs to become more evolutionary—insect color vision as a case in point. In: Barth, F. G. and Schmid, A. (eds.). *Ecology of sensing*. Springer, Berlin, Heidelberg, Germany, pp 19-37.

Chittka, L., Spaethe, J., Schmidt, A. and Hickelsberger, A. (2001). Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision. In: Chittka, L., Thompson, J. D. (eds.). *Cognitive ecology of pollination*. Cambridge University Press, Cambridge, UK, pp 106-126.

Chittka, L., Dyer, A. G., Bock, F. and Dornhaus, A. (2003). Psychophysics: bees trade off foraging speed for accuracy. *Nature*, 424, 388.

Chittka, L. and Wells, H. (2004). Color vision in bees: mechanisms, ecology and evolution. In: Prete, F. R. (ed.). *Complex worlds from simpler nervous systems.* MIT Press, Boston, USA, pp 165-191.

Chittka, L., Ings, T. C. and Raine, N. E. (2004). Chance and adaptation in the evolution of island bumblebee behaviour. *Population Ecology*, 46, 243-251.

Chittka, L., Skorupski, P. and Raine, N. E. (2009). Speed-accuracy tradeoffs in animal decision making. *Trends in Ecology and Evolution*, 24, 400-407.

Clement, J. S. and Mabry, T. J. (1996). Pigment Evolution in the Caryophyllales: a Systematic Overview. *Botanica Acta*, 109, 360-367.

Collias, N. E. and Collias, E. C. (1968). Anna's Hummingbirds trained to select different colors in feeding. *Condor*, 70, 273-274.

Comba, L., Corbet, S. A., Hunt, H., Outram, S., Parker, J. S. and Glover, B. J. (2000). The role of genes influencing the corolla in pollination of *Antirrhinum majus*. *Plant, Cell and Environment*, 23, 639-647.

Crepet, W. L. (1983). The role of insect pollination in the evolution of the angiosperms. In: Real, L. (ed). *Pollination biology*. Academic Press, Orlando, USA, pp 29-50.

Cronk, Q. and Ojeda, I. (2008). Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, 59, 715-727.

Cuthill, I. C., Partridge, J. C., Bennett, A. T., Church, S. C., Hart, N. S. and Hunt, S. (2000). Ultraviolet vision in birds. *Advances in the Study of Behavior*, 29, 159-214.

Dafni, A. (1983). Pollination of *Orchis caspia*-a nectarless plant which deceives the pollinators of nectariferous species from other plant families. *The Journal of Ecology*, 71, 467-474.

Dafni, A., Bernhardt, P., Shmida, A., Ivri, Y., Greenbaum, S., O'Toole, C. and Losito, L. (1990). Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. *Israel Journal of Botany*, 39, 81-92.

Dalsgaard, B., González, A. M. M., Olesen, J. M., Ollerton, J., Timmermann, A., Andersen, L. H. and Tossas, A. G. (2009). Plant-hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. *Oecologia*, 159, 757-766.

Danieli-Silva, A., de Souza, J. M. T., Donatti, A. J., Campos, R. P., Vicente-Silva, J., Freitas, L. and Varassin, I. G. (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands?. *Oikos*, 121, 35-43.

Darwin, C. (1862). *On the various contrivances by which British and foreign orchids are fertilised by insects*. John Murray, London, UK.

Darwin, C. (1876). *The effects of cross and self fertilisation in the vegetable kingdom*. John Murray, London, UK.

Daumer, K. (1956). Reizmetrische Untersuchung des Farbensehens der Bienen. *Zeitschrift für vergleichende Physiologie*, 38, 413-478.

Davies K. (2004). *Plant pigments and their manipulation. Annual plant reviews Vol. 14*. Blackwell Publishing Ltd., Oxford, UK.

Delaplane, K. S., Mayer, D. R. and Mayer, D. F. (2000). *Crop pollination by bees*. CABI Publishing, New York, USA.

Delph, L. F. and Lively, C. M. (1989). The evolution of floral color change: pollinator attraction versus physiological constraints in *Fuchsia excorticata*. *Evolution*, 43, 1252-1262.

Dressler, R. L. (1968). Pollination by euglossine bees. *Evolution*, 22, 202-210.

Dyer, A. G. (1999). Broad spectral sensitivities in the honeybee's photoreceptors limit colour constancy. *Journal of Comparative Physiology A*, 185, 445-453.

Dyer, A. G. and Chittka, L. (2004). Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A*, 190, 759-763.

Dyer, A. G., Whitney, H. M., Arnold, S. E., Glover, B. J. and Chittka, L. (2007). Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of *Antirrhinum majus* flower colour. *Arthropod-Plant Interactions*, 1, 45-55.

Dyer, A. G., Paulk, A. C. and Reser, D. H. (2010). Colour processing in complex environments: insights from the visual system of bees. *Proceedings of the Royal Society B: Biological Sciences*, 278, 952-959.

Endler, J. A. and Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*, 86, 405-431.

Exner, F. and Exner, S. (1910). Die physikalischen Grundlagen der Blütenfärbungen. *Sitzungsberichte der Akademie der Wissenschaften in Wein. Mathematisch-naturwissenschaftliche Klasse, Abteilung 1*, 119, 191-245.

Faegri, K. and Pijl, L. van der (1979). *The principles of pollination ecology*. Pergamon Press, Oxford, UK.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. and Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35, 375-403.

Forrest, J. and Thomson, J. D. (2009). Background complexity affects colour preference in bumblebees. *Naturwissenschaften*, 96, 921-925.

Foster, D. H. (2011). Color constancy. *Vision Research*, 51, 674-700.

Free, J. B. (1963). The flower constancy of honeybees. *The Journal of Animal Ecology*, 32, 119-131.

Free, J. B. (1970a). Effect of flower shapes and nectar guides on the behaviour of foraging honeybees. *Behaviour*, 37, 269-285.

Free, J. B. (1970b). The flower constancy of bumblebees. *The Journal of Animal Ecology*, 39, 395-402.

Frisch, K. von (1914). Der Farbensinn und Formensinn der Biene. *Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere*, 35, 1-188.

Frisch, K. von, Lindauer, M., Daumer, K. (1960). Über die Wahrnehmung polarisierten Lichtes durch das Bienenauge. *Experientia*, 16, 289-301.

Galen, C. and Cuba, J. (2001). Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. *Evolution*, 55, 1963-1971.

Galsterer, S., Musso, M., Asenbaum, A. and Fürnkranz, D. (1999). Reflectance measurements of glossy petals of *Ranunculus lingua* (Ranunculaceae) and of non-glossy petals of *Heliopsis helianthoides* (Asteraceae). *Plant Biology*, 1, 670-678.

Gegear, R. J. and Burns, J. G. (2007). The birds, the bees, and the virtual flowers: can pollinator behavior drive ecological speciation in flowering plants?. *The American Naturalist*, 170, 551-566.

Gegenfurtner, K. R. and Kiper, D. C. (2003). Color vision. *Neuroscience*, 26, 181-206.

Giger, A. and Srinivasan, M. (1997). Honeybee vision: analysis of orientation and colour in the lateral, dorsal and ventral fields of view. *Journal of Experimental Biology*, 200, 1271-1280.

Gill, F. B. (1988). Trapline foraging by hermit hummingbirds: competition for an undefended, renewable resource. *Ecology*, 1933-1942.

Giurfa, M. (1991). Colour generalization and choice behaviour of the honeybee *Apis mellifera ligustica*. *Journal of Insect Physiology*, 37, 41-44.

Giurfa, M., Núñez, J. and Backhaus, W. (1994). Odour and colour information in the foraging choice behaviour of the honeybee. *Journal of Comparative Physiology A*, 175, 773-779.

Giurfa, M., Nunez, J., Chittka, L. and Menzel, R. (1995). Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A*, 177, 247-259.

Giurfa, M., Vorobyev, M., Kevan, P. and Menzel, R. (1996). Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *Journal of Comparative Physiology A*, 178, 699-709.

Giurfa, M., Vorobyev, M., Brandt, R., Posner, B. and Menzel, R. (1997). Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology A*, 180, 235-243.

Giurfa, M. and Vorobyev, M. (1998). The angular range of achromatic target detection by honey bees. *Journal of Comparative Physiology A*, 183, 101-110.

Glover, B. J. and Martin, C. (1998). The role of petal cell shape and pigmentation in pollination success in *Antirrhinum majus*. *Heredity*, 80, 778-784.

Glover, B. J. and Whitney, H. M. (2010). Structural colour and iridescence in plants: the poorly studied relations of pigment colour. *Annals of Botany*, 105, 505-511.

Goldsmith, T. H. and Ruck, P. R. (1958). The spectral sensitivities of the dorsal ocelli of coackroaches and honeybees. *Journal of General Physiology*, 41, 1171-1185.

Goldsmith, T. H. and Goldsmith, K. M. (1979). Discrimination of colors by the black-chinned hummingbird, *Archilochus alexandri*. *Journal of Comparative Physiology A*, 130, 209-220.

Goldsmith, T. H. (1980). Hummingbirds see near ultraviolet light. *Science*, 207, 786-788.

Goldsmith, T. H., Collins, J. S. and Perlman, D. L. (1981). A wavelength discrimination function for the hummingbird *Archilochus alexandri*. *Journal of Comparative Physiology A*, 143, 103-110.

Goldsmith, K. M. and Goldsmith, T. H. (1982). Sense of smell in the Black-chinned Hummingbird. *Condor*, 84, 237-238.

Goldsmith, T. H. (1990). Optimization, constraint, and history in the evolution of eyes. *Quarterly Review of Biology*, 65, 281-322.

Goldsmith, T. H. (1991). The evolution of visual pigments and colour vision. *The Perception of Colour*, 6, 62-89.

Goodwin, T. W. (1984). *The biochemistry of the carotenoids. Vol. 2*. Chapman and Hall, New York, USA.

Gorton, H. L., Vogelmann, T. C. (1996). Effects of epidermal cell shape and pigmentation on optical properties of *Antirrhinum* petals at visible and ultraviolet wavelengths. *Plant Physiology*, 112, 879-888.

Gottsberger, G. and Gottlieb, O. R. (1981). Blue flower pigmentation and evolutionary advancement. *Biochemical Systematics and Ecology*, 9, 13-18.

Grant, V. (1950). The flower constancy of bees. *The Botanical Review*, 16, 379-398.

Grant, K. A. (1966). A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. *American Naturalist*, 100, 85-97.

Grant, K. A. and Grant, V. (1968). *Hummingbirds and their flowers*. Columbia University Press, New York, USA.

Grant, V. and Témeles, E. J. (1992). Foraging ability of rufous hummingbirds on hummingbird flowers and hawkmoth flowers. *Proceedings of the National Academy of Sciences USA*, 89, 9400-9404.

Greggers, U. and Mauelshagen, J. (1997). Matching behavior of honeybees in a multiple-choice situation: the differential effect of environmental stimuli on the choice process. *Animal Learning and Behavior*, 25, 458-472.

Gribakin, F.G. (1975). Functional morphology of the compound eye of the bee. In: Horridge, G. A. (ed). *The compound eye and vision of insects*. Clarendon Press, Oxford, UK, pp 154-176.

Gronquist, M., Bezzerides, A., Attygalle, A., Meinwald, J., Eisner, M. and Eisner, T. (2001). Attractive and defensive functions of the ultraviolet pigments of a flower (*Hypericum calycinum*). *Proceedings of the National Academy of Sciences USA*, 98, 13745-13750.

Grotewold, E. (2006). The genetics and biochemistry of floral pigments. *Annual Review of Plant Biology*, 57, 761-780.

Guldberg, L. D. and Atsatt, P. R. (1975). Frequency of reflection and absorption of ultraviolet light in flowering plants. *American Midland Naturalist*, 93, 35-43.

Gumbert, A. (2000). Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology*, 48, 36-43.

Hainsworth, F. R. and Wolf, L. L. (1972). Crop volume, nectar concentration and hummingbird energetics. *Comparative Biochemistry and Physiology Part A: Physiology*, 42, 359-366.

Haisworth, F. R. and Wolf, L. L. (1976). Nectar characteristics and food selection by hummingbirds. *Oecologia*, 25, 101-113.

Hanley, M. E., Lamont, B. B. and Armbruster, W. S. (2009). Pollination and plant defence traits co-vary in Western Australian *Hakeas*. *The New Phytologist*, 182, 251-260.

Harborne, J. B. (1993). *Introduction to ecological biochemistry*. Gulf Professional Publishing, London, UK.

Harborne, J. B. and Grayer, R. J. (1994). Flavonoids and insects. In: Harborne, J. B. (ed.). *The Flavonoids*, *Advances in Research since 1986*. Chapman and Hall, London, UK, pp 589-618.

Harder, L. D. (1986). Effects of nectar concentration and flower depth on flower handling efficiency of bumble bees. *Oecologia*, 69, 309-315.

Hart, N. S. (2001). Variations in cone photoreceptor abundance and the visual ecology of birds. *Journal of Comparative Physiology A*, 187, 685-697.

Hart, N. S. and Vorobyev, M. (2005). Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. *Journal of Comparative Physiology A*, 191, 381-392.

Hart, N. S. and Hunt, D. M. (2007). Avian visual pigments: characteristics, spectral tuning, and evolution. *The American Naturalist*, 169, 7-26.

Healy, S. D. and Hurly, T. A. (2001). Foraging and spatial learning in hummingbirds. In: Chittka, L. and Thomson, J. D. (eds.). *Cognitive ecology of pollination*. Cambridge University Press, Cambridge, UK, 127-147.

Healy, S. D. and Hurly, T. A. (2004). Spatial learning and memory in birds. *Brain, Behavior and Evolution*, 63, 211-220.

Heinrich, B. (1976). The foraging specializations of individual bumblebees. *Ecological Monographs*, 46, 105-128.

Heinrich, B. (2004). *Bumblebee economics*. Harvard University Press, Cambridge, UK.

Helversen, O. von (1972). Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. *Journal of Comparative Physiology A*, 80, 439-472.

Helversen, O. von and Edrich, W. (1974). Der Polarisationsempfänger im Bienenauge: ein Ultraviolettrezeptor. *Journal of Comparative Physiology A*, 94, 33-47.

Hempel de Ibarra, N., Giurfa, M. and Vorobyev, M. (2001). Detection of coloured patterns by honeybees through chromatic and achromatic cues. *Journal of Comparative Physiology A*, 187, 215-224.

Hempel de Ibarra, N., Giurfa, M. and Vorobyev, M. (2002). Discrimination of coloured patterns by honeybees through chromatic and achromatic cues. *Journal of Comparative Physiology A*, 188, 503-512.

Hempel de Ibarra, N., Vorobyev, M. and Menzel, R. (2014). Mechanisms, functions and ecology of colour vision in the honeybee. *Journal of Comparative Physiology A*, 200, 411-433.

Herrera, C. M. (1996). Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd, D. G. and Barrett, S. C. H. (eds.). *Floral biology: Studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York, USA, pp 65-87.

Herrera, C. M., Medrano, M., Rey, P. J., Sánchez-Lafuente, A. M., García, M. B., Guitián, J. and Manzaneda, A. J. (2002). Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism-and antagonism-related traits. *Proceedings of the National Academy of Sciences USA*, 99, 16823-16828.

Herrera, C. M., Castellanos, M. C. and Medrano, M. (2006). Geographical context of floral evolution: towards an improved research programme in floral diversification. In: Harder, L. D. and Barrett, S. C. H. (eds.). *Ecology and evolution of flowers*. Oxford University Press, Oxford, UK, pp 278-294.

Herrera, G., Zagal, J. C., Diaz, M., Fernández, M. J., Vielma, A., Cure, M., Martinez, J., Bozinovic, F. and Palacios, A. G. (2008). Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). *Journal of Comparative Physiology A*, 194, 785-794.

Heuschen, B., Gumbert, A. and Lunau, K. (2005). A generalised mimicry system involving angiosperm flower colour, pollen and bumblebees' innate colour preferences. *Plant Systematics and Evolution*, 252, 121-137.

Hill, P. S., Wells, P. H. and Wells, H. (1997). Spontaneous flower constancy and learning in honey bees as a function of colour. *Animal Behaviour*, 54, 615-627.

Hisatomi, O., Kayada, S., Aoki, Y., Iwasa, T. and Tokunaga, F. (1994). Phylogenetic relationships among vertebrate visual pigments. *Vision Research*, 34, 3097-3102.

Hopkins, R. and Rausher, M. D. (2012). Pollinator-mediated selection on flower color allele drives reinforcement. *Science*, 335, 1090-1092.

Horovitz, A. and Cohen, Y. (1972). Ultraviolet reflectance characteristics in flowers of crucifers. *American Journal of Botany*, 59, 706-713.

Horridge, G. A. and Zhang, S. W. (1995). Pattern vision in honeybees (*Apis mellifera*): Flower-like patterns with no predominant orientation. *Journal of Insect Physiology*, 41, 681-688.

Hurly, A. T. and Healy, S. D. (1996). Memory for flowers in rufous hummingbirds: location or local visual cues?. *Animal Behaviour*, 51, 1149-1157.

Hurvich, L. M. (1981). *Color vision*. Sinauer Associates, Sunderland, UK.

Ings, T. C., Montoya, J. M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C. F., Edwards F., Figueroa, D., Jacob, U., Jones, J. I., Lauridsen, R. B., Ledger, M. E., Lewis, H. M., Olesen, J. M., van Veen, F. J. F., Warren, P. H. and Woodward, G. (2009). Review: Ecological networks–beyond food webs. *Journal of Animal Ecology*, 78, 253-269.

Inouye, D. W. (1980). The terminology of floral larceny. *Ecology*, 61, 1251-1253.

Inouye, D. W. (1983). The ecology of nectar robbing. In: Bentley, B. and Elias, T. (eds). *The biology of nectaries*. Columbia University Press, New York, USA, pp 153-173.

Irwin, R. E. and Brody, A. K. (1998). Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. *Oecologia*, 116, 519-527.

Irwin, R. E. and Brody, A. K. (1999). Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology*, 80, 1703-1712.

Irwin, R. E. (2000). Hummingbird avoidance of nectar-robbed plants: spatial location or visual cues. *Oikos*, 91, 499-506.

Irwin, R. E. and Brody, A. K. (2000). Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology*, 81, 2637-2643.

Irwin, R. E., Brody, A. K. and Waser, N. M. (2001). The impact of floral larceny on individuals, populations, and communities. *Oecologia*, 129, 161-168.

Irwin, R. E. and Maloof, J. E. (2002). Variation in nectar robbing over time, space, and species. *Oecologia*, 133, 525-533.

Irwin, R. E. (2003). Impact of nectar robbing on estimates of pollen flow: conceptual predictions and empirical outcomes. *Ecology*, 84, 485-495.

Irwin, R. E., Adler, L. S. and Brody, A. K. (2004). The dual role of floral traits: pollinator attraction and plant defense. *Ecology*, 85, 1503-1511.

Irwin, R. E. (2006). The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *The American Naturalist*, 167, 315-328.

Irwin, R. E. (2009). Realized tolerance to nectar robbing: compensation to floral enemies in *Ipomopsis aggregata*. *Annals of Botany*, 103, 1425-1433.

Irwin, R. E., Bronstein, J. L., Manson, J. S. and Richardson, L. (2010). Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution, and Systematics*, 41, 271-292.

Jander, U. and Jander, R. (2002). Allometry and resolution of bee eyes (Apoidea). *Arthropod Structure and Development*, 30, 179-193.

Johnson, S. D. and Bond, W. J. (1994). Red flowers and butterfly pollination in the fynbos of South Africa. In: Arianoutsou, M. and Groves, R. H. (eds.). *Plant-animal interactions in Mediterranean-type ecosystems.* Kluwer Academic Publishers, Dordrecht, Netherlands, pp 137-148.

Johnson, S. D. and Steiner, K. E. (2000). Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution*, 15, 140-143.

Johnson, S. D., Hargreaves, A. L. and Brown, M. (2006). Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*, 87, 2709-2716.

Jones, C. E. and Buchmann, S. L. (1974). Ultraviolet floral patterns as functional orientation cues in hymenopterous pollination systems. *Animal Behaviour*, 22, 481-485.

Jordano, P. (1987). Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. *American Naturalist*, 129, 657-677.

Jordano, P., Bascompte, J. and Olesen, J. M. (2003). Invariant properties in coevolutionary networks of plant-animal interactions. *Ecology Letters*, 6, 69-81.

Junker, R. R. and Blüthgen, N. (2008). Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research*, 10, 295-308.

