The Journal of Experimental Biology 216, 285-291

doi:10.1242/jeb.075374

RESEARCH ARTICLE

Social scent marks do not improve avoidance of parasites in foraging bumblebees

Bertrand Fouks* and H. Michael G. Lattorff

Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg Hoher Weg 4, 06099 Halle (Saale), Germany

*Author for correspondence (bertrand.fouks@zoologie.uni-halle.de)

SUMMARY

Foraging is a result of innate and acquired mechanisms, and is optimized in order to increase fitness. During foraging, an animal faces many threats, such as predation and infection. The uptake of parasites and diseases while foraging is common and an individual should be adapted to detect and avoid such threats, using cues from either the abiotic environment or the parasite. Social animals possess an additional cue to detect such contaminated food sources: information provided by conspecifics. Bumblebees avoid contaminated flowers, but the cues used by the bees to distinguish contamination remain unknown. Under controlled laboratory conditions, we tested the use of scent marks derived from other foragers in choosing between a contaminated (by *Crithidia bombi*) and an uncontaminated flower. As a positive control we tested the bee's choice between two flowers, one scented with geraniol and containing a highly rewarding sugar solution, and the other not scented and containing a poorer reward. The bees mainly chose the uncontaminated and the rewarding scented flowers. Scent marks did not increase the efficiency of the bumblebees in choosing the better flower. The bees from both experiments behaved similarly, showing that the main and most relevant cue used to choose the uncontaminated flower is the odour from the parasite itself. The adaptation of bumblebees to avoid flowers contaminated by *C. bombi* arose from the long-term host–parasite interaction between these species. This strong adaptation results in an innate behaviour of bees and a detection and aversion of the odour of contaminated flower nectar.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/2/285/DC1

Key words: *Bombus terrestris*, *Crithidia bombi*, host–parasite interaction, social cue, social immunity, social learning.

Received 25 May 2012; Accepted 16 September 2012

INTRODUCTION

Foraging behaviour and its optimization was and still remains a centre of evolutionary, ecological and neuroscience research. Solitary animals rely on environmental cues and their experience to forage; in social animals, an additional level appears that is composed of the signals, cues and information given by conspecifics in order to choose a resource patch. While foraging, many threats appear such as predators and parasites, leading to a drastic decrease of the fitness of an organism. Thus, organisms should have evolved in order to detect and avoid such threats. In the case of parasitism, the first barrier against it is the avoidance of parasites, which may be less costly than immune responses. The incidence of parasites is of great importance for foraging behaviour and has even been implemented into the optimal foraging models (Lozano, 1991).

In order to detect parasitic threats, an organism can rely on evidence from the environment and also from the parasite itself (Hart, 1990). When living in a society, animals can cooperate to avoid parasites. Indeed, ants and termites avoid any direct contact with parasitic flies, helminths and fungi (reviewed in Cremer et al., 2007). This is called social immunity, as this avoidance depends on the cooperation of a social group. Other levels of social immunity exist, such as hygienic behaviour in honeybees (Wilson-Rich et al., 2009) and allogrooming, where social groups cooperate or behave altruistically to reduce the effect of the parasite on the whole group (Cremer et al., 2007). Moreover, living in a group facilitates learning *via* conspecifics, known as social learning, which may lead to the evolution of culture in many vertebrate species (Heyes and Galef, 1996). Social learning appears to be of a great importance in honeybees, bumblebees and even in fruitflies and crickets (Battesti et al., 2012; Chittka and Leadbeater, 2005; Coolen et al., 2005; Kawaguchi et al., 2006). The combination of social learning and social immunity has been observed in mammals, e.g. primates (Huffman et al., 2010). However, in invertebrates this has never been studied.

The bumblebee *Bombus terrestris* (Linnaeus 1758) is a model species for investigating foraging mechanisms (Hodges, 1985). Bumblebees use both innate and learning mechanisms to find resource patches (Plowright et al., 2006), and the social cues allow them to optimize their foraging efficiency (Goulson, 1999). They are able to learn which flowers are the most rewarding with the help of the flower, social cues and experience (Hudon and Plowright, 2011; Kawaguchi et al., 2006; Leadbeater and Chittka, 2009; Plowright et al., 2011).

Bumblebees are eusocial insects with an annual life cycle, whose colonies are founded by a single, once-mated queen in early spring. Their social life and the low genetic diversity within a colony make them a prime target for parasites. Their social organisation provides parasites with a stable and rich environment (Schmid-Hempel, 1998). The low genetic variability within a colony, due to the single mated and unique queen, allows parasites to easily infect every individual within it (Baer and Schmid-Hempel, 1999; Baer and Schmid-Hempel, 2001). However, their social life also provides them with a different way to fight against a parasite or disease, so-called social immunity (Cremer et al., 2007). There are different

levels of social immunity, from the uptake of the parasite to its transmission to the next generation (Cremer et al., 2007). Social immunity may occur in the presence of a parasite (activated response) but also in the absence of parasites (prophylactic response) (Cremer et al., 2007; Richter et al., 2012).

Bumblebees are parasitized by *Crithidia bombi* (Lipa and Triggiani, 1988) (Trypanosomatida), a well-adapted gut parasite of bumblebees (Schmid-Hempel, 2001). This parasite decreases drastically the chance for a future queen to found a new colony, and also the size and the efficiency of new colonies (Brown et al., 2003). According to the Red Queen hypothesis (Bell, 1982; Decaestecker et al., 2007), this long-term relationship leads to an arms race. Recently, Fouks and Lattorff (Fouks and Lattorff, 2011) discovered an avoidance behaviour in foraging bumblebees of flowers contaminated either by a specific parasite (*C. bombi*) or by a common microorganism (*Escherichia coli*: Bacteria).

The combination of activated social immunity during foraging behaviour exhibited in bumblebees is of importance as parasites might be taken up on shared food patches (Durrer and Schmid-Hempel, 1994). The foraging behaviour of the bees is influenced by parasites (Fouks and Lattorff, 2011) and as such the fitness of flowers might be influenced indirectly.

