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Effects of aposematic coloration on predation risk in bumblebees? A comparison between differently coloured populations, with consideration of the ultraviolet

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Abstract

It has been proposed that sympatric bumblebee species form mimicry rings to profit from learnt avoidance behaviour by predators. This hypothesis can be tested by comparing the predation rates of local bumblebees with those of imported non-native bumblebees, whose coat colour is different from that of local bees, so that their coloration is unfamiliar to local predators. To test whether populations of non-native bumblebees suffer higher worker loss rates during foraging, we conducted transplant experiments in the UK, Germany and Sardinia. The loss rates of foraging workers of four *Bombus terrestris* populations (*Bombus terrestris canariensis*, *Bombus terrestris terrestris*, *Bombus terrestris sassaricus* and *Bombus terrestris dalmatinus*) were compared, evaluating data from 989 foragers, whose flight times were monitored precisely (over 8258 h of foraging). While all of these workers display a bright UV-reflecting abdominal tip, the colours in other body parts differ strongly to the eyes of avian predators. The hypothesis that foragers from the non-native bumblebee populations, which differ in coloration from the local native population, would suffer higher predation risk was not upheld. In contrast, in one location (Sardinia) the native population had the highest loss rate. The consistent population rank order we found in terms of forager losses indicates that such losses are more prominently affected by factors other than the familiarity of local predators with aposematic colour patterns.

Introduction

In common with many other toxic or venomous animals, the majority of bumblebee species display characteristically bright and visible colour patterns (Plowright & Owen, 1980; Goulson, 2003; Williams, 2007). Typically, these patterns have high contrast between bands of bright colours, such as yellow, white, orange or red and regions of black on their thorax and/or abdomen. Wallace (1879) suggested that such conspicuous coloration could in fact benefit animals by allowing them to directly advertize their unpalatibility as prey items to potential predators. If a predator gets stung or poisoned by a characteristically coloured potential prey item, it should learn to associate the specific coloration pattern with the painful and unpleasant experience and hence avoid it in future (Howse & Allen, 1994; Ruxton, Sherratt & Speed, 2004; Gilbert, 2005; Mappes, Marples & Endler, 2005; Chittka & Osorio, 2007). Indeed, birds (Mostler, 1935) as well as toads (Brower, Brower & Westcott, 1960) have been shown to make such negative associations with bumblebees, and avoid them as potential prey items once they have become experienced with their noxiousness. Potentially, the effect of such warning (aposematic) coloration could expand beyond prey species boundaries if more than one unpalatable or venomous species display the same, or similar, warning coloration (Müllerian mimicry: Mallet & Joron, 1999).

To date, several mimicry rings have been suggested among bumblebee faunas worldwide, including at least four in Europe (Plowright & Owen, 1980; Prys-Jones & Corbet, 1991; Gilbert, 2005; Williams, 2007). The proposed European mimicry rings display the following patterns of body coloration: (1) entirely black except for a red or an orange tip to the abdomen (tail); (2) broad yellow-and-black bands with a white tail; (3) broad yellow-and-black bands with a red, orange, yellow or brown tail; or (4) entirely tawny brown. However, these sets of species were assembled based entirely on human visual assessments of similarity, when it is much more appropriate to consider similarity as perceived by the visual systems of the animal predators that commonly eat bumblebees (Cuthill & Bennett, 1993; Endler & Mielke, 2005). Because of the often pronounced differences in colour vision between species, some signals that appear distinct for human observers will not be so for other animal species and vice versa – hence, any exploration of colour

mimicry requires consideration of the receiver receptor system.

Comparing the coat coloration and patterning of workers from different populations (subspecies) of the common European bumblebee species *Bombus terrestris* (Linnaeus 1758), there are substantial differences between several distinct populations (Vogt, 1911; Estoup *et al.*, 1996; Velthuis & van Doorn, 2006; Rasmont *et al.*, 2008). For example, *Bombus terrestris terrestris* (Linnaeus 1758) from Central Europe, *Bombus terrestris dalmatinus* (Dalla Torre 1882) from the eastern Mediterranean region and *Bombus terrestris audax* (Harris 1776) from Great Britain all have a very similar appearance. Workers from all three populations are predominantly black with two yellow bands, one each on the thorax and abdomen, with a white tip to their abdomen (Fig. 1a). Workers of the Sardinian population, *Bombus terrestris sassaricus* (Tournier 1890), differ in appearance as they lack the yellow band on the thorax, and have reddish-brown legs. Workers from both the Canary Island *Bombus terrestris canariensis* (Pérez 1895) and Corsican *Bombus terrestris xanthopus* (Kriechbaumer 1870) populations entirely lack all yellow bands. Reflectance in the ultraviolet, which is an essential component of the vision of avian insectivores (Cuthill & Bennett, 1993), has not been explored so far, and we endeavour to fill this gap here.