Junker, R. R. and Blüthgen, N. (2010). Floral scents repel facultative flower visitors, but attract obligate ones. *Annals of Botany*, 105, 777-782.

Kaczorowski, R. L., Blumenfeld, G., Koplovich, A. and Markman, S. (2014). Color and side preferences in Palestine sunbirds (*Nectarinia osea*). *Israel Journal of Ecology and Evolution*, 60, 35-40.

Kay, Q. O. N., Daoud, H. S. and Stirton, C. H. (1981). Pigment distribution, light reflection and cell structure in petals. *Botanical Journal of the Linnean Society*, 83, 57-83.

Kearns, C. A., Inouye, D. W. and Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, 29, 83-112.

Kelber, A., Vorobyev, M. and Osorio, D. (2003). Animal colour vision-behavioural tests and physiological concepts. *Biological Reviews*, 78, 81-118.

Kelber, A. and Osorio, D. (2010). From spectral information to animal colour vision: experiments and concepts. *Proceedings of the Royal Society B*, 277, 1617-1625.

Kevan, P. G. and Baker, H. G. (1983). Insects as flower visitors and pollinators. *Annual Review of Entomology*, 28, 407-453.

Kevan, P. G. and Lane, M. A. (1985). Flower petal microtexture is a tactile cue for bees. *Proceedings of the National Academy of Sciences USA*, 82, 4750-4752.

Kevan, P. G., Chittka, L. and Dyer, A. G. (2001). Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. *Journal of Experimental Biology*, 204, 2571-2580.

Kimler, L., Larson, R. A., Messenger, L., Moore, J. B. and Mabry, T. J. (1971). Betalamic acid, a new naturally occurring pigment. *Journal of the Chemical Society D: Chemical Communications*, 21, 1329-1330.

Kingston, A. B. and Quillan, P. B. (2000). Are pollination syndromes useful predictors of floral visitors in Tasmania?. *Austral Ecology*, 25, 600-609.

Knudsen, J. T., Tollsten, L., Groth, I., Bergström, G. and Raguso, R. A. (2004). Trends in floral scent chemistry in pollination syndromes: floral scent composition in hummingbird-pollinated taxa. *Botanical Journal of the Linnean Society*, 146, 191-199.

Knuth, P. E. O. W. (1908). *Handbook of Flower Pollination. Volume II. Translated by JR Ainsworth Davis.* Clarendon Press, Oxford, UK.

Kourounioti, R. L. A., Band, L. R., Fozard, J. A., Hampstead, A., Lovrics, A., Moyroud, E., Vignolini, S., King, J. R., Jensen, O. E. and Glover, B. J. (2013). Buckling as an origin of ordered cuticular patterns in flower petals. *Journal of the Royal Society Interface*, 10, 20120847.

Kühn, A. (1924). Versuche über das Unterscheidungsvermögen der Bienen und Fische für Spektrallichter. *Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen, Mathematisch-Physikalische Klasse*, 1924, 66-71.

Kühn, A. (1929). Farbenunterscheidungsvermögen der Tiere. In: Bethe, A., v. Bergmann, G., Embden, G. and Ellinger, A. (eds.). *Handbuch der normalen und pathologischen Physiologie*. Springer, Berlin, Heidelberg, Germany, 720-741.

Labhart, T. and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microscopy Research and Technique*, 47, 368-379.

Land, M. F. and Nilsson, D. E. (2012). *Animal eyes*. Oxford University Press, Oxford, UK.

Lara, C. and Ornelas, J. (2001). Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. *Oecologia*, 128, 263-273.

Laverty, T. M. (1994). Bumble bee learning and flower morphology. *Animal Behaviour*, 47, 531-545.

Lázaro, A., Hegland, S. J. and Totland, Ø. (2008). The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia*, 157, 249-257.

Lehrer, M. and Bischof, S. (1995). Detection of model flowers by honeybees: the role of chromatic and achromatic contrast. *Naturwissenschaften*, 82, 145-147.

Lehrer, M., Horridge, G. A., Zhang, S. W. and Gadagkar, R. (1995). Shape vision in bees: innate preference for flower-like patterns. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 347, 123-137.

Lehrer, M. (1998). Looking all around: honeybees use different cues in different eye regions. *Journal of Experimental Biology*, 201, 3275-3292.

Leonard, A. S. and Papaj, D. R. (2011). 'X' marks the spot: the possible benefits of nectar guides to bees and plants. *Functional Ecology*, 25, 1293-1301.

Lubbock, J. (1888). *On the senses, instincts and intelligence of animals with special reference to insects*. Kegan Paul, London, UK.

Lunau, K. (1990). Colour saturation triggers innate reactions to flower signals: flower dummy experiments with bumblebees. *Journal of Comparative Physiology A*, 166, 827-834.

Lunau, K. (1991). Innate flower recognition in bumblebees (*Bombus terrestris*, *B. lucorum*; Apidae): optical signals from stamens as landing reaction releasers. *Ethology*, 88, 203-214.

Lunau, K. (1992). A new interpretation of flower guide colouration: absorption of ultraviolet light enhances colour saturation. *Plant Systematics and Evolution*, 183, 51-65.

Lunau, K. (1993). Interspecific diversity and uniformity of flower colour patterns as cues for learned discrimination and innate detection of flowers. *Experientia*, 49, 1002-1010.

Lunau, K. and Maier, E. J. (1995). Innate colour preferences of flower visitors. *Journal of Comparative Physiology A*, 177, 1-19.

Lunau, K., Wacht, S. and Chittka, L. (1996). Colour choices of naive bumble bees and their implications for colour perception. *Journal of Comparative Physiology A*, 178, 477-489.

Lunau, K. (2000). The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution,* 222, 89-111.

Lunau, K. (2004). Adaptive radiation and coevolution—pollination biology case studies. *Organisms Diversity and Evolution*, 4, 207-224.

Lunau, K. (2006). Stamens and mimic stamens as components of floral colour patterns. *Botanische Jahrbücher*, 127, 13-41.

Lunau, K., Fieselmann, G., Heuschen, B. and Loo, A. van de (2006). Visual targeting of components of floral colour patterns in flower-naive bumblebees (*Bombus terrestris*; Apidae). *Naturwissenschaften*, 93, 325-328.

Maier, E. J. and Bowmaker, J. K. (1993). Colour vision in the passeriform bird, *Leiothrix lutea*: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. *Journal of Comparative Physiology A*, 172, 295-301.

Maloof, J. E. and Inouye, D. W. (2000). Are nectar robbers cheaters or mutualists?. *Ecology*, 81, 2651-2661.

Matheson, A., Buchmann, S. L., O'toole, C., Westrich, P. and Williams, I. H. (1996). *The conservation of bees*. Academic Press, New York, USA.

McCall, C. and Primack, R.B. (1992). Influence of flower characteristics, weather, time of day and season on insect visitation rates in three plant communities. *American Journal of Botany*, 79, 434-442.

McDade, L. A. (1983). Long-tailed hermit hummingbird visits to inflorescence color morphs of *Heliconia irrasa*. *Condor*, 85, 360-364.

Meléndez-Ackerman, E., Campbell, D. R. and Waser, N. M. (1997). Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. *Ecology*, 78, 2532-2541.

Melendez-Ackerman, E. and Campbell, D. R. (1998). Adaptive significance of flower color and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution*, 52, 1293-1303.

Memmott, J. (1999). The structure of a plant-pollinator food web. *Ecology Letters*, *2*, 276-280.

Menzel, R. (1967). Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). *Zeitschrift für vergleichende Physiologie*, 56, 22-62.

Menzel, R. (1974). Spectral sensitivity of monopolar cells in the bee lamina. *Journal of Comparative Physiology*, 93, 337-346.

Menzel, R. and Blakers, M. (1976). Colour receptors in the bee eye—morphology and spectral sensitivity. *Journal of Comparative Physiology A*, 108, 11-13.

Menzel, R. (1985). Learning in honey bees in an ecological and behavioral context. *Experimental Behavioral Ecology*, 31, 55-74.

Menzel, R. and Backhaus, W. (1991). Color vision in insects. In: Gouras, P. (ed.). *Vision and visual dysfunction. VI. Perception of color*. Macmillan, London, UK, pp 262-293.

Menzel, R. and Shmida, A. (1993). The ecology of flower colours and the natural colour vision of insect pollinators: the Israeli flora as a study case. *Biological Reviews*, 68, 81-120.

Miller, R. S. and Miller, R. E. (1971). Feeding activity and color preference of ruby-throated hummingbirds. *Condor*, 73, 309-313.

Miller, R. S., Tamm, S., Sutherland, G. D. and Gass, C. L. (1985). Cues for orientation in hummingbird foraging: color and position. *Canadian Journal of Zoology*, 63, 18-21.

Mol, J., Grotewold, E. and Koes, R. (1998). How genes paint flowers and seeds. *Trends in Plant Science*, 3, 212-217.

Morehouse, N. I. and Rutowski, R. L. (2009). Comment on “Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators”. *Science*, 325, 1072-1072.

Morris, W. F. (1996). Mutualism denied? Nectar-robbing bumble bees do not reduce female or male success of bluebells. *Ecology*, 77, 1451-1462.

Müller, H. (1873). On the fertilisation of flowers by insects and on the reciprocal adaptations of both. *Nature*, 8, 187-189.

Navarro, L. (2001). Reproductive biology and effect of nectar robbing on fruit production in *Macleania bullata* (Ericaceae). *Plant Ecology*, 152, 59-65.

Neumeyer, C. (1981). Chromatic adaptation in the honeybee: successive color contrast and color constancy. *Journal of Comparative Physiology A*, 144, 543-553.

Neumeyer, C. (1991). Evolution of colour vision. *Evolution of the Eye and Visual System*, 1, 284-305.

Neumeyer, C. (1992). Tetrachromatic color vision in goldfish: evidence from color mixture experiments. *Journal of Comparative Physiology A*, 171, 639-649.

Nicolson, S. W. (2011). Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two: review article. *African Zoology*, 46, 197-204.

Nilsson, L. A. (1998). Deep flowers for long tongues. *Trends in ecology and evolution*, 13, 259-260.

Noda, K. I., Glover, B. J., Linstead, P. and Martin, C. (1994). Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. *Nature*, 369, 661-664.

Oberrath, R. and Böhning-Gaese, K. (1999). Floral color change and the attraction of insect pollinators in lungwort (*Pulmonaria collina*). *Oecologia*, 121, 383-391.

Ödeen, A. and Håstad, O. (2010). Pollinating birds differ in spectral sensitivity. *Journal of Comparative Physiology A*, 196, 91-96.

Olesen, J. M. and Jordano, P. (2002). Geographic patterns in plant-pollinator mutualistic networks. *Ecology*, 83, 2416-2424.

Olesen, J. M., Bascompte, J., Dupont, Y. L. and Jordano, P. (2006). The smallest of all worlds: pollination networks. *Journal of Theoretical Biology*, 240, 270-276.

Olesen, J. M., Bascompte, J., Dupont, Y. L. and Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences USA*, 104, 19891-19896.

Ollerton, J. (1996). Reconciling ecological processes with phylogenetic patterns: The apparent paradox of plant-pollinator systems. *Journal of Ecology*, 84, 767-769.

Ollerton, J. (1998). Pollination: sunbird surprise for syndromes. *Nature*, 394, 726-727.

Ollerton, J., Alarcón, R., Waser, N. M., Price, M. V., Watts, S., Cranmer, L., Hingston, A. Peter, C. I. and Rotenberry, J. (2009). A global test of the pollination syndrome hypothesis. *Annals of Botany*, 103, 1471-1480.

Ollerton, J., Winfree, R. and Tarrant, S. (2011). How many flowering plants are pollinated by animals?. *Oikos*, 120, 321-326.

Orbán, L. L. and Plowright, C. M. (2013). The effect of flower-like and non-flower-like visual properties on choice of unrewarding patterns by bumblebees. *Naturwissenschaften*, 100, 621-631.

Ortega-Jimenez, V. M. and Dudley, R. (2012). Flying in the rain: hovering performance of Anna's hummingbirds under varied precipitation. *Proceedings of the Royal Society B: Biological Sciences*, 279, 3996-4002.

Osche, G. (1979). Zur Evolution optischer Signale bei Blütenpflanzen. *Biologie in unserer Zeit*, 9, 161-170.

Osorio, D., Vorobyev, M. and Jones, C. D. (1999). Colour vision of domestic chicks. *The Journal of Experimental Biology*, 202, 2951-2959.

Ostler, W. K. and Harper, K. T. (1978). Floral ecology in relation to plant species diversity in the Wasatch Mountains of Utah and Idaho. *Ecology*, 59, 848-861.

Owen, C. R. and Bradshaw, H. D. (2011). Induced mutations affecting pollinator choice in *Mimulus lewisii* (Phrymaceae). *Arthropod-Plant Interactions*, 5, 235-244.

Parkin, J. (1928). The glossy petal of *Ranunculus*. *Annals of Botany*, 3, 739-755.

Peitsch, D., Fietz, A., Hertel, H., de Souza, J., Ventura, D. F. and Menzel, R. (1992). The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology A*, 170, 23-40.

Peter, C. I. and Johnson, S. D. (2008). Mimics and magnets: the importance of color and ecological facilitation in floral deception. *Ecology*, 89, 1583-1595.

Pleasants, J. M. and Waser, N. M. (1985). Bumblebee foraging at a" hummingbird" flower: reward economics and floral choice. *American Midland Naturalist*, 114, 283-291.

Pohl, N., Carvallo, G., Botto-Mahan, C. and Medel, R. (2006). Nonadditive effects of flower damage and hummingbird pollination on the fecundity of *Mimulus luteus*. *Oecologia*, 149, 648-655.

Pohl, M., Watolla, T. and Lunau, K. (2008). Anther-mimicking floral guides exploit a conflict between innate preference and learning in bumblebees (*Bombus terrestris*). *Behavioral Ecology and Sociobiology*, 63, 295-302.

Porsch, O. (1931). Grellrot als Vogelblumenfarbe. *Biologia Generalis*, 2, 647-674.

Primack, R. B. (1982). Ultraviolet patterns in flowers, or flowers as viewed by insects. *Arnoldia*, 42, 139-146.

Raine, N. E. and Chittka, L. (2007). The adaptive significance of sensory bias in a foraging context: floral colour preferences in the bumblebee *Bombus terrestris*. *PLoS ONE*, 2, e556.

Ramsey, J., Bradshaw, H. D. and Schemske, D. W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution*, 57, 1520-1534.

Rands, S. A., Glover, B. J. and Whitney, H. M. (2011). Floral epidermal structure and flower orientation: getting to grips with awkward flowers. *Arthropod-Plant Interactions*, 5, 279-285.

Rausher, M. D. (2008). Evolutionary transitions in floral color. *International Journal of Plant Sciences*, 169, 7-21.

Raven, P. H. (1972). Why are bird-visited flowers predominantly red. *Evolution*, 26, 674.

Reisenman, C. E. and Giurfa, M. (2008). Chromatic and achromatic stimulus discrimination of long wavelength (red) visual stimuli by the honeybee *Apis mellifera*. *Arthropod-Plant Interactions*, 2, 137-146.

Rocca, M. A. and Sazima, M. (2010). Beyond hummingbird-flowers: the other side of ornithophily in the Neotropics. *Oecologia Australis*, 14, 67-99.

Rodríguez-Gironés, M. A. and Santamaría, L. (2004). Why are so many bird flowers red?. *PLoS Biology*, 2, e350.

Roubik, D. W. (1982). The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. *Ecology*, 63, 354-360.

Roubik, D. W., Holbrook, N. M. and Parra, G. V. (1985). Roles of nectar robbers in reproduction of the tropical treelet *Quassia amara* (Simaroubaceae). *Oecologia*, 66, 161-167.

Santamaría, L. and Rodríguez-Gironés, M. A. (2007). Linkage rules for plant-pollinator networks: trait complementarity or exploitation barriers?. *PLoS Biology*, 5, e31.

Schaefer, H. M., Levey, D. J., Schaefer, V. and Avery, M. L. (2006). The role of chromatic and achromatic signals for fruit detection by birds. *Behavioral Ecology*, 17, 784-789.

Schemske, D. W. and Horvitz, C. C. (1984). Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science*, 225, 519-521.

Schemske, D. W. and Bradshaw, H. D. (1999). Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences USA*, 96, 11910-11915.

Schiestl, F. P. and Johnson, S. D. (2013). Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution*, 28, 307-315.

Scogin, R., Young, D. A. and Jones Jr, C. E. (1977). Anthochlor pigments and pollination biology. II. The ultraviolet floral pattern of *Coreopsis gigantea* (Asteraceae). *Bulletin of the Torrey Botanical Club*, 104, 155-159.

Scogin, R. (1980). Floral pigments and nectar constituents of two bat-pollinated plants: coloration, nutritional, and energetic considerations. *Biotropica*, 12, 273-276.

Scogin, R. (1983). Visible floral pigments and pollinators. In: Jones, C. E. and Little, R. J. (eds.). *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, USA, pp 60-172.

Scogin, R. (1988). Floral anthocyanidins of bird-visited flowers. *Botanical Gazette*, 149, 437-442.

Seeley, T. D. (1985). *Honeybee ecology: a study of adaptation in social life*. Princeton University Press, Princeton, USA.

Silberglied, R. E. (1979). Communication in the ultraviolet. *Annual Review of Ecology and Systematics*, 10, 373-398.

Simonds, V. and Plowright, C. M. S. (2004). How do bumblebees first find flowers? Unlearned approach responses and habituation. *Animal Behaviour*, 67, 379-386.

Skorupski, P., Spaethe, J. and Chittka, L. (2006). Visual search and decision making in bees: time, speed, and accuracy. *International Journal of Comparative Psychology*, 19, 342-357.

Skorupski, P., Döring, T. F. and Chittka, L. (2007). Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. *Journal of Comparative Physiology A*, 193, 485-494.

Skorupski, P. and Chittka, L. (2010). Differences in photoreceptor processing speed for chromatic and achromatic vision in the bumblebee, *Bombus terrestris*. *The Journal of Neuroscience*, 30, 3896-3903.

Smith, C. E., Stevens, J. T., Temeles, E. J., Ewald, P. W., Hebert, R. J. and Bonkovsky, R. L. (1996). Effect of floral orifice width and shape on hummingbird-flower interactions. *Oecologia*, 106, 482-492.

Spaethe, J., Tautz, J. and Chittka, L. (2001). Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences USA*, 98, 3898-3903.

Spaethe, J. and Briscoe, A. D. (2005). Molecular characterization and expression of the UV opsin in bumblebees: three ommatidial subtypes in the retina and a new photoreceptor organ in the lamina. *Journal of Experimental Biology*, 208, 2347-2361.

Sprengel, C. K. (1793). *Das entdeckte Geheimnifs der Natur im Bau und in der Befuchtung der Blumen*. Vieweg, Berlin, Germany.

Srinivasan, M. V. and Lehrer, M. (1988). Spatial acuity of honeybee vision and its spectral properties. *Journal of Comparative Physiology A*, 162, 159-172.

Stebbins, G. L. (1970). Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics*, 1, 307-326.

Stiles, F. G. (1976). Taste preferences, color preferences, and flower choice in hummingbirds. *Condor*, 78, 10-26.

Strauss, S. Y. and Agrawal, A. A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, 14, 179-185.

Strauss, S. Y. and Irwin, R. E. (2004). Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and Systematics*, 35, 435-466.

Strauss, S. Y. and Whittall, J. B. (2006). Non-pollinator agents of selection on floral traits. In: Harder, L. D. and Barrett, S. C. H. (eds.). *Ecology and evolution of flowers*. Oxford University Press, Oxford, UK, pp 120-138.

Sutherland, S. D. and Vickery Jr, R. K. (1993). On the relative importance of floral color, shape, and nectar rewards in attracting pollinators to *Mimulus*. *Western North American Naturalist*, 53, 107-117.

Temeles, E. J. (1996). A new dimension to hummingbird-flower relationships. *Oecologia*, 105, 517-523.

Tepedino, V. J. (1979). The importance of bees and other insect pollinators in maintaining floral species composition. *Great Basin Naturalist Memoirs*, 3, 139-150.

Thomson, J. D., Wilson, P., Valenzuela, M. and Malzone, M. (2000). Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biology*, 15, 11-29.

Thomson, J. D. and Wilson, P. (2008). Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. *International Journal of Plant Sciences*, 169, 23-38.

Totland, Ø. (1994). Influence of climate, time of day and season, and flower density on insect flower visitation in alpine Norway. *Arctic and Alpine Research*, 26, 66-71.

