Title: **COLOUR PREFERENCES IN RELATION TO THE FORAGING PERFORMANCE AND FITNESS OF THE BUMBLEBEE *Bombus Terrestris***

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COLOUR PREFERENCES IN RELATION TO THE FORAGING PERFORMANCE AND FITNESS OF THE BUMBLEBEE *Bombus Terrestris*

*Bombus terrestris'*in Yayılma Performansı, Çiçek Tercihi ile İlişkisi ve Doğadaki Uyumu

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Abstract: Bumblebee (*Bombus terrestris*) colonies showed significant variation in their unlearned preference for violet (bee UV-blue) over blue (bee blue) flowers. Bumblebee colonies with a higher average innate preference for violet (over blue) in the laboratory harvested more nectar per unit time under field conditions. Although this correlation was strong (r_s=0.82), it narrowly missed statistical significance at the 5% level (p=0.089), but was significant at the 10% level. This increase in foraging performance appears to make evolutionary sense because, on average, violet flower species contain around four times the amount of sugar (in nectar) as flowers of any other colour in the local area. Interestingly, although colonies with a stronger preference for violet were more effective at nectar foraging, this increase in colony food availability was not predictably translated into investment in fitness, quantified as gyne (new queen) production.

Key Words unlearned colour preferences, innate, adaptive significance, bumble bee, colour naïve, color.

Özet: Bombus kolonileri mor (arı UV-mavi) ve mavi (arı-mavi) çiçekler üzerinde öğrenilmemiş tercihleri konusunda önemli farklılıklar göstermektedir. Laboratuvar'da maviye göre mor'u doğuştan daha çok tercih eder. Bombus kolonileri doğada alan koşullarında verilen zaman diliminde daha fazla nektar işlemiştir. Bu korelasyon (r_s = 0.82) güçlü olsa da %5 (p = 0.089) seviyesinde istatistiki olarak önemli olmamış, fakat %10 seviyesinde önemli görülmüştür. Bu yayılma performansındaki artış evrimsel açıdan anlamlıdır, çünkü çalışmanın yapıldığı lokal bölgede mor renkli çiçekler diğer renk çiçeklerden 4 kat daha fazla şeker (nektarın içinde) içermektedir. Gerçi mor çiçekleri çok tercih eden koloniler nektar toplamada daha etkili olmasına rağmen, besin bulma durumunda bu artış sayısal olarak kraliçe üretimine ve doğadaki genel uyuma tahmin edilir şekilde yansımamıştır.

Anahtar Kelimeler: Öğrenilmemiş renk tercihleri, doğuştan, uyumsal önem, Bombus arısı, öğrenilmemiş renk, renk.

INTRODUCTION

Bees forage in a ''pollination market" typically containing dozens of flower species that differ considerably in their nectar and pollen rewards. An individual bee visits hundreds, perhaps even thousands, of flowers each day–so it quickly builds up experience of which flower species are the most profitable and when. But how do bees know which flowers to visit when they leave the nest for the first time? Newly emerged bees that have never seen flowers show distinct preferences for certain colours over others (Lunau et al.; 1996; Briscoe & Chittka, 2001; Chittka & Wells, 2004). For example, the bumblebee (*Bombus terrestris* L.) shows strong preferences for violet or blue throughout its geographic range (Briscoe & Chittka, 2001; Chittka et al., 2004). Such innate colour preferences might help naïve insects to find food, and, possibly even, to select the most profitable flowers from those available. Floral colour preferences can be modified or even overwritten

to some degree by learning, but there is evidence that in some situations (for example when rewards are similar across a range of flower species), bees will revert to their initial, unlearned preferences (Heinrich et al., 1977; Banschbach, 1994; Gumbert, 2000). Our hypothesis is that these innate colour preferences reflect the traits of local flowers that are most profitable for bees. If so, this could mean that when bees leave the nest for the first time, they do so with an inbuilt, unlearned, preference for particular colours which help them to find the most profitable flowers in the local area. Local variation in flower traits could therefore drive selection for particular innate colour preferences in bees, because they would forage more efficiently than bees with different preferences. Plant species in any given location produce flowers of many different colours. These colour differences appear to be linked to both the reliability of finding high nectar rewards (Giurfa et al., 1995), and average amount of sugar available in particular flowers (Chittka et al., 2004). For example, in the area of this study (near Würzburg, Germany) violet flowers were on average four times more productive than blue flowers– the next most rewarding flower colour (Chittka et al., 2004).