If it is true that predators learn to avoid bumblebee workers with local, familiar coloration, it is predicted that workers of visually distinct, non-native populations face a higher local predation risk. In order to test this hypothesis, we evaluated the results from several transplant experiments, to compare the loss rate of workers from native and non-native populations. Choosing a central-place forager like bumblebees has a major advantage compared with previous transplant studies, which addressed this question using butterflies and mark–recapture techniques (Mallet & Barton, 1989; Kapan, 2001): bumblebee workers return to the nest after each foraging bout, whereas members of many other species have no particular motivation to remain near a location where they have been released; hence differences in recapture rates might in fact reflect differences in propensity to disperse. Using bumblebees, we were able to record the total amount of time each worker spent foraging outside the nest and therefore, crucially, the total amount of time each colour morph was actually exposed to potential predators. We could then compare the loss rates of workers from populations with different colour patterns. Our setup allowed us to reach large sample sizes, recording data for 989 foragers exposed to predators for over 8258 h outside the nest (see Table 1).

Materials and methods

Spectral reflectance measurements of bee colour coats

The spectral reflectance curves of freshly freeze-killed bees were measured using a spectrophotometer (AvaSpec-2048, Avantes, Eerbeek, the Netherlands) in the UV and visible range, and a calibrated light source (DH2000, Ocean Optics, Dunedin, FL, USA); the setup is described in Chittka & Kevan (2005). In addition, we inserted a special attenuator (Inline Fibre Optic Attenuator, 0–100%; 200–2000 nm; Avantes) into the light path from the light source to the probe to allow spectral reflectance measurements of small

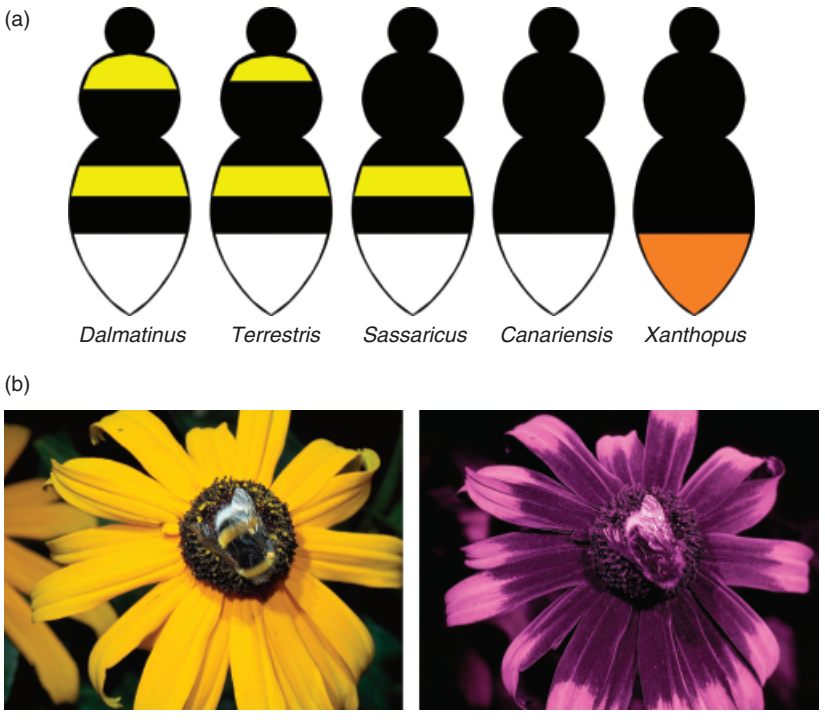

Figure 1 Colour patterns of workers of different *Bombus terrestris* populations. (a) Colour patterns of workers of five *B. terrestris* populations as they appear to human eyes. (b) Worker of *Bombus terrestris dalmatinus*, on a *Rudbeckia fulgida* flower, photographed in the visible (left) and ultraviolet light (right) revealing strong UV reflectance of the abdomen.

