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

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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 either from 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. We tested under controlled laboratory conditions the use of scent marks derived from other foragers in choosing between a contaminated and uncontaminated flower. As a positive control we tested the bee’s choice towards 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 flower. 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 by them to choose the uncontaminated flower is the odour from the parasite itself. The adaptation of bumblebees to avoid flowers contaminated by Crithidia 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.

**Key-words** *Bombus terrestris, Crithidia bombi*, host-parasite interactions, social cues, social immunity, social learning.

**Introduction**

Foraging behaviour and its optimization was and still remains a centre of evolutionary, ecological and neuroscience research. When investigating foraging behaviour in social animals an additional level appears which 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 avoidance of parasites is the first barrier against it, which could be less costly than immune responses. Theory incorporates the role of parasites into the optimal foraging models (Lozano, 1991).

In order to detect such 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 directly any contact with parasitic flies, helminths and fungi (reviewed in Cremer et al, 2007). This is called social immunity, since 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), or 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 an individual to learn via his conspecifics, known as social learning, which may lead to the evolution of culture in many vertebrate species (Heyes and Galef, 1996). This 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
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, 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 presence of a parasite (activated response) but also in absence of parasites (prophylactic response) (Cremer et al., 2007; Richter et al., 2012).

Bumblebees are parasitized by *Crithidia bombi*, 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). This long term relationship leads, according to the red queen theory hypothesis (Bell, 1982), to an arms race. Recently, Fouks and Lattorff (2011) discovered an avoidance behaviour of contaminated flowers, either by a specific parasite (*Trypanosoma: Crithidia bombi*) or by a common micro-organism (*Bacteria: Escherichia coli*), in foraging bumblebees.

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 resulting from that 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 know which cues the bumblebees use for choosing the rewarding (non-contaminated) flower, we record the flower choice of bumblebees during 6 days with two different 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, we use a positive control with the same setup without contamination but where the most rewarding flower was scented with geraniol, to investigate the mechanism used by the bees to distinguish both flowers.

Materials and methods

Bumblebees

Bumblebees from 3 different colonies were used for the experiment (Koppert). One colony was used for the Geraniol experiment, while two other colonies were used for C. bombi experiment in order to avoid any peculiar behaviour from a colony. From each original colony, 2 batches of 25 marked bumblebees (with Opalithplättchen) were housed in a metal cage (14.5cm x 12cm x 2.5cm) 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 minutes, 3 times a day during a 3 day trial period. The flower consisted of a blue foam paper (Ø 6cm) glued on a piece of wood placed on a plastic cylinder (Ø 2.8cm, 4.5cm), in the center an Eppendorf tube (0.2 mL) was placed. The artificial flower was filled 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 (terrarium of 1m x 0.4m x 0.5m, the ground was covered by a green Kraft paper) with the flower placed towards the light source. After these 3 days of training, only the bumblebees who were feeding were kept for the experiment. All the bumblebees were flower naive before the training.

For the experiment, each bee was placed in a flight arena and was given a choice between two artificial flowers (as described above), 10 cm apart from each others and equidistant from the bumblebee entrance. Each group of bees was tested 4 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 allow no cues to help the bees in choosing between the two flowers (referred to as the Individual setup later on), 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 later on). The position of flowers was switched regularly between the trials in order to avoid any side bias. The duration before the bee landed, where she landed, the time period of feeding and switching between flowers after the first landing or after feeding were recorded. When the bee spent more than 3 minutes without landing on a flower, she was put back to 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 (>90%, Carl Roth®) (5µL:50ml) 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 and the sub-colony “Group setup” was composed of 12 individuals, and the “Individual setup” was composed of 11 individuals.

**C. bombi experiment**

The *Crithidia* experiment consisted of one flower with a sucrose solution (50:50, v:v; below referred to as the rewarding flower), and the other flower containing the same sucrose solution (50:50, v:v) including a concentration of 3000 cells/mL of *Crithidia bombi* (strain 076 provided by P. Schmid-Hempel, ETH Zurich) (below referred to as the unrewarding flower). *C. 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 2 sub-colonies “Group setup” contained 13 and 12 individuals, and the 2 sub-colonies “Individual setup” contained 14 and 12 individuals.