In order to test if floral colour preference, or indeed any foraging related trait, is adaptive, one would ultimately want to show that the trait confers greater fitness to its bearers, compared to animals lacking the trait, or that have it in a modified form (Chittka & Briscoe, 2001). In the social bees, matters are somewhat more complicated because reproduction is restricted to a subset of individuals: thus the unit of selection is not the individual, but the entire colony, which works together to maximize the contribution of sexually active individuals to the next generation. Hence, for bumblebees, inter-colony, rather than inter-individual, trait variation allows us to test the adaptive benefits of foraging behaviours within a given ecological framework. One indirect measure of biological fitness conferred on a colony by a trait is foraging performance (Alcock, 1996), as the amount of food available to a bumblebee colony is positively correlated with the production of males and new queens (Schmid-Hempel & Schmid-Hempel, 1998; Pelletier & Mc Neil, 2003). Here we explore inter-colony variation of floral colour preference, a heritable foraging related trait (Chittka & Wells, 2004), to measure the extent to which such preferences can be regarded as adaptive, i.e. improving the foraging performance of the individual bees within a colony, and hence, potentially, overall colony fitness.

METHODS

Fig. 1.
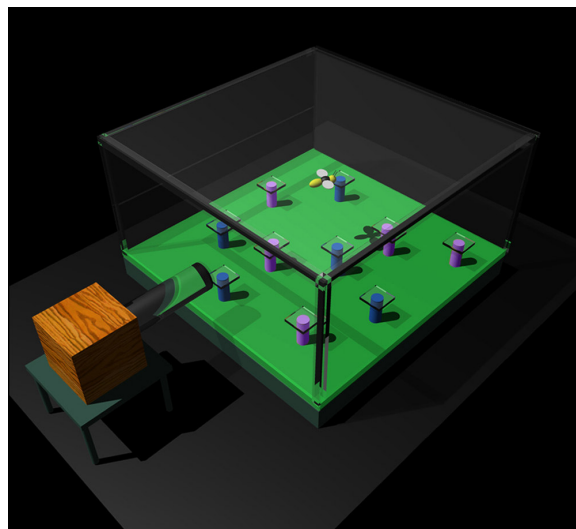
Fig. 1. Schematic representation of the basic setup used for laboratory colour preference experiments. The wooden nestbox (left), containing the bumblebee colony, is connected to a flight arena (right) via a clear Plexiglas tube. The experimenter can control the traffic of bees passing from the nest into the arena (and vice versa) using shutters along the length of the connecting tube. The flight arena contains one or more types of artificial flowers, which differ in colour and presence of reward depending on precise training and test conditions (see methods for full details). Figure design courtesy of R. Beau Lotto.

Bee colour preferences

We tested the innate (unlearned) colour preferences of bumblebees (*B. terrestris*) using artificial flowers presented to them in a laboratory flight arena. The five bumblebee colonies used in this study were obtained from commercial bee breeders. Colonies were kept in darkness (except during necessary observations made under dim red light), under controlled temperature and humidity conditions (27°C and 60% relative humidity), and fed pollen-honey paste *ad libitum* prior to laboratory colour preference and field foraging performance tests.

This rearing procedure minimises the risk that any observed inter-colony differences were caused by non-genetic factors. Bees were never exposed to flower colours prior to experiments–hence they began our colour preference test entirely colour naïve. Nest boxes were connected to a flight arena (120 x 100 x 35cm: see Figure 1) in which workers were allowed to forage for