Table 1 Overview of the data collected during the transplant experiments

Location	Population	Foragers	Lost foragers	Flight time (h)	Loss rate (% h^{-1})
(a) Sardinia 2000					
	B. t. sassaricus	51	4	270.2	0.029
	B. t. terrestris	65	6	776.0	**0.012**
	B. t. canariensis	79	20	581.0	0.044
(b) Sardinia 2001					
Block A	*B. t. sassaricus*	50	21	197.0	0.213
	B. t. terrestris	40	20	642.6	0.078
	B. t. canariensis	58	18	646.5	**0.048**
Block B	*B. t. sassaricus*	40	7	90.0	0.194
	B. t. terrestris	60	28	459.0	0.102
	B. t. canariensis	52	9	455.0	**0.038**
Block C	*B. t. sassaricus*	33	2	183.7	0.033
	B. t. terrestris	42	5	276.5	0.043
	B. t. canariensis	33	2	231.3	**0.026**
(c) Germany 2001					
Block A	*B. t. sassaricus*	18	6	51.4	0.648
	B. t. terrestris	38	10	157.8	0.167
	B. t. canariensis	37	5	98.3	**0.138**
Block B	*B. t. sassaricus*	28	2	72.0	0.099
	B. t. terrestris	16	1	89.2	0.070
	B. t. canariensis	42	2	120.9	**0.039**
Block C	*B. t. sassaricus*	37	5	160.0	0.084
	B. t. terrestris	16	1	76.9	0.081
	B. t. canariensis	11	0	58.9	**0**
(d) UK 2004					
	B. t. canariensis	19	3	94.5	0.167
	B. t. dalmatinus	23	4	239.1	**0.073**
(e) UK 2005					
	B. t. canariensis	70	29	1475.1	0.028
	B. t. dalmatinus	31	4	755.6	**0.017**

Foraging data and loss rates of 25 *Bombus terrestris* colonies from four populations (*Bombus terrestris sassaricus, Bombus terrestris terrestris, Bombus terrestris canariensis and Bombus terrestris dalmatinus*) in (a) Sardinia 2000, (b) Sardinia 2001, (c) Germany 2001, (d) UK 2004 and (e) UK 2005. Data presented in each column are: (1) location where the transplant experiment took place; (2) *Bombus terrestris* population used; (3) the total number of bees which left the nest (foragers); (4) the total number of bees which left the nest and did not return; (5) the total recorded flight time (hours) of bees which left the nest (and returned); (6) loss rate (proportion of foragers lost per hour). For each colony the loss rate was calculated using the formula: loss rate = (number of lost foragers/total number of foragers)/total flight time of foragers [h]. The population with the lowest loss rate in each independent population comparison is shown in bold. The local native coloration in each experimental location is represented by *B. t. sassaricus* in Sardinia, *B. t. terrestris* in Germany and *B. t. dalmatinus* in the UK (see 'Methods' for further explanation). The mean loss rates of the populations at the different locations are plotted in Fig. 3.

target areas ($\varnothing$ 2 mm). To calculate colour receptor signals, we used the blue tit *Cyanistes caeruleus* as a representative avian insectivore. Blue tit colour vision has input from four photoreceptor types (single cones), whose sensitivity is determined by the opsin visual pigment as well as oil droplets and ocular media that filter incoming light; the receptors are maximally sensitive in the UV ([ultraviolet sensitive (UVS); λ_{max} = 374 nm], the blue [short wavelength sensitive (SWS); λ_{max} = 455 nm], the green [medium wavelength sensitive (MWS); λ_{max} = 539 nm] and the red [long wavelength sensitive (LWS); λ_{max} = 607 nm: Hart, 2001; Hart & Vorobyev, 2005). The so-called double cones consist of a large principal cone, filtered by an oil droplet whose absorptance varies somewhat between dorsal and ventral eye regions, and a smaller accessory cone which, in blue tits, does not have an oil droplet (Hart *et al.*, 2000). Double cones are thought not to contribute to colour vision, but to be important in motion and shape vision (Hart & Hunt, 2007); hence, we also calculated their responses to bumblebee colour patterns. As the extent to which principal and accessory members are optically and electrically coupled is not fully known, we calculated separate quantum catches for the two types; we also calculated principal cone quantum catches for the two subtly different types of oil droplets present in the dorsal and ventral eye regions. The spectral sensitivity curves for single and double cones in conjunction with their respective oil droplets and filtering by ocular media have been kindly provided by Nathan Hart (University of Western Australia).

