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Phorid parasitoid attack triggers specific defensive behaviours and collaborative responses in leaf-cutting ants

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ABSTRACT

Being social adds another level of defence for organisms: social defences. Beside individual defensive behaviours, social organisms can limit parasite infections by using collective and collaborative behaviours. We evaluated whether the social defence of the leaf-cutting ant *Atta cephalotes* is specific against phorid parasitoids and the occurrence of collaborative responses depends on the context, i.e. ant activity in foraging trails and number of phorid attacks. We exposed workers to freshly dead specimens of phorids, non-phorid flies or a control without flies in different ant flux conditions and number of attacks and evaluated ant response. We found that workers responded more frequently to phorids than to non-phorid flies and controls suggesting that specific chemical or visual cues of phorids are recognized by leaf-cutting ants triggering a behavioural response. Although the probability of collaborative defences was similar in different ant flux conditions and number of attacks, they occurred more frequently when ants were attacked by a phorid than when they were attacked by the other treatments. Therefore, we demonstrated that leaf-cutting ants differentiate parasitoid flies from other flies, showing a collaborative response against them, in contrast to the other flies and the control, for which almost no collaborative responses were displayed.

1. Introduction

Benefits conferred by group living are considered one of the main reasons why social species have become dominant in many habitats. Cooperation can increase the fitness of individuals by increasing the efficiency of brood care, foraging and enemy defences. However, living in groups or societies has been also associated with increased parasitism risk because frequent and intimate contacts between individuals may facilitate parasite spread and because close genetic relatedness between group members make them more susceptible to parasites (Otterstatter and Thomson, 2007; Stroeymeyt et al., 2014). Empirical studies about whether parasite load increases in group living organisms offer mixed evidence (Schmid-Hempel, 2017). Therefore, for a full understanding of the evolution of group living we need to elucidate contexts that limit and favour parasite infectious for group living individuals.

Despite having a higher risk of infection, being social adds another level of defence for these organisms: social defences. In addition to the individual physiological immune response, social organisms also express 'social immunity' (Cotter and Kilner, 2010; Cremer et al., 2007; Meunier, 2015; Wilson-Rich et al., 2009). Social immunity is expressed through a variety of individual, collective and collaborative behaviours that limit parasite uptake from the environment, its establishment into the nest habitat and its transmission between group members (Cremer et al., 2007; Meunier, 2015). Individual behaviours that qualifies as mediators of social immunity are illustrated, for instance, by foragers that avoid habitats containing entomopathogenic fungi, such as in the termite *Macrotermes michaelseni* (Mburu et al., 2009), and infected individuals avoiding contact with the brood (Bos et al., 2012). While collective and collaborative behaviours (i.e. defensive behaviours that include all group members or only some, respectively) can be exhibited by guards of the honeybee *Apis mellifera* when they prevent infected nestmates from entering the hive (Waddington and Rothenbuhler, 1976), and by *Colobopsis truncatus* when they form a living "plug" in the nest entrance to avoid attacks by enemies or predators (Anderson and Franks, 2001). Although mechanisms of social immunity have been studied during the last 20 years in several eusocial species (Meunier, 2015), the context of using an individual or social defence against parasites has been poorly studied.

Leaf-cutting ants and parasitoid phorid flies (Diptera: Phoridae) offer a good model to evaluate which factors influence the use of individual and social behavioural strategies against parasitoids. Phorid flies use their ovipositors to insert an egg into the body of worker ants

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engaged in different tasks, although most phorid parasitoid species attack ants at foraging trails (Elizalde and Folgarait, 2012). As development take place, the larvae feed from their hosts tissues, eventually killing them at the time of pupation (Elizalde and Folgarait, 2011). The response behaviours of leaf-cutting ants against phorids include dropping their load, retreating to the nest, moving legs, antennae, and mandibles, outrunning the phorid, or adopting body postures such as lowering the tip of the abdomen, having a C posture, or making a ball with their whole body (Movie A.1) (Bragança et al., 2009; Elizalde and Folgarait, 2012; Feener and Moss, 1990; Feener and Brown, 1993; Orr, 1992; Tonhasca, 1996). Furthermore, small workers ride or “hitchhike” on leaf fragments carried back to the nest by larger foragers defending them from parasitoid attack (Vieira-Neto et al., 2006). In several instances, the ants capture and kill the ovipositing parasitoid (Elizalde and Folgarait, 2012). These behavioural responses can occur at both individual and social levels; i.e. they may include the worker being attacked (individual response) or the attacked worker as well nearby non-attacked workers (collaborative response) (Elizalde and Folgarait, 2012). Since many leaf-cutting ant species may become pests by inflicting significant economics damage on crops (Montoya-Lerma et al., 2012), a detailed understanding of defensive behaviours employed by ants against natural enemies is of relevance for practical reasons in order to use it for the biological pest control of leaf-cutting ants (Guillade and Folgarait, 2014). In addition, this knowledge will contribute to the theoretical framework aiming to understand the maintenance of group living.