Kooi, C. J. van der, Wilts, B. D., Leertouwer, H. L., Staal, M., Elzenga, J. T. M. and Stavenga, D. G. (2014a). Iridescent flowers? Contribution of surface structures to optical signaling. *The New Phytologist*, 203, 667-673.

Kooi, C. J. van der, Dyer, A. G. and Stavenga, D. G. (2014b). Is floral iridescence a biologically relevant cue in plant-pollinator signaling?. *The New Phytologist*. DOI: 10.1111/nph.13066

Pijl, L. van der (1961). Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution*, 15, 44-59.

Varela, F. G. and Porter, K. R. (1969). Fine structure of the visual system of the honeybee (*Apis mellifera*): I. The retina. *Journal of Ultrastructure Research*, 29, 236-259.

Varela, F. G. and Wiitanen, W. (1970). The optics of the compound eye of the honeybee (*Apis mellifera*). *The Journal of General Physiology*, 55, 336-358.

Vignolini, S., Thomas, M. M., Kolle, M., Wenzel, T., Rowland, A., Rudall, P. J., Baumberg, J. J. Glover, B. J. and Steiner, U. (2012a). Directional scattering from the glossy flower of *Ranunculus*: how the buttercup lights up your chin. *Journal of The Royal Society Interface*, 9, 1295-1301.

Vignolini, S., Davey, M. P., Bateman, R. M., Rudall, P. J., Moyroud, E., Tratt, J., Malmgren, S., Steiner, U. and Glover, B. J. (2012b). The mirror crack'd: both pigment and structure contribute to the glossy blue appearance of the mirror orchid, *Ophrys speculum*. *The New Phytologist*, 196, 1038-1047.

Vignolini, S., Moyroud, E., Glover, B. J. and Steiner, U. (2013). Analysing photonic structures in plants. *Journal of the Royal Society Interface*, 10, 20130394.

Vignolini, S., Moyroud, E., Hingant, T., Banks, H., Rudall, P. J., Steiner, U. and Glover, B. J. (2014a). The flower of *Hibiscus trionum* is both visibly and measurably iridescent. *The New Phytologist*. DOI: 10.1111/nph.12958.

Vignolini, S., Moyroud, E., Hingant, T., Banks, H., Rudall, P. J., Steiner, U. and Glover, B. J. (2014b). Is floral iridescence a biologically relevant cue in plant-pollinator signalling? A response to van der Kooi et al. (2014). *The New Phytologist*. DOI: 10.1111/nph.13178

Vogel, S. (1954). *Blütenbiologische Typen als Elemente der Sippengliederung: dargestellt anhand der Flora Südafrikas*. Fischer, Jena, Germany.

Vogel, S. (1971). Ölproduzierende Blumen, die durch ölsammelnde Bienen bestäubt werden. *Naturwissenschaften*, 58, 58-58.

Vogelmann, T. C. (1993). Plant tissue optics. *Annual Review of Plant Biology*, 44, 231-251.

Vorobyev, M. and Brandt, R. (1997). How do insect pollinators discriminate colors?. *Israel Journal of Plant Sciences*, 45, 103-113.

Vorobyev, M. and Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 351-358.

Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J. and Cuthill, I. C. (1998). Tetrachromacy, oil droplets and bird plumage colours. *Journal of Comparative Physiology A*, 183, 621-633.

Vorobyev, M., Marshall, J., Osorio, D., Hempel de Ibarra, N. and Menzel, R. (2001). Colourful objects through animal eyes. *Color Research and Application*, 26, S214-S217.

Vorobyev, M. (2003). Coloured oil droplets enhance colour discrimination. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270, 1255-1261.

Wakakuwa, M., Kurasawa, M., Giurfa, M. and Arikawa, K. (2005). Spectral heterogeneity of honeybee ommatidia. *Naturwissenschaften*, 92, 464-467.

Waser, N. M. (1979). Pollinator availability as a determinant of flowering time in ocotillo (*Fouquieria splendens*). *Oecologia*, 39, 107-121.

Waser, N. M. and Price, M. V. (1981). Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution*, 35, 376-390.

Waser, N. M. and Price, M. V. (1983). Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. In: Jones, C. E., Little, R. J. (eds.). *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, USA, pp 341-359.

Waser, N. M. and Price, M. V. (1985). The effect of nectar guides on pollinator preference: experimental studies with a montane herb. *Oecologia*, 67, 121-126.

Waser, N. M. (1986). Flower constancy: definition, cause, and measurement. *American Naturalist*, 127, 593-603.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. and Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043-1060.

Waser, N. M. (2006). Specialization and generalization in plant-pollinator interactions: a historical perspective. In: Waser, N. M. and Ollerton, J. (eds.). *Plant-pollinator* *interactions: From specialization to generalization*. University of Chicago Press, Chicago, USA, pp 3-17.

Weevers, T. H. (1952). Flower colours and their frequency. *Acta Botanica Neerlandica*, 1, 81-92.

Wehner, R. and Bernard, G. D. (1993). Photoreceptor twist: a solution to the false-color problem. *Proceedings of the National Academy of Sciences USA*, 90, 4132-4135.

Weiss, M. R. (1995). Floral color change: a widespread functional convergence. *American Journal of Botany*, 82, 167-185.

Weiss, M. R. and Lamont, B. B. (1997). Floral color change and insect pollination: a dynamic relationship. *Israel Journal of Plant Sciences*, 45, 185-199.

Westerkamp, C. (1991). Honeybees are poor pollinators—why?. *Plant Systematics and Evolution*, 177, 71-75.

Whitney, H. M., Chittka, L., Bruce, T. J. and Glover, B. J. (2009a). Conical epidermal cells allow bees to grip flowers and increase foraging efficiency. *Current Biology*, 19, 948-953.

Whitney, H. M., Federle, W. and Glover, B. J. (2009b). Grip and slip. *Communicative and Integrative Biology*, 2, 505-508.

Whitney, H. M., Kolle, M., Alvarez-Fernandez, R., Steiner, U. and Glover, B. J. (2009c). Contributions of iridescence to floral patterning. *Communicative and Integrative Biology*, 2, 230-232.

Whitney, H. M., Bennett, K. V., Dorling, M., Sandbach, L., Prince, D., Chittka, L. and Glover, B. J. (2011a). Why do so many petals have conical epidermal cells?. *Annals of Botany*, 108, 609-616.

Whitney, H. M., Poetes, R., Steiner, U., Chittka, L. and Glover, B. J. (2011b). Determining the contribution of epidermal cell shape to petal wettability using isogenic *Antirrhinum* lines. *PloS ONE*, 6, e17576.

Whitney, H. M., Glover, B. J., Walker, R. and Ellis, A. G. (2011c). The contribution of epidermal structure to flower colour in the South African flora. *Curtis's Botanical Magazine*, 28, 349-371.

Whitney, H. M., Rands, S. A., Elton, N. J. and Ellis, A. G. (2012). A technique for measuring petal gloss, with examples from the Namaqualand Flora. *PloS ONE*, 7, e29476.

Wilson, P., Castellanos, M. C., Hogue, J. N., Thomson, J. D. and Armbruster, W. S. (2004). A multivariate search for pollination syndromes among penstemons. *Oikos*, 104, 345-361.

Winkel, W. (1969). Experimentelle Untersuchungen an Zuckervögeln (Coerebidae) im Funktionskreis der Nahrungssuche: Über die Bedeutung von Farben, Formen und Zuckerkonzentrationen. *Zeitschrift für Tierpsychologie*, 26, 573-608.

Wittmann, D., Radtke, R., Cure, J. R. and Schifino-Wittmann, M. T. (1990). Coevolved reproductive strategies in the oligolectic bee *Callonychium petuniae* (Apoidea, Andrenidae) and three purple flowered *Petunia* species (Solanaceae) in southern Brazil. *Journal of Zoological Systematics and Evolutionary Research*, 28, 157-165.

Wolf, L. L., Hainsworth, F. R. and Stiles, F. G. (1972). Energetics of foraging: rate and efficiency of nectar extraction by hummingbirds. *Science*, 176, 1351-1352.

Wyler, H. and Dreiding, A. S. (1961). On betacyanins, the nitrogen containing pigment of centrospermae. Preliminary report. *Experientia*, 17, 23-25.

Wyszecki, G. and Stiles, W. S. (1982). *Color science Vol. 8*. Wiley, New York, USA.

Zufall, R. A. and Rausher, M. D. (2004). Genetic changes associated with floral adaptation restrict future evolutionary potential. *Nature*, 428, 847-850.

CHAPTER 11

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CHAPTER 12

APPENDIX

LIST OF PUBLICATIONS

Thesis is based on the following manuscripts:

Rohde, K., Papiorek, S. and Lunau, K. (2013). Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. *Journal of Comparative Physiology A*, 199, 197-210. DOI: 10.1007/s00359-012-0783-5

Lunau, K., Papiorek, S., Eltz, T. and Sazima, M. (2011). Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *The Journal of Experimental Biology*, 214, 1607-1612. DOI: 10.1242/jeb.052688

Papiorek, S., Junker, R. R., Alves-dos-Santos, I., Melo, G. A. R., Peixoto Amaral-Neto. L-, Sazima, M., Wolowski, M., Freitas, L. and Lunau, K. (*Manuscript under review in Plant Biology*). Bees, birds and yellow flowers: Pollinator-dependent convergent evolution of UV-patterns.

Papiorek, S., Rohde, K. and Lunau, K. (2013). Bees` subtle colour preferences: How bees respond to small changes in pigment concentration. *Naturwissenschaften*, 100, 633-643. DOI: 10.1007/s00114-013-1060-3

Papiorek, S., Junker, R. R. and Lunau, K. (2014). Gloss, colour and grip: Multifunctional epidermal cell shapes in bee- and bird-pollinated flowers. *PLoS ONE*, 9, e112013. DOI: 10.1371/journal.pone.0112013

THE AUTHORS' CONTRIBUTION

Chapter 2 - Rohde, K., Papiorek, S. and Lunau, K. (2013). Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. Journal of Comparative Physiology A, 199, 197-210

- Visual data analyses
- Statistical analyses
- Writing the manuscript

I hereby confirm that the above-given information are correct

K. Lunau

Prof. Dr. Klaus Lunau

Chapter 3 - Lunau, K., Papiorek, S., Eltz, T. and Sazima, M. (2011). Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *The Journal of Experimental Biology*, 214, 1607-1612

- Sampling of flowers in the field and in botanical gardens
- Spectrophotometric data collection of flower colours
- Visual data analysis
- Behavioural studies with hummingbirds and euglossine bees and data analyses
- Statistical analyses
- Writing the manuscript together with K. Lunau

I hereby confirm that the above-given information are correct

K. Lunau

Prof. Dr. Klaus Lunau

Chapter 4 - Papiorek, S., Junker, R. R., Alves-dos-Santos, I., Melo, G. A. R., Peixoto Amaral-Neto, L., Sazima, M., Wolowski, M., Freitas, L. and Lunau, K. (Manuscript under review in *Plant Biology*). Bees, birds and yellow flowers: Pollinator-dependent convergent evolution of UV-patterns.

- Study design
- Sampling of flowers in the field and in botanical gardens
- Spectrophotometric data collection and processing of flower colours
- Visual data analyses
- Behavioural studies with bees
- Statistical analyses together with R. Junker
- Writing the manuscript

I hereby confirm that the above-given information are correct

K. Lunau

Prof. Dr. Klaus Lunau

Chapter 5 - Papiorek, S., Rohde, K. and Lunau, K. (2013). Bees` subtle colour preferences: How bees respond to small changes in pigment concentration. *Naturwissenschaften*, 100, 633-643

- Visual data analyses
- Statistical analyses
- Writing the manuscript

I hereby confirm that the above-given information are correct

K. Lunau

Prof. Dr. Klaus Lunau

Chapter 6 - Papiorek, S., Junker, R. R. and Lunau, K. (2014). Gloss, colour and grip: Multifunctional epidermal cell shapes in bee- and bird-pollinated flowers. *PLoS ONE*, 9, e112013

- Study design together with K. Lunau
- Sampling of flowers in the field and in botanical gardens
- Spectrophotometric data collection
- Visual data analyses
- Microscopic data collection
- Analyses of microscopic data together with R. R. Junker
- Statistical analyses
- Writing the manuscript

I hereby confirm that the above-given information are correct

K. Lunau

Prof. Dr. Klaus Lunau

DECLARATION OF SELF-CONTAINED WORK

Herewith, I confirm that I composed the dissertation

“**Behavioural end ecological dynamics in competitive pollination networks**”,

single-handed without utilizing illegitimate resources. I used no other than the cited references and facilities. This work has not been previously handed in to another university and was not subject to miscellaneous examinations.

Düsseldorf, 15. Dezember 2014

S. Papiorek

Sarah Papiorek

SUPPLEMENTAL MATERIAL CHAPTER 2

This supplemental material is part of the publication:

Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours.

Rohde, K., Papiorek, S. and Lunau, K.

Journal of Comparative Physiology A (2013) 199, 197-210

Figure S1

Supplemental material available online at
http://link.springer.com/article/10.1007/s00359-012-0783-5

Bumblebees exhibiting dual choice between two artificial flowers offering a centripetal or centrifugal 3-step-gradient of the colours H1, H2, and H3 and making antennal contact at the colour of highest spectral purity (H3). The 4 photos show two individual bumblebees responding to both types of artificial flowers.

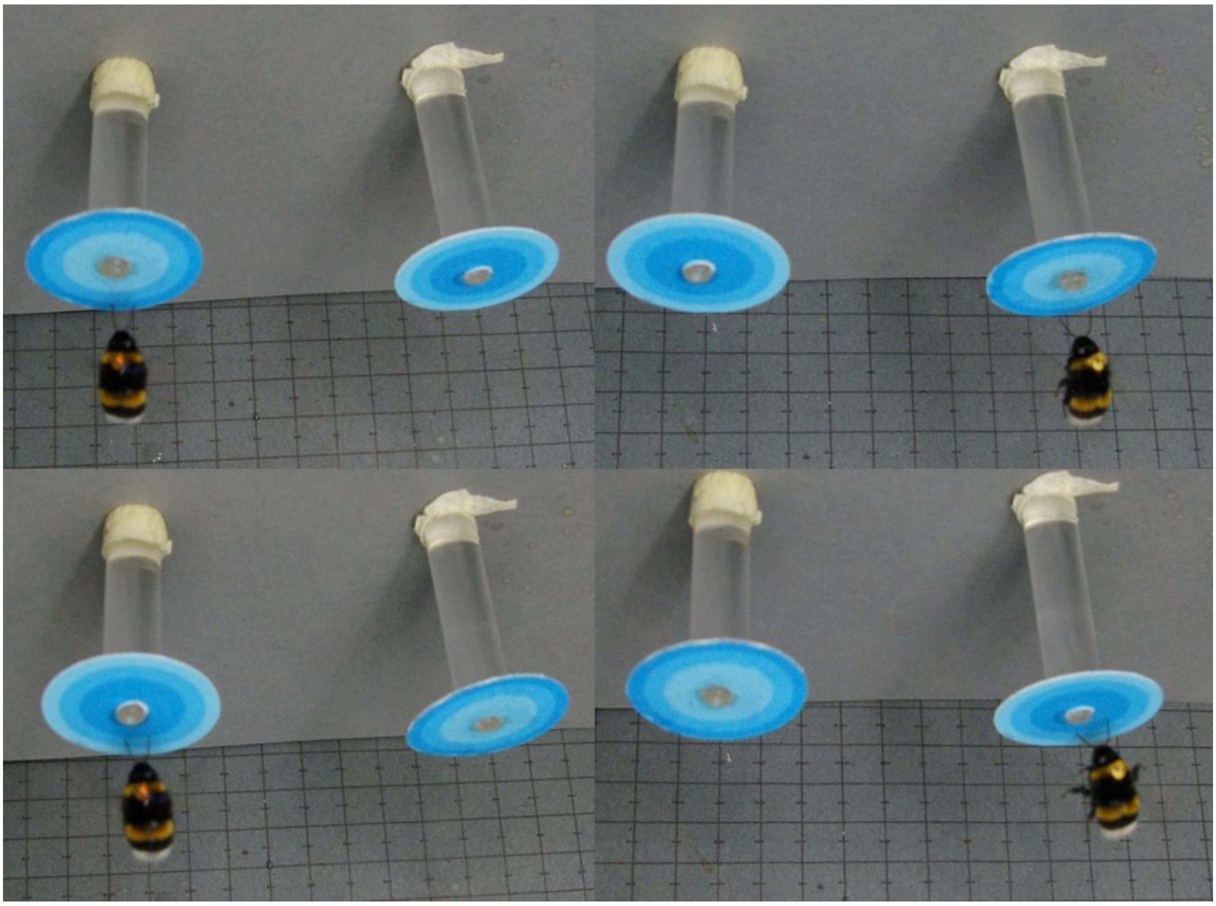
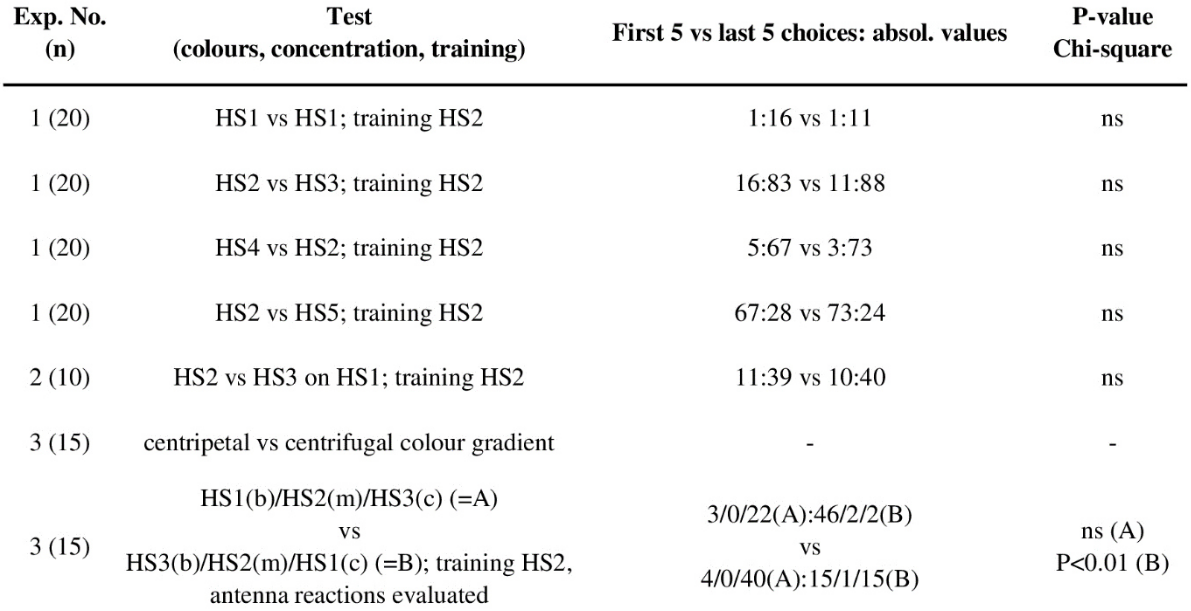


Table S1

Supplemental Material Table S1 has been modified for a better representation.
The original version is available online at
http://link.springer.com/article/10.1007/s00359-012-0783-5

Choice preferences of less and more experienced bumblebees. Statistical evaluation of the first 5 as compared to the last 5 out of 10 choices of naïve bumblebees (*Bombus terrestris*) using a Sign test or *Chi*-square test. Different letters associated with experiment number indicate separate tests. The concentric areas of artificial flower of Experiment 3 are indicated by b = border, m = middle, c = central; *Chi*-square test: ns= not significant.



Exp. No. (n)	**Test (colours, concentration, training)**	**First 5 vs last 5 choices: absol. values**	**P-value Chi-square**
1 (20)	HS1 vs HS1; training HS2	1:16 vs 1:11	ns
1 (20)	HS2 vs HS3; training HS2	16:83 vs 11:88	ns
1 (20)	HS4 vs HS2; training HS2	5:67 vs 3:73	ns
1 (20)	HS2 vs HS5; training HS2	67:28 vs 73:24	ns
2 (10)	HS2 vs HS3 on HS1; training HS2	11:39 vs 10:40	ns
3 (15)	centripetal vs centrifugal colour gradient	-	-
3 (15)	HS1(b)/HS2(m)/HS3(c) (=A) vs HS3(b)/HS2(m)/HS1(c) (=B); training HS2, antenna reactions evaluated	3/0/22(A):46/2/2(B) vs 4/0/40(A):15/1/15(B)	ns (A) P<0.01 (B)

SUPPLEMENTAL MATERIAL CHAPTER 3

This supplemental material is part of the publication:

Avoidance of achromatic colours by bees provides a private niche for hummingbirds.