All passeriform birds studied so far possess a tetrachromatic set of single cones, with limited interspecific variation in the tuning of photopigments (Bowmaker *et al.*, 1997;

Hart, 2001). Among the 12 different passerines studied, for example, the wavelengths of maximum absorbance ranged from 355 to 380 nm for the UV pigment, 440 to 454 nm for the short-wave pigment, 497 to 504 nm for the medium-wave pigment and 557 to 567 nm for the long-wave pigment (Hart, 2001). The blue tit thus serves as a typical example for a passerine bird. The relative amount of light absorbed by each spectral photoreceptor type (i) is

$$P_i = R_i \int_{300}^{700} I_S(\lambda) S_i(\lambda) D(\lambda) \mathrm{d}\lambda$$

where $I_S(\lambda)$ is the spectral reflectance function of the stimulus and $S_i(\lambda)$ is the spectral sensitivity function of the receptor. The spectral sensitivity curves take into account the filtering effects on incoming light of the oil droplets and ocular media (Hart & Vorobyev, 2005). $D(\lambda)$ is the illuminant (daylight normfunction D65: Wyszecki & Stiles, 1982) and R_i is a sensitivity factor which for each receptor (i) is adjusted so that a quantum catch corresponding to a white surface equals unity.

For UV photography (Fig. 1b), we used a Nikon D70 digital SLR camera (Tokyo, Japan) fitted with a Nikon UV Nikkor f4.5/105 mm lens and a Baader U-Filter (Baader Planetarium, Germany: 310–390 nm UV transmission) mounted on a Nikon AF-1 gel filter holder. The transmission function of the Baader U-filter can be found in Verhoeven & Schmitt (2010), confirming that this lens transmits the UV exclusively, with no significant transmittance in any other spectral domain. To completely prohibit any long-wave contamination (including in the IR), we used a high power chip type UV LED ($\lambda_{max} = 365$ nm; model: NCSU033A(T), Nichia Corporation, Tokushima, Japan) as a light source. First we took a white light comparison shot, with the lens stopped down at f11 for sufficient depth of field. Then, we flipped up the Baader U-filter mounted on the AF-1 filter holder. Exposure was 25 s at f11 and ISO400. The raw digital images were developed using Bibble Pro (© Bibble Labs Inc., Austin, TX, USA) to remove digital noise, sharpen and white-balance images.

Locations and study populations

Five transplant experiments were performed in total. They were carried out in two separate locations on the island of Sardinia (Costa Rei, autumn 2000, and Monte Padru, spring 2001), one in Germany (Würzburg, summer 2001: for details see Ings, Schikora & Chittka, 2005b) and two in Britain (London, summer 2004 and late spring 2005). Four commercially available *Bombus terrestris* populations were chosen: *B. t. canariensis* from the Canary Islands, *B. t. sassaricus* from Sardinia and *B. t. terrestris* from Central Europe were used in Sardinia and Germany, whilst *B. t. canariensis* and *B. t. dalmatinus* (the native population of south-eastern Europe and Turkey) were used in London. *Bombus terrestris dalmatinus* was chosen for use in London because the native British population (*B. t. audax*) is not supplied by commercial breeders, but the workers of both populations (*B. t. dalmatinus* and *B. t. audax*) are extremely similar in appearance (Ings, Raine & Chittka, 2005a). In total, 25 colonies were used, which were distributed across the individual experiments as follows: Sardinia 2000: one colony each of *B. t. sassaricus*, *B. t. terrestris* and *B. t. canariensis* (three colonies in total); Sardinia 2001: three colonies each of *B. t. sassaricus*, *B. t. terrestris* and *B. t. canariensis* (nine colonies in total); Germany 2001: three colonies each of *B. t. sassaricus*, *B. t. terrestris* and *B. t. canariensis* (nine colonies in total); UK 2004: one colony each of *B. t. dalmatinus* and *B. t. canariensis* (two colonies in total); UK 2005: one colony each of *B. t. dalmatinus* and *B. t. canariensis* (two colonies in total). Bumblebee colonies were purchased from Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands), except the *B. t. terrestris* for the German experiment (2001), which were obtained from Bunting Brinkman Bees (Tilburg, Belgium).