sucrose solution (50% by volume) from 16 colourless, artificial flowers (UV-transmittent Plexiglas plastic squares: 25 x 25mm). These colourless, rewarding training flowers were placed on vertical transparent glass cylinders (diameter = 10mm; height = 40mm) to raise them above the floor of the flight arena. The flowers (and their cylinder supports) were arranged randomly over the floor of the flight arena. The sucrose solution reward on these colourless training flowers was replenished using a micropipette as soon as it was consumed by foraging bees. All workers in each test colony were marked on the thorax with individually numbered tags (*Opalith Plättchen,* Christian Graze KG, Weinstadt-Endersbach, Germany) so they could be accurately identified. We observed the number of foraging trips (bouts) made into the flight arena by each bee to ensure we only tested the colour preference of bees which were strongly motivated to forage. To test bee colour preference, the 16 rewarding, colourless flowers were replaced by 16 unrewarding, coloured flowers: 8 violet (bee UV-blue) and 8 blue (bee blue: see Figure 2). Thus test (coloured) and training (colourless) flowers differed only in colour, and whether (or not) they provided rewards. The colour preference of each forager was tested individually during a single foraging bout in the flight arena. All flowers were changed between foraging bouts to ensure that subsequent test bees received no odour cues from the previously tested forager. We obtained colony colour preferences from all five colonies by averaging across the 12 forager bees tested per colony (i.e. 60 bees were tested in total). The number of flower choices evaluated per forager ranged from 22 to 55 (mean = 32,9), depending on how long each bee was willing to choose unrewarded flowers. A total of 1978 choices were recorded.

Fig. 2.
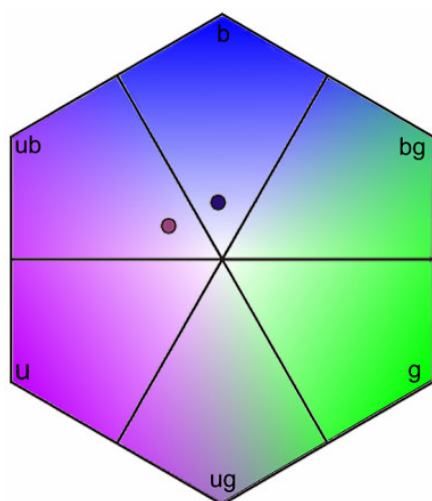
Fig. 2. Bee colour hexagon, which describes how bees perceive coloured objects. The point generated by a coloured object within the hexagon informs us how bees will perceive the object through their ultraviolet, blue and green photoreceptors, and through further processing of receptor signals in the central nervous system. Each object, such as a flower, is categorised into one of the six bee-subjective colour categories defined by the colour hexagon (ultraviolet (u), UV-blue (ub), blue (b), blue-green (bg), green (g), and UV-green (ug)), depending on which of the three colour receptors of bees (UV, blue or green) they stimulated most strongly (Chittka, 1992; Chittka & Kevan, 2005). Hence, colours are categorised as bee-blue if they stimulate the bees' blue receptors substantially more strongly than the UV and green receptors, and are categorised as blue-green if they stimulated the blue and green receptors more or less equally strongly, but stimulated the UV receptor very little, etc. The bee-subjective colour loci of the two artificial flower colours used in the laboratory preference tests, violet (bee UV-blue) and blue (bee-blue), are indicated by circles coloured as they would appear to humans.

Foraging performance

We placed the same five bumblebee colonies, for which we had tested colour preference, in the field (near Gieshügel, Würzburg) to measure their nectar foraging performance over a 3 week period in July 2001. The area is typical central European bumblebee habitat, giving colonies access to multiple flower species in bloom in dry grassland, deciduous forest and farmland. A colourless Plexiglas tunnel with a system of shutters, attached to the entrance of each colony, allowed the observer to control the movements of bees into and out of the nest. Hence the observer could monitor the flow of forager traffic, and record the time and mass of each individual forager when it departed, and returned to, the nest from each foraging bout. We determined the foraging rate of individual workers by dividing the difference in body mass (i.e. return minus outgoing mass) by the duration of the foraging trip (Spaethe & Weidenmüller, 2002; Ings et al., 2005). A total of 649 foraging trips were evaluated for the five colonies. Only trips longer than 10 minutes were considered foraging trips in an effort to exclude orientation and defecation flights (Capaldi & Dyer, 1999; Spaethe & Weidenmüller, 2002).

The five test colonies were left in the field for five weeks after the foraging tests to allow us to quantify the colony production of new queens (gynes). The number of gynes produced by a colony provides a more direct measure of

biological fitness than colony foraging performance. We reduced the nest entrance diameter to 7mm to prevent the escape of new emerged gynes from their natal nest, whilst allowing the smaller foraging workers to pass freely (Goulson et al., 2002).