**Molecular analyses**

After the experiment all bees were snap-frozen. Their guts were removed and crushed in 300µl of aqua dest. DNA was extracted from a 100µl 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. (2012). Fragment lengths were determined by means of capillary DNA sequencer Megabace 1000 (Amersham Biosciences). 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) is correlated to the intensity of infection (B. Fouks and H.M.G. Lattorff, unpublished). In order to determine the infection intensity we used the peaks of the microsatellite locus Cri 1.B6, which gives the most reliable estimate (B. Fouks and H.M.G. Lattorff, 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). In order 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 done using Image J ® software.

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 realized 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 the 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 generalized linear mixed effect model (GLMM) (Bates, 2008) including the individual I.D. as a random factor to account for pseudo-replication within individuals. The reward/contamination 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. (2008) and Crawley (2005).

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

We assigned the value 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 I.D. as a random factor to account for pseudo-replication within individuals.

For switching between flowers, both after landing and after feeding, we assigned the value 1 when a bee switched from one flower to the other and the 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 as fixed factors: the reward of the flower (rewarding or unrewarding), the setup (Group and Individual), the position (left or right), the day of recording; and individual I.D. as a random factor to account for pseudo-replication.

**Results**
**Behavioural assays**

**Geraniol setup**

As expected, bees fed longer and more often on the most rewarding and geraniol-scented flowers (Fig. 1A, GLMM: $P < 0.001$; Fig. 1B, GLMM: $P < 0.001$, Table S1 in supplementary material). Over days the bees show a decreased efficiency feeding on the scented flower: showing a loss of flower constancy, the position of the flower influences 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 Table S1 in supplementary material). In addition, the bees switch from one flower to the other more often when landing and feeding first on the unrewarding flower (Fig. 1C, GLMM: $P < 0.001$; Fig. 1D, GLMM: $P < 0.001$, see Table S1 in supplementary material). This indicates that bees are more attracted to flowers with the odour of geraniol, and when landing or feeding on the unrewarding flower, potentially due to mistake, they change to the most rewarding flower.

**Crithidia bombi setup**

We found that bumblebees fed longer and more often on the uncontaminated flower than on the one containing the parasite (Fig. 2A, GLMM: $P < 0.001$; Fig. 2B, GLMM: $P < 0.001$, see Table S1 in supplementary material). The bees behave similarly, but less efficiently as 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 do not affect the efficiency of the bees to choose the non-contaminated flower (Fig. 3B). The bees are more efficient when the uncontaminated flower is on the left position (for the bee), and show a non significant difference over days on their efficiency to choose 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 Table S1 in supplementary material). For switching to the other flower, the bees react in the same way as for the geraniol experiment but less efficiently, they change from one flower to the other more often after landing or feeding first on the contaminated flower (Fig. 2C, GLMM: $P < 0.001$; Fig. 2D, GLMM: $P < 0.05$; see Table S1 in supplementary material).

**Molecular assays**

First, we confirmed that the infection of the bees is due only to the strain of *C. bombi* applied to the flowers. The multilocus genotypes are 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 decreases the degree of infection of the bees (Fig. 4, Mann-Whitney U test: Z= 2.14, p < 0.05). 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 an individual to the other inside the nest (linear regression: r²= 0.018, p=0.17).

**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 to choose the uncontaminated flower (linear regression: r²= 0.001, p= 0.31). In addition, the intensity of infection is not correlated with the size of the bee (linear regression: r²= -0.019, p=0.82).

**Discussion**

As previously shown, worker bees exhibit an avoidance behaviour towards flowers contaminated by *C. bombi* (Fouks and Lattorff, 2011). Bees react to 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 (Figs 1A and 2A). Furthermore, they avoid the contaminated flower due to the odour from contamination since they visit the uncontaminated one more often without any other clue differentiating either flower (Fig. 3). They show also no clear learning over days to choose the flower without contamination indicating that the avoidance of the contaminated flower is an innate response. Finally, bees more often change to the rewarding flower when landing on the non-rewarding, contaminated one (Fig. 2D); emphasising the repellent effect of contamination for the bees.