Lunau, K., Papiorek, S., Eltz, T. and Sazima, M.

The Journal of Experimental Biology (2011) 214, 1607-1612

Table S1

Supplemental material available online at
http://jeb.biologists.org/cgi/content/full/214/9/1607/DC1

List of plant species in which the spectral reflection of the flowers was measured, and references about pollination.

Table S1. List of plant species in which the spectral reflection of the flowers was measured, and references about pollination

Genus	Species	Family	Flower colour	Pollination syndrome	Literature
Justicia	*brandegeana*	Acanthaceae	w	Tro	1
Justicia	*lutea*	Acanthaceae	w	Tro	1
Sanchezia	*nobilis*	Acanthaceae	r(b)	Tro	2,3
Baccharis	*trimera*	Asteraceae	w	Mel	4
Emilia	*fosbergii*	Asteraceae	r	Mel	4,5
Mutisia	*coccinea*	Asteraceae	r	Tro	6,7
Begonia	*coccinea*	Begoniaceae	r	Mel	8
Begonia	*cucullata alba*	Begoniaceae	w	Mel	9
Bilbergia	*amoena*	Bromeliaceae	r, w(b,s)	Tro	10
Canistropsis	*seidelii*	Bromeliaceae	w	Tro	6
Nidularium	*innicentii*	Bromeliaceae	r, w(b)	Tro	4,10,11
Vriesia	*rodigasiana*	Bromeliaceae	r(b)	Tro	6,12,13
Vriesia	*carinata*	Bromeliaceae	r	Tro	14
Centropogon	*spec.*	Campanulaceae	r	Tro	15
Lobelia	*cardinalis*	Campanulaceae	r(s)	Tro	2,16
Lobelia	*siphilitica*	Campanulaceae	w	Mel	17
Dichorisandra	*thyrsiflora*	Commelinaceae	w	Mel	4,8,18
Ipomoea syn. Quamoclit	*purpurea*	Convolvulaceae	w	Mel	4,19,20
Ipomoea syn. Quamoclit	*quamoclit*	Convolvulaceae	r, w	Tro	21,22,23
Costus	*pulverulentus*	Costaceae	r	Tro	24
Gaultheria	*procumbens*	Ericaceae	w	Mel	25
Macleania	*insiginis syn. ovata*	Ericaceae	r, w	Tro	15
Escallonia	*macrantha syn. rubra*	Escalloniaceae	w	Tro	26
Erythrina	*speciosa*	Fabaceae	r	Tro	3,6,11,13
Swartzia	*oblata*	Fabaceae	w	Mel	27
Fouquieria	*macdougalli*	Fouquieriaceae	r	Tro	28
Columnea	*gloriosa*	Gesneriaceae	r	Tro	29
Columnea	*magnifica*	Gesneriaceae	r	Tro	30,31
Mimulus	*cardinalis*	Gesneriaceae	r	Tro	32,33,34,35
Nematanthus	*crassifolius*	Gesneriaceae	r	Tro	36
Nematanthus	*fritschii*	Gesneriaceae	w	Tro	6
Nematanthus	*strigillosus*	Gesneriaceae	r(s)	Tro	36,37
Sinningia	*canescens*	Gesneriaceae	r	Tro	38
Sinningia	*cardinalis alba*	Gesneriaceae	w	Tro	38
Sinningia	*eumorpha*	Gesneriaceae	w	Mel	38,39
Sinningia	*macropoda*	Gesneriaceae	r	Tro	38,39
Heliconia	*angusta*	Heliconiaceae	r, w(b)	Tro	11
Heliconia	*latispatha*	Heliconiaceae	r(b)	Tro	40
Heliconia	*velloziana*	Heliconiaceae	r(b)	Tro	11,13,36
Salvia	*coccinea*	Lamiaceae	r, w	Tro	41
Salvia	*leucantha*	Lamiaceae	w	Tro	42
Salvia	*microphylla*	Lamiaceae	r	Tro	42
Erythronium	*oregonum*	Liliaceae	w	Mel	43
Desfontainia	*spinosa*	Loganiaceae	r	Tro	26,44
Hibiscus	*rosa- sinensis*	Malvaceae	r	Tro	3, 45
Malvaviscus	*arboreus*	Malvaceae	r	Tro	15,46
Napaea	*dioica*	Malvaceae	w	Mel	47
Clidemia	*hirta*	Melastomataceae	w	Mel	48
Fuchsia	*magellanica alba*	Onagraceae	w(p,s,st)	Tro	26,44,49
Maxillaria	*spec.*	Orchidaceae	r, w	Mel	50
Passiflora	*caerulea*	Passifloraceae	w	Mel	51
Passiflora	*edulis*	Passifloraceae	w(s)	Mel	4,5,52
Chelone	*obliqua alba*	Plantaginaceae	w	Mel	53
Penstemon	*digitalis*	Plantaginaceae	w	Mel	54,55
Geum	*rivale*	Rosaceae	r	Mel	56
Bouvardia	*ternifolia*	Rubiaceae	r, w	Tro	57
Ixora	*coccinea*	Rubiaceae	r	Mel	8
Manettia	*luteo-rubra*	Rubiaceae	r	Tro	58
Psychotria	*nuda*	Rubiaceae	r(s)	Tro	11,13,59
Digitalis	*alba*	Scrophulariaceae	w	Mel	53
Acnistus	*arborescens*	Solanaceae	w	Mel	60

r,red; w, white; tro, trochilophilous; mel, melittophilous. Unless otherwise stated, flower petals were measured (b, bract; p, petal; s, sepal; st, stamina).

1. **Schmidt-Lebuhn, A. N., Schwerdtfegera, M., Kesslera, M. and Lohaus, G.** (2007). Phylogenetic constraints vs. ecology in the nectar composition of Acanthaceae. *Flora* **202**, 62-69.

2. **Burr, B., Rosen, D. and Barthlott, W.** (1995). Untersuchungen zur Ultraviolettreflexion von Angiospermen blüten III. Dilleniidae und Asteridae. *Trop. Subtrop. Pflanzenwelt* **93,** 1-185.

3. **Mendonça, L. B. and dos Anjos, L.** (2005). Beija-flores (Aves, Trochilidae) e seus recursos florais em uma área urbana do sul do Brasil. *Rev. Bras. Zool.* **22**, 51-59.

4. **Steiner, J., Zillikens, A., Kamke, R., Pickbrenner Feja, B. and de Barcellos Falkenberg, D.** (2010) Bees and melittophilous plants of secondary Atlantic forest habitats at Santa Catarina Island, Southern Brasil. *Oecol. Aus.* **14**, 16-39.

5. **Locatelli, E., Machado, I. C. and Medeiros, P.** (2004). Riqueza de abelhas e a flora apícola em um fragmento da Mata Serrana (brejo de altitude) em Pernambuco, Nordeste do Brasil. In *Brejos de altitude em Pernambuco e Paraíba - História Natural, Ecologia e Conservação* (ed. K. C. Pôrto, J. J. P. Cabral and M. Tabarelli), pp. 153-177. Brazil: Ministério do Meio Ambiente.

6. **Buzato, S., Sazima, M. and Sazima, I.** (2000). Hummingbird-pollinated floras at three Atlantic forest sites. *Biotropica* **32**, 824-841.

7. **Sazima, M. and Machado, I.** (1983). Biologia floral de *Mutisia coccinia* St. Hil. (Asteraceae). *Rev. Bras. Bot.* **6**, 103-108.

8. **Pemberton, R. W. and Wheeler, G. S.** (2006). Orchid bees don't need orchids: Evidence from the naturalization of an orchid bee in Florida. *Ecology* **87**, 1995-2001.

9. **Pinheiro, M., Espindola de Abrao, B., Harter-Marques, B. and Miotto, S. T. S.** (2008). Floral resources used by insects in a grassland community in Southern Brazil. *Rev. Bras. Bot.* **31**, 469-489.

10. **Wendt, T., Coser, T. S., Matallana, G. and Guilherme, F. A. G.** (2008). An apparent lack of prezygotic reproductive isolation among 42 sympatric species of Bromeliaceae in Southeastern Brazil. *Plant Syst. Evol.* **275**, 31-41.

11 **Sazima, I., Buzato, S. and Sazima, M.** (1995) The saw-billed hermit *Ramphodon naevius* and its flowers in Southeastern Brazil. *J. Ornithol.* **136**, 195-206.

12 **Siqueira Filho, J. A. and Tabarelli, M.** (2006). Bromeliad species of the Atlantic forest of North-East Brazil: losses of critical populations of endemic species. *Oryx* **40**, 218-224.

13 **de Queiroz Piacentini, V. and Galarda Varassin, I.** (2007). Interaction network and the relationship between bromeliads and hummingbirds in an area of secondary Atlantic rain forest in Southern Brazil. *J. Trop. Ecol.* **23**, 663-671.

14 **Araujo, A.C., Fischer, E.A. and Sazima, M.** (1994). Floração sequencial e polinização de três espécies de *Vriesea* (Bromeliaceae) na região da Juréia, Sudeste do Brasil. *Rev. Bras. Bot.* **17**, 113-118.

15 **Borgella Jr., R., Snow, A. A. and Gavin, T. A.** (2001). Species richness and pollen loads of hummingbirds using forest fragments in Southern Costa Rica. *Biotropica* **33**, 90-109.

16 **Grant, V.** (1994). Modes and origins of mechanical and ethological isolation in angiosperms. *Proc. Natl. Acad. Sci. USA* **91**, 3-10.

17 **Johnston, M. O.** (1991). Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. *Ecology* **72**, 1500-1503.

18 **Biedinger, N. and Barthlott, W.** (1993). Untersuchungen zur Ultraviolettreflexion von Angiospermen-blüten I. Monocotyledonae. *Trop. subtrop. Pflanzenwelt* **86**, 1-122.

19 **Sargent, R. D. and Otto, S. P.** (2004). A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms. *Evol. Ecol. Res.* **6**, 1183-1199.

20 **Stucky, J. M.** (1984). Forager attraction by sympatric *Ipomoea hederacea* and *I. purpurea* (Convolvulaceae) and corresponding forager behavior and energetics. *Am. J. Bot.* **71**, 1237-1244.

21 **des Marais, D. L. and Rausher, M. D.** (2010). Parallel evolution at multiple levels in the origin of hummingbird pollinated flowers in *Ipomoea*. *Evolution* **64**, 2044-2054.

22 **Machado, I.C.S. and Sazima, M.** (1987). Estudo comparativo da biologia floral em duas espécies invasoras: *Ipomoea hederifolia* e *I. quamoclit* (Convolvulaceae). *Rev. Bras. Biol.* **47**, 425-436.

23 **Wolfe, L. M. and Sowell, D. R.** (2006). Do pollination syndromes partition the pollinator community? A test using four sympatric Morning Glory species. *Int. J. Plant Sci.* **167**, 1169-1175.

24 **Kay, K. M. and Schemske, D. W.** (2008). Natural selection reinforces speciation in a radiation of Neotropical rainforest plants. *Evolution* **62**, 2628–2642.

25 **Mirick, S. and Quinn, J. A.** (1981). Some observations on the reproductive biology of *Gaultheria procumbens* (Ericaceae). *Am. J. Bot.* **68**, 1298-1305.

26 **Huapi, N. and Fraga, A. R. M.** (1997). Interacciones entre el picaflor rubi *Shephanoides sephaniodes* y plantas del bosque subantártico en el Parque Nacional. *Hornero* **14**, 224-234.

27 **Pinheiro, M. & Sazima, M.** (2007). Visitantes florais e polinizadores de seis espécies arbóreas de Leguminosae melitófilas na Mata Atlântica no Sudeste do Brasil. *Rev. Bras. Biociências* **5**, 447-499.

28 **Schultheis, L. M. and Baldwin, B. G.** (1999). Molecular phylogenetics of Fouquieriaceae: evidence from nuclear rDNA ITS studies. *Am. J. Bot.* **86**, 578-589.

29 **Morley, B. D.** (1971). A hybrid swarm between two hummingbird-pollinated species of *Columnea* (Gesneriaceae) in Jamaica. *Bot. J. Linn. Soc.* **64**, 81-96.

30 **Murray, K. G., Feinsinger, P., Busby, W. H., Linhart, Y. B., Beach, J. H., Kinsman, S.** (1987). Evaluation of character displacement among plants in two Tropical pollination guilds. *Ecology* **68**, 1283-1296.

31 **Temeles, E. J., Linhart, Y. B., Masonjones, M. and Masonjones, H. D.** (2002). The role of flower width in hummingbird bill length–flower length relationship. *Biotropica* **34**, 68-80.

32 **Schemske, D. W. and Bradshaw Jr., H. D.** (1999). Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc. Natl. Acad. Sci. USA* **96**, 1910-1915.

33 **Ramsey, J., Bradshaw Jr., H. D. and Schemske, D. W.** (2003). Components of reproductive isolation between the Monkey flowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57**, 1520-1534.

34 **Bradshaw, H. D., Wilbert, S. M., Otto, K. G. and Schemske, D. W.** (1995). Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature* **376**, 762-765.

35 **Beardsley, P. M., Yen, A. and Olmstead, R. G.** (2003). AFLP Phylogeny of *Mimulus* section Erythranthe and the evolution of hummingbird. *Evolution* **57**, 1397-1410.

36 **Snow, D. W. and Teixeira, D. L.** (1982). Hummingbirds and their flowers in the coastal mountains of Southeastern Brazil. *J. Ornithol.* **123**, 446-450.

37 **Ferreira de Vasconcelos, M., Lombardi, J. A.** (1999). Padrão sazonal na ocorrencia de seis espécies de beija-flores (Apodiformes: Trochilidae) em uma localidade de campo rupestre na Serra do Curral, Minas Gerais. *Ararajuba* 7, 71-79.

38 **Perret, M., Chautems, A., Spichiger, R., Peixoto, M. and Savolainen, V.** (2001). Nectar sugar composition in relation to pollination syndromes in Sinningiae (Gesneriaceae). *Ann. Bot.* **87**, 267-273.

39 **Perret, M., Chautems, A., Spichiger, R., Kite, G. and Savolainen, V.** (2003). Systematics and evolution of tribe Sinningiae (Gesneriaceae): Evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS1. *Am. J. Bot.* **90**, 445-460.

40 **Stiles, F. G.** (1975). Ecology, flowering phenology and hummingbird pollination of some Costa Rican *Heliconia* species. *Ecology* **56**, 285-301.

41 **Grases, C. and Ramírez, N.** (1998). Biología reproductiva de cinco especies ornitófilas en un fragmento de bosque caducifolio secundario en Venezuela. *Rev. Biol. Trop.* **46**, 1095-1108.

42 **Wester, P. and Claßen-Bockhoff, R.** (2007). Floral diversity and pollen transfer mechanisms in bird-pollinated *Salvia* species. *Ann. Bot.* **100**, 401-421.

43 **Parachnowitsch, A. L. and Elle, E.** (2005). Insect visitation to wildflowers in the endangered Garry oak, *Quercus garryana*, ecosystem of British Columbia. *Can. Field Nat.* **119**, 245-253.

44 **Aizen, M. A. and Vázquez, D. P.** (2006). Flowering phenologies of hummingbird plants from the temperate forest of Southern South America: is there evidence of competitive displacement? *Ecography* **29**, 357-366.

45 **Gill, F. B., Stokes, F. J. and Stokes, C.** (1973). Contact zones and hybridization in the Jamaican hummingbird, *Trochilus polytmus* (L.). *Condor* **75**, 170-178.

46 **George, M. W.** (1980). Hummingbird foraging behavior at *Malvaviscus arboreus* var. *drummondii*. *Auk* **97**, 790-794.

47 **Iltis, H. H.** (1963). *Napaea dioica* (Malvaceae): Whence came the type? *Am. Midl. Nat.* **70**, 90-109.

48 **Ferreira, S. A. N., Cohen, I. A. and Jansen, M. R. A.** (1994). Reproductive biology of *Clidemia hirta* (L.) D. Don (Melastomataceae). *Acta Amazonica* **24**, 183-188.

49 **Traveset, A., Willson, M. F. and Sabag, C.** (1998). Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra Del Fuego. A disrupted mutualism. *Funct. Ecol.* **12**, 459-464.

50 **Roubik, D. W.** (1999). Deceptive orchids with Meliponini as pollinators. *Plant Syst. Evol.* **222**, 271-279.

51 **Galetto, L. and Bernardello, G.** (2003). Nectar sugar compositions in angiosperms from Chaco and Patagonia (Argentina): an animal visitor's matter? *Plant Syst. Evol.* **238**, 69–86.

52 **Yamamoto, M., Barbosa, A. A. A.,and de Oliveira, P. E. A. M.** (2010). A polinização em cultivos agrícolas e a conservação das áreas naturais: o caso do Maracuja-amarelo (*Passiflora edulis*). *Oecologia Aus.* **14**, 174-192.

53 **Ghebrehiwet, M., Bremer, B. and Thulin, M.** (2000). Phylogeny of the tribe Antirrhineae (Scrophulariaceae) based on morphological and ndhF sequence data. *Plant Syst. Evol.* **220**, 223-242.

54 **Wilson, P., Castellanos, M. C., Hogue, J. N., Thomson, J. D. and Armbruster, W. S.** (2004). A multivariate search for pollination syndromes among penstemons. *Oikos* **104**, 345-361.

55 **Dieringer, G. and Cabrera, L. R.** (2002). The interaction between pollinator size and the bristle staminode of *Penstemon digitalis* (Scrophulariaceae). *Am. J. Bot.* **89**, 991-997.

56 **Jennersten, O., Berg, L. and Lehman, C.** (1988). Pollen deposition and seed set in the sticky catchfly, *Viscaria vulgaris*. *J. Ecol.* **76**, 1111-1132.

57 **Faivre, A. E. and McDade, L. A.** (2001). Population-level variation in the expression of heterostyly in three species of Rubiaceae: does reciprocal placement of anthers and stigmas characterize heterostyly? *Am. J. Bot.* **88**, 841-853.

58 **Passos, L. and Sazima, M.** (1995). Reproductive biology of the distylous *Manettia luteo-rubra* (Rubiaceae). *Bot. Acta* **108**, 309-313.

59 **Cardoso de Castro, C. and Cardoso Araujo, C.** (2004). Distyly and sequential pollinators of *Psychotria nuda* (Rubiaceae) in the Atlantic Rain Forest, Brazil. *Plant Syst. Evol.* **244**, 131-139.

60 **DeWitt Smith, S., Hall, S. J., Izquierdo, P. R. and Baum, D. A.** (2008). Comparative pollination biology of sympatric and allopatric Andean *Iochroma* (Solanaceae). *Ann. Mo. Bot. Gard.* **95**, 600-617.

61 **D'Arcy, W. G.** (1999). Red-flowered *Cestrum* and red-loving hummingbirds. In *Solanaceae IV: advances in biology and utilization* (eds. M. Nee, D. E. Symon, R. N. Lester and J. P. Jessop), pp. 33-43. Kew, UK: Royal Botanic Gardens.

62 **Pérez, F., Arroyo, M. T. K., Medel, R. and Hershkovitz, M. A.** (2006). Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). *Am. J. Bot.* **93**, 1029-1038.

Table S2

Supplemental material available online at
http://jeb.biologists.org/cgi/content/full/214/9/1607/DC1

List of plant species in which the spectral reflection of the flowers was measured and references which established a mixed pollination syndrome out of bees and birds as pollinators.

Table S2. List of plant species in which the spectral reflection of the flowers was measured and references which established a mixed pollination syndrome out of bees and birds as pollinators

Genus	Species	Family	Flower colour*	Pollination syndrome	Literature
Impatiens	*noli-tangere*	Balsaminaceae	r	tro, mel	1,2
Schlumbergera	*truncata*	Cactaceae	w	tro, mel	2,3
Costus	*malortieanus*	Costaceae	w	tro, mel	4
Euphorbia	*pulcherrima*	Euphorbiaceae	r	tro, mel	2,5
Bauhinia	*variegata*	Fabaceae	w	tro, mel	6,7
Cajanus	*cajan*	Fabaceae	r, w	tro, mel	2,8,9
Erythrina	*crista-galli*	Fabaceae	r	tro, mel	10,11
Salvia	*splendens*	Lamiaceae	r, w	tro, mel	3,12
Hamelia	*patens*	Rubiaceae	r	tro, mel	13,14,15

r, red; w, white; tro, trochilophilous; mel, melittophilous. Unless otherwise stated, flower petals were measured (b=bract, p=petal, s=sepal).