Here, we investigate the interaction of social information and innate preference in avoiding unrewarding or contaminated flowers. In order to determine which cues the bumblebees use to choose the rewarding (non-contaminated) flower, we recorded the flower choice of bumblebees over a period of 6 days with two different experimental setups: one where the flowers were cleaned in order to remove scent cues left by conspecifics, and the other where the flowers were not cleaned. In addition, to investigate the mechanism used by the bees to distinguish both flowers, we used a positive control with the same setup without contamination but where the most rewarding flower was scented with geraniol.

MATERIALS AND METHODS

Bumblebees

Bumblebees from three different colonies were used for the experiment (Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands). One colony was used for the geraniol experiment, while two other colonies were used for the *C. bombi* experiment in order to control for any colony-specific effects. From each original colony, two batches of 25 marked bumblebees (with Opalithplättchen, ApisPro, Hoher Neuendorf, Germany) were housed in a metal cage (14.5×12×2.5 cm) containing empty honey pots on a wax frame, and were provided with pollen *ad libitum*. Each bee was trained to fly and feed on an artificial flower for 5 min, three times a day during a 3 day trial period. The flower consisted of a blue foam paper (Ø 6 cm) glued onto a piece of wood placed on a plastic cylinder (Ø 2.8 cm, 4.5 cm length); an Eppendorf tube (0.2 ml) was placed in the centre of the flower. The artificial flower was filled with a solution of honey water and washed after each trial with ethanol (50%) (Leadbeater and Chittka, 2009). The foraging trial and experiment occurred in a flight arena (1×0.4×0.5 m terrarium, with the ground covered by green Kraft paper) with the flower placed towards the light source. After these 3 days of training, only the bumblebees that were feeding were kept for the experiment. All the bumblebees were flower naive before training.

For the experiment, each bee was placed in a flight arena and given a choice between two artificial flowers (as described above), 10 cm apart from each other and equidistant from the bumblebee entrance. Each group of bees was tested four times a day over a period of 6 days. In one flight arena, the flower was washed after every trial with ethanol (50%) in order to eliminate any cues that would help the bees choose between the two flowers (referred to as the individual setup), and in the other flight arena the artificial flowers were not washed in order to allow the bees to use the scent marks left on the flower by their conspecifics (referred to as the group setup). The position of flowers was switched regularly between the trials in order to avoid any position bias.

The duration before the bee landed, where she landed, the time period of feeding, and whether she switched between flowers after the first landing or after feeding were recorded. When the bee spent more than 3 min without landing on a flower, she was put back with her sub-colony.

Geraniol experiment

As a positive control we used a strong odour to indicate the rewarding flower to the bee. We used a sponge to apply a diluted solution of geraniol (5 µl:50 ml, >90%, Carl Roth, Karlsruhe, Germany) on the flower containing the most rewarding 'nectar' consisting of sucrose water (50:50, v:v), while the other flower contained a more diluted sucrose solution (30:70, v:v). One colony was used; the 'group setup' sub-colony was composed of 12 individuals, and the 'individual setup' sub-colony was composed of 11 individuals.

Crithidia bombi experiment

The *C. bombi* experiment consisted of one flower with a sucrose solution (50:50, v:v; referred to as the rewarding flower), and the other flower containing the same sucrose solution (50:50, v:v) but including a concentration of 3000 cells ml^{-1} of *C. bombi* (strain 076 provided by P. Schmid-Hempel, ETH Zurich) (referred to as the unrewarding flower). *Crithida bombi* was cultivated in cell cultures and cell number was quantified according to a standard method (Popp and Lattorff, 2011). In order to avoid any odour or cue from the medium, *C. bombi* cells were washed two times with pure water before preparation of the sucrose solution. Two colonies were used for this experiment; the two 'group setup' sub-colonies contained 13 and 12 individuals, and the two 'individual setup' sub-colonies contained 14 and 12 individuals.

Molecular analyses

After the experiment, all bees were snap-frozen. Their guts were removed and crushed in 300 µl of Aqua Dest laboratory water (J. T. Baker, Deventon, The Netherlands). DNA was extracted from a 100 µl aliquot of the homogenate using the Chelex method (Walsh et al., 1991). DNA was used to genotype samples using a multiplex PCR with the microsatellite primers Cri 4, Cri 4G9, Cri 1.B6 and Cri 2F10 (Schmid-Hempel and Reber Funk, 2004) according to the method described by Erler et al. (Erler et al., 2012). Fragment lengths were determined by means of a Megabace 1000 capillary DNA sequencer (Amersham Biosciences, Freiburg, Germany). The area of the peaks for each microsatellite allele was calculated using the software Fragment Profiler (Amersham Biosciences).

The intensity of the fluorescence signal of the microsatellite alleles (peak height/area in electropherogram) determined by a capillary sequencer (MegaBace 1000, Amersham Biosciences) has been shown to be correlated to the intensity of infection (B.F. and H.M.G.L., unpublished). Thus to determine the infection intensity, we used the peaks of the microsatellite locus Cri 1.B6, which gives the most reliable estimate (B.F. and H.M.G.L., unpublished). The area of the peaks was compared between the different setups (group and individual) using a Mann–Whitney *U*-test. Additionally, a linear regression between the overall proportion of visits on the

uncontaminated flower of every bee and the area of the peak was performed.

Allometry analysis

The size of bumblebees is well known to have an effect on their foraging efficiency and learning ability (Chittka and Niven, 2009; Spaethe et al., 2007; Spaethe and Weidenmüller, 2002). To rule out any potential bias between the different setups for the *C. bombi* experiment, the size of the bees was determined by quantifying the length between two junctions of veins on their forewings, as wing length is highly correlated to body size (Hunt et al., 1998; Klingenberg et al., 2001; Müller et al., 1996; Muller and Schmid-Hempel, 1992). Wings were removed, mounted on object slides and digitised. Calculations were performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Using wing size as a proxy for body size of the bees, we tested for the influence of body size comparing the setups (group and individual) using a Mann–Whitney *U*-test. We performed a linear regression between the overall proportion of visits on the uncontaminated flower of every bee and their size. Furthermore, we realised a linear regression between the peak's area of the microsatellite Cri 1.B6 (the intensity of infection of an individual) and the size of the bee.