Scent marks and their significance have been well studied (Goulson et al., 2001; Goulson et al., 1998; Goulson et al., 2000; Leadbeater and Chittka, 2009, 2011; Saleh and Chittka, 2006; Saleh et al., 2006; Saleh et al., 2007; Witjes and Eltz, 2007, 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., 2001; Goulson et al., 1998). On the other hand, some studies report the contrary (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 the one unvisited (Leadbeater and Chittka, 2011). Scent marks are mainly composed by 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 are detectable by social insects, which accumulate on the flower after each visit and remain unchanged over a period of 24 hours (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 to choose the rewarding flower. This could be due to the fact that both flowers were visited. Even so they should have accumulated more on the uncontaminated flower and allowed the bees to choose it more easily. The other possibility is that scent marks are not really useful to facilitate the choice of bees between contaminated or uncontaminated flower, due to the strong cue given by the odour of the parasite (Fig. 3). 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). 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 from the metabolic secretion of the parasite. Indeed, the presence of yeasts inside the nectar of flowers might produce specific odours (Raguso, 2004).

Moreover, scent marks are used by the bees through experience and learning; the latter might be impaired by an immune challenge and/or C. bombi infection, as it is known to decrease learning ability (Alghamdi et al., 2008; Gegear et al., 2006). However, a decrease of learning ability has been observed only when given visual cues: while for the odour cues the immune response does not decrease the learning ability of the bees (Gegear et al., 2006); this corroborates our results with the efficiency of a bee to choose the uncontaminated flower in regard to their infection load. Nonetheless, bees having a supplementary cue upon which to choose the flower feed not significantly more on the uncontaminated flower than the bee with only the odour of the “nectar”, but our sample size is big enough to significantly show this
kind of preference (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). This would thus be possible if the odour from honey pots and/or
conspecifics can be repellent for bees, since only the parasite possesses an odour in
our experiment.

In a previous experiment (Fouks and Lattorff, 2011) we found that a bee at a social
and individual level seems to learn to forage preferentially on the uncontaminated
flower over a period of days. In this previous 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. Here we do not find such a
significant pattern, but our sample size per day and bee is 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 by observation to eat medicinal leaves (Huffman et al, 2010), in
crickets learning from others to avoid predation (Coolen et al, 2005). Indeed, copying
behaviour is a really important cue for naïve bees to choose certain flowers (Grüter et
al., 2010; Kawaguchi et al., 2006; Leadbeater and Chittka, 2005; Worden and Papaj,
2005). Furthermore, the infected bees have an impaired learning for visual cues
(Alghamdi et al., 2008; Gegear et al., 2006) and reduce their foraging activity after
infection due to the immune challenge (Otterstatter et al., 2005). For naïve bees this
could lead to rely 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 on the flower. Indeed, it has already
been observed that bees transmit C. bombi via the flower (Durrer and Schmid-
Hempel, 1994); in our experiment we confirmed that. Since the only difference
between the two groups is the washing of the flower which might kill, or remove the
parasite, in addition we directly observed the bees defecating on the flower.

As previously shown, the bees have, in the C. bombi experiment, 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 stays unclear. Bumblebees have a better ability to learn an odour using
their right antenna than their left one (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 the preference to visit a certain position without even choosing the flower. Here, the better ability to visit uncontaminated flowers on the left position could be due to the higher rejection rate combined with higher visitation rate on contaminated flowers on the right position.

Another surprising result is the decreased efficiency of the bee to feed on the geraniol scented flower over time. Even if the reward of the unscented flower was lower, it might still high enough for the bees to select this flower. This 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, in the first place bumblebees were attracted strongly by the scented flower, but over time this attractiveness could have decreased realizing that the other flower is 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. This is not so surprising given that their ability to distinguish an odour is weak compared to visual cues (Gegear et al., 2006; Milet-Pinheiro et al., 2012).

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References


Figures

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

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

Figure 3: Proportion of rewarding/uncontaminated flower visitation with and without scent marks for both geraniol and C. bombi experiment. A) The proportion of the most rewarding flower visitation between the 2 setups for geraniol experiment (Group: n= 203, Individual: n= 165), B) The proportion of the uncontaminated flower
visitation between the 2 setups for *C. bombi* experiment. The bars represent the means between the different colonies and their 95% confidence interval. The use of the scent marks did not significantly improve the efficiency of the bees to feed on the most 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, Table S1).

**Figure 4:** Intensity of infection in regard to the presence or absence of scent marks (n=51). Box plots depict median, interquartile range and non-outlier range; the dots represent the outliers. The washing of the flower decrease the chance of a bee to be reinfected (Mann-Whitney U test: Z=2.14, *p* < 0.05).