Here we evaluated whether individual and collaborative defences of leaf-cutting ants are context dependent, i.e. whether the use of individual or collaborative defences were triggered by circumstances related by different contexts including different nestmate densities or parasite behaviour. On one hand, the number of ants in a foraging trail may influence whether ants respond individually or collaboratively because when ant flux is high there is a higher probability that a phorid will be detected by nearby ants. On the other hand, the number of times that phorids attack ants in an oviposition attempt may increase the probability that ants respond collaboratively because it may increase phorid detection by ants. Therefore, we expect that as higher the ant flux conditions in the foraging trail or the number of phorid attacks per worker, the probability of collaborative defences will be higher. First, we evaluated experimentally and at field conditions whether leaf-cutting ants specifically respond to phorid flies and whether they collaboratively respond to them. Then, we tested our hypothesis that the occurrence of collaborative responses depends on ant activity in foraging trails and the number of attacks by phorids. Finally, we analysed whether the type of defensive behaviour (i.e. adopt a C posture, bite or run away) depends on ant activity and number of phorid attacks. As we mentioned earlier, leaf-cutting ants have an array of defensive behaviours and their effectiveness might vary with the context (Feener and Moss, 1990). We propose that when the ant activity in the trail is low, running away from the threat would be more advantageous because there is less chance that nestmates respond and shoo the parasitoid. Regarding the number of parasitoid attacks, we proposed that behavioural defences such as C postures and biting would be more advantageous when the phorids are less active because the probability of effectively catching the phorid will be higher; meanwhile when parasitoids attack several times ants might prefer to run away in order to avoid an aggressive parasitoid.

2. Material and methods

2.1. Study area and organisms

We conducted this study during 2016 at La Selva Biological Station of the Organization for Tropical Studies, Puerto Viejo, Sarapiquí, Heredia Province, Costa Rica (10°26´ N, 83°59´ O). To determine how leaf-cutting ants respond to phorid attack and what factors affect individual and collaborative defensive strategies, we worked with 11 nests of the leaf-cutting ant *Atta cephalotes*, the most common leaf-cutting ant species in the region (Farji-Brener 2001). The most common phorid flies attacking *A. cephalotes* are females of *Eibesfeldtphora curvinervis* (Disney et al., 2008; Feener and Brown, 1993), which are active along their foraging trails during daylight hours (ca. 0630–1800) and attack ants with a head width of 1.6 mm or larger (Feener and Brown, 1993). This phorid perches on leaves or twigs along the sides of the major foraging trail of ants. In these perches, female parasitoids direct their head toward the trail watching the passage of ant foragers. Once leaving its perch, a female pursues the forager it selected for no more than 1 m: if the female does not reach the forager over that distance, it either returns to its perch or briefly searches on the wing for another acceptable ant. A new sequence of pursue and attack behaviour begun if the fly encounters another suitable forager (Feener and Brown, 1993).