Statistical analyses

All statistics were realised with R software (R Development Core Team, 2011).

Behavioural assays

The avoidance behaviour exhibited by bumblebees was expected to increase with the presence of scent marks on flowers and over days as a result of social and associative learning.

The data for feeding duration for each experiment were log transformed and analysed with a generalised linear mixed model (GLMM) (Bates et al., 2008), including individual as a random factor to account for pseudo-replication within individuals. The reward/contamination status of the flower (rewarding/uncontaminated or unrewarding/contaminated), the position (left or right) and the setup (group or individual) were included as fixed factors in the models. For all GLMMs, the distribution of all response variables and their residuals were inspected for symmetry and overdispersion. For model building and simplification (backward stepwise deletion), we followed the practical guide developed by Bolker et al. (Bolker et al., 2009) and Crawley (Crawley, 2005).

The number of visits was analysed for both experiments (geraniol and *C. bombi*) by a GLMM with a Poisson distribution including reward and position as explanatory factors and individual and day of recording as random factors in order to account for pseudo-replication within individuals.

We assigned a value of 1 for a visit on the uncontaminated flower and 0 for a visit on the contaminated flower. The proportion of visits on the rewarding flower was analysed by a GLMM with a binomial distribution including setup (group and individual) and position (left or right) and day as fixed factors and individual as a random factor to account for pseudo-replication within individuals.

For switching between flowers, both after landing and after feeding, we assigned a value of 1 when a bee switched from one flower to the other and 0 when the bee stayed on the first flower. The proportion of switches to the other flower after landing and after feeding were analysed for both experiments (geraniol and *C. bombi*) by a GLMM with a binomial distribution including flower reward (rewarding or unrewarding), setup (group and individual), position (left or right) and day of recording as fixed factors, and individual as a random factor to account for pseudo-replication.

RESULTS

Behavioural assays

Geraniol experiment

As expected, bees fed longer and more often on the most rewarding and geraniol-scented flowers (GLMM: $P<0.001$; Fig. 1A,B, supplementary material Table S1). Over days, the bees showed a decreased efficiency feeding on the scented flower, showing a loss of flower constancy; the position of the flower influenced the choice of the bees but not significantly (GLMM: the best model is the model containing the position and day as explanatory factors, position: $P=0.144$, day: $P<0.05$; see supplementary material Table S1). In addition, the bees switched from one flower to the other more often when landing and feeding first on the unrewarding flower (GLMM: $P<0.001$; Fig. 1C,D, see supplementary material Table S1). This indicates that bees are more attracted to flowers with the odour of geraniol, and when landing or feeding on the unrewarding flower,

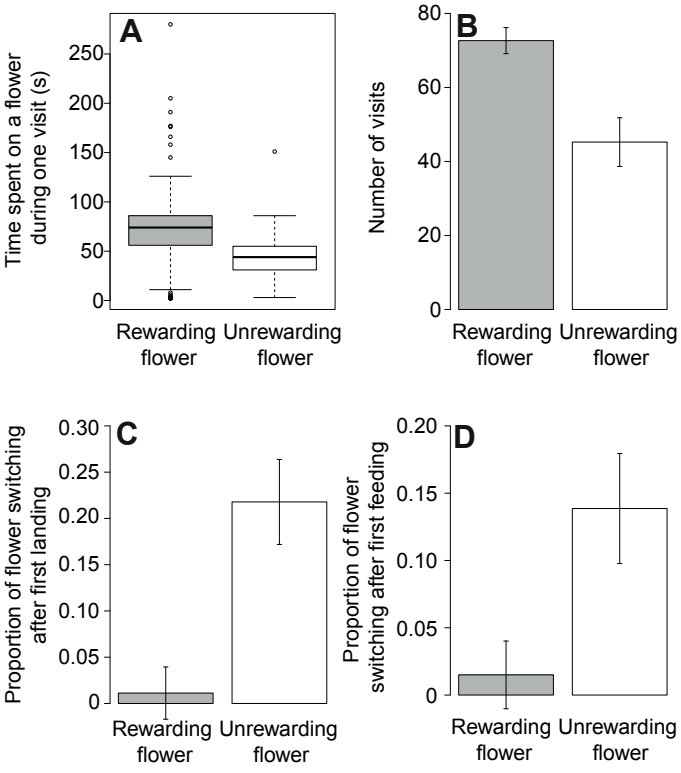

Fig. 1. Feeding duration, flower preference and flower switching after landing and feeding for the geraniol experiment. (A) Feeding duration on flowers with and without the presence of geraniol (N=368). (B) Visit frequency on flowers with and without the presence of geraniol for each individual for the overall trial (N=368). (C) Proportion of flower switching after the first landing on the unrewarding or rewarding flower (N=18). (D) Proportion of flower switching after the first feeding on the non-rewarding or rewarding flower (N=25). For feeding duration, box plots depict medians, interquartile range and non-outlier range; the dots represent the outliers. The bars represent the means between the different colonies and their 95% confidence intervals. Foragers feed longer on the most rewarding flower (GLMM: $P<0.001$), and visit preferentially the scented flower (GLMM: $P<0.001$). The proportion of flower switching is higher when landing and feeding first on the less rewarding flower (GLMM: $P<0.001$ and $P<0.001$, respectively).

potentially due to a mistake, they change to the most rewarding flower.