1 **Pojar, J.** (1975). Hummingbird flowers of British Columbia. *Syesis* **8**, 25-28.

2 **Burr, B. and Barthlott, W.** (1993). Untersuchungen zur Ultraviolettreflexion von Angiospermenblüten II. Magnoliidae, Ranunculidae, Hamamelididae, Caryophyllidae, Rosidae. *Trop. Subtrop. Pflanzenwelt* **87**, 1-193.

3 **Steiner, J., Zillikens, A., Kamke, R., Pickbrenner Feja, B. and de Barcellos Falkenberg, D.** (2010). Bees and melittophilous plants of secondary Atlantic forest habitats at Santa Catarina Island, Southern Brasil. *Oecologia Aus.* **14**, 16-39.

4 **Kay, K. M. and Schemske, D. W.** (2003). Pollinator assemblages and visitation rates for 11 species of neotropical *Costus* (Costaceae). *Biotropica* **35**, 198-207.

5 **Mendonça, L. B. and dos Anjos, L.** (2005). Beija-flores (Aves, Trochilidae) e seus recursos florais em uma área urbana do sul do Brasil. *Rev. Bras. Zool.* **22**, 51-59.

6 Santos, G. M. T., Machado, V. L. L. and Giannotti, E. (1993). Flowering entomofauna in *Bauhinia variegate* L. (Leguminosae, Caesalpiniaceae). *Rev. Bras. Entomol.* **37**, 737-750.

7 Corlett, R. T. (2005) Interactions between birds, fruit bats and exotic plants in urban Hong Kong, South China. *Urban Ecosystems*, **8**, 275-283.

8 **Gill, F. B., Stokes, F. J. and Stokes, C.** (1973). Contact zones and hybridization in the Jamaican hummingbird, *Trochilus polytmus* (L.). *Condor* **75**, 170-178.

9 **Wetmore, A.** (1916). The Birds of Vieques Island, Porto Rico. *Auk* **33**, 403-419.

10 **Galetto, L., Bernardello, G., Isele, I. C., Vesprini, J., Speroni, G. and Berduc, A.** (2000). Reproductive biology of *Erythrina crista-galli* (Fabaceae). *Ann. Mo. Bot. Gard.* **87**, 127-145.

11 **Galetto, L. and Bernardello, G.** (2003). Nectar sugar compositions in angiosperms from Chaco and Patagonia (Argentina): An animal visitor's matter? *Plant Syst Evol* **238**, 69-86.

12 **Wester, P. and Claßen-Bockhoff, R.** (2007). Floral diversity and pollen transfer mechanisms in bird-pollinated *Salvia* species. *Ann. Bot.* **100**, 401-421.

13 **Feinsinger, P., Wolfe, J. A. and Swarm, L. A.** (1982). Island ecology: reduced hummingbird diversity and the pollination biology of plants, Trinidad and Tobago, West Indies. *Ecology* **63**, 494-506.

14 **Kodric-Brown, A., Brown, J. H., Byers, G. S. and Gori, D. F.** (1984). Organization of a tropical island community of hummingbirds and flowers. *Ecology* **65**, 1358-1368.

15 **Pemberton, R. W. and Wheeler, G. S.** (2006). Orchid bees don´t need orchids: Evidence from the naturalization of an orchid bee in Florida. *Ecology* **87**, 1995-2001.

SUPPLEMENTAL MATERIAL CHAPTER 4

This supplemental material is part of the manuscript:

Bees, birds and yellow flowers: Pollinator-dependent convergent evolution of UV-patterns.

Papiorek, S., Junker, R. R., Alves-dos-Santos, I., Melo, G. A. R., Peixoto Amaral-Neto, L., Sazima, M., Wolowski, M., Freitas, L. and Lunau, K.

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Supplemental Material 1

List of analysed plant species with reference of their habitat (OW= Old World, NW=New World), literature with pollinator reference and measured flower parts for centre and periphery (o=orifice, c=corolla, pc=petal centre, pp=petal periphery, df=disc floret, rf=ray floret, it=inner tepal, ot=outer tepal, flc=flag centre, flp=flag periphery). The mean spectral reflectance in the UV, blue, green and red wavelength region are given for each flower part.

Genus	Species epiphet	Author abbreviation	Family	Pollinator	Habitat	Literature	Flower par	Flower orgar	Mean spectral reflectance			
									UV	Blue	Green	Red
Aphelandra	*squarrosa*	Nees	Acanthaceae	bird	NW	1	'center'	o	0.035	0.070	0.353	0.424
							'periphery	c	0.051	0.084	0.461	0.541
Barleria	*oenotheroides* syn. *micans*	Dum.Cours.	Acanthaceae	bird	OW	2	'center'	o	0.022	0.029	0.096	0.154
							'periphery	c	0.133	0.074	0.327	0.412
Justicia	*aurea*	Schltdl.	Acanthaceae	bird	NW	3-6	'center'	o	0.104	0.122	0.550	0.814
							'periphery	c	0.033	0.153	0.420	0.461
Schaueria	*calicotricha*	(Link & Otto) Nees	Acanthaceae	bird	NW	2, 7	'center'	o	0.036	0.102	0.264	0.286
							'periphery	c	0.035	0.109	0.278	0.291
Allamanda	*cathartica*	L.	Apocynaceae	bee	NW	8-10	'center'	p_c	0.037	0.057	0.338	0.473
							'periphery	p_p	0.100	0.074	0.499	0.629
Aloe	*swynnertonii*	Rendle	Asphodelaceae	bird	OW	11	'center'	o	0.028	0.088	0.243	0.243
							'periphery	c	0.050	0.093	0.221	0.213
Bidens	*ferulifolia*	(Jacq.) Sweet	Asteraceae	bee	NW	12, 13	'center'	df	0.024	0.012	0.103	0.144
							'periphery	rf	0.270	0.062	0.398	0.477
Heterotheca	*villosa*	(Pursh) Shinners	Asteraceae	bee	NW	14-17	'center'	df	0.038	0.025	0.230	0.302
							'periphery	rf	0.030	0.013	0.245	0.283
Coreopsis	*grandiflora*	Hogg ex Sweet	Asteraceae	bee	NW	18	'center'	df	0.025	0.022	0.178	0.265
							'periphery	rf	0.059	0.023	0.508	0.739
Solidago	*canadensis*	L.	Asteraceae	bee	NW	19	'center'	df	0.040	0.025	0.180	0.231
							'periphery	rf	0.092	0.032	0.194	0.210
Impatiens	*cristata*	Wall.	Balsaminaceae	bee	OW	20, 21	'center'	p_c	0.025	0.061	0.174	0.236
							'periphery	p_p	0.111	0.115	0.279	0.324
Berberis	*darwinii*	Hook.	Berberidaceae	bee	NW	22, 23	'center'	it	0.056	0.040	0.173	0.266
							'periphery	ot	0.065	0.049	0.190	0.275
Epimedium	*versicolor*	E.Morren	Berberidaceae	bee	OW	24	'center'	it	0.108	0.128	0.309	0.321
							'periphery	ot	0.244	0.198	0.408	0.413
Tecoma	*fulva* subsp. *garrocha*	(Hieron.) J.R.I.Wood	Bignoniaceae	bird	NW	25	'center'	o	0.032	0.088	0.290	0.384
							'periphery	c	0.032	0.092	0.284	0.358
Tecoma	*stans*	(L.) Juss. ex Kunth	Bignoniaceae	bee	NW	26-28	'center'	p_c	0.031	0.056	0.550	0.773
							'periphery	p_p	0.302	0.091	0.670	0.848
Cochlospermum	*vitifolium*	(Willd.) Spreng.	Bixaceae	bee	NW	29, 30	'center'	a	0.027	0.014	0.133	0.201
							'periphery	p_p	0.131	0.034	0.373	0.488
Dyckia	*velascana*	Mez	Bromeliaceae	bird	NW	31, 32	'center'	o	0.030	0.039	0.421	0.482
							'periphery	c	0.017	0.036	0.405	0.443
Arachis	*hypogaea*	L.	Fabaceae	bee	NW	33	'center'	fl_c	0.020	0.120	0.591	0.736
							'periphery	fl_p	0.220	0.034	0.420	0.528
Cajanus	*cajan*	(L.) Millsp.	Fabaceae	bee	OW	35-37	'center'	fl_c	0.032	0.040	0.090	0.108
							'periphery	fl_p	0.344	0.078	0.441	0.600
Senna	*alata*	(L.) Roxb.	Fabaceae	bee	NW	27, 38-40	'center'	p_c	0.240	0.076	0.370	0.458
							'periphery	p_p	0.220	0.061	0.334	0.432
Senna	*alexandrina*	Mill.	Fabaceae	bee	OW	39, 40, 41	'center'	p_c	0.036	0.034	0.077	0.150
							'periphery	p_p	0.308	0.076	0.349	0.503
Senna	*artemisioides*	Isely	Fabaceae	bee	OW	39	'center'	p_c	0.177	0.059	0.358	0.449
							'periphery	p_p	0.108	0.030	0.240	0.313
Senna	*bicapsularis*	(L.) Roxb.	Fabaceae	bee	NW	39, 40, 41, 42	'center'	p_c	0.269	0.047	0.445	0.638
							'periphery	p_p	0.242	0.069	0.406	0.563
Senna	*fistula*	L.	Fabaceae	bee	OW	39, 40, 41	'center'	p_c	0.044	0.049	0.253	0.266
							'periphery	p_p	0.297	0.100	0.480	0.639
Cytisus	*scoparius*	(L.) Link	Fabaceae	bee	OW	43, 44	'center'	fl_c	0.056	0.021	0.153	0.244
							'periphery	fl_p	0.139	0.060	0.361	0.425
Lotus	*alpinus*	(DC.) Ramond	Fabaceae	bee	OW	45, 46	'center'	fl_c	0.053	0.012	0.570	0.703
							'periphery	fl_p	0.038	0.010	0.264	0.456
Mucuna	*japira*	A.M.G.Azevedo, K.Agostini & Sazima	Fabaceae	bird	NW	47, 48	'center'	fl_c	0.024	0.022	0.090	0.101
							'periphery	fl_p	0.017	0.020	0.093	0.103
Sophora	*macrocarpa*	Sm.	Fabaceae	bird	NW	49	'center'	fl_c	0.069	0.045	0.302	0.299
							'periphery	fl_p	0.057	0.028	0.346	0.373
Nematanthus	*fluminensis*	(Vell.) Fritsch	Gesneriaceae	bird	NW	50-52	'center'	o	0.037	0.066	0.229	0.257
							'periphery	c	0.065	0.098	0.276	0.288
Wachendorfia	*thyrsiflora*	Burm.	Haemodoraceae	bee	OW	53	'center'	p_c	0.039	0.079	0.400	0.446
							'periphery	p_p	0.250	0.159	0.324	0.373
Lachenalia	*aloides* var. *aurea*	(Lindl.) Engl.	Hyazinthaceae	bird	OW	54-55	'center'	o	0.040	0.033	0.106	0.100
							'periphery	c	0.032	0.035	0.091	0.079
Phlomis	*fruticosa*	L.	Lamiaceae	bee	OW	56-57	'center'	p_c	0.026	0.104	0.462	0.472
							'periphery	p_p	0.172	0.130	0.497	0.497
Heteropteris	sp.		Malphigiaceae	bee	NW	58	'center'	a	0.034	0.062	0.159	0.202
							'periphery	p_p	0.304	0.088	0.610	0.694
Ensete	*lasiocarpum*	(Franch.) Cheesman	Musaceae	bee	OW	59	'center'	o	0.024	0.033	0.086	0.122
							'periphery	c	0.049	0.063	0.182	0.220
Lysimachia	*ciliata*	L.	Myrsinaceae	bee	NW	60	'center'	p_c	0.035	0.082	0.384	0.413
							'periphery	p_p	0.176	0.045	0.310	0.341
Digitalis	*grandiflora*	Mill.	Plantaginaceae	bee	OW	61	'center'	o	0.027	0.081	0.210	0.266
							'periphery	c	0.036	0.181	0.343	0.368
Asphodeline	*liburnica*	(Scop.) Rchb.	Xanthorrhoeacea	bee	OW	62	'center'	p_c	0.013	0.013	0.054	0.062
							'periphery	p_p	0.018	0.013	0.086	0.088
Asphodeline	*lutea*	(L.) Rchb.	Xanthorrhoeacea	bee	OW	63	'center'	p_c	0.199	0.043	0.384	0.446
							'periphery	p_p	0.189	0.044	0.366	0.427

1: McDade LA. 1985. Breeding systems of Central American *Aphelandra* (Acanthaceae). American Journal of Botany 72: 1515-1521.
2: Schmidt-Lebuhn AN, Schwerdtfeger M, Kessler M, Lohaus G. 2007. Phylogenetic constraints vs. ecology in the nectar composition of Acanthaceae. Flora 202: 62–69.
3: Feinsinger P, Beach JH, Linhart YB, Busby WH, Murray KG. 1987. Disturbance, pollinator predictability, and pollination success among Costa Rican Cloud Forest plants. Ecology 68: 1294-1305.
4: Murray KG, Feinsinger P, Busby WH, Linhart YB, Beach JH, Kinsman S. 1987. Evaluation of character displacement among plants in two tropical pollination guilds. Ecology 68: 1283-1293.
5: Willmer PG, Corbet SA. 1981. Temporal and microclimatic partitioning of the floral resources of *Justicia aur*ea amongst a concourse of pollen vectors and nectar robbers. Oecologia 51: 67-78.
6: Temeles EJ, Linhart YB, Masonjones M, Masonjones HD. 2002. The role of flower width in hummingbird bill length-flower length relationships. Biotropica 34: 68-80.
7: Daniel TF, McDade LA, Manktelow M, Kiel CA. 2008. The "Tetramerium Lineage" (Acanthaceae: Acanthoideae: Justicieae): Delimitation and intra-lineage relationships based on cp and nrITS sequence data. Systematic Botany 33: 416-436.
8: Skov C, Wiley J. 2005. Establishment of the neotropical orchid bee *Euglossa viridissima* (Hymenoptera: Apidae) in Florida. Florida Entomologist 88: 225-227.
9: Evoy WH, Jones BP. 1971. Motor patterns of male euglossine bees evoked by floral fragrances. Animal Behaviour 19: 583-588.
10: Toledo VDAAD, Fritzen AEDT, Neves CA, Ruvolo-Takasusuki MCC, Sofia SH, Terada Y. 2003. Plants and pollinating bees in Maringá, State of Paraná, Brazil.
Brazilian Archives of Biology and Technology 46: 705-710.
11: Brandt HD. 1960. Birds of Potchefstroom and district. Ostrich 31: 6-19.
12: Pawelek JC, Frankie GW, Thorp RW, Przybylski M. 2009. Modification of a community garden to attract native bee pollinators in urban San Luis Obispo. California. Cities and the Environment 2: 1-20.

13: Frankie G, Thorp R, Hernandez J, Rizzardi M, Ertter B, Pawelek, JC, Witt SL, Schindler M, Coville R, Wojcik VA. 2009. Native bees are a rich natural resource in urban California gardens. California Agriculture 63: 113-120.

14: Thomson JD. 1981. Field measures of flower constancy in bumblebees. American Midland Naturalist 105: 377-380.

15: Thomson JD. 1982. Patterns of visitation by animal pollinators. Oikos 39: 241-250.

16: Hobbs GA, Lilly CE. 1954. Ecology of species of *Megachil*e Latreille in the mixed prairie region of southern Alberta with special reference to pollination of alfalfa. Ecology 35: 453-462.

17: Inouye DW. 1980. The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. Oecologia 45: 197-201.

18: Wojcik VA, Frankie GW, Thorp RW, Hernandez JL. 2008. Seasonality in bees and their floral resource plants at a constructed urban bee habitat in Berkeley, California. Journal of the Kansas Entomological Society 81: 15-28.

19: Gross RS, Werner PA. 1983. Relationships among flowering phenology, insect visitors, and seed-set of individuals: experimental studies on four co-occurring species of goldenrod (Solidago: Compositae). Ecological Monographs 53: 95-117.

20: Kumar A, Lall A. 1998. Bumble bee species and flowering plant relationships at high altitude in north west India. Indian Journal of Ecology 25: 1-7.

21: Saini MS, Raina RH, Khan ZH. 2012. Taxonomy and pollination ecology of *Bombus rufofasciatus* (Hymenoptera: Apidae) from the Indian Himalaya. Polish Journal of Entomology 81: 347-363.

22: Chalcoff VR, Aizen MA, Galetto L. 2006. Nectar concentration and composition of 26 species from the temperate forest of South America. Annals of Botany 97: 413-421.

23: Smith-Ramírez C, Martinez P, Nunez M, González C, Armesto JJ. 2005. Diversity, flower visitation frequency and generalism of pollinators in temperate rain forests of Chiloé Island, Chile. Botanical Journal of the Linnean Society 147: 399-416.

24: Suzuki K. 1984. Pollination system and its significance on isolation and hybridization in Japanese E*pimedium* (Berberidaceae). The Botanical Magazine 97: 381-396.

25: Galetto L. 1995. Nectary structure and nectar characteristics in some Bignoniaceae. Plant-Systematics and Evolution 196: 99-121.

26: Silva CI, Augusto SC, Sofia SH, Moscheta IS. 2007. Diversidade de abelhas em *Tecoma stans* (L.) Kunth (Bignoniaceae): importância na polinização e produção de frutos. Neotropical Entomology 36: 33

27: Pemberton RW, Wheeler GS. 2006. Orchid bees don't need orchids: evidence from the naturalization of an orchid bee in Florida. Ecology 87: 1995-2001.

28: Raju AS, Rao SP. 2006. Nesting habits, floral resources and foraging ecology of large carpenter bees (*Xylocopa latipes* and *Xylocopa pubescens*) in India. Current Science-Bangalore 90: 1210.

29: Snow AA, Roubik DW. 1987. Pollen deposition and removal by bees visiting two tree species in Panama. Biotropica 19: 57-63.

30: Roubik DW, Ackerman JD, Copenhaver C, Smith BH. 1982. Stratum, tree, and flower selection by tropical bees: implications for the reproductive biology of outcrossing *Cochlospermum vitifolium* in Panama. Ecology 63: 712-720.

31: Galetto L, Bernardello G. 2003. Nectar sugar composition in angiosperms from Chaco and Patagonia (Argentina): an animal visitor's matter? Plant Systematics and Evolution 238: 69-86.

32: Galetto L. 1991. Floral nectar, nectary structure and pollinators in some Argentinean Bromeliaceae. Annals of Botany 67: 401-411.

33: Hammons RO, Leuck DB. 1966. Natural cross-pollination of the peanut, *Arachis hypogaea* L., in the presence of bees and thrips. Agronomy Journal 58: 396-396.

34: Gill FB, Stokes FJ, Stokes C. 1973. Contact zones and hybridization in the Jamaican hummingbird, *Trochilus polytmus* (L.). The Condor 75: 170-176

35: Milet-Pinheiro P, Schlindwein C. 2008. Comunidade de abelhas (Hymenoptera, Apoidea) e plantas em uma área do Agreste pernambucano, Brasil. Revista Brasileira de Entomologia 52: 625-636.

36: Bhatia GK, Gupta SC, Green JM, Sharma D. 1980. Estimates of natural cross-pollination in *Cajanus cajan* (L.) Millsp.: Several Experimental Approaches. International Workshop on Pigeonpeas 129.

37: Williams IH. 1977. Behaviour of insects foraging on pigeon pea (*Cajanus cajan* (L.) Millsp.) in India. Tropical Agriculture 54: 353-363.

38: Dulberger R, Smith MB, Bawa KS. 1994. The stigmatic orifice in *Cassia*, *Senna*, and *Chamaecrista* (Caesalpiniaceae): morphological variation, function during pollination, and possible adaptive significance. American Journal of Botany 81: 1390-1396.

39: Marazzi B, Conti E, Endress PK. 2007. Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiinae). International Journal of Plant Sciences 168: 371-391.

40: Tucker SC. 1996. Trends in evolution of floral ontogeny in *Cassia* sensu stricto, *Senna*, and *Chamaecrista* (Leguminosae: Caesalpinioideae: Cassieae: Cassiinae); a study in convergence. American Journal of Botany 83: 687-711.

41: Marazzi B, Endress PK. 2008. Patterns and development of floral asymmetry in *Senna* (Leguminosae, Cassiinae). American Journal of Botany 95: 22-40.