2.2. Experiment

To evaluate if ants specifically respond to phorid flies, we experimentally evaluated the ant behaviour against phorids. We manipulated the presence and attack of phorid and non-phorid flies using freshly dead specimens which allow us to control fly size, behaviour, activity and abundance. As non-phorid flies, we used specimens of Thephritidae family (fruit fly). Phorid flies were collected at nest entrances. All specimens were collected with a vacuum aspirator and frozen for one hour. Then, we glued them at the tip of a thin thread with a non-toxic adhesive and manipulated them by tying the thread to a stick. In addition to the phorid and non-phorid fly treatments, we used a thread without flies as thread-effect control (Movie A.2). We evaluated the behaviour of ants against: (1) the phorid fly, (2) the non-phorid fly, and (3) the thread without flies (control), by placing the treatments in the middle of different trails or trail sectors of 11 nests, sequentially and randomly at intervals of one hour among them (i.e. treatments were chosen at random until completing them). We placed treatments in the first 10 m from the nest. When nests trails were not enough to complete each treatment on a different trail, we used different trail sectors 1 m apart. To avoid bias on sampling, we chose phorid and non-phorid fly treatments blindly, i.e. once the treatments were finished we associated data sample with its corresponding treatment (Kardish et al., 2015). To determine the effect of phorid attack behaviour, we placed treatments over trails above 1 cm from the ground without touching ants during 1 min (still behaviour), or we chose randomly one ant of similar size and touched its head (where this parasitoid species oviposit the ants, (Feener and Brown, 1993)) three or six times (attacking behaviour) on 1 s intervals (i.e. attacking behaviour treatments lasted ˜3 or 6 s, respectively). To control intensity of the attacks, the same person always performed the attacks. We observed if ants responded to the treatments and how many ants responded in a trail sector of 5 cm around the treatment or the attacked ant. We categorized ant defensive behaviours as individual when only one ant responded (for all cases the attacked ant) and as collaborative when more than one ant responded (between 2 and 4 ants with 64% of collaborative responses including 2 ants, mean ± SD 2.43 ± 0.63 ants). We also registered what kind of behaviour ants adopted: C posture, bite or run away. In C posture, the ant pulls its abdomen completely through its legs and stands stilted on its back four legs with the front two legs in the air; the mandibles are open. The bite behaviour was defined when ants bite by opening and closing fast their mandibles. In the run-away behaviour an increase of speed close to the parasitoid area was evident. In collaborative defences, all workers participating in the defence adopted the same behaviour. To evaluate the effect of ant activity, we worked at different moments along the day obtaining different ant flux conditions (low and high ant flux, from 27 to 123 and from 143 to 404 ants/min respectively). We estimated the ant flux by counting for 1 min the number of unloaded and loaded ants in both directions in a point of the trail before starting each treatment.

2.3. Statistical analysis

To determine whether ants responded more frequently to phorids than to non-phorid flies and controls, we used a generalized linear mixed model (GLMM) where the response variable was the ant response with Binomial distribution (i.e. with or without ant response), the fixed factor was the treatment (phorid fly, non-phorid fly and control) and the random factor was the nest. To determine whether phorids caused collaborative responses more frequently than other threats, we used a GLMM where the response variable was the defensive strategy with Binomial distribution (i.e. individual or collaborative defences), the fixed factor was the treatment (phorid fly, non-phorid fly and control) and the random factor was the nest. To determine whether ant flux and phorid attack behaviour (i.e. still, three or six attacks) affected the type of ant response, i.e. individual or collaborative, we used a GLMM where the response variable was the defensive strategy with Binomial distribution (i.e. individual or collaborative strategies), the fixed factor was the ant flux (low and high) and the attack behaviour (still, three or six attacks) and the random factor was the nest. Finally, to determine whether treatments affected the type of behavioural response employed by ants to defend themselves, we used a Multinomial Linear Regression (MLR) where the response variable was the ant behaviour with three categories: C posture, bite or run away; and the predictor variables were the treatment, with ant flux and attack behaviour as co-variable. Also, we evaluated whether the type of ant behavioural response against phorids depended on ant flux and/or attack behaviour, using an MLR where the response variable was the ant behaviour with three categories: C posture, bite or run away; and the predictor variables were the ant flux and attack behaviour. Because MLR do not account differences among nests, we evaluated whether treatments, ant flux and number of attacks affected the type of behavioural response employed by ants for each nest with Chi square tests. Statistical analyses were performed in the R environment (R Development Core Team, 2013) using the package 'lme4' (Bates et al., 2015) and 'nnet' (Venables and Ripley, 2002).