Crithidia bombi experiment

We found that bumblebees fed longer and more often on the uncontaminated flower than on the one containing the parasite (GLMM: $P<0.001$; Fig. 2A,B, see supplementary material Table S1). The bees behaved similarly, but less efficiently than in the geraniol experiment. When examining the proportion of workers foraging on the uncontaminated flower according to the setup, it appears that the scent marks did not affect the efficiency of the bees in choosing the non-contaminated flower (Fig. 3B). The bees were more efficient when the uncontaminated flower was on the left (for the bee), and showed a non-significant difference over days in their efficiency in choosing the uncontaminated flower (GLMM: the best model is the model containing the position and day as explanatory factors, position: $P<0.05$, day: $P=0.117$; see supplementary material Table S1). For switching to the other flower, the bees reacted in the same way as for the geraniol experiment but less efficiently; they changed from one flower to the other more often after landing or feeding first on the contaminated flower (GLMM: $P<0.001$, Fig. 2C; GLMM: Fig. 2D, $P<0.05$; see supplementary material Table S1).

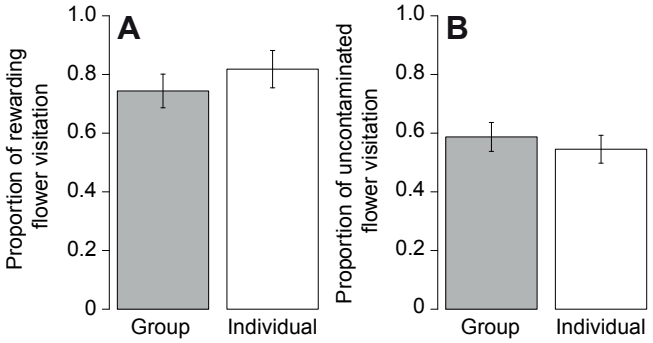

Fig. 3. Proportion of rewarding/uncontaminated flower visitation with and without scent marks for both geraniol and *C. bombi* experiments. (A) Proportion of rewarding flower visitation between the two setups for the geraniol experiment (group: $N=203$; individual: $N=165$). (B) Proportion of uncontaminated flower visitation between the two setups for *C. bombi* experiment. The bars represent the means between the different colonies and their 95% confidence intervals. The use of the scent marks did not significantly improve the efficiency of the bees in feeding on the rewarding flower (geraniol: the best model does not include the setup as a fixed factor; *C. bombi*: no model was better than the model containing no explanatory factor; supplementary material Table S1).

Molecular assays

First, we confirmed that the infection of the bees was due only to the strain of *C. bombi* applied to the flowers. The multilocus genotypes were identical between the cultivated strain and the infection determined in the bee guts. When comparing the infection intensity between the two setups, it seems that the washing of the flower decreased the degree of infection of the bees (Mann–Whitney *U*-test: $Z=2.14$, $P<0.05$; Fig. 4). The ability of the bees to choose the uncontaminated flower did not affect the intensity of infection, showing a transmission of the parasites directly from one individual to another inside the nest (linear regression: $r^2=0.018$, $P=0.17$).

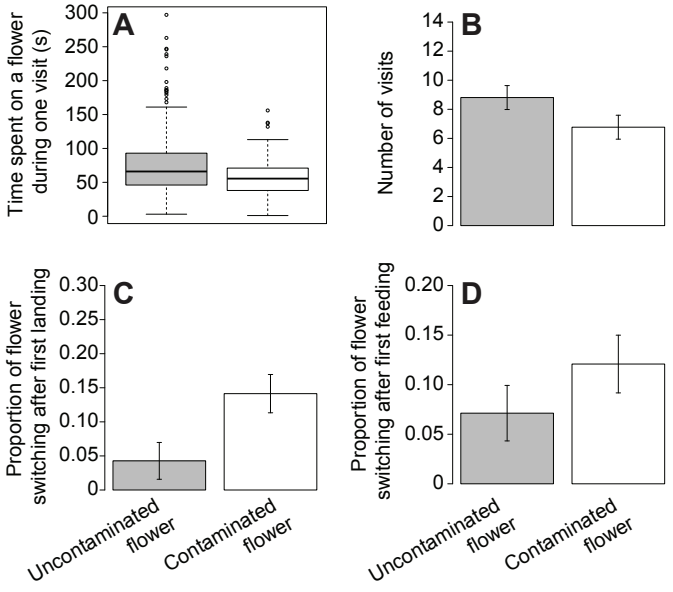

Fig. 2. Feeding duration, flower preference and flower switching after landing and feeding for the *Crithidia bombi* experiment. (A) Feeding duration on flowers with and without the presence of the parasite ($N=810$). (B) Visit frequency on flowers with and without the presence of the parasite for each individual for the overall trial ($N=810$). (C) Proportion of flower switching after the first landing on the uncontaminated or contaminated flower ($N=77$). (D) Proportion of flower switching after the first feeding on the uncontaminated or contaminated flower ($N=73$). For feeding duration, box plots depict medians, interquartile range and non-outlier range; the dots represent the outliers. The bars represent the means between the different colonies and their 95% confidence intervals. Foragers feed longer on the uncontaminated flower (GLMM: $P<0.001$), and visit preferentially the uncontaminated flower (GLMM: $P<0.001$). The proportion of flower switching is higher when landing and feeding first on the contaminated flower (GLMM: $P<0.001$ and $P<0.05$, respectively).

Allometry assays

No bias between setups was found for the size distribution of the bees (Mann–Whitney *U*-test: $Z=0.47$, $P=0.65$). There was also no correlation between the size of a bee and their performance in choosing the uncontaminated flower (linear regression: $r^2=0.001$, $P=0.31$). In addition, the intensity of infection was not correlated with the size of the bee (linear regression: $r^2=-0.019$, $P=0.82$).