General methods of data collection

The colonies were housed in the field in specially designed bipartite plywood nest boxes, whose entrance consisted of a long transparent Plexiglas tunnel with a system of shutters to enable movements of bees in and out of the nest to be controlled by observers. All workers in each colony were marked with individually numbered tags (*Opalith Plättchen*, Christian Graze KG, Weinstadt-Endersbach, Germany), which allowed us to obtain a complete foraging record for every forager. During each observation period, all marked bees were allowed to leave and enter the nest at will; the departure and arrival time for each bee was recorded. A completed trip outside the nest is referred to as a foraging bout. Outside these observation periods, shutters were closed. Males and newly emerged queens were never allowed to leave the colonies, to prevent any non-native bees from establishing themselves as a result of our experiments.

The mass of all workers was measured on each departure from and arrival to the nest (see Ings *et al.*, 2005*b* for methods). One hour before the end of the daily observation period, further workers were prevented from leaving the nest, thus minimizing the chances of foragers returning to the nest outside the observation period. Bees that returned outside observation periods were returned to their colony the next morning. Before placement in the field, all colonies were fed pollen and artificial nectar *ad libitum*. The colonies were also fed in the field during poor weather when no observations took place.

Experiments in Sardinia and Germany

In experiments conducted in Sardinia and Germany in 2001, three sets (blocks) of observations were carried out consecutively (for further details see Ings *et al.*, 2005*b*). Each block consisted of one colony from each of the three populations: *B. t. sassaricus*, *B. t. terrestris* and *B. t. canariensis* (an additional block, i.e. three more colonies, was observed in Sardinia 2000). New colonies were used for each block. All three colonies within each block were placed simultaneously in the field within 5 m of each other. Observations began

immediately and were carried out simultaneously on all three populations. All colonies were monitored continuously between 08:00–19:00 h during dry weather. The total duration of observations varied between blocks depending upon the weather and ranged from 4 to 16 days.

Experiments in the UK

One colony of each population (*B. t. canariensis* and *B. t. dalmatinus*) was placed on the roof of the Fogg Building, Queen Mary University of London in 2004 and 2005. In 2004, both colonies were monitored continuously between 1000–1700 h on 20 days (between 2 July and 3 August 2004) during dry weather. In 2005, both colonies were monitored continuously between 07:00–21:00 h for 10 consecutive days (20–29 May 2005). Colonies were kept inside the building overnight to protect them from harsh weather conditions. Observations began 10 min after the colonies were placed outside each day. Outside the stated observation hours, colonies were replaced by empty nest boxes to provide returning workers with overnight shelter. Empty nest boxes were also placed outside for two days after the observation period and checked regularly for returning foragers.

Data analysis

We calculated the following variables for each test colony: (1) number of workers leaving the nest, that is potential foragers; (2) number of bees not returning from a foraging bout (henceforth 'lost' bees); (3) the total flight time of all completed foraging bouts. For each colony, the loss rate (proportion of foragers lost per hour) was calculated using the formula: loss rate = (number of lost foragers/total number of foragers)/total flight time of foragers (h). Foragers that did not return on the last day of observations, or on a day before a break in non-consecutive observations, were excluded from analyses. This is because such workers might not have been lost, but returned after the termination of experiments. Population loss rates were compared using a mixed general linear model, using colony as a random factor.

We also explored whether variation in body mass affected mortality. Paired *t*-tests were used to assess potential differences in body mass between lost bees versus bees that returned to the colony (21 colonies). Body masses of the foragers tested during the experiment in Sardinia 2000 (from three colonies) were not available; thus only masses of 22 (of 25) colonies were available for this analysis. A further colony was excluded from this analysis as no bees were lost during the entire experiment. Body mass in *B. terrestris* is strongly correlated with body size (Goulson *et al.*, 2002; Spaethe & Weidenmüller, 2002). For consistency across lost and returning bees, we used the departure mass of each bee on its first foraging bout. The numbers of bees tested and the total flight times analysed are presented for each colony in Table 1.