3. Results

We found that workers responded more frequent to phorid fly treatments than to non-phorid fly treatments and controls (i.e. the thread without flies) (GLMM, $\chi^2 = 72.68$, $p < 0.0001$, random effect: $\chi^2 = 68.01$, $p < 0.0001$, Tukey post-hoc test, phorid and non-phorid fly: $z = 6.89$, $p < 0.0001$, phorid and control: $z = 7.71$, $p < 0.0001$, non-phorid and control: $z = 0.90$, $p = 0.64$; Fig. 1). Furthermore, strategies used to defend themselves depended on treatments; collaborative strategies were more frequent when ants were attacked by a phorid fly than when they were attacked by non-phorid fly or for the control to (GLMM, $\chi^2 = 16.45$, $p = 0.0003$, random effect: $\chi^2 = 4.49$, $p = 0.03$, Tukey post-test, phorid and non-phorid fly: $z = 3.24$,

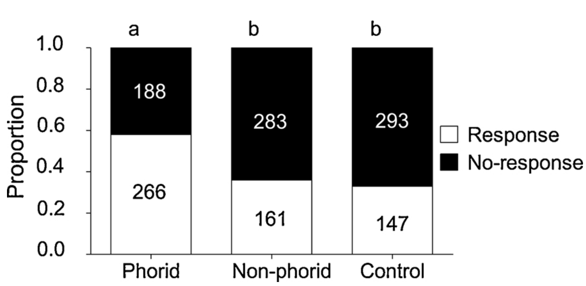

Fig. 1. Proportion of ant response and non-response to phorid, non-phorid fly and control (i.e. thread without flies). Different letters denote statistically different groups (GLMM, $\chi^2 = 72.68$, $p < 0.0001$, random effect: $\chi^2 = 68.01$, $p < 0.0001$, Tukey post-hoc test, phorid and non-phorid fly: $z = 6.89$, $p < 0.0001$, phorid and control: $z = 7.71$, $p < 0.0001$, non-phorid and control: $z = 0.90$, $p = 0.64$). Numbers inside bar indicate the number of events.

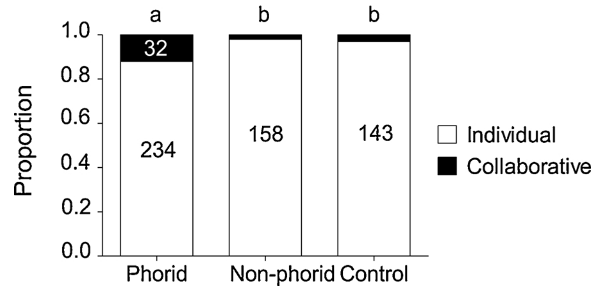

Fig. 2. Proportion of individual and collaborative responses to phorid, non-phorid fly and control (i.e. thread without flies). Different letters denote statistically different groups (GLMM, $\chi^2 = 16.45$, $p = 0.0003$, random effect: $\chi^2 = 4.49$, $p = 0.03$, Tukey post-test, phorid-non phorid fly: $z = 3.24$, $p = 0.003$, phorid-control: $z = 2.72$, $p = 0.02$, non-phorid fly-control: $z = 0.66$, $p = 0.79$). Numbers inside bar indicate the number of events, in case of non-phorid fly and control, 3 and 4 collaborative response were observed, respectively.

$p = 0.003$, phorid and control: $z = 2.72$, $p = 0.02$, non-phorid fly and control: $z = 0.66$, $p = 0.79$; Fig. 2).

Regarding whether a higher ant flux or number of phorid attacks caused that ants defend collaboratively, we found no-effect (GLMM, ant flux: $\chi^2 = 0.005$, $p = 0.98$, attack number: $\chi^2 = 0.53$, $p = 0.76$, random effect: $\chi^2 = 1.72$, $p = 0.19$; Fig. 3). The probability of collaborative responses was similar during low and high ant flux in the trail (Fig. 3A), as well as if phorids were still or attacking three or six times (Fig. 3B).