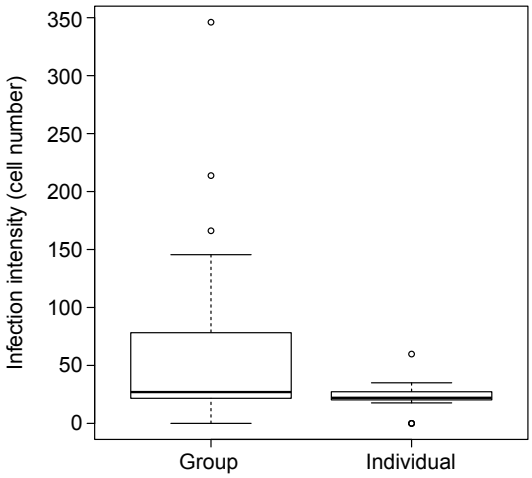

Fig. 4. Intensity of infection with regard to the presence (group) or absence (individual) of scent marks ($N=51$). Box plots depict medians, interquartile range and non-outlier range; the dots represent the outliers. Washing of the flower decreases the chance of a bee being reinfected (Mann–Whitney *U*-test: $Z=2.14$, $P<0.05$).

DISCUSSION

In accordance with previous results (Fouks and Lattorff, 2011), worker bees in the present study exhibited avoidance behaviour of flowers contaminated by *C. bombi*. Bees treated contamination as a decrease of the reward of the 'nectar'. Indeed, the same pattern between the geraniol and *C. bombi* experiments has been observed for the number of visits and their duration (Fig. 1A, Fig. 2A). Furthermore, the bees avoided the contaminated flower as a result of the odour from contamination because they visited the uncontaminated flower more often without any other clue differentiating the flowers (Fig. 3). There was also no clear indication that the bees learned to choose the flower without contamination over the duration of the study period, indicating that the avoidance of the contaminated flower is an innate response. Finally, bees more often switched to the rewarding flower after landing on the non-rewarding, contaminated flower (Fig. 2D), emphasising the repellent effect of contamination for the bees.

Scent marks and their significance have been well studied (Goulson et al., 1998; Goulson et al., 2000; Goulson et al., 2001; Leadbeater and Chittka, 2009; Leadbeater and Chittka, 2011; Saleh and Chittka, 2006; Saleh et al., 2006; Saleh et al., 2007; Witjes and Eltz, 2007; Witjes and Eltz, 2009). On the one hand, some studies have shown that scent marks act as repellents for experienced bees, allowing them to choose rewarding flowers more efficiently, as previous visitors might have reduced the available nectar (Goulson et al., 1998; Goulson et al., 2001). On the other hand, some studies report the contrary (e.g. Witjes and Eltz, 2007). Finally, other studies showed that bees react to scent marks as a function of their previous experience (Leadbeater and Chittka, 2009; Saleh and Chittka, 2006). Recently, it has been shown that naive bees have no preference, neither for flowers already visited nor for those unvisited (Leadbeater and Chittka, 2011). Scent marks are mainly composed of cuticular hydrocarbons, and they correspond to footprint cues rather than pheromone signals (Goulson et al., 2000; Saleh et al., 2007; Wilms and Eltz, 2008; Witjes and Eltz, 2009). These substances are non-volatile and even tiny differences in their quantities – which accumulate on the flower after each visit and remain unchanged over a period of 24 h – are detectable by social insects (D'Ettorre, 2008; Saleh et al., 2007; Witjes and Eltz, 2009). In our experiment, the scent marks do not increase or decrease the efficiency of the bees in choosing the rewarding flower. This could be due to the fact that both flowers were visited. Even so, the scent marks should have accumulated more on the uncontaminated flower, allowing the bees to choose it more easily. The other possibility is that scent marks are not really useful in facilitating the choice of bees between contaminated or uncontaminated flowers because of the strong cue given by the odour of the parasite (Fig. 3). For example, some ungulates avoid fields contaminated by feces containing parasites (Fankhauser et al., 2008; Fleurance et al., 2007). It has also been shown that leaf-cutter ants can discriminate the fungus strain and reject foreign fungus by the odour of the fungus (Ivens et al., 2008). And recently, it has been shown that *Drosophila* avoid bad smells (Wasserman et al., 2012). The smell might not be directly produced by the parasite, but could be an unavoidable interaction of the parasite and the substrate or stem from the metabolic secretion of the parasite. Indeed, the presence of yeasts inside the nectar of flowers might produce specific odours (Raguso, 2004).

Moreover, bees use scent marks through experience and learning; the latter might be impaired by an immune challenge and/or *C. bombi* infection, as both are known to decrease learning ability (Alghamdi et al., 2008; Gegear et al., 2006). However, a decrease of learning ability has been observed only when bees are given visual cues, whereas for the odour cues the immune response does not decrease the learning ability of the bees (Gegear et al., 2006). This corroborates our results regarding the efficiency of bees in choosing the uncontaminated flower based on its infection load. Nonetheless, bees having a supplementary cue with which to choose the flower do not feed significantly more on the uncontaminated flower than bees presented only with the odour of the 'nectar' (Plowright et al., 2011). Other social cues could have been gathered by the bees in the 'individual' setup, such as the odour from the honey pots or from conspecifics (Battesti et al., 2012; Dornhaus and Chittka, 2005; Renner and Nieh, 2008). Bees use the odour from honey pots and/or conspecifics to find the same flower species as their conspecifics (e.g. lavender nectar will produce a different honey odour than geranium nectar). So the odour from honey pots and/or conspecifics is an attractant and is not repellent. This allows us to conclude that bees did not use the odour from honey pots and/or conspecifics because only the parasite possesses an repellent odour in our experiment.

In a previous experiment (Fouks and Lattorff, 2011) we found that bees at the social and individual levels seem to learn to forage preferentially on uncontaminated flowers over a period of days. In this experiment, entire colonies were placed in the foraging arena; bees were allowed to forage simultaneously on the flowers, and so could rely on their nest-mates to choose the flower. In the present study, we did not find such a significant pattern, but the number of trials per day and bee was lower and might not be sufficient to detect a significant learning pattern. It is likely that this learning is strengthened due to social learning *via* copying behaviour, which has been observed in primates who learn to eat medicinal leaves by observation (Huffman et al., 2010), and in crickets learning from others to avoid predation (Coolen et al., 2005). Indeed, copying behaviour is very important for naive bees, which copy more experienced bees in order to choose certain flowers (Grüter et al., 2010; Kawaguchi et al., 2006; Leadbeater and Chittka, 2005; Worden and Papaj, 2005). Furthermore, infected bees demonstrate impaired learning of visual cues (Alghamdi et al., 2008; Gegear et al., 2006) and reduce their foraging activity after infection because of the immune challenge (Otterstatter et al., 2005). For naive bees this could lead to reliance on conspecifics, which have better learning efficiency and so should feed more often on the uncontaminated flower.