Results

Colour analysis

It was found that the white tip of the abdomen in all populations reflects UV light strongly, except the Corsican *B. t. xanthopus*, whose tail is orange-red and UV absorbing (Figs 1a and 2a). The receptor signals in an insectivorous bird's eye of the black, yellow and white body parts were indistinguishable between populations (Kruskal–Wallis test; $P > 0.1$ for all comparisons). Black body parts generate low quantum catches in all receptors (Fig. 2b), whereas white parts stimulate all receptors, although signals fall somewhat from long to short wave photoreceptors. Note that the relatively strong UV signals in these white body regions is in marked contrast with most flowers that appear white to humans – such flowers typically absorb all UV light (below *c*. 400 nm: Kevan, Giurfa & Chittka, 1996). The white segments of the abdomen did not produce any between-population differences in visual appearance to birds for the populations for which we collected data on loss rates. In future, it would be interesting to test *B. t. xanthopus*, whose coloration, including UV reflectance, differs entirely from all other populations of the species (Figs 1a and 2). Other body parts in all populations are UV absorbing, but between-population differences in the distribution of colours in the (human) visible light spectrum are clearly discriminable to avian predators. The quantum catches for principal and accessory members of double cones are presented in Table 2.

Analysis of loss rates

In Sardinia and Germany, there were significant differences in loss rate (proportion of foragers lost per hour) among *B. terrestris* populations ($F_{2,4.769} = 7.903$, $P = 0.031$: Fig. 3). In neither location did the native population have the lowest loss rate (Table 1), and in both Sardinia and Germany the same relative pattern of mean loss rates was observed among the three tested populations (*B. t. sassaricus* > *B. t. terrestris* > *B. t. canariensis*: Fig. 3). In fact, there was no significant effect of location on the relative losses of the three tested populations ($F_{1,2} = 0.313$, $P = 0.632$). In Sardinia, the native population (*B. t. sassaricus*) actually suffered the *highest* mean loss rate (mean ± SE = 0.117 ± 0.050% of foragers lost per hour). That is more than twice that of *B. t. terrestris* (0.059 ± 0.020%) and three times that of *B. t. canariensis* (0.039 ± 0.005%) at that location. In Germany, *B. t. sassaricus* again suffered the highest mean loss rate (0.277 ± 0.185%), followed by the native population *B. t. terrestris* (0.106 ± 0.031%) and *B. t. canariensis* (0.059 ± 0.041%). In contrast, results from the UK experiments were less clear cut. Although the population representing the local native coloration (*B. t. dalmatinus*) had a lower mean loss rate (0.045 ± 0.028%: Fig. 3) than *B. t. canariensis* (0.098 ± 0.070%), this difference was not statistically significant ($F_{1,1} = 1.597$, $P = 0.426$). Considering potential size differences, we found no difference in the