Finally, we found that the behaviour of ants used to defend themselves varied with treatments (MLR, treatment effect: $\chi^2 = 38.06$, $p < 0.0001$ MLR, ant flux: $\chi^2 = 14.48$, $p = 0.0007$, attacks: $\chi^2 = 38.87$, $p < 0.0001$; Fig. 4). When ants were attacked by phorids they adopted C postures more frequently and bite less frequently than when they were attacked by non-phorid flies or with the control (Tukey post-test, run-away behaviour: phorid and non-phorid fly: t-ratio =

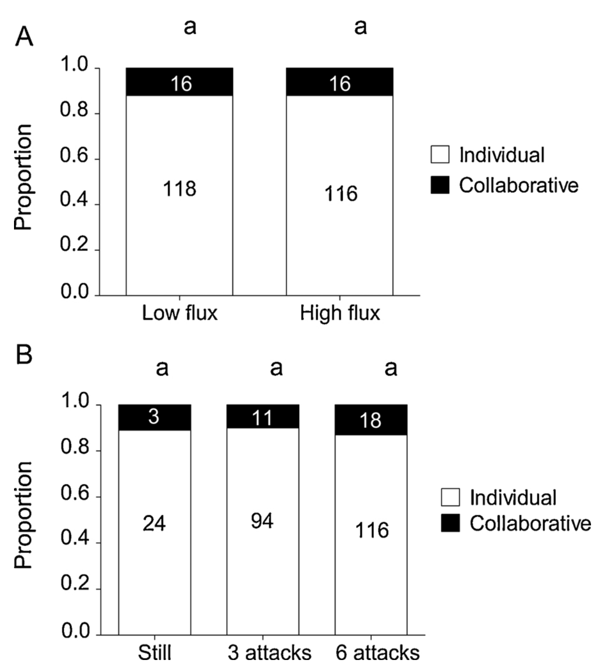

Fig. 3. Proportion of individual and collaborative response to phorid during low and high ant flux (panel A) and when phorid was still over the trail, attacked 3 and 6 times. Equal letters denote no-statistically different groups (GLMM, ant flux: $\chi^2 = 0.005$, $p = 0.98$, attack number: $\chi^2 = 0.53$, $p = 0.76$, random effect: $\chi^2 = 1.72$, $p = 0.19$). Numbers inside bar indicate the number of events.

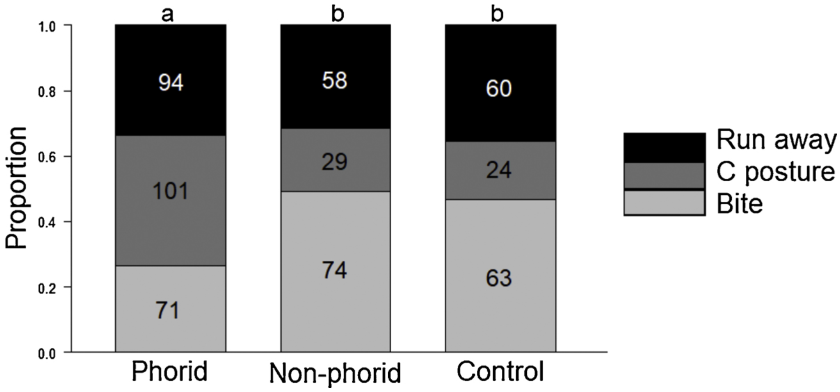

Fig. 4. Proportion of ant responses (C posture, bite and run away) against phorids, non-phorid flies and control. Different letters denote statistically different groups (MLR, treatment effect: $\chi^2 = 38.06$, $p < 0.0001$; Tukey post-test, run-away behaviour: phorid and non-phorid fly: t-ratio = 0.41, p = 0.91, phorid and control: t-ratio = 0.41, p = 0.91, non-phorid fly and control: t-ratio = 0.74, p = 0.75, bite behaviour: phorid and non-phorid fly: t-ratio = 4.95, p = 0.0009, phorid and control: t-ratio = 4.56, p = 0.0002, non-phorid fly and control: t-ratio = 0.27, p = 0.96, and C posture behaviour: phorid and non-phorid fly: t-ratio = 4.92, p = 0.0001, phorid and control: t-ratio = 5.22, p = 0.0006, non-phorid fly and control: t-ratio = 0.33, p = 0.94). Numbers inside bar indicate the number of events.