The higher infection intensity in the group of bees foraging on scented flowers is probably due to novel infections directly obtained from the flower. Indeed, it has already been observed that bees transmit *C. bombi via* the flower (Durrer and Schmid-Hempel, 1994), and this was confirmed in our experiment. We directly observed bees defecating on the flower, which in the case of infected bees might lead to a deposit of new *C. bombi* cells. In the individual setup, the flower was washed with ethanol, killing *C. bombi* cells, whereas in the group setup, we did not wash the flower, allowing bees to infect themselves directly from the faeces of infected bees.

As previously shown, in the *C. bombi* experiment the bees have a better ability to recognise the uncontaminated flower when it is on their left side (right side for the observer) (Anfora et al., 2011; Fouks and Lattorff, 2011). The explanation for the side preference remains unclear. Bumblebees have a better ability to learn an odour using their right antenna than their left antenna (Anfora et al., 2011). They also show preferences in the direction of circling (Kells and Goulson, 2001). This combination of left–right asymmetries could result in a preference for visiting a certain position without even choosing the flower in that position. Here, the preference for visiting uncontaminated flowers on the left could be due to the higher

rejection rate combined with the higher visitation rate to contaminated flowers on the right.

Another surprising result is the decreased efficiency of the bee in feeding on the geraniol-scented flower over time. Even if the reward of the unscented flower was lower, it might still be high enough for the bees to select this flower. This choice might be determined by the internal sucrose responsiveness threshold of every bee, a feature that is strongly influenced by genetic factors, at least in honeybees (Rueppell et al., 2006). Thus, bumblebees were first attracted strongly by the scented flower, but over time this attractiveness could have decreased as they realised that the other flower was also rewarding.

In conclusion, scent marks did not help the bees to choose the rewarding flower. The odour from the contaminated sucrose solution is sufficient for the bees to avoid it, despite a quite high error rate. The high error rate is not so surprising given that their ability to distinguish an odour is weak compared with their ability to use visual cues (Gegear et al., 2006; Milet-Pinheiro et al., 2012).

ACKNOWLEDGEMENTS

The authors thank V. Nehring for some advice with the statistics, J. H. Kidner for help with language editing, and two anonymous reviewers for helpful comments.

FUNDING

This work was supported by a Bundesministerium für Bildung und Forschung (BMBF) (Federal Ministry of Education and Research, Germany) grant (FKZ: 0315126 to H.M.G.L.).