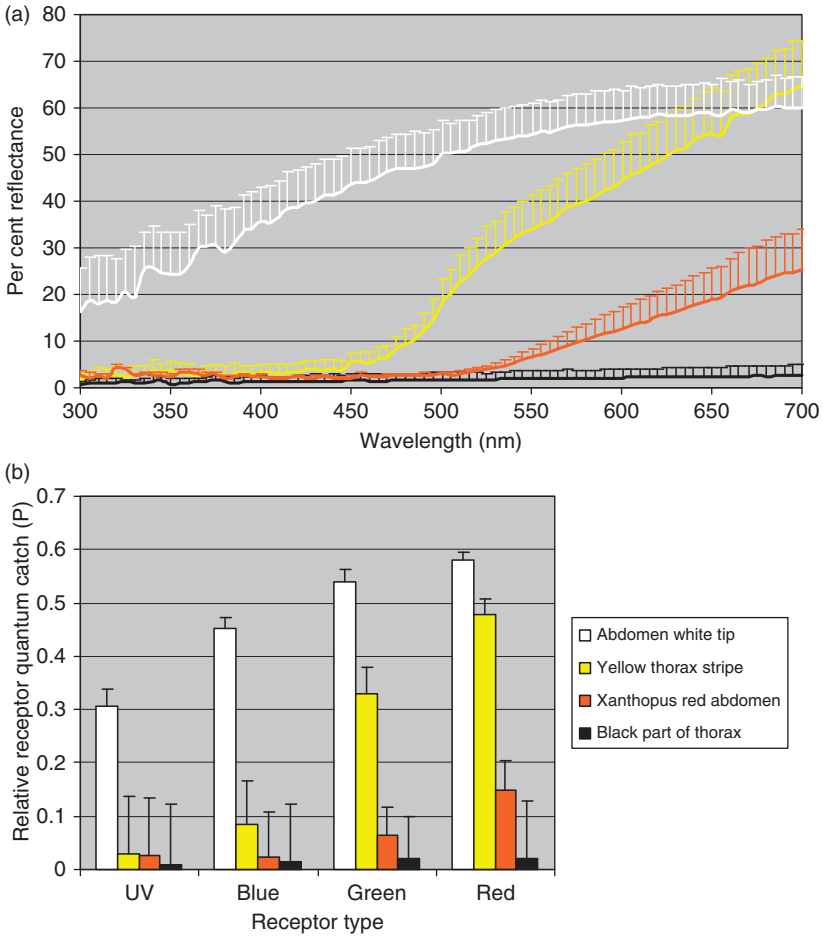

Figure 2 Reflectance properties of bee colour coat viewed by avian predators. (a) Average spectral reflectance functions (± SD) of the various body regions of *Bombus terrestris* workers. 100% reflectance is defined as the light reflected from 'white standard' (barium sulphate). (b) Relative receptor quantum catches (P) in birds' UV (UV-sensitive), blue (short wavelength sensitive), green (medium wavelength sensitive) and red (long wavelength sensitive) receptors of the various body regions, where a 100% (ideal white) reflector will generate a quantum catch of one.

Table 2 Relative receptor quantum catches in double cones for the differently coloured body parts of bumblebees *Bombus terrestris*

	Principal cone	Accessory cone
Abdomen white tip	0.539	0.523
Yellow thorax stripe	0.335	0.313
Bombus terrestris xanthopus red abdomen	0.082	0.078
Black part of thorax	0.019	0.019

Values are given separately for principal and accessory cones. Principal cones have slightly different oil drop filters in dorsal and ventral eye regions; however as the relative quantum catches for these regions did not differ when measured to the third decimal place values are therefore not reported separately for distinct eye regions. White, yellow and black regions did not differ between populations and are therefore given as average quantum catches for all populations. Only Corsican *Bombus terrestris xanthopus* bees have a red abdomen, so values for this region are given separately. Receptor sensitivities are scaled so that the response to an ideal white stimulus (100% reflectance over the entire spectrum) equals 1.

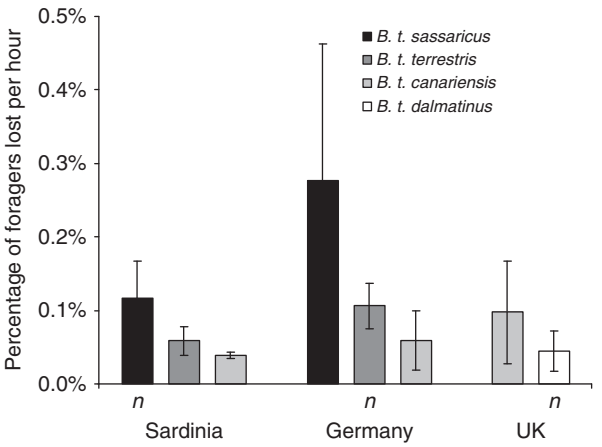

Figure 3 Comparison of forager loss rates among the four *Bombus terrestris* populations (*Bombus terrestris sassaricus*, *Bombus terrestris terrestris*, *Bombus terrestris canariensis* and *Bombus terrestris dalmatinus*) in Sardinia, Germany and the UK. Columns represent the mean (± 1 SE) loss rate per population (the proportion of foragers lost per hour) in each study location. Populations with the local native coloration for experimental location are indicated by an *n* under the appropriate column.

departure weight of lost bees compared with those that returned to the nest (paired *t*-test, $t = 1.17$, d.f. = 20, $P = 0.256$).

Discussion

Our spectral analysis found UV reflectance of the white abdominal segments of all *Bombus terrestris* populations except the Corsican one, although some authors have dismissed the possibility of such reflectance (Williams, 2007). However, even though this UV reflectance did not differ among the bumblebee populations used in our transplant experiments, there are clear and highly visible differences in the colour patterns of the tested populations from the perspective of a potential avian predator, and more importantly, between the respective native populations and some of the ones we introduced experimentally. This is especially true for the black and white *B. t. canariensis*, which is visually dissimilar from any native species or bumblebee population in all of the habitats tested: there are simply no similarly patterned large, plainly black-and-white insect species in Sardinia, South Germany or England – hence these bees' appearance should have been wholly unfamiliar to local insectivores.

