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ORIGINAL PAPER

Socially peaceful: foragers of the eusocial bee *Lasioglossum malachurum* are not aggressive against non-nestmates in circle-tube arenas

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Abstract Due to the universally found nestmate recognition in eusocial insects, it is predictable that non-nestmates interact aggressively. In sweat bees (Hymenoptera: Halictidae), this trend was largely shown for queen–queen interactions, but data on worker–worker interactions are still scarce and somehow controversial. We studied behavioural interactions between foragers of the eusocial and ground-nesting bee *Lasioglossum malachurum* within circle-tubes. Independently of colony membership, bees exhibited high frequencies of cooperative behaviours, together with lack of aggression and moderate avoidance of social interactions. The cooperative mutual passing was the most frequently recorded behaviour. Size difference between the opponents had no effects on cooperation or avoidance. In a heterospecific experiment, bee foragers were observed to react more aggressively and to pass very rarely towards cuckoo bees, suggesting that our results were not biased by the circle-tube methodology. Our results and comparisons with other bee species suggest that studying worker interactions may be not enough to predict the social organisation in bees. Whatever the evolutionary meaning of this generalised tolerance towards conspecifics, the present findings are somehow in agreement with recent studies showing that *L. malachurum* colonies may have imperfect nestmate recognition and often include a mixture of related and unrelated workers.

Keywords Halictidae · Circle-tube experiment · Sociality · Worker behaviour · Nestmate discrimination

Introduction

Sweat bees (family Halictidae) show an impressively broad range of social behaviours, from solitary nesting (Wcislo 1997, McConnell-Garner and Kukuk 1997) to communal nesting (non-relatives share nests but do not cooperate in the brood care) (Kukuk 1992), semisociality (relatives of a single generation share nests and division of labour is present) (Packer 2006) and eusociality (defined by overlapping generations, cooperative brood care and reproductive division of labour) (Richards and Packer 1998, Wyman and Richards 2003). Different social behaviours can be even found in different populations of the same species (Packer 1990, Richards et al. 2003). Eusociality, in particular, evolved independently three times within the family, with many reversals to solitary life-style (Brady et al. 2006). Given such enormous social variability and flexibility, great attention was paid in the past to study how different social organisations are reflected in the behaviour expressed by females in intra-specific interactions (reviewed in Richards and Packer (2010) and Boesi and Polidori (2011)).

The types and variance in behavioural interactions of bees can be investigated through observations of dyadic encounters between females in circle-tube arenas, e.g., plastic tubes fashioned into circles believed to roughly reproduce nest conditions (Breed et al. 1978). This technique was used in the past for a number of bee species, including sweat bees (e.g., Paxton et al. 1999, Palaban et al.

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2000, Boesi et al. 2009), and to some extent, it can be used to predict the social organisation of biologically unknown or poorly known species (Packer 2006).

In particular, it was shown that within circle-tubes communal species are characterised by a high degree of cooperation and a low level of aggression both between nestmates and non-nestmates, while solitary species (and apparently semisocial ones, based on limited evidence) show a higher degree of avoidance and a moderate to high degree of aggression between non-nestmates (McConnell-Garner and Kukuk 1997, Paxton et al. 1999, Packer 2006).

For eusocial bee species, on the other side, high levels of aggression between females belonging to different colonies and high cooperation between individuals from the same colony could be predicted. This prediction is justified by the fact that eusocial species evolved the capacity to discriminate individuals based on familiarity (nestmate recognition), in order to defend nests from the intrusion of alien conspecifics (Fletcher and Michener 1987). Many studies on social bees support this prediction, since workers from different colonies were observed to interact aggressively in a broad range of taxa (e.g., honeybees: Breed 1998; bumblebees: Gamboa et al. 1987; stingless bees: Suka and Inoue 1993, Inoue et al. 1999).

Nestmate recognition is obviously also present in eusocial sweat bees. For example, nests of *Lasioglossum zephyrum* (Smith) are guarded and strangers are not allowed to enter (Kukuk et al. 1977), and such differential treatment is based on chemical differences of Dufour's gland content (Smith and Wenzel 1988).

However, in eusocial sweat bees, the behavioural expression of nestmate recognition is still not clear. For example, previous circle-tube experiments showed that queens/gynes and guards are typically aggressive with each other (Palaban et al. 2000, McConnell-Garner and Kukuk 1997), while non-nestmate foragers were observed to be highly tolerant (*Halictus ligatus* Say, Palaban et al. 2000), moderately aggressive (*L. zephyrum*, McConnell-Garner and Kukuk 1997), or even strongly aggressive (*Halictus lanei* (Moure), Packer et al. 2003). Moreover, experiments involving nestmate workers are still lacking for eusocial sweat bees. Such observations highlight the importance to further test for worker–worker interactions in eusocial sweat bee species, in order to add new data that can be used to find out general patterns through comparative analyses.