42: Janzen DH. 1966. Notes on the behavior of the carpenter bee *Xylocopa fimbriata* in Mexico (Hymenoptera: Apoidea). Journal of the Kansas Entomological Society 39: 633-641.

43: Parker IM. 1997. Pollinator limitation of *Cytisus scoparius* (Scotch Broom), an invasive exotic shrub. Ecology 78: 1457-1470.

44: Stout JC. 2000. Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L. (Fabaceae). Apidologie 31: 129-140.

45: Ollerton J, Lack A. 1998. Relationships between flowering phenology, plant size and reproductive success in *Lotus corniculatus* (Fabaceae). Plant Ecology 139: 35-47.

46: Rasmussen IR, Brodsgaard B. 1992. Gene flow inferred from seed dispersal and pollinator behaviour compared to DNA analysis of restriction site variation in a patchy population of *Lotus corniculatus* L.. Oecologia 89: 277-283.

47: Agostini K, Sazima M, Sazima I. 2006. Bird pollination of explosive flowers while foraging for nectar and caterpillars. Biotropica 38: 674-678.

48: Rocca MA, Sazima M. 2010. Beyond hummingbird-flowers: The other side of ornithophily in the Neotropics. Oecologia Australis 14: 67-99.

49: Fernández MJ, López-Calleja M, Bozinovic F. 2002. Interplay between the energetics of foraging and thermoregulatory costs in the green-backed firecrown hummingbird *Sephanoides sephaniodes*. Journal of Zoology 258: 319-326.

50: Sazima I, Buzato S, Sazima M. 1995. The saw-billed hermit *Rhamphodon naevius* and its flowers in Southeastern Brazil. Journal für Ornithologie 136: 195-206.

51: Buzato S, Sazima M, Sazima I. 2000. Hummingbird-pollinated floras at three Atlantic Forest sites. Biotropica 32: 824-841.

52: Snow DW, Teixeira DL. 1982. Hummingbirds and their flowers in the coastal mountains of southeastern Brazil. Journal für Ornithologie 123: 446-450.

53: Jesson LK, Barrett SC. 2002. Enantiostyly in *Wachendorfia* (Haemodoraceae): the influence of reproductive systems on the maintenance of the polymorphism. American Journal of Botany 89: 253-262.

54: Duncan GD, Edwards TJ, Mitchell A. 2005. Character variation and a cladistic analysis of the genus *Lachenalia* Jacq. F. ex Murray (Hyacinthaceae). Acta Horticulturae 673: 113-120.

55: Robinson J, Robinson CSC, Winterbottom JM. 1957. Notes on the birds of the Cape L'Agulhas region. Ostrich 28: 147-163.

56: Westerkamp C, Claßen-Bockhoff R. 2007. Bilabiate flowers: The ultimate response to bees? Annals of Botany 100: 361-374.

57: Brantjes NBM. 1981. Floral mechanics in *Phlomis* (Lamiaceae). Annals of Botany 47: 279-282.

58: Sazima M, Sazima I. 1989. Oil-gathering bees visit flowers of glandular morphs of the oil-producing Malpighiaceae. Botanica Acta 102: 106-111.

59: Liu A-Z, Kress WJ, Wang H, Li D-Z. 2002. Insect pollination of *Musella* (Musaceae), a monotypic genus endemic to Yunnan, China. Plant Systematics and Evolution 235: 135-146

60: Simpson BB, Neff JL. 1983. Floral biology and floral rewards of *Lysimachia* (Primulaceae). American Midland Naturalist 110: 249-257.

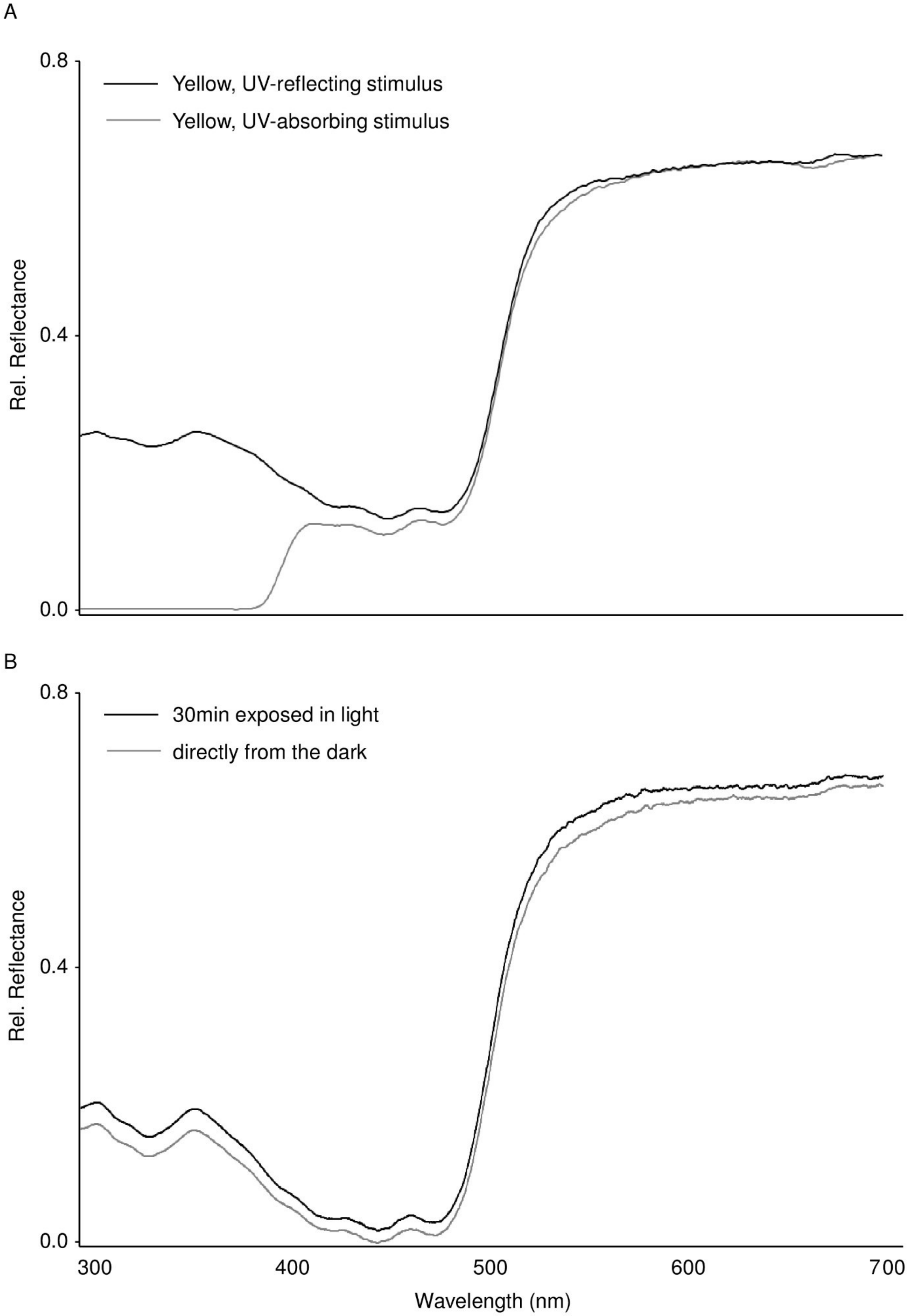
61: Nazir R, Reshi Z, Ahmad B. 2008. Reproductive ecology of medicinally important Kashmir Himalayan species of *Digitalis* L.. Plant Species Biology 23: 59-70

62: Teppner H. 2005. Pollinators of Tomato, *Solanum lycopersicum* (Solanaceae), in Central Europe. Phyton 45: 217-235.

63: Lifante ZD. 1996. Reproductive biology of *Asphodelus aestivus* (Asphodelaceae). Plant Systematics and Evolution 200: 177-191.

Supplemental Material 2

Spectral reflectance of test stimuli made by carotenoids. (A): Spectral reflectance of test stimuli made by carotenoids on filter paper covered with either UV-transmitting or UV-absorbing foil. (B): Differences in spectral reflectance between test stimuli, which are directly obtained from the dark and exposed to light for 30min.


A
0.8
0.4
0.0
Rel. Reflectance
Yellow, UV-reflecting stimulus
Yellow, UV-absorbing stimulus
B
0.8
0.4
0.0
Rel. Reflectance
30min exposed in light
directly from the dark
300
400
500
600
700
Wavelength (nm)

This supplemental material is part of the publication:

Gloss, colour and grip: Multifunctional epidermal cell shapes in bee- and bird-pollinated flowers

Papiorek, S., Junker, R. R. and Lunau, K.

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Figure S1

Supplemental material available online at
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0112013#s5

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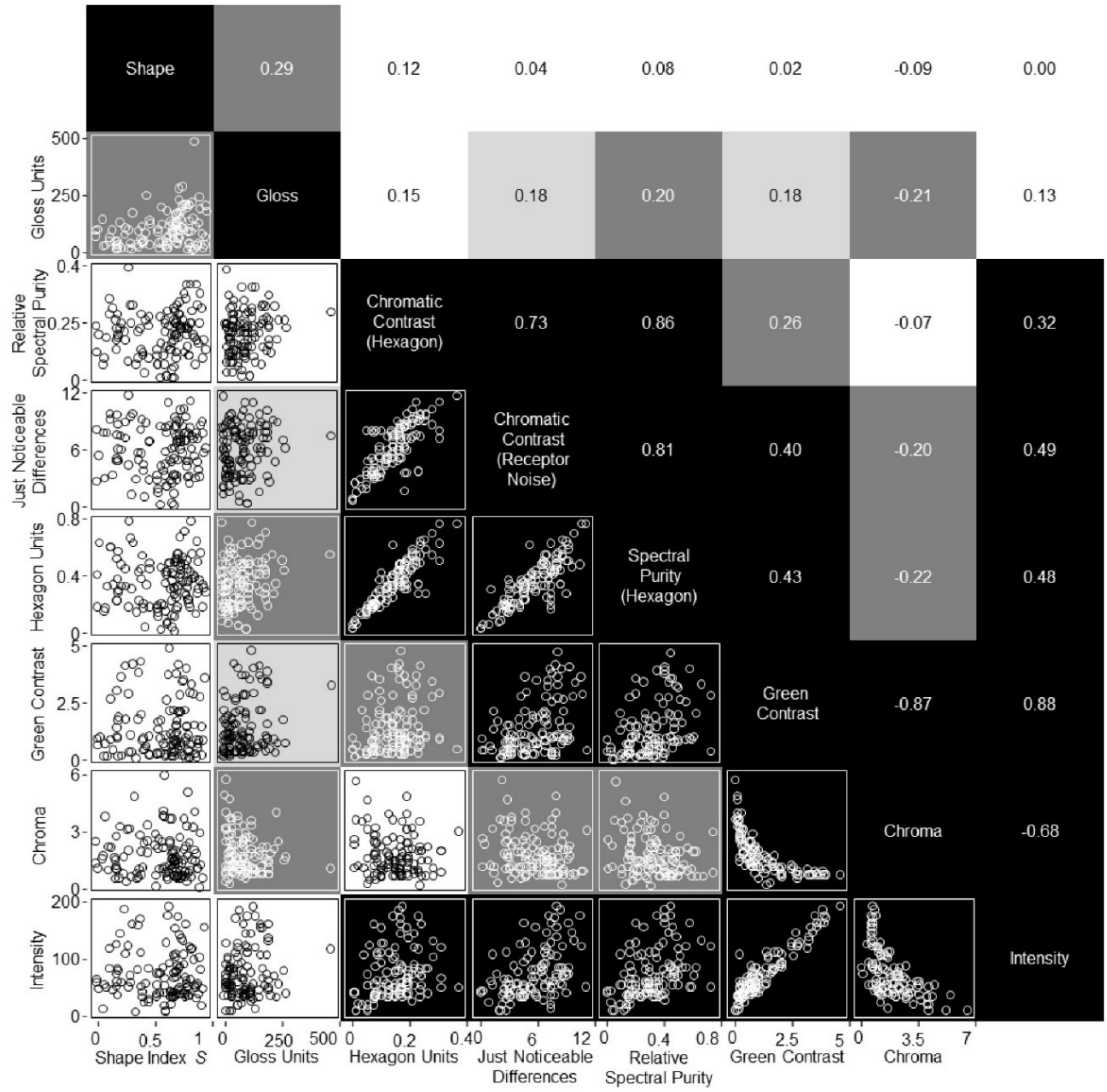
Heatmap visualizing the results of Spearman's rho correlation between epidermal cell shape S, floral gloss, and all investigated colour parameters. The significance level of correlation is colour-coded with black for $p<0.001$, dark grey for $p<0.05$, light grey for $p>0.05$, and white for $p>0.1$. In the upper triangle of the symmetric matrix, rho-values are given. In the lower triangle, corresponding scatter plots are presented.



Text S1

Supplemental Material Text S1 has been modified for a better representation.
The original version is available online at
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0112013#s5

DOI:10.1371/journal.pone.0112013.s002

Excel file including a list of studied plant species with literature reference about pollination mode, measured flower parts, floral colour, cell shape parameters and shape index S, floral gloss values and colour parameters.

									cell shape parameters										colour parameters					
genus	species epiphet	family	order	pollinator	literature	flower organ	colour	flower part	h	w	α1	α2	β1	β2	γ1	γ2	*S*	gloss	chromatic contrast (Hexagon)	chromatic contrast (Receptor Noise)	spectral purity (Hexagon)	green contrast	chroma	intensity
Brillantaisia	*lamium*	Acanthaceae	Lamiales	bee	1	corolla basal outside	purple	robbing-sensitive	23.2±2.5	22.4±3.4	69±6	66.8±5.7	76.2±7.2	80±8.3	77.8±7	75.8±4.8	0.61	211.00	0.23	8.68	0.46	1.06	1.45	38.75
						lip inside	purple	visual-active	34.4±3	18±1.9	49.2±7.7	54.4±5.8	71.8±6.9	98.2±3.2	78.8±8.2	86±4.3	0.39	16.80	0.40	11.94	0.78	0.47	1.94	27.08
Hypoestes	*phyllostachya*	Acanthaceae	Lamiales	bee	2	corolla basal outside	purple	robbing-sensitive	17.8±2.4	17.4±6.3	76.8±6.1	80.6±5.5	87±6.5	88.8±7.5	78.8±7.6	80.4±9.8	0.76	218.60	0.20	7.38	0.41	0.87	1.06	26.68
						lip inside	purple	visual-active	34.6±2.6	13±1.6	36.6±3	33.8±9.3	75.2±3.3	106.4±10.6	84.6±6.9	77.2±4.3	0.25	67.50	0.24	9.42	0.60	2.76	0.89	61.40
Jacobinia	*pauciflora*	Acanthaceae	Lamiales	bird	3	corolla basal outside	red	robbing-sensitive	23±8.1	18.6±4.6	68.2±10.1	62±10.3	75.4±12.5	84.4±11.2	84.4±6.8	80.4±8	0.59	105.20	0.10	3.10	0.20	0.40	3.61	42.94
						lip inside	yellow	visual-active	28±1.2	25.6±2.9	69.8±7	75.8±11.4	70.4±3.8	87.8±9.7	86.2±1.9	85±7.2	0.62	47.60	0.14	4.60	0.21	1.85	0.91	39.31
Ruellia	*chartacea*	Acanthaceae	Lamiales	bird	4	corolla basal outside	red-orange	robbing-sensitive	23±1.2	18.4±2.8	83.4±7	87.8±4.8	86.8±4.7	89.8±5.4	78.6±6.8	82.8±9.1	0.82	234.70	0.10	2.80	0.19	0.49	2.53	37.89
						lip inside	orange	visual-active	42.2±2.2	57±6.9	79.2±6.6	81.6±3	89±7.2	85.8±5.2	83.2±4.3	85.6±4.2	0.81	220.40	0.13	3.45	0.24	1.58	0.85	32.89
Ruellia	*malacosperma*	Acanthaceae	Lamiales	bee	5	corolla basal outside	purple	robbing-sensitive	12.2±1.8	9.8±3.7	91.8±4.1	49.9±2.1	86±6.2	85.8±3.3	67.6±6.7	72.2±7.8	0.72	101.00	0.21	8.61	0.49	1.20	1.19	34.66
						lip inside	purple	visual-active	33.6±2.6	26.6±2.9	41.2±6.5	49.8±4.3	40.8±12.9	120.6±27.1	74.4±13.7	86.2±11.6	0.15	72.80	0.20	8.34	0.41	1.04	1.06	31.43
Thunbergia	*grandiflora*	Acanthaceae	Lamiales	bee	6, 7	corolla basal outside	white	robbing-sensitive	24±4.7	32±5.3	89.8±3.4	91.2±1.9	81.8±14	83.6±18.6	70.6±23.5	73.4±7	0.79	209.10	0.11	7.99	0.28	0.33	0.53	27.98
						lip inside	purple	visual-active	70.4±5.7	43.8±2.8	48.4±5.1	42.4±5.8	57.6±3.3	114.2±2.9	85.2±9.5	74.2±15.7	0.23	31.00	0.16	8.02	0.33	0.28	2.26	26.17
Agapanthus	*africanus ´Albus`*	Amaryllidaceae	Asparagales	bee	8	petal outside	white	robbing-sensitive	32.8±2.6	35.2±4.4	86.2±1.8	59.5±2.5	85.2±7.5	86.1±4.4	77.8±8.8	77.1±4.7	0.77	226.00	0.22	8.67	0.53	3.51	0.34	35.04
						petal inside	white	visual-active	37.2±3.5	20.8±5	87.6±4.8	67.8±6.9	88.4±11.9	85±5.5	70.6±10.6	76.4±12.8	0.68	144.00	0.20	8.77	0.49	5.01	0.36	49.01
Clytostoma	*callistegioides*	Bignoniaceae	Lamiales	bee	9, 10	corolla basal outside	light-purple	robbing-sensitive	24±1.9	28±3.9	67.8±5.5	48.2±2.9	60.6±13.7	60.2±12.7	88±3.5	90.2±11.9	0.45	99.40	0.09	5.23	0.23	3.90	0.34	40.99
						lip inside	purple	visual-active	35.6±8.8	23.6±5.1	47±6.9	49±10.6	68.4±7.6	100.8±7.3	91.6±2.3	87.6±9.2	0.36	114.00	0.15	7.51	0.33	1.88	1.22	56.78
Pandorea	*jasminoides*	Bignoniaceae	Lamiales	bee	11, 12	corolla basal outside	white	robbing-sensitive	22.8±0.4	16.2±1.6	76.2±7.5	45.1±3.6	88.4±6.7	86.8±4.9	76±7.9	81.1±4.2	0.73	182.00	0.21	8.78	0.50	3.87	0.37	39.51
						lip inside	white	visual-active	23.4±3.3	37.8±6.1	82.4±6	84±3.7	68.4±8.1	81.2±8.2	84±3.2	88.6±6.1	0.75	153.00	0.16	7.80	0.39	4.25	0.34	44.70
Tecomaria	*capensis*	Bignoniaceae	Lamiales	bird	13	corolla basal outside	orange	robbing-sensitive	38.2±2.2	29.6±1.9	48.4±3.7	37.8±1.9	76.2±5.9	67.7±7.1	81.8±4.1	83.1±3.2	0.51	267.60	0.22	9.09	0.34	0.71	1.58	30.24
						lip inside	orange	visual-active	27±13.3	20.4±2.9	64.8±18.5	61.6±19.2	80.2±4.8	112±28.8	88.8±2.9	86±1.7	0.49	45.50	0.10	4.73	0.18	0.52	1.36	18.62
Echium	*candicans*	Boraginaceae	unplaced	bee	14	petal outside	blue	robbing-sensitive	35.8±3.3	38.4±7.8	72.6±5	53.1±4.7	75.2±5.4	75.3±4	80.4±3.8	82.6±3.6	0.66	74.70	0.13	8.04	0.43	2.71	0.97	66.13
						petal inside	blue	visual-active	31.4±2.7	34±4.4	63.4±8.4	63.8±2.8	48±9.9	110.6±27.5	87.6±7.4	86.2±6.4	0.30	48.20	0.22	9.88	0.52	1.50	1.21	38.56
Pitcairnia	*angustifolia*	Bromeliacaee	Poales	bird	15	petal outside	red	robbing-sensitive	20.4±5.9	31.8±6.1	82.8±5.3	54.9±4.9	73.4±6.4	80±5.8	76.4±13	82.9±7.7	0.65	66.30	0.07	3.59	0.16	0.25	4.58	22.37
						petal inside	red	visual-active	24.8±6.1	19.8±4.5	72±4.1	91.2±6.4	85.8±6.2	88.6±12.7	75.2±12.1	67.6±14.1	0.70	66.50	0.05	2.85	0.13	0.25	4.42	23.48
Canarina	*canariensis*	Campanulaceae	Asterales	bird	16, 17	petal outside	red	robbing-sensitive	37.8±5	69.2±19.3	88±3.7	76.5±10.6	85.4±11.1	87.4±4.9	89.6±12.6	88.8±6.2	0.88	203.10	0.27	7.44	0.50	0.64	1.12	28.36
						petal inside	red-orange	visual-active	40.8±3.3	61.8±19.5	91.2±5	87.2±5.9	92.8±27.2	71.8±16.7	83.6±6.3	90.2±9.3	0.74	177.60	0.20	5.20	0.33	0.65	1.24	24.48
Cistus	*albidus*	Cistaceae	Malvales	bee	18, 19	petal outside	white	robbing-sensitive	24.4±2.3	25.6±3.8	55.6±3.8	40.4±1.6	54.4±5.9	54.6±5.5	80.8±5.9	88.5±6.3	0.78	223.00	0.31	10.48	0.71	3.58	0.48	45.02
						petal inside	white	visual-active	28.8±1.3	20.6±1.3	32±1.6	40.8±6.7	48.6±10	111.4±7.6	75.6±12.6	79±6.4	0.41	185.00	0.27	10.00	0.63	4.28	0.47	54.92
Ipomoea	*purpurea*	Convolvulaceae	Solanales	bee	20	corolla apical inside	white	robbing-sensitive	18±2.3	17.4±2.5	80.6±3.9	48.1±3.8	85.2±7	87±4.1	77.4±18.4	80.4±15.8	0.83	160.00	0.34	11.33	0.78	3.03	0.55	41.95
						corolla basal outside	purple	visual-active	36±7.2	35.6±5.1	81.4±4.2	82.8±3.9	82.8±4.1	86±2	87.6±3.3	82.4±6.7	0.83	4.80	0.21	8.54	0.43	0.22	2.87	19.61
Kalanchoe	*blossfeldiana*	Crassulaceae	Saxifragales	bee	21	petal outside	red	robbing-sensitive	28.6±0.9	40.4±3.6	87.8±1.9	62.2±3	65.8±9.3	75.7±5.3	87.4±9.6	84.2±8.7	0.73	63.10	0.11	3.17	0.19	1.59	1.15	56.23
						petal inside	red	visual-active	56.4±4.3	43±3.7	72.2±4	77±7.8	76.8±5.3	88.2±7.5	75.8±4.3	83.2±4.2	0.64	27.50	0.10	4.10	0.20	0.82	3.25	88.85
Erica	*versicolor*	Ericaceae	Ericales	bird	22	corolla basal outside	red	robbing-sensitive	26±4.8	32.6±8.6	90.6±2.9	60.4±4.9	89.8±3.3	87.6±3	74.6±6.7	78.3±4.6	0.76	310.00	0.19	6.35	0.44	0.75	1.16	20.64
						lip inside	green	visual-active	22.6±1.5	67.8±7.9	91.8±1.3	87.8±1.3	86.2±4	79±13.2	62.4±5	73.8±9.1	0.69	134.00	0.12	3.13	0.23	1.25	0.57	15.90
Escallonia	*rubra var. macrantha*	Escalloniaceae	Escalloniales	bird	23, 24	corolla basal outside	pink	robbing-sensitive	18±3.4	14.2±3.4	89.4±2.2	50.8±2.4	81.8±9	85.5±4.5	70.4±9.8	77.7±3.5	0.72	179.00	0.17	6.50	0.41	0.51	1.35	18.51
						petal inside	pink	visual-active	50.4±7.9	44.2±7.7	63.6±6.9	67.2±14.8	72.4±1.8	87±10.4	79.6±10.5	79.2±3.8	0.53	32.20	0.20	7.07	0.49	1.14	1.33	43.11