However, in our study, native populations did not consistently have the lowest loss rates. On the contrary, in Sardinia, the native population actually had the highest losses. This suggests that a pattern of body coloration unfamiliar to local predators did not appear to expose bumblebees to a higher predation risk at the three sites studied here. Therefore, at these locations, there appears to be no evidence of strong selection pressure towards the convergence of bumblebee colour pattern, mediated by predators. This is surprising, given that local mimicry rings are currently the most commonly accepted explanation for why bumblebees at mid latitudes exhibit particular colour patterns (Plowright & Owen, 1980; Williams, 2007). Nonetheless, we are confident in the power of our data. First, there is no risk of subconscious experimenter bias: the data were collected with an objective that was entirely different from the study subject here (Chittka, Ings & Raine, 2004; Ings *et al.*, 2005*b*). Second, our sample sizes of almost 1000 foragers completing more than 8258 h of foraging flights (Table 1) are considerably larger than all other transplant or release/recapture studies of which we are aware. Collecting data from a larger number of bees would further increase confidence in our results; however, for the study sites where we observed significant population differences in loss rate, our sample sizes were already large (Sardinia: 603 foragers, from 12 colonies, completed over 4808 h of foraging flights; Germany: 243 foragers, from nine colonies, completed over 885 h of foraging flights), and we found no evidence of any specific colony exerting high leverage on our dataset. Finally, because we have used a central-place forager, we have a complete record of times spent in flight and numbers of foragers lost, which avoids many of the typical complications with mark–recapture studies where the animals' activities over a relevant time period remain unknown and the possibility that there might be differences in the animals' propensity to leave the observation area, or the ability to hide from the experimenters' view. It is important to point out that it is not the number of colonies tested that matters for statistics, but the number of occasions that each colour pattern was potentially presented to predators – so it is the product of the number of foragers tested with the time that these foragers spent in the field that matters for assessments of predation risk.

The predators presumed to drive selection towards such colour pattern convergence are insectivorous birds because they rely strongly on visual, particularly colour, cues to identify prey items (Mostler, 1935; Gilbert, 2005). However, it is currently unknown whether birds will only avoid prey that are extremely similar to items that they have experienced as noxious, or whether they will form broad categories by shape, flight behaviour and sound; therefore, including bumblebees of all colour patterns (Chittka & Osorio, 2007; Chittka, Skorupski & Raine, 2009), which would not give native bumblebees in any one location a particular advantage.

One possibility is that it is not the familiarity of local predators with local aposematic patterns that determines predation risk, but the overall efficiency of aposematic coloration. This might explain why the rank order of loss rates of three populations is the same in Germany and Sardinia, where *B. t. sassaricus* consistently suffered the highest loss rates and *B. t. canariensis* suffered the lowest (with the German *B. t. terrestris* at intermediate levels). However, inspection of the banding patterns (Fig. 1) of these three populations shows the highest number of contrasting boundaries in *B. t. terrestris*, and the lowest in *B. t. canariensis*, with *B. t. sassaricus* being intermediate, and thus not matching the rank order of loss rates by a conspicuousness ranking.

Other reasons for the absence of an effect of local predator familiarity on differences in mortality between the tested bee populations could be that different causes of mortality, that is ones unrelated to visual appearance of the bumblebees, might be more significant at these study sites. Crab spiders, waiting on a flower to ambush foraging bees (Chittka, 2001), or robberflies (Goulson, 2003) are unlikely to distinguish potential prey items based on differences in their coloration. Other natural enemies such as parasitoid conopid flies could also infect bees outside the nest, modifying their subsequent post-infection behaviour (Müller & Schmid-Hempel, 1993; Müller, 1994), ultimately affecting predation and other risk factors.

There remains the question of what causes the apparent similarity in appearance between bumblebee species (Plowright & Owen, 1980; Williams, 2007) and local populations of distinct species (Rasmont *et al.*, 2008) in several locations. In our view, the hypothesis of mimicry rings remains plausible, but it is also clear from our data that factors other than similarity with locally common species can substantially overshadow any effects that would be in line with the mimicry hypothesis. Perhaps avian predator pressure (and the resulting selection on bee similarity) is only strong in

some years but not others, or it acts more on gynes than on workers, but our data clearly defy a simple explanation of the local convergence of bumblebee colour patterns.

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