Here, we present data on the behavioural repertoire and on the frequencies of the different behaviours performed by workers of the eusocial sweat bee *Lasioglossum malachurum* Kirby (Hymenoptera: Halictidae) in circle-tube arenas. In the past, circle-tube experiments showed high levels of aggression between *L. malachurum* queens (Smith and Weller 1989), while no behavioural studies were carried out to date on the interactions between workers.

Furthermore, in order to study deeper the reaction capacity of bee workers within the artificial arenas, we also studied the worker behaviour towards a cleptoparasitic bee, which, representing a risk for the colonies, should elicit aggression by the resident bees.

Materials and methods

Study species and site

L. malachurum is an obligate eusocial sweat bee (Paxton et al. 2002). Queens establish their colonies in subterranean nests in spring, and then produce one (in Northern Europe) to three (in Southern Europe) worker phases and a last phase composed of males and gynes (Knerer 1992). These phases are separated by several days during which no foraging activity takes place (Mitesser et al. 2006). Mated gynes overwinter and found new colonies in the following spring, often competing for already dug tunnels (nest usurpation, Zobel and Paxton 2007). Colonies include as few as about four workers per nest on average in the spring phase (Paxton et al. 2002), but they can become populous in the summer phases (up to 80 workers per nest) (Knerer 1992). Generally, one queen monopolises reproduction (Richards et al. 2005), but colonies with a mix of related and unrelated workers are not uncommon (Soro et al. 2009).

Field observations were carried out on a large population (>1,000 nests) of *L. malachurum* located near Alberese, a small town in Maremma Regional Park, a Mediterranean natural reserve in Central Italy (Tuscany, Grosseto province) (42°40′5″N, 11°6′23″E). The studied nest aggregation was placed on a trail bounded by cultivated fields. At this site, *L. malachurum* is on flight from early April to late September (Polidori et al. unpublished data), forages mainly on Compositae (Polidori et al. 2010), and is victim of a wide spectrum of dipteran, hymenopteran and coleopteran natural enemies (Polidori et al. 2005, 2009, and unpublished data).

Circle-tube experiments

Behavioural trials using circle-tube arenas were performed from 15 May and 15 June 2006 on days of good weather conditions and high bee activity. Circle-tube arenas consisted of 44-cm-long pieces of clear sterile plastic tubes of 0.7 cm inner diameter fashioned into circles. Three kinds of experiments were carried out: (1) nestmate *L. malachurum* foragers (NM), (2) non-nestmates *L. malachurum* foragers (NNM), and (3) *L. malachurum* forager vs. cuckoo bee female (CB). Cuckoo bees used in the tests belong to *Sphecodes monilicornis* (Kirby), the most abundant cleptoparasitic bee at the nesting site (Polidori et al. 2009).

Bees were netted while exiting from their nests or when attempting to enter nest with pollen load, in order to be sure of their nest membership. Cuckoo bees were collected while patrolling the host nesting site. Immediately after collection, bees were brought directly to the place where trials were performed, about 15 m away in a shaded place, and they then were kept for a maximum of 10 min in a plastic box. If no other females were captured before this time, the bee was released to avoid possible stress due to a long handling time. When a pair of individuals was successfully provided for a circle-tube experiment, they were put in the arena in the following way: the first bee entered and stayed 2 min before the second one was inserted. This method, which roughly resumes nest conditions (Breed et al. 1978), was used because we explicitly wanted to study the reaction of bees either if they were the nest owner (the first bee) or an intruder (the second bee); an "owner effect" on bee behaviour is in fact predictable (Wcislo 1997). Cuckoo bees always entered after the host bees. Each circle-tube (each trial) lasted 15 min, a period within which behavioural differences are generally readily detected (Palaban et al. 2000).