Cassia	*artemisioides*	Fabaceae	Fabales	bee	25	petal outside	yellow	robbing-sensitive	21.8±1.3	33.8±5.5	81.8±6	56.3±5.8	66.6±8.7	75.4±5.4	88±6.9	84.8±4.3	0.49	55.10	0.11	4.12	0.21	1.05	0.51	15.38
						petal inside	yellow	visual-active	25.4±3	23.2±2.9	58.8±3.8	62.2±4	61.2±3.6	116.8±18	90.6±3	84.8±4.1	0.31	33.10	0.17	6.34	0.30	1.96	0.60	27.71
Cassia	*bicapsularis*	Fabaceae	Fabales	bee	25	petal outside	yellow	robbing-sensitive	21±2.4	33.2±1.5	76.8±3.9	51.9±2.1	69±5.1	76.2±3	83.4±5.5	79.3±7.3	0.76	112.40	0.24	10.42	0.47	2.95	0.50	49.00
						petal inside	yellow	visual-active	25.4±2.5	24±4.3	71.8±7.8	74.6±5.5	76.8±12.7	82±6	77.6±5.1	80.8±7.5	0.67	121.00	0.25	11.14	0.49	3.83	0.55	60.06
Cajanus	*cajan*	Fabaceae	Fabales	bee	26	banner inside	orange	robbing-sensitive	24.2±2.3	25.2±3.9	91.4±3.8	89±4.1	78.4±12.4	79.8±10.9	80.4±7.4	80.2±16.3	0.88	259.30	0.15	5.04	0.28	0.23	4.76	35.11
						banner basal outside	yellow	visual-active	24.6±1.3	19.8±2.3	63.6±4.3	40.4±2.2	72.4±7.6	67.4±4.3	76.6±5.9	80.6±9	0.50	88.40	0.04	1.42	0.05	0.60	0.96	13.55
Collaea	*argentina*	Fabaceae	Fabales	bee	27	banner inside	rose	robbing-sensitive	25.6±3	27.8±4.1	79.8±3	50.6±3.5	76.6±10.1	78.3±3.2	76.4±9.8	74.5±11.2	0.68	89.40	0.12	5.18	0.31	2.27	0.43	32.16
						banner basal outside	white	visual-active	26.4±1.3	23.6±2.9	85.8±1.9	86.8±3.3	83.8±3	86.8±5.9	70.8±10.5	77±5	0.76	116.00	0.20	7.04	0.49	2.20	0.48	30.44
Lablab	*purpureus*	Fabaceae	Fabales	bee	28	banner inside	purple	robbing-sensitive	21.8±2.4	28.8±6.7	84.8±3.6	56±2.9	93.6±7.7	88.9±3.5	80.2±4.6	78.9±4.7	0.73	67.30	0.17	7.73	0.25	1.63	1.11	53.63
						banner basal outside	light-purple	visual-active	30.4±5.1	29.8±0.8	64±6.6	73.2±5.4	66.4±10.8	92.8±10.4	77.2±8.2	79.2±15.8	0.47	121.00	0.12	5.91	0.38	2.09	0.92	57.42
Lotus	*berthelotii*	Fabaceae	Fabales	bird	16	banner inside	red	robbing-sensitive	26.2±3.3	17.4±1.8	71.2±12	43.6±6.3	85.4±3.4	82.1±3.6	76.8±12.5	82.7±8	0.66	16.80	0.03	2.51	0.08	0.12	6.64	11.57
						banner basal outside	blue	visual-active	13.6±0.9	13.6±1.5	55.6±5.6	66.8±5.4	58.2±10.3	79±14.8	77±9	80.4±8.3	0.44	32.10	0.07	3.19	0.15	0.19	5.53	10.81
Aesschynanthus	*radicans*	Gesneriaceae	Lamiales	bird	29	corolla basal outside	red	robbing-sensitive	59.4±10.1	81.4±10.4	73.6±14	76.6±3.4	91.6±8.1	87.5±5	87±6.9	86.2±5.1	0.80	41.10	0.20	5.09	0.36	0.19	4.41	37.17
						lip inside	red, yellow	visual-active	34.4±3.4	44±14.7	84.6±4.7	87.4±5.9	80.6±8.5	83.6±10.5	88.4±3.1	82.6±3.2	0.88	136.00	0.34	8.01	0.64	0.98	1.40	26.84
Chirita	*gueilinensis*	Gesneriaceae	Lamiales	bee	30	corolla basal outside	white	robbing-sensitive	37.6±2.4	37±6.8	87.8±6.1	60.3±6.2	91±6.4	88.7±4.8	76.6±4.8	81.7±5.8	0.80	220.40	0.19	8.07	0.45	4.09	0.47	54.75
						lip inside	purple	visual-active	61±8.7	18.8±0.4	47±4.4	44±8.7	76±5.1	105.4±7.9	85±3.5	75.6±6.2	0.33	138.00	0.19	7.73	0.48	3.72	0.46	49.68
Columnea	*gloriosa*	Gesneriaceae	Lamiales	bird	31, 32	corolla basal outside	red	robbing-sensitive	29.4±4.9	34±7.6	83.2±8.3	57.2±6.8	73.2±1.6	75.5±4.2	92.2±5.7	91.7±3.7	0.70	87.10	0.02	0.73	0.05	0.45	1.57	22.75
						lip inside	red	visual-active	44.6±9.4	56.6±15	83.4±6.5	89±1	90±12.1	68.2±14.7	79±16.3	73.4±16.4	0.61	131.20	0.02	0.61	0.03	0.41	1.69	22.07
Columnea	*magnifica*	Gesneriaceae	Lamiales	bird	32	corolla basal outside	red	robbing-sensitive	24.2±3.9	29.4±1.5	85±5.6	84.6±5	81.2±8.6	76.4±10.2	59.2±16.9	60.6±20.6	0.59	93.80	0.12	3.70	0.18	0.35	3.04	37.70
						lip inside	red	visual-active	19.2±2.4	19.8±4.1	82±13.5	49.3±7.1	84.4±12.4	82.8±6.2	70.6±17.8	76.8±7.3	0.73	37.30	0.09	3.70	0.17	0.83	0.95	19.78
Gloxinia	*sylvatica*	Gesneriaceae	Lamiales	bird	33, 34	corolla basal outside	red	robbing-sensitive	29±4.6	47.2±11.3	80.6±8.4	60.9±5.4	84.4±10.1	86.1±5.2	85.8±6.8	85.4±1.8	0.87	39.00	0.07	3.35	0.14	0.39	1.99	28.38
						lip inside	red	visual-active	69±18.3	17.6±5.5	34.4±3.4	43.6±3.8	78.6±8.2	106.2±10.3	84±5.8	82.6±5.2	0.30	30.00	0.15	5.06	0.23	1.13	2.28	55.41
Kohleria	*spicata*	Gesneriaceae	Lamiales	bird	35, 36	corolla basal outside	white	robbing-sensitive	30.4±3.3	24.4±4.4	93.8±6.9	58.1±3.3	90±3.5	86.9±1.9	83.8±2.8	84.6±3	0.84	13.70	0.21	6.38	0.38	0.80	1.59	34.25
						lip inside	white	visual-active	57.6±4.4	25.4±3.8	41.2±4	27.8±6.9	57.8±10.6	129.2±8.8	84.6±5	78.4±4.3	0.16	101.00	0.11	3.01	0.19	0.77	1.62	33.82
Mitraria	*coccinea*	Gesneriaceae	Lamiales	bird	24, 37	corolla basal outside	red-orange	robbing-sensitive	83.4±5.8	83.2±12	84.2±6.9	79.2±7.8	86.6±3.5	83.1±2.8	85.2±1.9	84.5±2.7	0.80	91.00	0.34	7.20	0.51	0.94	1.41	35.47
						lip inside	orange	visual-active	82±4.3	52.6±7.6	58±7.6	55.6±9.8	53.6±12.6	111.8±21.6	85±6.2	75±8.8	0.27	103.00	0.26	3.64	0.34	0.54	1.47	24.72
Sinningia	*cardinalis*	Gesneriaceae	Lamiales	bird	38	corolla basal outside	red	robbing-sensitive	30±5.4	29.8±6.7	75.8±5.8	51.1±6.3	79.4±3	81.4±3	85.4±3.4	86.2±3.9	0.74	66.00	0.24	7.41	0.55	2.20	0.56	30.73
						lip inside	orange	visual-active	47±6.4	35.2±10.1	66.8±5.2	63.2±14.4	78.6±13.3	92.2±8	82.2±7.2	73.4±8.2	0.53	149.10	0.09	3.16	0.19	0.38	3.29	32.37
Sinningia	*macropoda*	Gesneriaceae	Lamiales	bird	38	corolla basal outside	red	robbing-sensitive	35.6±3.6	20.4±5.9	87.4±9.9	53.5±5.2	89.4±15.3	90.1±6.2	85.6±15.1	86.7±13.7	0.89	36.10	0.14	4.21	0.31	0.83	1.31	30.10
						lip inside	red	visual-active	46.6±4.6	31±4.5	48.2±8.2	51.6±11.8	77±10.7	91±4.8	89±5.2	84.6±2.3	0.45	21.90	0.18	5.96	0.43	1.89	0.54	28.27
Sinningia	*pusilla*	Gesneriaceae	Lamiales	bee	39	corolla basal outside	purple	robbing-sensitive	21±1.9	22.4±7	87.6±4.4	53.6±3.3	91.6±3.6	88.2±1.7	74.4±7.4	75.3±5.8	0.69	111.00	0.17	6.39	0.42	0.73	1.00	18.87
						lip inside	purple	visual-active	65.4±4.5	27±2.4	40.4±8.9	38.8±7	72.6±4.4	117.4±4.7	80.2±3.3	81±6.2	0.31	51.20	0.24	8.93	0.57	1.84	1.00	43.47
Sinningia	*reitzii*	Gesneriaceae	Lamiales	bird	38	corolla basal outside	pink	robbing-sensitive	22.2±2.2	25±3.9	87.2±8.6	56.3±4.1	90±1.9	90.5±2.3	58.8±7	73.7±5.3	0.69	95.20	0.14	4.97	0.37	0.29	1.63	18.17
						lip inside	pink	visual-active	33.6±2.9	26.6±3.6	57.4±5.2	52.8±9.2	56±9.8	98±10.9	81.2±9.3	75.8±5.7	0.31	19.10	0.16	5.14	0.29	0.27	1.76	20.07
Sinningia	*sellovii*	Gesneriaceae	Lamiales	bird	38	corolla basal outside	pink	robbing-sensitive	22.2±1.3	24.6±3.4	82.2±7.7	83±3.2	81.6±6.3	82.6±5.6	85.4±7.5	85.6±10.5	0.86	26.40	0.16	5.64	0.39	1.64	1.14	51.29
						lip inside	pink	visual-active	31.4±4.2	24.2±1.6	43.4±11.2	36.3±6.9	56±5.1	55.8±5.5	84.2±4.6	107.9±10.1	0.21	26.30	0.09	3.31	0.18	0.82	1.26	29.47
Streptocarpus	*grandis*	Gesneriaceae	Lamiales	bee	40	corolla basal outside	white	robbing-sensitive	40.2±4.8	59.6±9	87±2	70.2±5.4	87.8±5.2	87.6±2.5	80.8±4.7	80.7±2.6	0.85	118.00	0.17	7.38	0.41	2.97	0.47	40.92