RESULTS

In the laboratory colour preference tests, there was appreciable variation in the extent to which bumblebee colonies preferred either blue (bee blue) or violet (bee UV-blue) flowers. In the five colonies tested, the average percentage preference for violet over blue ranged from 41% to 56%. This heterogeneity among colonies in their colour preferences was highly statistically significant (Kruskal-Wallis-test; $H = 12.96$; $p = 0.0115$). Those bumblebee colonies with a higher average innate preference for violet in the laboratory harvested more nectar per unit time in the field (Figure 3). This is as one might expect, given that the violet flowers in the local area provide substantially more nectar sugar than blue flowers (Chittka et al., 2004). Although this is a strong positive correlation ($r_s=0.82$) it narrowly misses statistical significance at the 5% level ($p=0.089$), possibly because of the relatively small number of colonies tested (n=5). Although not statistically significant, this positive correlation suggests that colonies with a stronger unlearned preference for violet accumulate, on average, more nectar (energy) during colony development which they could invest in gyne (new queen) production for the next generation.

Fig. 3.
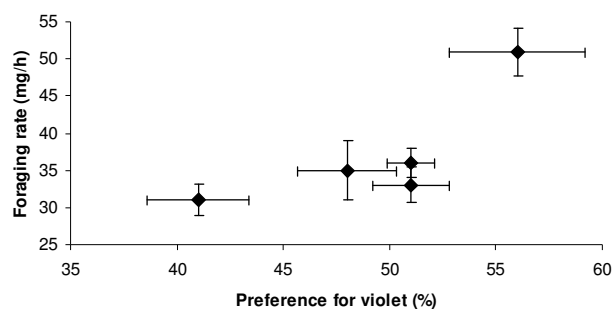
Fig. 3. *Correlation of unlearned (innate) floral colour preference and foraging performance in the wild, measured for five bumblebee colonies (B. terrestris) near Würzburg ($r_s = 0.82$; n = 5; p = 0.089). Each data point represents mean (± 1 S.E.) colony performance, for each of these traits, for one test colony.*

Gyne production per colony ranged from 4 to 39, with the highest number of queens being produced by the

colony with the strongest violet preference (Figure 4). However, any overall positive correlation between colony violet preference and increased queen production is weak ($r_s = 0.46$) and far from statistically significant (p=0.43). Indeed, the colony with the weakest violet preference and the lowest nectar foraging success (Figure 3), produced a relatively large number of gynes (Figure 4). In conclusion, while there is an overall trend for colonies with a stronger violet preference to perform better in an environment with highly rewarding violet flowers, we need more data to ascertain whether this trend is actually biologically meaningful.

Fig. 4.
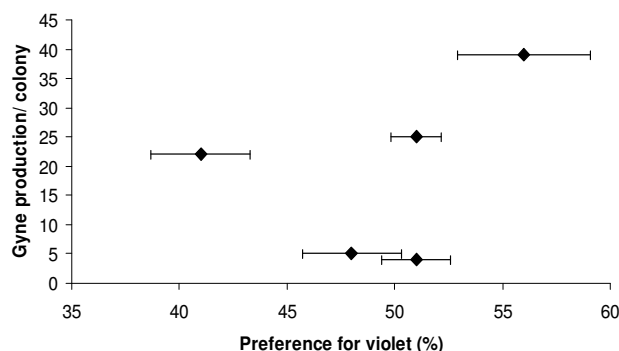
Fig. 4. *Correlation of unlearned floral colour preference and production of new queens (gynes) in five bumblebee colonies ($r_s = 0.46$; n = 5; p = 0.43). Colour preference data represent mean (± 1 S.E.) colony performance, whilst gyne production represents the actual number of new queens produced by each colony.*

DISCUSSION

Previous studies have investigated the potential adaptive significance of bee colour preferences by correlating variation in these preferences amongst either bumblebee species (Chittka & Wells, 2004), or amongst populations within a single bumblebee species (Briscoe & Chittka, 2001; Chittka & Briscoe, 2001; Chittka et al., 2004), with differences in their respective foraging environments. This approach has proven useful - for instance, it provides convincing support for an adaptive explanation the red preference in the American bumblebee species, *Bombus occidentalis* (Greene). Whilst a strong preference for blue and violet appears ubiquitous amongst bumblebee species tested, *B. occidentalis* alone showed a distinct secondary preference for red (Chittka & Wells, 2004). Interestingly, this species collects significant quantities of nectar by

robbing the long corolla tubes of red hummingbird pollinated flowers (Chittka & Waser, 1997; Irwin & Brody, 1999), therefore this derived red preference could represent an adaptation to exploit these red flowers. Whilst such a correlative approach can be revealing, perhaps a more direct way to test the adaptive significance of any trait is to exploit within population trait variation, and investigate if this variation translates into differences in fitness under natural conditions. For bumblebees, the biological fitness of the colony appears to be closely linked to the amount of food available (Schmid-Hempel & Schmid-Hempel, 1998; Pelletier & McNeil, 2003). Hence, we investigated how inter-colony variation in colour preferences might affect both nectar foraging performance (an indirect measure) and colony gyne production (a direct measure of colony fitness).