During the trials, all activities and behaviours of the two bees after a frontal encounter (bees at <2 mm distant from each other, FE) were recorded on a tape-recorder. Since behaviour of marked individuals has been shown to be, to some extent, influenced by paints (Packer 2005), workers were marked by cutting the apical parts of the wings of one side but never more than the maximum observed wear (Boesi et al. 2009). In the congeneric *Lasioglossum majus* (Nylander), the behaviour of individuals with worn wings does not alter their intraspecific interactions (Boesi et al. 2009), so we assume this would also occur in *L. malachurum*. Very worn wings were not cut because individual recognition was possible. Bees were all handled with plastic gloves to avoid human finger traces (e.g., sweat, dust), which could affect the bees chemical odour. Since already used tubes may contain odours from previous occupant bees (Smith and Weller 1989), each circle-tube was used only once. Forty different *L. malachurum* foragers were used in 20 conspecific trials, and 24 *L. malachurum* foragers and 24 *S. monilicornis* females were used in 24 heterospecific trials. After the experiment, individuals were removed from the tube and placed in ethanol (70%) to confirm species identification and to obtain head width (measured under a stereomicroscope to the nearest 0.02 mm), used in the analysis as a proxy estimate of body size (e.g., Rust 1991).

Behaviour identification and categorisation

Behavioural patterns and categories followed those used in past circle-tube studies on different species of bees (Palaban et al. 2000, Wcislo 1997, Paxton et al. 1999, Boesi et al. 2009). Ten behavioural patterns distributed in three behavioural categories (cooperation/tolerance, aggression, avoidance/withdrawal) were observed. Aggression was performed with "C-posture" (CP: a female curls her abdomen under the thorax so her body forms a C-shape with mandibles and sting pointed at the other female) and with "mandibular hold" (MH: the mandibles of one bee are clamped around the neck, limbs or antenna of the other). Preference to not interact (avoidance/withdrawal) was expressed with "withdrawing" (WHD: a bee makes a 180°± turn away from the other individual or backs quickly away from it), "Synchronised Back and/or Reverse" (SBR: both bees do tandem reversals or backing) and with "stop without contact" (STO: bees in a frontal encounter stop before coming in contact). Cooperation/tolerance was expressed by "mutual passing" (PASS: bees meet and manoeuvre to accommodate each other while they pass in opposite directions), "following" (FOW: a forward movement by a bee toward another), "stop in contact" (STOC: bees in a frontal encounter stop in contact and touch each other slowly with antennae and mandibles), "going and backing" (GOB: a bee moves to come close or in contact with the other, and then walks quickly backward waiting for the other following), and with "push and lunge" (PUL: a bee lunges forward usually with mandibles open, and then a cooperative posture follows). Due to the very low frequency of many of the above-reported behaviours (see "Results"), most of the analyses were performed pooling together the behaviours of each category, while for PASS, which was abundantly observed (see "Results"), a separate analysis was also done. Frequency of behaviours performed in each trial was calculated as the number of times a given behaviour was expressed divided by the total number of FE.

Statistical analysis

Bee head widths were not normally distributed even after log-transformation (as $\ln(x)$) (Shapiro–Wilk test, W=0.87, P<0.001), and numbers and frequencies of behavioural interactions were also not normally distributed after log- or arcsin-transformation (Shapiro–Wilk test, $0.21>W>0.90$, P<0.05 for all samples). Thus, comparisons and correlations were all tested through non-parametric statistics (Mann–Whitney test and Kendall correlation test). In the text and tables, average numbers are given±standard deviation, and medians are also shown.

In order to contrast the behaviour of *L. malachurum* with comparable data for other sweat bee species studied with the circle-tube apparatus, we plotted the relative frequency of PASS (the most cooperative of the behaviours) exhibited by a pair (averaged across pairs) against the frequency of aggressive interactions, as this is an easy way to visualise differences among species (Packer 2006).

All the statistics were performed with XLSTAT 2008.

Results

Conspecific circle-tube trials

The mean number of interactions (FE) performed *per* trial was very variable, ranging from 2 to 21 in NM trials (8.8±6.3 on average, median=8.5) and from 2 to 22 in NNM trials (8.9±6.2 on average, median=8), with no differences between the two types of experiment (Mann–Whitney test, *U*=195, *n*=10, *P*=0.91). Within experiments, on the contrary, intruders appeared to be less active (NM, 6±4.2, median=4.5; NNM, 4.9±3.8, median=3) than owners (NM, 11.6±6.9, median=12.5; NNM, 12.9±5.6, median=11.5) in both kinds of trials (although such difference was only close to significance for NM trials) (Mann–Whitney test, NM, *U*=74, *n*=10, *P*=0.07; NNM, *U*=90.5, *n*=10, *P*=0.001).