0.41, p = 0.91, phorid and control: t-ratio = 0.41, p = 0.91, non-phorid fly and control: t-ratio = 0.74, p = 0.75, bite behaviour: phorid and non-phorid fly: t-ratio = 4.95, p = 0.0009, phorid and control: t-ratio = 4.56, p = 0.0002, non-phorid fly and control: t-ratio = 0.27, p = 0.96, and C posture behaviour: phorid and non-phorid fly: t-ratio = 4.92, p = 0.0001, phorid and control: t-ratio = 5.22, p = 0.0006, non-phorid fly and control: t-ratio = 0.33, p = 0.94). Considering ant behaviour in each nest, we found the same differentiated response to phorids in 7 of the 11 nests studied (Fig. A1). Furthermore, regardless the ant flux in the trail ants used the same behaviours to defend themselves against phorids, but when phorids were still over the trail ants run away less frequently and bite more than when ants were attacked three or six times (MLR, ant flux: $\chi^2 = 1.51$, p = 0.47, attacks: $\chi^2 = 10.82$, p = 0.03, interaction: $\chi^2 = 2.49$, p = 0.65, Tukey post-test, run-away behaviour: still and 3 attacks: t-ratio = 6.25, p = 0.0001, still and 6 attacks: t-ratio = 6.37, p = 0.0001, 3 and 6 attacks: t-ratio = 0.29, p = 0.95, bite behaviour: still and 3 attacks: t-ratio = 2.99, p = 0.03, still and 6 attacks: t-ratio = 3.29, p = 0.02, 3 and 6 attacks: t-ratio = 0.43, p = 0.90, and C posture behaviour: still and 3 attacks: t-ratio = 0.51, p = 0.87, still and 6 attacks: t-ratio = 0.11, p = 0.99, 3 and 6 attacks: t-ratio = 0.68, p = 0.78; Fig. 5). Considering ant behaviours in each nest, although only 3 nests showed the same differentiated response when phorids were still or attacking, ants from those nests with non-significant results showed a trend like the general pattern (Fig. A2).

4. Discussion

The ability to distinguish between parasitic and non-parasitic threats allows animals to decrease costs associated with antiparasitic behaviours. We found that *A. cephalotes* responds with higher probability against phorid flies than against non-phorid flies and the thread without flies. This result suggests that specific chemical or visual cues of phorid flies are recognized by leaf-cutting ants triggering a behavioural response. Ants, and insects in general, use a wide range of chemical cues and signals (e.g. trail pheromones) for nestmate recognition, defence, colony founding and identifying resources (Cardé and Bell, 1995). Beside chemical communication, ants use visual cues for example to orient themselves and travel from nest to resources and vice versa (Vilela et al., 1987) and stridulatory vibration signals to food recruitment, alarm communication and attract nestmates to join excavation (Pielström and Roces, 2012; Roces and Hölldobler, 1995). Although some authors have proposed that ant defensive behaviours are triggered by the detection of parasitoid wing buzzing at short range (Hickling and Brown, 2000; Porter et al., 1995), previous studies have shown that vibrations are sensed only as substrate-borne signals, since ants are unable to perceive airborne sound (Roces and Tautz, 2001). In agreement with this, we used dead specimens of phorids that preserve chemical (they were frozen only for one hour) and visual but not vibrational cues (i.e. parasitoid wing buzzing), and found that ants responded against them. Therefore, ants might recognize phorids by some chemical and/or visual cue triggering a specific defensive behaviour. Although leaf-cutting ants are the major pest in the Neotropics and phorid parasitoids have been proposed as a management tool for them (Guillade and Folgarait, 2015), we know little about how leaf-cutting ants recognize these parasites. Our results offer bases for future studies evaluating the importance of chemical and visual cues used by leaf-cutting ants to recognize and respond against phorid flies.

We found that the defensive behaviour adopted by ants depended on the kind of treatment that ants were exposed to, i.e. phorids, non-phorid flies or threads alone. C postures were more common in presence of phorid flies than in presence of the other treatments. C postures are known to be assumed by ants after phorid attack (Folgarait, 2013), even in other ant species such as *Solenopsis invicta* (Wuellner et al., 2002). Proximate causes about why ants assume postures after attack are unclear. Considering that workers open their mandibles during C posture and alarm pheromones are secreted by the mandibular gland (Hughes and Goulson, 2001; Norman et al., 2014) and the Dufour and venom gland located in the gaster tip (Hölldobler and Wilson, 1986), workers might release alarm pheromones that attract nearby workers. These attracted workers might help with defence or might attend the attacked ant afterwards (Wuellner et al., 2002). Another explanation is that ants adopting this position are prepared and in a better position for an upcoming attack (Scharf et al., 2011). Whatever the reason why leaf-cutting ants adopt this posture against phorid flies, this ability of recognizing a parasitoid and responding adequately against it (and not to any other threat) can provide a benefit to them by, for example, increasing the amount of time available for foraging (Feener and Brown, 1992; Guillade and Folgarait, 2015; Morrison, 1999; Porter et al., 1995).