						lip inside	white	visual-active	47±2.2	42.8±3.2	65±4	68.4±4.2	65.4±7.5	96±5.8	83.6±9.2	84.2±5.6	0.47	61.20	0.19	8.38	0.45	4.39	0.47	58.20
Phlomis	*fruticosa*	Lamiaceae	Lamiales	bee	41, 42	corolla basal outside	yellow	robbing-sensitive	27±2	33.8±3.4	88.8±1.9	59.8±2.4	89.6±13.6	90.2±7.1	84.8±2	85.6±3.6	0.90	20.30	0.31	8.79	0.56	2.86	0.93	53.14
						lip inside	yellow	visual-active	37.2±2.8	26.4±2.7	41.6±4.2	53.8±4	57.6±8.8	97.8±20.9	80.6±6.7	91.2±10.2	0.30	13.00	0.12	5.39	0.23	3.12	0.45	43.24
Plectranthus	cf. *barbatus*	Lamiaceae	Lamiales	bee	43	corolla basal outside	purple	robbing-sensitive	27.8±3.8	28±5.3	88.8±0.8	57.9±1.7	83.8±9.5	86.5±4.5	85.2±8.2	86.5±7	0.91	106.00	0.17	5.71	0.63	1.12	0.92	25.69
						lip inside	light-purple	visual-active	27.6±2.8	29.4±4.2	39.2±4.1	49.4±8.4	39.6±5.8	109.6±16.9	89.6±7.2	83±7.6	0.17	32.40	0.26	9.17	0.34	1.56	0.43	42.70
Salvia	*canariensis*	Lamiaceae	Lamiales	bee	44	corolla basal outside	purple	robbing-sensitive	22.2±3.8	24.4±4.5	77.2±8.4	49.9±4.8	79.8±10.8	76.8±8.1	86±4.3	82.7±5.8	0.79	10.60	0.19	7.24	0.48	2.27	0.49	31.47
						lip inside	purple	visual-active	32±3.2	17.2±0.8	46±4.2	48.2±2.3	71.8±7.5	112.2±11.6	77.6±3.6	84.4±7.1	0.30	10.90	0.19	7.82	0.38	1.08	0.93	31.77
Salvia	*microphylla*	Lamiaceae	tro	bird	45	corolla basal outside	red	robbing-sensitive	42.8±4.7	40±8.9	89.2±0.4	62.4±3.9	94.4±7.4	91.4±3.4	76.8±5.1	76.7±4.5	0.71	133.00	0.26	3.74	0.27	0.26	4.39	36.19
						lip inside	red	visual-active	67±5.1	27.8±3.8	55.2±4.8	64.4±7.3	72.8±5.6	108.8±12.4	78.4±2.9	85±2.1	0.40	12.20	0.21	1.66	0.31	0.47	1.95	31.27
Salvia	*rutilans*	Lamiaceae	Lamiales	bird	46	corolla basal outside	red	robbing-sensitive	28.6±3.4	30.8±6.3	84.6±4.5	55.7±2.6	78.8±6.2	82.6±4.7	78.2±11.8	80±6.1	0.77	104.70	0.19	5.89	0.36	0.30	3.46	49.21
						lip inside	red	visual-active	30.8±1.9	24.4±2.5	52±3.7	50.6±8.6	62.8±12.1	93.4±10.6	81.4±7	74.6±5.8	0.33	17.10	0.21	6.28	0.33	0.31	4.00	61.79
Abutilon	*megapotamicum*	Malvacea	Malvales	bird	47, 48	sepal outside	red	robbing-sensitive	20.2±2.5	42.4±14.4	86.6±7.2	84.8±2	68.8±14.5	74±7.5	81.2±6.9	84±4.1	0.81	41.30	0.19	5.10	0.36	0.55	2.99	52.71
						petal inside	yellow	visual-active	20.4±1.1	17±2.4	84.4±7.9	50±4.7	86.6±3.6	84.2±4	84.6±6.5	80.6±3.3	0.72	180.00	0.06	1.79	0.12	2.27	0.61	37.12
Proboscidea	*fragrans*	Martyniaceae	Lamiales	bee	49	corolla basal outside	light-purple	robbing-sensitive	16.8±2.7	22.8±6.3	91.4±5	56.9±1.6	93.6±15.8	90.5±8.4	73.4±8.2	74.5±6.9	0.73	300.00	0.22	7.25	0.52	1.82	0.70	27.45
						lip inside	purple	visual-active	33.2±2	19.4±1.5	56.6±4.4	52.6±4.4	76.4±5.8	102.8±18.1	78±7.5	80.6±8.1	0.40	41.60	0.27	9.67	0.66	1.19	1.40	37.39
Melastoma	*candidum*	Melastomataceae	Myrtales	bee	50	petal outside	purple	robbing-sensitive	67.4±4.2	46.6±8.6	87.8±2.9	64.1±4.1	86.8±9.4	88.8±5.4	91.8±5.7	89.7±5.5	0.94	192.00	0.12	6.47	0.29	3.67	0.68	79.90
						petal inside	purple	visual-active	53.6±3.3	22.4±3.4	47.6±3.8	61.8±6.1	77±3.8	108.4±9.1	70±7.8	79.4±12.5	0.36	80.20	0.18	9.11	0.45	4.20	0.79	95.39
Tibouchina	*urvilleana*	Melastomataceae	Myrtales	bee	51-54	petal outside	purple	robbing-sensitive	19.8±3.6	32.4±8	89.8±1.6	59.3±3.3	89.8±17.7	88.3±9.5	87±11	79.4±7.4	0.73	247.20	0.27	9.34	0.55	0.93	1.38	36.37
						petal inside	purple	visual-active	48±4.3	33.6±1.5	48.6±5.9	53.2±5.4	53.2±10.8	114.6±8.4	87.4±4.1	82.6±7.9	0.25	47.20	0.29	10.02	0.60	1.00	1.48	43.11
Acca	*sellowiana*	Myrtaceae	Myrtales	bird	55	sepal outside	white	robbing-sensitive	51.8±15.1	47.8±6.6	89.4±1.9	89.4±2.3	77.2±14.7	104.6±17.7	74.6±15.8	71.8±13.8	0.60	16.90	0.07	2.25	0.15	1.78	0.23	19.55
						petal inside	green	visual-active	15.4±2.7	14.6±2.3	87.8±2.8	50.4±1.9	81±2.7	84.8±4	78.2±7.6	78.6±0.5	0.82	21.40	0.10	3.58	0.21	1.19	0.31	14.01
Callistemon	*citrinus*	Myrtaceae	Myrtales	bird	56	petal outside	green	robbing-sensitive	8.6±0.9	12±1	82±6.5	46.2±3.2	62.6±11.9	68.6±8.9	83.8±6.4	79±4.8	0.65	45.70	0.18	4.81	0.35	1.21	0.61	14.67
						stamina	red	visual-active	18.8±1.8	16.2±2	80.4±3.9	76.4±4.3	82.4±2.3	77.8±4.1	74.2±7.1	70.2±9.9	0.66	37.90	0.03	2.45	0.07	0.12	4.50	11.30
Calothamnus	*quadrifidus*	Myrtaceae	Myrtales	bird	57, 58	petal outside	red	robbing-sensitive	15.4±2.1	13.6±1.5	79.8±3.6	45.5±2	77.8±8	78.2±3.5	71.8±7.9	74.9±4.9	0.71	137.20	0.15	5.39	0.32	0.46	2.61	40.93
						petal inside	red	visual-active	18.4±2.3	14.8±2.5	71.2±5.2	81±5.8	77.6±7.2	76.8±3.7	68.6±11.7	71.4±9.1	0.65	193.50	0.22	6.05	0.41	0.70	2.92	70.45
Fuchsia	*magellanica*	Onagraceae	Myrtales	bird	59	sepal outside	pink	robbing-sensitive	13.2±1.1	18.8±1.5	82.8±3.1	50±1.6	86±5.7	85.6±5.6	77±4.2	81.7±5.1	0.82	97.00	0.10	7.96	0.24	0.18	4.01	38.28
						petal inside	purple	visual-active	13.4±1.1	15.2±2.9	86.8±3.4	87.4±1.7	78.4±7.6	67±9.4	67.8±9.9	73.6±7.1	0.65	15.20	0.21	8.56	0.43	0.36	2.41	28.77
Polygala	*myrtifolia*	Polygalaceae	Fabales	bee	52, 60	lower petal outside	purple	robbing-sensitive	57.6±6.5	53.2±6.4	85.2±7.5	66.4±4.5	91.2±2.7	90.7±3	69±9	78.6±4.4	0.72	141.30	0.25	9.64	0.52	0.91	1.40	39.57
						lower petal inside	purple	visual-active	32±5.1	52.4±9.8	86.4±4.2	91.8±3	90.4±2.3	60.8±20	72.4±12.2	72.2±11.2	0.53	132.40	0.23	7.15	0.45	0.32	2.03	14.13
Passiflora	*caerulea*	Passifloraceae	Malphigiales	bee	61	petal outside	white	robbing-sensitive	30±4.1	23±2.7	91±4.2	56.7±2.9	84.8±0.8	86.4±2.6	79±8.6	84.7±4.7	0.84	523.00	0.25	7.73	0.55	3.42	0.58	40.56
						petal inside	light-purple	visual-active	40±4.8	18.6±2.2	50.8±1.9	60.8±2.3	82.6±2.9	97.8±6.4	84±5.5	80.8±7.1	0.48	40.10	0.24	9.40	0.54	0.96	1.79	27.84
Penstemon	*digitalis*	Plantaginaceae	Lamiales	bee	62	corolla basal outside	white	robbing-sensitive	30.4±2.8	21.8±6.9	74.8±5	46.3±4	81.4±7.2	83.4±4.2	89±6.4	87.6±5.6	0.75	192.00	0.28	9.22	0.67	2.24	0.47	29.03
						lip inside	white	visual-active	49.6±4.2	51.4±7.1	54.8±6.5	50.2±3.4	61.4±11.5	80.6±18.6	72.4±8.6	82.2±6.3	0.38	104.00	0.08	4.71	0.19	2.98	0.30	31.71
Tetranema	*roseum*	Scophulariaceae	Lamiales	bee	63	corolla basal outside	purple	robbing-sensitive	18.8±2.5	18.8±2.9	91±2.2	54.9±1.7	87.2±4.4	89.7±3.6	89.6±2.3	91.8±3.2	0.93	219.20	0.15	5.95	0.32	0.95	1.02	25.46
						lip inside	purple	visual-active	37.4±1.1	44.2±3.4	80.8±2.4	83.8±3.3	90.4±6.3	81.8±8.1	86.2±5.3	73.4±1.5	0.73	100.90	0.17	7.00	0.33	1.30	1.13	40.66
Juanulloa	*aurantiaca* syn. *mexicana*	Solanaceae	Solanales	bird	64	sepal outside	orange	robbing-sensitive	18.8±5.3	32.6±14.3	81.6±5	58.7±3.2	83.2±5.4	85±3.7	81±13.2	82.7±11	0.90	82.90	0.21	3.52	0.37	0.96	0.92	20.80
						petal inside	orange	visual-active	41.2±3.8	31±1.7	81.6±6.4	84.8±5.8	83.8±3.3	88.4±6.8	74.2±7.8	72.6±9.4	0.71	42.90	0.22	3.54	0.31	0.79	1.18	21.77
Strelitzia	*reginae*	Strelitziaceae	Zingiberales	bird	65	petal outside	blue	robbing-sensitive	20.4±3.6	24±3.4	87.2±6.6	54.6±4.3	89.8±2.9	86.5±4.9	89.4±5.2	88.2±3.7	0.92	70.70	0.09	7.99	0.19	0.33	1.84	10.46
						petal inside	blue	visual-active	20±3.2	19.2±7.2	49.4±6.4	42.4±6.7	51±11.9	120.6±16.9	86.6±1.3	82.8±5.4	0.20	153.10	0.07	8.02	0.16	0.28	1.17	8.32
Aloe	*vera*	Xanthorrhoeaceae	Asparagales	bird	66	tepal outside	yellow	robbing-sensitive	43±1.7	37.6±5.4	87.6±3	59.7±1.4	83.4±5.3	81.3±4	72.2±12.8	76.2±13	0.71	125.30	0.02	0.53	0.02	0.95	0.91	22.70
						tepal inside	yellow	visual-active	46±5.1	38.2±3.9	89.4±1.1	89.8±0.8	74.8±14	86.8±4.5	75±8.8	92.2±4.3	0.80	97.50	0.29	9.68	0.27	1.08	1.14	21.22

Aloe	*vogtsii*	Xanthorrhoeaceae	Asparagales	bird	67	tepal outside	yellow	robbing-sensitive	49.8±10.2	74.6±9.7	91±1.9	78.8±6.3	98.8±10.9	92.5±5	77.8±17.8	74.6±10.7	0.60	44.20	0.07	1.86	0.12	1.45	0.75	28.92
						tepal inside	yellow	visual-active	45.4±13.1	64±11.3	88.2±2.8	90.4±2.7	79±13.2	86.4±11.3	80.4±6.9	79.6±5.5	0.81	146.00	0.17	4.59	0.32	1.57	0.86	30.07

literature

1 Bartoš M, Janeček Š, Padyšáková E, Patáčová E, Altman J, Pešata M, Kantorová J, Tropek R. 2012. Nectar properties of the sunbird-pollinated plant Impatiens sakeriana: A comparison with six other co-flowering species. South African Journal of Botany 78: 63-74.

2 White D, Cribb BW, Heard TA. 2001. Flower constancy of the stingless bee *Trigona carbonaria* Smith (Hymenoptera: Apidae: Meliponini). Australian Journal of Entomology 40: 61-65. DOI: 10.1046/j.1440-6055.2001.00201.x

3 Buzato S, Sazima M, Sazima I. 2000. Hummingbird-pollinated floras at three Atlantic Forest sites. Biotropica 32: 824-841.

4 Rodríguez-Flores CI, Stiles FG. 2005. Ánalisis ecomorfológico de una comunidad de colibríes ermitaños (Trochilidae, Phaethorninae) y sus flores en la amazonía colombiana. Ornitología Colombiana 3: 7-27.

5 Tripp EA, Manos PS. 2008. Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruellia* (Acanthaceae). Evolution 62: 1712-1737.

6 Fiala B, Krebs A, Barlow HS, Maschwitz U. 1996. Interactions between the climber *Thunbergia grandiflora*, its pollinator *Xylocopa latipes* and the ant *Dolichoderus thoracicus*: the 'nectar-thief hypothesis' refuted? Malayan Nature Journal 50: 1-14.

7 Young AM. 1983. Nectar and pollen robbing of *Thunbergia grandiflora* by *Trigona* bees in Costa Rica. Biotropica 15: 78-80.

8 Ying S, Li-Huan Z. 2009. Study on pollinating insects of *Agapanthus africanus* and their foraging behavior. Acta Agriculturae Shanghai 25: 87-91.

9 Rivera GK. 2000. Nuptial nectary structure of Bignoniaceae from Argentina. Darwiniana 38: 227-239.

10 Wilms W, Imperatriz-Fonseca VL, Engels W. 1996. Resource partitioning between highly eusocial bees and possible impact of the introduced africanized honey bee on ative stingless bees in the Brazilian Atlantic Rainforest. Studies on Neotropical Fauna and Environment 31: 137-151. DOI: 10.1076/snfe.31.3.137.13336

11 Pemberton RW, Liu H. 2008. Potential of invasive and native solitary specialist bee pollinators to help restore the rare cowhorn orchid (*Cyrtopodium punctatum*) in Florida. Biological Conservation 141: 1758-1764.

12 James EA, Knox RB. . Reproductive-Biology of the Australian Species of the Genus *Pandorea* (Bignoniaceae). Australian Journal of Botany 41: 611-626.

13 Scott-Elliot GF. 1890. Ornithophilous flowers in South Africa. Annals of Botany 4: 265-280.

14 Vicidomini S. 2006. Biologia di *Xylocopa violacea* (Linnè, 1758) (Hymenoptera: Apidae): repertorio floristico europeo. Atti del Museo Civico di Storia Naturale di Trieste 53: 71-86.

15 Fumero-Cabán JJ, Meléndez-Ackerman EJ. 2007. Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). American Journal of Botany 94: 419-424

16 Olesen JM. 1985. The Macaronesian bird-flower element and its relation to bird and bee opportunists. Botanical Journal of the Linnean Society 91: 395-414.

17 Ollerton J, Cranmer L, Stelzer RJ, Sullivan S, Chittka L. 2009. Bird pollination of Canary Island endemic plants. Naturwissenschaften 96: 221-232. DOI 10.1007/s00114-008-0467-8

18 Bosch J. 1992. Floral biology and pollinators of three co-occurring *Cistus* species (Cistaceae). Botanical Journal of the Linnean Society 109: 39-55.

19 Diaz-Losada E, Ricciardelli-D'Albore G, Saa-Otero MP. 1998. The possible use of honeybee pollen loads in characterising vegetation. Grana 37: 155-166.

20 Brown BA, Clegg MT. 1984. Influence of flower color polymorphism on genetic transmission in a natural population of the common morning glory, *Ipomoea purpurea*. Evolution 38: 796-803.

21 Kawaguchi LG, Ohashi K, Toquenaga Y. 2007. Contrasting responses of bumble bees to feeding conspecifics on their familiar and unfamiliar flowers. Proceedings of the Royal Society B 274: 2661-2667. DOI:10.1098/rspb.2007.0860

22 Geerts S, Pauw A. 2011. Easy technique for assessing pollination rates in the genus *Erica* reveals road impact on bird pollination in the Cape fynbos, South Africa. Austral Ecology 36: 656-662.

23 Aizen MA, Vázquez DP. 2006. Flowering phenologies of hummingbird plants from the temperate forest of southern South America: is there evidence of competitive displacement? Ecography 29: 357-366.

24 Aizen MA, Rovere AE. 2010. Reproductive interactions mediated by flowering overlap in a temperate hummingbird-plant assemblage. Oikos 119: 696-706.

25 Duncan DH, Nicotra AB, Wood JT, Cunningham SA. 2004. Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. Journal of Ecology 92: 977-985.

26 Williams IH. 1977. Behaviour of insects foraging on pigeonpea (*Cajanus cajan* (L .) Millsp.) in India. Tropical Agriculture 54: 353-363.

27 Silvina A. Córdoba SA, Cocucci AA. 2011. Flower power: its association with bee power and floral functional morphology in papilionate legumes. Annals of Botany 108: 919-931. DOI:10.1093/aob/mcr196

28 Garcia Neto M, Couto RHN, Malheiros EB. 1988. Polinização em *Dolichos lab-lab*. Ciência Zootécnica 3: 3-4.

29 McClure HE. (1966). Flowering, fruiting and animals in the canopy of a tropical rain forest. Malayan Forester 29: 182-203.

30 Gao-Zhong P, Yu-Mei P, Sai-Chun T, Chun-Qiang W, Qiu-Xia C. 2009. Pollination biology and reproductive allocation of *Chirita gueilinensis* (Gesneriaceae). Bulletin of Botanical Research 29: 169-175.

31 Feinsinger P, Beach YH, Linhart YB, Busby WH, Murray KG. 1987. Disturbance, pollinator predictability, and pollination success among Costa Rican cloud forest plants. Ecology 68: 1294-1305.

32 Murray KG,Feinsinger P, Busby WH, Linhart YB, Beach YH Kinsman S. 1987. Evaluation of character displacement among plants in two tropical pollination guilds. Ecology 68: 1283-1293.

33 Faria RR, Araújo AC. 2010. Flowering phenology and pollination of ornithophilous species in two habitats of Serra da Bodoquena, Mato Grosso do Sul, Brazil. Anais da Academia Brasileira de Ciências 82: 843-855.

34 Camargo E, Rodrigues LC, Araujo AC. 2011. Pollination biology and reproduction of *Seemannia sylvatica* (Kunth) Hanstein (Gesneriaceae) in the Serra da Bodoquena National Park, Mato Grosso do Sul. Biota Neotropica 11: 125-130.

35 Feinsinger P. 1978. Ecological interactions between plants and hummingbirds in a successional tropical community. Ecological Monographs 48: 269-287.

36 Feinsinger P. 1976. Organization of a tropical guild of nectarivorous birds. Ecological Monographs 46: 257-291.

37 Magrach A, Santamaría L, Larrinaga AR. 2011. Differential effects of anthropogenic edges and gaps on the reproduction of a forest-dwelling plant: The role of plant reproductive effort and nectar robbing by bumblebees. Austral Ecology 37: 600-609. DOI: 10.1111/j.1442-9993.2011.02320.x

38 Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V. 2001. Nectar sugar composition in relation to pollination syndromes in Sinningiae (Gesneriaceae). Annals of Botany 87: 267-273.

39 SanMartin-Gajardo I, Sazima M. 2004. Non-euglossine bees also function as pollinators of *Sinningia* species (Gesneriaceae) in southeastern Brazil. Plant Biology 6: 506-513.

40 Harrison CJ, Möller M, Cronk QCB. 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. Annals of Botany 84: 49-60

41 Westerkamp C, Claßen-Bockhoff R. 2007. Bilabiate flowers: The ultimate response to bees? Annals of Botany 100: 361-374. DOI:10.1093/aob/mcm123;

42 Brantjes NBM. 1981. Floral Mechanics in *Phlomis* (Lamiaceae). Annals of Botany 47: 279-282.

43 Potgieter CJ, Edwards TJ, Miller RM,Van Staden J. 1999. Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. Plant Sysematics and Evolution 218: 99-112.

44 Thimm SG. 2008. Pollen-placement and pollen-portioning in diverse *Salvia*-species. PhD Thesis, Universität Mainz, Germany.

45 Wester P, Claßen-Bockhoff R. 2007. Floral diversity and pollen transfer mechanisms in bird-pollinated *Salvia* species. Annals of Botany 100: 401-421. DOI:10.1093/aob/mcm036

46 Wester P, Claßen-Bockhoff R. 2011. Pollination syndromes of New World *Salvia* species with special reference to bird pollination. Annals of the Missouri Botanical Garden 98: 101-155.

47 Pickens AL. 1931. Some flowers visited by birds. The Condor 33: 23-28.

48 Gottsberger G, Schrauwen J, Linskens HF. 1984. Amino acids and sugars in nectar, and their putative evolutionary significance. Plant Systematics and Evolution 145: 55-77.

49 Anderson F. 1922. The development of the flower and embryogeny of *Martynia louisiana*. Bulletin of the Torrey Botanical Club 49: 141-157.

50 Liu SC, Wen HH, Chen MY, Yang YT. 2008. Study on pollination ecology of four species of Melastomataceae in Taiwan. Formosan Entomologist 28: 67-85.

51 Steiner J, Zillikens A, Kamke R, Feja PE, Falkenberg DB. 2010. Bees and melittophilous plants of secondary Atlantic forest habitats at Santa Catarina island, southern Brazil. Oecologia Australis 14: 16-39. DOI:10.4257/oeco.2010.1401.01

52 Pemberton RW, Wheeler GS. 2006. Orchid bees don´t need orchids: Evidence from the naturalization of an orchid bee in Florida. Ecology 87: 1995-2001.

53 Cortopassi-Laurino M, Zillikens A, Steiner J. 2009. Pollen sources of the orchid bee *Euglossa annectans* Dressler 1982 (Hymenoptera: Apidae, Euglossini) analyzed from larval provisions. Genetics and Molecular Research 8: 546-556.

54 enner SS. 1989. A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. Annals of the Missouri Botanical Garden 76: 496-518.

55 Ducroquet JPHJ, Hickel ER. 1997. Birds as pollinators of Feijoa (*Acca sellowiana* Berg). Acta Horticulturae 452: 37-40.

56 Hansen DM, Olesen JM, Jones CG. 2002. Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? Journal of Biogeography 29: 721-734.

57 Byrne M, Elliott CP, Yates C, Coates DJ. 2007. Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. Molecular Ecology 16: 1303-1314. DOI: 10.1111/j.1365-294X.2006.03204.x

58 Collins BG, Newland C, Briffa P. 1984. Nectar utilization and pollination by Australian honeyeaters and insects visiting *Calothamnus quadrifidus* (Myrtaceae). Australian Journal of Ecology 9: 353-365.

59 Traveset A, Willson, MF, Sabag C. 1998. Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra Del Fuego: a disrupted mutualism. Functional Ecology 12: 459-464.

60 Westerkamp C, Weber A. 1997. Secondary and tertiary pollen presentation in *Polygala myrtifolia* and allies (Polygalaceae, South Africa). South African Journal of Botany 63: 254-258.

61 Amela Garcia MT, Hoc PS. 1997. Floral biology and reproductive system of *Passiflora caerulea* (Passifloraceae). Beiträge zur Biologie der Pflanzen 70: 1-20.

62 Dieringer G, Cabrera RL. 2002. The interaction between pollinator size and the bristle staminode of *Penstemon digitalis* (Scrophulariaceae). American Journal of Botany 89: 991-997. 2002.

63 Christenhusz MJM. 2010. Nomenclatural corrections in Mesoamerican Plantaginaceae and a new species of *Tetranema* from Honduras. Phytotaxa 14: 56-60.

64 Abrahamczyk S, Kessler M. 2010. Hummingbird diversity, food niche characters, and assemblage composition along a latitudinal precipitation gradient in the Bolivian lowlands. Journal of Ornithology 151: 615-625.

65 Skead CJ. 1975. Weaverbird pollination of *Strelitzia reginae*. Ostrich 46: 183-185.

66 Ghadirian T, Qashqaei AT, Dadras M. 2007. Notes on feeding and breeding abits of the Purple Sunbird *Nectarinia asiatica* (*Cinnyris asiaticus*) in Bandar Abbas, Hormozgan, Southern Iran. Podoces 2: 122-126.

67 Brandt HD. 1960. Birds of Potchefstroom and district. Journal of African Ornithology 31: 6-19. DOI: 10.1080/00306525.1960.9632645