Overall, the cooperative behaviours more often recorded in trials were mutual passing (PASS) (45.7±21.8% of FE, median=46.4%, from 19.4% to 70.4% depending on the kind of experiment) and moving back after contact eliciting following (GOB) (23.9±14.7% of FE, median=25.5%, from 6.1% to 38.3%). The withdrawal/avoidance interactions were primarily expressed stopping without contact (STO) (9.4±5.7% of FE, median=8.4%, from 6.6% to 16.9%). All the other recorded behaviours had frequencies lower than 5%.

Foragers in general behaved in similar ways either in NM or in NNM dyadic encounters (Fig. 1 and Table 1). On the whole, both owners and intruders were highly cooperative in both NM and NNM trials, showing moderate levels of withdrawal/avoidance behaviour and almost complete lack of aggression towards conspecifics (Fig. 1 and Table 1). Frequency of behavioural categories expressed by owners and intruders did not differ in each kind of trials, except owners in NNM which actually avoided more than intruders (Fig. 1 and Table 1). Owners in NM trials were not more cooperative, and did not avoid more, than in NNM trials; intruders also behaved similarly in NM and in NNM trials (Fig. 1 and Table 1).

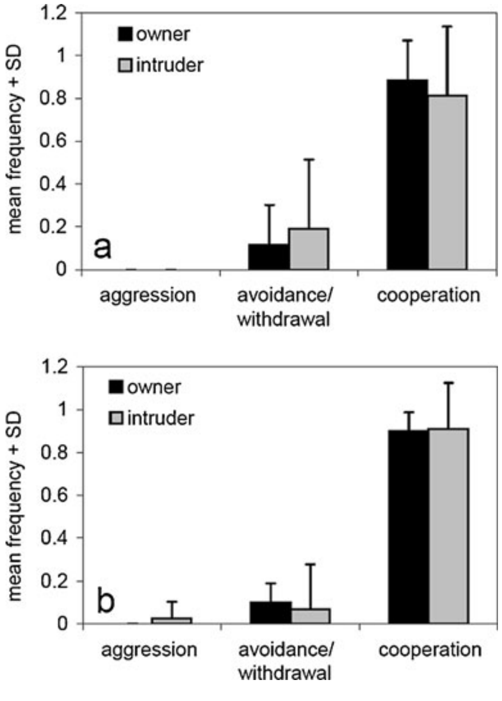

Fig. 1 Mean frequency±SD of aggressive, avoidance/withdrawal and cooperative behaviours per FE expressed by *L. malachurum* recorded in circle-tube experiments. **a** NM trials; **b** NNM trials

When analysing more in detail the expression of cooperation/tolerance, the most important behavioural category recorded for all trials, few differences appeared between NM and NNM trials. In fact, the contribution of PASS, the most commonly used cooperative posture, was marginally higher in NM trials (50.5±32.1, median=52.3) than in NNM trials (29.3±18.9, median=33.2) (Fig. 2) (Mann–Whitney test, *U*=75, *n*=10, *P*=0.059). However, PASS was in general avoided in the first interaction despite being the most abundant behaviour. In fact, nestmate females more often performed the cooperative GOB as first behaviour (70% of trials), while withdrawal/avoidance was performed first in 20% of trials and PASS only in 10% (i.e., one case because *n*=10). Non-nestmates also performed more often GOB (60%) as first behaviour, and then withdrawal/avoidance (40%).

Difference between female sizes (larger-smaller) did not affect the frequency of cooperative interactions per trial in any experiment (Kendall correlation test, NM, $\tau=-0.045$, *n*=10, *P*=0.85, NNM, $\tau=0.16$, *n*=10, *P*=0.52). The same absence of relationship was found for withdrawal/avoidance interactions, with a single weak exception (Kendall correlation test, NM, $\tau=0.028$, *n*=10, *P*=0.91, NNM, $\tau=-0.51$, *n*=10, *P*=0.039).

Heterospecific circle-tube trials

In the experiments involving *S. monilicornis* the mean number of behaviours performed by *L. malachurum per* trial was highly variable, ranging from 2 to 36 (14±8.4 on average, median=13.5). Overall, the cooperative behaviours by *L. malachurum* more often recorded in trials were moving back after contact eliciting following (GOB) (41% of FE) and stop into contact (STOC) (30.2% of FE), followed by the aggressive C-posture (CP) (11.3% of FE). The withdrawal/avoidance interactions were rare and exclusively expressed with withdrawal behaviour (WHD) (10.4%). Cuckoo bees more often were aggressive, both doing C-posture (CP) (18.7%) and holding the opponent with mandibles (MH) (13.5%). All the other recorded behaviours had frequencies lower than 5%.