REFERENCES

Alghamdi, A., Dalton, L., Phillis, A., Rosato, E. and Mallon, E. B. (2008). Immune response impairs learning in free-flying bumble-bees. *Biol. Lett.* **4**, 479-481.
Anfora, G., Rigosi, E., Frasnelli, E., Ruga, V., Trona, F. and Vallortigara, G. (2011). Lateralization in the invertebrate brain: left-right asymmetry of olfaction in bumble bee, *Bombus terrestris*. *PLoS ONE* **6**, e18903.
Baer, B. and Schmid-Hempel, P. (1999). Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature* **397**, 151-154.
Baer, B. and Schmid-Hempel, P. (2001). Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. *Evolution* **55**, 1639-1643.
Bates, D., Maechler, M. and Dai, B. (2008). lme4: Linear mixed-effects models using 449 S4 classes. R Package Version 0.999375-18. Available at http://lme4.r-forge.r-project.org/.
Battesti, M., Moreno, C., Joly, D. and Mery, F. (2012). Spread of social information and dynamics of social transmission within *Drosophila* groups. *Curr. Biol.* **22**, 309-313.
Bell, G. (1982). *The Masterpiece of Nature: the Evolution and Genetics of Sexuality*. London, UK: Croom Helm.
Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J.-S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127-135.
Brown, M. J. F., Schmid-Hempel, R. and Schmid-Hempel, P. (2003). Strong context-dependent virulence in a host–parasite system: reconciling genetic evidence with theory. *J. Anim. Ecol.* **72**, 994-1002.
Chittka, L. and Leadbeater, E. (2005). Social learning: public information in insects. *Curr. Biol.* **15**, R869-R871.
Chittka, L. and Niven, J. (2009). Are bigger brains better? *Curr. Biol.* **19**, R995-R1008.
Coolen, I., Dangles, O. and Casas, J. (2005). Social learning in noncolonial insects? *Curr. Biol.* **15**, 1931-1935.
Crawley, M. (2005). *Statistics: An Introduction Using R*. Chichester, UK: John Wiley & Sons Ltd.
Cremer, S., Armitage, S. A. O. and Schmid-Hempel, P. (2007). Social immunity. *Curr. Biol.* **17**, R693-R702.
D'Ettorre, P. A. and Hughes D. P. (2008). *Sociobiology of Communication: an Interdisciplinary Perspective*. Oxford, UK: Oxford University Press.
Decaestecker, E., Gaba, S., Raeymaekers, J. A. M., Stoks, R., Van Kerckhoven, L., Ebert, D. and De Meester, L. (2007). Host–parasite 'Red Queen' dynamics archived in pond sediment. *Nature* **450**, 870-873.
Dornhaus, A. and Chittka, L. (2005). Bumble bees (*Bombus terrestris*) store both food and information in honeypots. *Behav. Ecol.* **16**, 661-666.
Durrer, S. and Schmid-Hempel, P. (1994). Shared use of flowers leads to horizontal pathogen transmission. *Proc. R. Soc. Lond. B* **258**, 299-302.
Erler, S., Popp, M., Wolf, S. and Lattorff, H. M. G. (2012). Sex, horizontal transmission, and multiple hosts prevent local adaptation of *Crithidia bombi*, a parasite of bumblebees (*Bombus* spp.). *Ecol. Evol.* **2**, 930-940.
Fankhauser, R., Galeffi, C. and Suter, W. (2008). Dung avoidance as a possible mechanism in competition between wild and domestic ungulates: two experiments with chamois *Rupicapra rupicapra*. *Eur. J. Wildl. Res.* **54**, 88-94.
Fleurance, G., Duncan, P., Fritz, H., Cabaret, J., Cortet, J. and Gordon, I. J. (2007). Selection of feeding sites by horses at pasture: testing the anti-parasite theory. *Appl. Anim. Behav. Sci.* **108**, 288-301.
Fouks, B. and Lattorff, H. M. G. (2011). Recognition and avoidance of contaminated flowers by foraging bumblebees (*Bombus terrestris*). *PLoS ONE* **6**, e26328.
Gegear, R. J., Otterstatter, M. C. and Thomson, J. D. (2006). Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proc. R. Soc. Lond. B* **273**, 1073-1078.
Goulson, D. (1999). Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. *Perspect. Plant Ecol. Evol. Syst.* **2**, 185-209.
Goulson, D., Hawson, S. A. and Stout, J. C. (1998). Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. *Anim. Behav.* **55**, 199-206.
Goulson, D., Stout, J. C., Langley, J. and Hughes, W. O. H. (2000). Identity and function of scent marks deposited by foraging bumblebees. *J. Chem. Ecol.* **26**, 2897-2911.
Goulson, D., Chapman, J. W. and Hughes, W. O. H. (2001). Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *J. Insect Behav.* **14**, 669-678.
Grüter, C., Leadbeater, E. and Ratnieks, F. L. W. (2010). Social learning: the importance of copying others. *Curr. Biol.* **20**, R683-R685.
Hart, B. L. (1990). Behavioral adaptations to pathogens and parasites: five strategies. *Neurosci. Biobehav. Rev.* **14**, 273-294.
Heyes, C. M. and Galef, B. G. (1996). *Social Learning in Animals: the Roots of Culture*. London, UK: Academic Press.
Hodges, C. M. (1985). Bumble bee foraging: energetic consequences of using a threshold departure rule. *Ecology* **66**, 188-197.
Hudon, T. and Plowright, C. (2011). Trapped: assessing attractiveness of potential food sources to bumblebees. *J. Insect Behav.* **24**, 144-158.
Huffman, M. A., Spiezio, C., Sgaravatti, A. and Leca, J. B. (2010). Leaf swallowing behavior in chimpanzees (*Pan troglodytes*): biased learning and the emergence of group level cultural differences. *Anim. Cogn.* **13**, 871-880.
Hunt, G. J., Guzmán-Novoa, E., Fondrk, M. K. and Page, R. E., Jr (1998). Quantitative trait loci for honey bee stinging behavior and body size. *Genetics* **148**, 1203-1213.
Ivens, A. B. F., Nash, D. R., Poulsen, M. and Boomsma, J. J. (2009). Caste-sapecific symbiont policing by workers of *Acromyrmex* fungus-growing ants. *Behav. Ecol.* **20**, 378-384.
Kawaguchi, L. G., Ohashi, K. and Toquenaga, Y. (2006). Do bumble bees save time when choosing novel flowers by following conspecifics? *Funct. Ecol.* **20**, 239-244.
Kells, A. R. and Goulson, D. (2001). Evidence for handedness in bumblebees. *J. Insect Behav.* **14**, 47-55.
Klingenberg, C. P., Badyaev, A. V., Sowry, S. M. and Beckwith, N. J. (2001). Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. *Am. Nat.* **157**, 11-23.
Leadbeater, E. and Chittka, L. (2005). A new mode of information transfer in foraging bumblebees? *Curr. Biol.* **15**, R447-R448.
Leadbeater, E. and Chittka, L. (2009). Bumble-bees learn the value of social cues through experience. *Biol. Lett.* **5**, 310-312.
Leadbeater, E. and Chittka, L. (2011). Do inexperienced bumblebee foragers use scent marks as social information? *Anim. Cogn.* **14**, 915-919.
Lipa, J. J. and Triggiani, O. (1988). *Crithidia bombi* sp. n., a flagellated parasite of a bumble-bee *Bombus terrestris* L. (Hymenoptera, Apidae). *Acta Protozool.* **27**, 287-290.
Lozano, G. A. (1991). Optimal foraging theory: a possible role for parasites. *Oikos* **60**, 391-395.
Milet-Pinheiro, P., Ayasse, M., Schlindwein, C., Dobson, H. E. M. and Dötterl, S. (2012). Host location by visual and olfactory floral cues in an oligolectic bee: innate and learned behavior. *Behav. Ecol.* **23**, 531-538.
Müller, C. B. and Schmid-Hempel, P. (1992). Variation in life-history pattern in relation to worker mortality in the bumble-bee, *Bombus lucorum*. *Funct. Ecol.* **6**, 48-56.
Müller, C. B., Blackburn, T. M. and Schmid-Hempel, P. (1996). Field evidence that host selection by conopid parasitoids is related to host body size. *Insectes Soc.* **43**, 227-233.
Otterstatter, M. C., Gegear, R. J., Colla, S. R. and Thomson, J. D. (2005). Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behav. Ecol. Sociobiol.* **58**, 383-389.
Plowright, C. M. S., Simonds, V. M. and Butler, M. A. (2006). How bumblebees first find flowers: habituation of visual pattern preferences, spontaneous recovery, and dishabituation. *Learn. Motiv.* **37**, 66-78.
Plowright, C. M. S., Evans, S. A., Leung, J. C. and Collin, C. A. (2011). The preference for symmetry in flower-naïve and not-so-naïve bumblebees. *Learn. Motiv.* **42**, 76-83.
Popp, M. and Lattorff, H. M. G. (2011). A quantitative in vitro cultivation technique to determine cell number and growth rates in strains of *Crithidia bombi* (Trypanosomatidae), a parasite of bumblebees. *J. Eukaryot. Microbiol.* **58**, 7-10.
R Development Core Team (2008). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
Raguso, R. A. (2004). Why are some floral nectars scented? *Ecology* **85**, 1486-1494.
Renner, M. and Nieh, J. (2008). Bumble bee olfactory information flow and contact-based foraging activation. *Insectes Soc.* **55**, 417-424.
Richter, J., Helbing, S., Erler, S. and Lattorff, H. M. G. (2012). Social context dependent immune gene expression in bumblebees (*Bombus terrestris*). *Behav. Ecol. Sociobiol.* **66**, 791-796.