Despite living in group might have a higher risk of parasitism, social organisms can express a variety of individual, collective and collaborative behaviours to limit parasitism (Cremer et al., 2007; Meunier, 2015). Here, we found that collaborative defences were more frequent when ants were exposed to phorid flies than to non-phorid flies or in control, possibly increasing the chances of ants to avoid parasitoid oviposition. It has been shown that collaborative behaviours have the benefit to perform tasks that exceed physical and cognitive abilities of individuals (Czaczkes and Ratnieks, 2013; Krause et al., 2010; Laland, 2004). However, the workforce required is higher for collaborative behaviours than for individual ones. Therefore, selective pressures may favour the use of collaborative behaviour in context of real danger, in this case the presence of a phorid parasitoid (Schaller et al., 2015). Several social organisms such as snapping shrimps, aphids, termites and bees use collaborative and/or collective defensive behaviours only when the individual response fails or when the threat (parasite, predator or intruder) is risky enough (Eisner et al., 1976; Foster, 1990; Tóth et al., 2005). Here, we demonstrated that leaf-cutting ants differentiate parasitoid flies from other flies, showing a collaborative response against them, in contrast to the other flies and the control string,

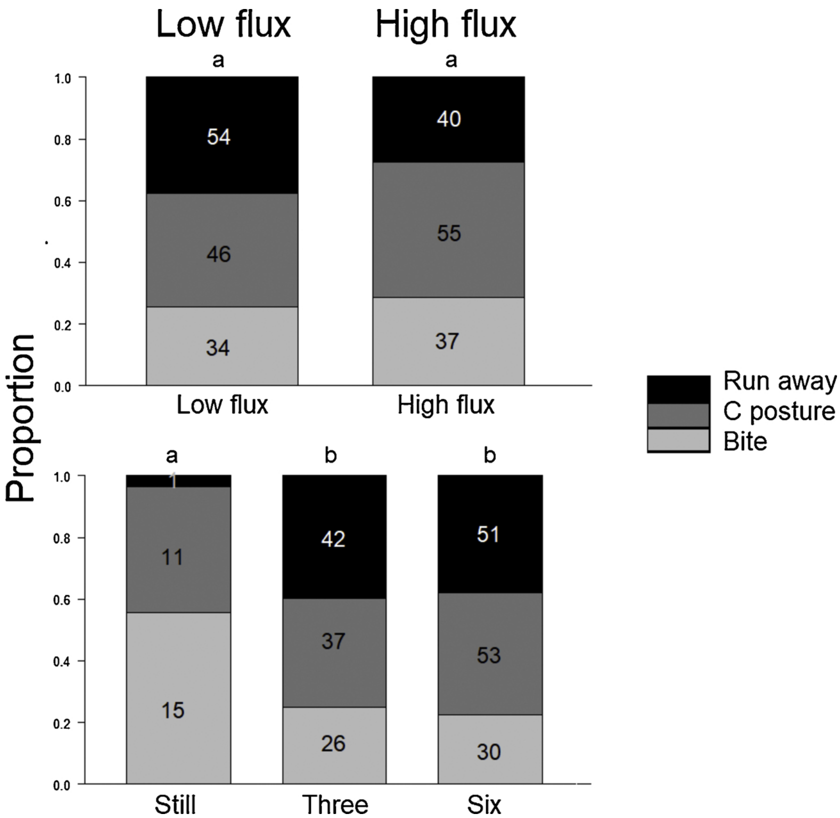

Fig. 5. Proportion of ant responses (C posture, bite and run-away) against phorids during low and high ant flux and with treatments still, attacking 3 and 6 times. Different letters denote statistically different groups (MLR, ant flux: $\chi^2 = 1.51$, $p = 0.47$, attacks: $\chi^2 = 10.82$, $p = 0.03$, interaction: $\chi^2 = 2.49$, $p = 0.65$, Tukey post-test, run-away behaviour: still and 3 attacks: t-ratio = 6.25, p = 0.0001, still and 6 attacks: t-ratio = 6.37, p = 0.0001, 3 and 6 attacks: t-ratio = 0.29, p = 0.95, bite behaviour: still and 3 attacks: t-ratio = 2.99, p = 0.03, still and 6 attacks: t-ratio = 3.29, p = 0.02, 3 and 6 attacks: t-ratio = 0.43, p = 0.90, and C posture behaviour: still and 3 attacks: t-ratio = 0.51, p = 0.87, still and 6 attacks: t-ratio = 0.11, p = 0.99, 3 and 6 attacks: t-ratio = 0.68, p = 0.78). Numbers inside bar indicate the number of events.

for which almost no collaborative responses were displayed.

However, we found that only a 12% of responses to phorids were collaborative and these were performed by 4 workers as maximum even when the ant flux raised to 400 ants per min. Although we cannot reject that some trait of alive phorids may be lost after the freezing process and manipulation, the observed frequency and the number of participating workers in collaborative behaviours was similar to that found during natural phorid attacks (Elizalde and Folgarait, 2012). For example, *Atta vollenweideri* collaboratively responds against *Eibesfeldtphora trilobata* in 16% of the situations and a similar number of ants participate in this (Elizalde and Folgarait, 2012). Also, using dead specimens of phorids allowed us to control fly size, behaviour, activity and abundance without an apparent effect on the response level of workers. Therefore, the observed frequency and number of participating ants in collaborative responses seems to represent well what happens in nature. Another possible explanation is that costs of collaborative defences may exceed their benefits; for example, if collaborative defences are not enough to deter phorids or nearby workers have higher risk of being parasitized by helping the attacked ant. However, not much is known about effectiveness of collaborative responses of leaf-cutting ants against phorid attack, which is surprising given the large literature on ant phorid interaction.