Frequency of behavioural categories expressed by *L. malachurum* and *S. monilicornis* somehow differed, *L. malachurum* being more cooperative than *S. monilicornis*

Table 1 Comparisons between the levels of aggressive, avoidance/withdrawal and cooperative behaviours performed by *L. malachurum* in NM and NNM circle-tube trials (conspecific encounters) and in CB circle-tube trials (heterospecific encounters)

	Percent aggressive		Percent avoidance/withdrawal		Percent cooperative				
Conspecifc encounters									
	Owner	Intruder	Owner	Intruder	Owner	Intruder	Owner vs. intruder statistics (Mann–Whitney test)		
							Aggressive	Avoidance/ withdrawal	Cooperative
2006 NM ($n=10$)	0	0	11.6±18.5, median=0	18.9±32.3, median=0	88.4±18.5, median=100	81±32.3, median=100	–	$U=47$, $P=0.81$	$U=53$, $P=0.81$
2006 NNM ($n=10$)	0	2.5±7.9, median=0	10±8.8, median=10.2	6.6±21, median=0	89.9±8.8, median=89.7	90.8±21.6, median=100	–	$U=76.5$, $P=0.02$	$U=32$, $P=0.15$
NM vs. NNM statistics (Mann–Whitney test)	–	–	$U=39$, $P=0.40$	$U=64$, $P=0.24$	$U=61$, $P=0.40$	$U=40.5$, $P=0.46$			
Heterospecific encounters									
	Lm	Sm	Lm	Sm	Lm	Sm	Lm vs. Sm statistics (Mann–Whitney test)		
							Aggressive	Avoidance/ withdrawal	Cooperative
2006 CB ($n=24$)	13.8±21.8, median=6.9	32.2±42.8, median=0	10.4±12.8, median=5.7	2.1±10.2, median=0	75.8±20.4, median=81.6	36.6±44.2, median=10	$U=314.5$, $P=0.57$	–	$U=396$, $P=0.02$

If at least one sample was composed only by null values or if only one value in a sample differed from zero, the test was not performed

(Table 1). Moreover, *L. malachurum* foragers were more aggressive (Mann–Whitney test, $U=35$, $n_1=10$, $n_2=24$, $P<0.0001$) and slightly less cooperative (Mann–Whitney test, $U=180$, $n_1=10$, $n_2=24$, $P=0.022$) in heterospecific encounters than the owners in NNM conspecific encounters, while no differences appeared in withdrawal/avoidance behaviour (Mann–Whitney test, $U=126.5$, $n_1=10$, $n_2=24$, $P=0.81$) (Table 1).

The contribution of PASS on cooperative behaviour was much lower in heterospecific encounters than in conspecific encounters (Fig. 2) (Mann–Whitney test, CB vs. NM, $U=204.5$, $n_1=10$, $n_2=24$, $P<0.0001$; CB vs. NNM, $U=198$, $n_1=10$, $n_2=24$, $P<0.0001$).

An aggressive behaviour by *L. malachurum* was recorded as the first performed one in a single trial, and GOB (70.8%) was more likely performed first in the trials. *S. monilicornis* performed the first behaviour in only two trials, attacking the resident bee with MH in both cases.

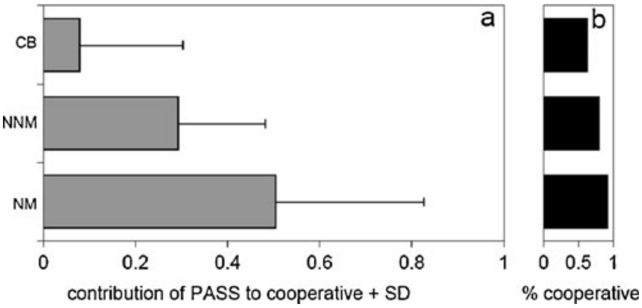

Fig. 2 Contribution of PASS±SD on cooperative behaviour performed per FE in circle-tube experiments, ranked in descendent order (**a**), and the corresponding overall frequency of cooperation/tolerance (**b**)

Discussion

Following a recent summary of circle-tube studies by Richards and Packer (2010), solitary sweat bee species are characterised by high rates of avoidance (withdrawals), communal ones by high rates of cooperation or tolerance (passing), and semisocial and eusocial ones by high rates of aggression, coupled with very low rates of passing. However, a peculiar species (*H. ligatus*) was not reviewed in that work, and almost only data on queens/gynes interactions were discussed (essentially because more data were available on such experiments), leading to unclear conclusions on the typical behaviour of eusocial species. In fact, previous studies on dyadic interactions in eusocial sweat bees show a certain variability that seems primarily due to the caste the opponents belong (e.g., Breed et al., 1978). Here, we provided the first data on the behavioural profile expressed by foragers in both nestmates (for the first time) and non-nestmates encounters for an additional eusocial halictid bee. We discuss below how our findings may help in drawing a general picture for bee behaviour in halictid bees, comparing our results with those obtained on other social sweat bees (communal, semisocial and eusocial).