In order to understand how such variation in colour preferences matters in the economy of nature, we need to quantify the rewards on offer in the floral market - the environment in which foraging bees operate. Have bee colour preferences actually evolved to match floral offerings? Plants with different coloured flowers also differ in the nectar rewards they provide. In a study of 154 flower species (near Berlin, Germany), violet (bee UV-blue) and blue (bee blue) flowers were the most likely floral colours to offer high nectar rewards (Giurfa et al., 1995). In addition to being regularly rewarding, blue and violet flowers commonly appear to be the most productive flower colours in terms of sugar availability (Chittka et al., 2004). Thus, the strong preference for violet and blue shown by at least 8 bumblebee species (Briscoe & Chittka, 2001; Chittka & Briscoe, 2001; Chittka & Wells, 2004), and 8 geographically distinct populations of *B. terrestris* across Europe (Chittka et al., 2004), appears to represent a widespread adaptive response to foraging across a wide range of different floral markets.

However, do local bumblebee populations respond to the specific rewards offered in their local floral market? In the area of our study (near Würzburg, Germany), violet flowers produced on average, four times as much nectar sugar as blue flowers – the next most rewarding flower colour (Chittka et al., 2004). We see a strong trend for colonies with the highest preference for violet (over blue) to forage more effectively under natural conditions. This intriguingly points us towards concluding that a higher preference for violet (over blue) is adaptive in this local area. However, although this is a strong trend, the relationship is not statistically significant at the 5% level: therefore we need more data to confirm our conjecture. It is also interesting that the correlation between the strength of violet preference and gyne production is

weak. This suggests that the improvements in nectar foraging performance related to stronger violet preferences, do not translate directly into gyne production. Potentially colony investment in both the number and size of gynes could be more tightly constrained by protein (from pollen), rather than energy (from nectar) availability. Colony production of sexually active individuals (gynes and males) is clearly a much more direct measure of its biological fitness, than foraging performance. Hence, future studies should also consider quantifying additional measures of fitness, such as the number of males, size of gynes, and the total biomass investment in sexuals made by the colony. In our study, colony fitness does not seem to be predictably influenced by the strength of its preference for violet over blue flowers.

This study clearly illustrates a number of general challenges faced when trying to quantify the fitness impacts of foraging-related traits in bees. First, the traits of interest, such as colour preference and foraging performance, must be measured for a large number of colonies, which requires a large and motivated workforce of experimenters. Indeed measuring the colour preference and foraging performance of five colonies simultaneously represents a large amount of work. Second, even if the traits under examination are somehow correlated with foraging performance, they may have no measurable impact on biological fitness within one generation. However, even if any fitness effect is difficult to measure within a single generation, the effects of that trait may still be important over evolutionary relevant time scales. Finally, other traits, notably parasite resistance (Baer & Schmid-Hempel, 1999), may be so important that they obscure the potential impact of the trait(s) under examination. This is further complicated by the fact that the parasite load may itself also affect foraging behaviour (König & Schmid-Hempel, 1995; Schmid-Hempel & Stauffer, 1998; Otterstatter et al., 2005) and learning performance (Mallon et al., 2003). Therefore, this is not just a lesson in the difficulties involved in *measuring* adaptive significance – it is also a lesson related to the evolution of foraging behaviour itself. If the effects of foraging related traits on biological fitness are relatively hard to measure, or are often obscured by other, unrelated traits, then selection on foraging strategies may itself be relatively weak. Thus foraging related traits may well be sitting on relatively broad adaptive peaks, where deviations from the optimum may not be strongly penalized in terms of fitness costs because of the shape of the adaptive landscape (Gilchrist & Kingsolver, 2001; Whitlock, 1997). If variation in foraging strategies is

indeed sometimes selectively neutral, evolutionary chance processes may play a greater role in between-species or between-population differences than is generally thought.

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