Rueppell, O., Chandra, S. B. C., Pankiw, T., Fondrk, M. K., Beye, M., Hunt, G. J. and Page, R. E. (2006). The genetic architecture of sucrose responsiveness in the honeybee (*Apis mellifera* L.). *Genetics* **172**, 243-251.
Saleh, N. and Chittka, L. (2006). The importance of experience in the interpretation of conspecific chemical signals. *Behav. Ecol. Sociobiol.* **61**, 215-220.
Saleh, N., Ohashi, K., Thomson, J. D. and Chittka, L. (2006). Facultative use of the repellent scent mark in foraging bumblebees: complex versus simple flowers. *Anim. Behav.* **71**, 847-854.
Saleh, N., Scott, A., Bryning, G. and Chittka, L. (2007). Distinguishing signals and cues: bumblebees use general footprints to generate adaptive behaviour at flowers and nest. *Arthropod–Plant Interact.* **1**, 119-127.
Schmid-Hempel, P. (1998). *Parasites in Social Insects*. Princeton, NJ: Princeton University Press.
Schmid-Hempel, P. (2001). On the evolutionary ecology of host–parasite interactions: addressing the question with regard to bumblebees and their parasites. *Naturwissenschaften* **88**, 147-158.
Schmid-Hempel, P. and Reber Funk, C. (2004). The distribution of genotypes of the trypanosome parasite, *Crithidia bombi*, in populations of its host, *Bombus terrestris*. *Parasitology* **129**, 147-158.
Spaethe, J. and Weidenmüller, A. (2002). Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insectes Soc.* **49**, 142-146.
Spaethe, J., Brockmann, A., Halbig, C. and Tautz, J. (2007). Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften* **94**, 733-739.
Walsh, P. S., Metzger, D. A. and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506-513.
Wasserman, S., Lu, P., Aptekar, J. W. and Frye, M. A. (2012). Flies dynamically anti-track, rather than ballistically escape, aversive odor during flight. *J. Exp. Biol.* **215**, 2833-2840.
Wilms, J. and Eltz, T. (2008). Foraging scent marks of bumblebees: footprint cues rather than pheromone signals. *Naturwissenschaften* **95**, 149-153.
Wilson-Rich, N., Spivak, M., Fefferman, N. H. and Starks, P. T. (2009). Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu. Rev. Entomol.* **54**, 405-423.
Witjes, S. and Eltz, T. (2007). Influence of scent deposits on flower choice: experiments in an artificial flower array with bumblebees. *Apidologie* **38**, 12-18.
Witjes, S. and Eltz, T. (2009). Hydrocarbon footprints as a record of bumblebee flower visitation. *J. Chem. Ecol.* **35**, 1320-1325.
Worden, B. D. and Papaj, D. R. (2005). Flower choice copying in bumblebees. *Biol. Lett.* **1**, 504-507.

Experiment	Variable analysed	models	Fixed factors in the model	Comparisons of the models
Geraniol	Feeding duration	Modelfull	Flower*setup*position*day	*F* test: χ^2=13.59, *p*= 0.48 (vs the bestmodel), χ^2=31.24, *p*< 0.01 (vs the modelnull)
		modelbest	flower	*F* test: χ^2=17.64, *p*< 0.001 (vs the modelnull)
		modelnull	None	
	Flower preference	Modelfull	Flower+position	AIC=513.96
		Modelbest	Flower	AIC=512.52
		Modelnull	None	AIC=630.26
	Proportion of rewarding flower visitation	Modelfull	setup*position*day	AIC=372.34
		modelbest	day+position	AIC=364.90
		modelsetup	setup	AIC=369.60
		modelnull	None	AIC=368.45
	Switch of flower after landing	Modelfull	Flower*setup*position*day	AIC=149.34
		modelbest	flower	AIC=127.51
		modelnull	None	AIC=144.66
	Switch of flower after feeding	Modelfull	Flower*setup*position*day	AIC=158.83
		modelbest	flower	AIC=139.44
		modelnull	None	AIC=183.70
C. bombi	Feeding duration	Modelfull	Flower*setup*position*day	*F* test: χ^2=21.09, *p*= 0.10 (vs the bestmodel), χ^2=50.87, *p*< 0.001 (vs the modelnull)
		modelbest	Flower	*F* test: χ^2=29.79, *p*< 0.001 (vs the modelnull)
		modelnull	None	
	Flower preference	Modelfull	Flower+position	AIC=1093.79
		modelbest	Flower	AIC=1093.22
		modelnull	None	AIC=1105.13
	Proportion of rewarding flower visitation	Modelfull	setup*position*day	AIC=1115.76
		modelbest	day+position	AIC=1108.95
		modelsetup	setup	AIC=1113.54
		modelnull	None	AIC=368.45
	Switch of flower after landing	Modelfull	Flower*setup*position*day	AIC=513.54
		modelbest	flower	AIC=493.74
		Modelnull	None	AIC=496.17

	Switch of flower after feeding	Modelfull	Flower*setup*position*day	AIC=480.86
		modelbest	flower	AIC=471.36
		modelnull	None	AIC=493.55

Table S1: Results of the GLMM for all the experiments. Modelfull represents the model including all the explanatory factors, modelbest is the model fitting the best the data, modelnull is the model including no explanatory factor. The comparison of models for the visit duration was tested using F-tests, while the Akaike Information Criterion (AIC) was used for the other models. The lower AIC is, the best the model is. * represents the interactions between fixed factors.