First of all, aggressive behaviour between conspecifics is common in halictid bees observed on field. For example, as single foundress nests are left unguarded during foraging trips and usurpation attempts may occur, conflict over nest ownership may arise in these nest aggregations (Kaitala et al., 1990). The duration of such conflict may be extended, as in *L. malachurum*, where prolonged fights between intruder and nest owner last from 10 to 26 min and can result in the damage to, or even the loss of, legs and other body parts (Smith and Weller, 1989). Thus, queens/gynes are clearly able to discriminate non-relatives.

Guards, which are workers devoted to protect the nest entrance (Michener 1974), also appear to be able in discriminating non-nestmates. The guard blocks the nest entrance with its abdomen or assumes a “C” posture with head and sting extended toward the intruder, while nestmate foragers are easily permitted to reenter the nest (Bell and Hawkins, 1974; Smith and Weller, 1989). An exception is that of *Lasioglossum versatum* (Robertson), in which guards admit individuals from any colony (Michener 1966). However, following Michener (1974), this species is not fully eusocial but is somewhere in between communal and semisocial organisation, with nest burrows sometimes even interconnecting underground (Michener 1966).

In contrast, our study shows that foragers may be not able to discriminate conspecifics based on familiarity, or alternatively, they can discriminate them, but this does not imply a change in behavioural treatment. In fact, *L. malachurum* foragers behaved in a strongly tolerant way towards both nestmates and non-nestmates, with only one difference in avoidance/withdrawal behaviour, more frequently recorded for owners in NNM trials. However, despite this possible weaker interest (higher avoidance frequency) of the owner to interact with a non-nestmate intruder, owners generally showed higher activity (mean number of FE per trial) relative to intruders in NNM trials. Not even PASS frequency differed between nestmates and non-nestmates, with just a weak tendency ($P=0.059$) of bees to pass less often in NNM trials. Thus, it seems that foragers cannot or do not care to treat nestmates and non-nestmates differently.

There are three other eusocial halictid bees in which forager encounters were studied with circle-tube apparatus, although only non-nestmates were used. In *H. ligatus*, foragers passed frequently and were not aggressive, similarly to what found for *L. malachurum* (Palaban et al. 2000). In *L. zephyrum* and *H. lanei*, on the contrary, foragers passed rarely and were aggressive (Breed et al. 1978, Packer et al. 2003). In these cases, results are in general agreement with the behaviour of non-nestmates workers in eusocial Hymenoptera (e.g., Gamboa 1996, Breed 1998).

One possibility to explain such difference among eusocial sweat bees is to try to find out difference in their eusocial organisation. *L. malachurum* and *H. ligatus* are both known to be obligate eusocial across their geographic range (Knerer 1992, Richards and Packer 1998). Overall relatedness may be somehow reduced in nests founded by multiple foundress (*H. ligatus*, Richards and Packer 1998) or through inter-nest worker drifting (*L. malachurum*, Soro et al. 2009). In such cases, a relatively weaker nest-mate behavioural discrimination and thus a high tolerance between non-nestmates foragers may explain the presence of alien workers within nests in both species (Paxton et al. 2002; Soro et al. 2009). In fact, when discrimination is not perfect, members of groups within which average relatedness is high, are predicted to switch from aggression to tolerance (Segoli et al. 2009). Accordingly, in *L. malachurum*, cuticular and Dufour's gland compounds varied significantly among colonies, providing the potential for nestmate discrimination, but the presence of unrelated individuals within nests that are not chemically different from their nestmates suggests that the discrimination system of *L. malachurum* is prone to acceptance errors (Soro et al. 2011). Our results may represent the behavioural expression of such a nest-recognition pattern. Interestingly, such behavioural profile resembles that observed for communal halictines (Fig. 3), so that we suggest that using only worker interactions to predict social organisation of a species may lead to important misunderstanding.

On the other side, *L. zephyrum* is known to live in small eusocial colonies (<14 females vs. a maximum of about 80 in *L. malachurum*) but also as solitary, thus being facultative eusocial (Michener et al. 1971). In this species, workers can be often unrelated to the queen and have a tendency to exhibit higher reproductive status, i.e., have developed ovaries and presumably lay eggs, when they are unrelated to the queen (Smith 1987). In this case, a higher aggression between non-nestmates workers may be explained by effective risk of reproductive competition between workers among nests. Supporting this hypothesis, a study on behavioural interactions showed that females of this species residing near one another behave as if they are more closely related than females residing at greater distances from one another (Kukuk and Decelles 1986).

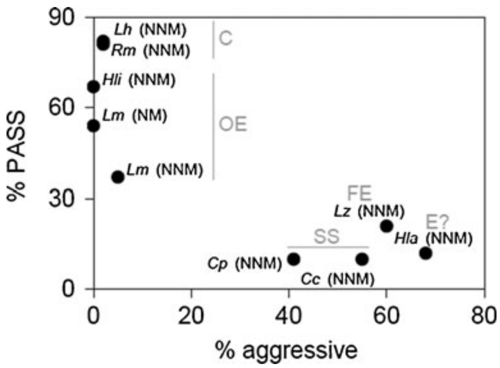

Fig. 3 Frequency of aggressive behaviours per FE plotted against frequency of PASS per FE for forager encounters in eight species of social halictid bees. *Lm L. malachurum* (this study), *Lz L. zephyrum* (Breed et al. 1978), *Lh L. hemichalceum* (Kukuk 1992), *Hli H. ligatus* (Pabalan et al. 2000), *Hla H. lanei* (Packer et al. 2003), *Cp C. patagoniga* (Packer 2006), *Cc Corynura chloris* (Spinola) (Packer 2006), *Rm R. mutabilis* (Packer 2006). In *grey*, there is the social organisation known for the species: *OE* obligate eusocial, *FE* facultative eusocial, *SS* semisocial, *C* communal, *E?* supposedly eusocial

The risk of reproductive competition would be in fact more severe in terms of indirect fitness if intruders are non-relatives. In accordance to the fact that competition could be higher for colonies where most of workers are able to reproduce (Michener 1974), *L. zephyrum* turns to be behaviourally similar to semisocial halictines studied with circle-tubes (Fig. 3).

The fourth species, *H. lanei*, is to date only supposed to be fully eusocial (in particular based on the great size dimorphism between queens and workers and comparison with sociality of relative species). Packer et al. (2003) observed in all but one case high aggression level among workers, suggesting that all but one experiment were done with non-nestmates and stating that this pattern is consistent with the species being eusocial. Although this could be a reasonable conclusion when looking at *L. zephyrum*, it sharply contrasts to what observed in *H. ligatus* and *L. malachurum*. Thus, we suggest that *H. lanei* is either similar to *L. zephyrum* for the colony social organisation (often unrelated workers with developed ovaries compete for reproduction) or it is not fully eusocial. In fact, based on what was observed for two semisocial halictines, its behavioural profile is also consistent with this social organisation (Fig. 3).

Again, such comparison suggests the weak power of worker interaction patterns alone in predicting social organisation.

Another hypothesis to explain why we do not observed aggression among nestmates is that in *L. malachurum* colonies, only guarding bees learn to discriminate relatives from non-relatives. For example, an experiment done with *L. zephyrum* guards showed that bees learn the odours of their nestmates, then accept or reject other bees on the basis of the similarity of the latters' odours to those of the guards' nestmates (Buckle and Greenberg 1981). Such recognition mechanism enables individuals with different odours to live together and may enhance kin selection by providing a more complete basis for discriminating relatives from non-relatives (Buckle and Greenberg 1981). Although we could not exclude such a possibility for our study species, it is also true that also foragers of *L. zephyrum* react aggressively to non-nestmates. However, in *H. ligatus*, guards were not tolerant towards non-nestmate but foragers passed frequently with non-nestmates, suggesting that its profile could be similar to *L. malachurum*.

Concerning avoidance/withdrawal behaviour, *L. malachurum* (with about 12% to 19% of frequency, see Table 1) behaved similarly to the supposed eusocial *H. lanei* (14%) and the communal *Ruizantheda mutabilis* (Spinola) (17%) and *Lasioglossum hemichalceum* (Cockerell) (15%) but differently from another semisocial species (*Corynura patagonica* (Cockerell), 49%). Thus, no very distinctive information can be taken from the observed pattern. Behaviours pooled in the avoidance/withdrawal category actually include also some that, in certain social contexts, may reflect more dominance than avoidance (e.g., Palaban et al. 2000; Arneson and Wcislo 2003), so it is often difficult to understand the meaning of such postures (see also Boesi and Polidori 2011) Also, in *H. ligatus*, the low frequency of withdrawals per encounter is less readily comprehensible when compared to that in other species (Palaban et al. 2000). In general, it seems at least that avoidance is higher for solitary species (about 50–60%, see Boesi and Polidori 2011 and Richards and Packer (2010) for recent reviews), while things are more unclear for social species.

Size does not account for variance observed in cooperative and avoidance/withdrawal behaviour. This contrasts with gyne–gyne dyadic interactions in the laboratory for *L. malachurum* and *Lasioglossum pauxillum* (Schenck) (Smith and Weller 1989), where size influenced the behaviour, but is consistent with other circle-tube studies in which no effect of size was detected (Breed et al. 1978, Wcislo 1997). One has also to note that an effect of size was found on aggression level, the larger of the two individuals generally being more aggressive; in our study, however, no aggression was observed, suggesting that size may influence agonistic, but not cooperative or avoidance patterns (Smith and Weller 1989).

Concerning interactions with cuckoo bees, results should be taken with some cautions. In fact, this is the first time, as far as we know, that circle-tube tests were performed with females of different species. The main problem with the interpretation of such tests is that we could not assume that *L. malachurum* uses the various behavioural postures with exactly the same meanings believed for conspecific encounters. The best way to limit this problem is to discuss only the behaviours whose meaning is most clear: PASS (an undoubtedly cooperative/tolerant posture) and CP/MH (two clear aggressive postures). Using these behaviours, it appeared that *L. malachurum* was much less tolerant towards cuckoo bees than towards conspecifics and more aggressive as well (although frequencies were on average not very high).

A further possible explanation for the lack of aggression between non-nestmates in *L. malachurum* involves the status of colonies at the time of the experiments. In fact, as colonies get larger during the nesting cycle, interactions between nestmates and non-nestmates might change, both between nestmates and non-nestmates. In a communal andrenid bee, *Andrena scotica* Perkins, high number of nestmates reduces the level of tolerance towards conspecifics (Paxton et al. 1999), so one might expect a similar trend in eusocial sweat bees. Despite new experiments that have to be carried out to explicitly test for this hypothesis in *L. malachrum*, circle-tube tests ($n=19$) performed with non-nestmates in July of 2008 (when colonies are typically more populous than in May) may not confirm this view. In fact, the frequency of cooperative interactions were still very high (about 78%), avoidance behaviour was moderate (about 22%), and no aggressive postures were recorded (Polidori et al., unpublished data). The contribution of PASS on cooperative behaviour was even higher than that here reported for spring colonies, reaching almost the 50% (Polidori et al., unpublished data). However, a direct comparison should be avoided because data come from two different years, and because the actual number of workers in the colonies was not recorded. In addition, no data are available to date to test if also nestmates retain the same behaviour in high-density colonies. Thus, we can only suggest at the moment that behaviour is not very density-dependent in *L. malachurum*. If this hypothesis turns to be valid, on the other hand, then that might make it more difficult to draw a general picture because different species may have been tested at different points in the colony cycle, and thus comparisons should be more cautious.

A last hypothesis that could explain our result concerns the methodology used for the observation (artificial arenas). Although this is an accepted method to study social interactions, it is also true that slightly different types of arenas and/or experimental set-up (e.g., circle-tubes vs. vertical-tubes; short vs. long duration) may affect behaviour of bees (Palaban et al. 2000, Jeanson et al. 2005). However, we can discard such possibility. In fact, (1) resident bees reacted aggressively and passed rarely in encounter with cuckoo bees (see above), and (2) in *L. malachurum*, queen behaviour did not change over time when their stay in the tube was extended from 15 to 20 min (Ayasse pers. comm., quoted in Pabalan et al. 2000). Thus, *L. malachurum* evolved a real behavioural strategy towards conspecifics, and the generalised tolerance was not caused by the artificial conditions of the experiments.

In conclusion, this is the first evidence of an indiscriminate and virtually identical level of tolerance between nestmates and non-nestmates in a eusocial sweat bee. Notably, a similar result was obtained recently for an ant (*Myrmecia nigriceps* Mayr): bioassays revealed no significant difference in the rare occurrence of aggression in trials involving workers from the same nest or from different nests (van Wilgenburg et al. 2007). As we hypothesised in our study, the authors suggest that workers of *M. nigriceps* are either unable to recognise alien conspecifics or that the costs of ignoring workers from foreign colonies are sufficiently low to favour low levels of inter-colony aggression. Further studies should be devoted in understanding why eusocial species such as *L. malachurum* behave so differently when compared to the general pattern known for eusocial insects.

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