Several studies have shown that ant defensive behaviours are context-depended (Norman et al., 2014; Parmentier et al., 2015; Tranter et al., 2015; Whitehouse and Jaffe, 1996). For example, *Atta laevigata* recruit soldiers in face of a vertebrate threat, but recruit small ants when is exposed to conspecific and interspecific ant threat (Whitehouse and Jaffe, 1996). Contrary to our prediction, we found that frequency of individual and collaborative responses was independent of ant activity in the trail and number of phorid attacks. This could be consequence of too close flux categories that dilute the effect. We fixed the two ant flux categories according to ant activity in the studied nests: a low and a high flux (from 27 to 123 and from 143 to 404 ants/min, respectively). However, we found the same result considering frequency of individual and collaborative responses in extreme ant flux conditions (27–62 ants per min for low versus 193–404 for high flux), but in this case ants tended to use collaborative responses more frequently in the highest flux than in the lowest flux (18% against 6% of the responses). This, in concert with the natural low frequency of collaborative defences (Elizalde and Folgarait, 2012), does not allow rejecting conclusively the possibility that collaborative responses depended on the number of workers around the area of parasitoid attack. Regarding the absence of effect of number of phorid attacks, one possibility is that three or six attacks were insufficient to elicit collaborative behaviours with higher probability. Collaborative and collective defences in other organisms are performed when individual responses fails in shooing the threat (Tóth et al., 2005). Therefore, it is plausible that three or six attacks over the same workers or the lack of movement of a dead phorid (e.g. there is no ovipositor injection or landing on the host ant) do not trigger a collaborative defence because the ant can defend itself and/or nearby ants do not perceive the phorid, which might also explain why so few ants participated in collaborative defence.

5. Conclusions

Ecological interactions between insect hosts and their parasitoids impose strong selective forces on behaviours and have the potential to shape the structure of communities (Hawkins, 2005). Here, we showed that leaf-cutting ants responded selectively to phorid attacks suggesting that they recognize specifically the parasitoid. Generally collaborative responses are more effective than individual responses, i.e. by collaborative responses social organisms perform tasks that exceed the physical and cognitive abilities of individuals. However, leaf-cutting ants used them only to face a known threat. Costs and benefits of collaborative defences as well as how collaborative defences arise whether by individual recognition or recruitment might clarify why leaf-cutting ants use them against phorids.

Declarations of interest

None

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Appendix A

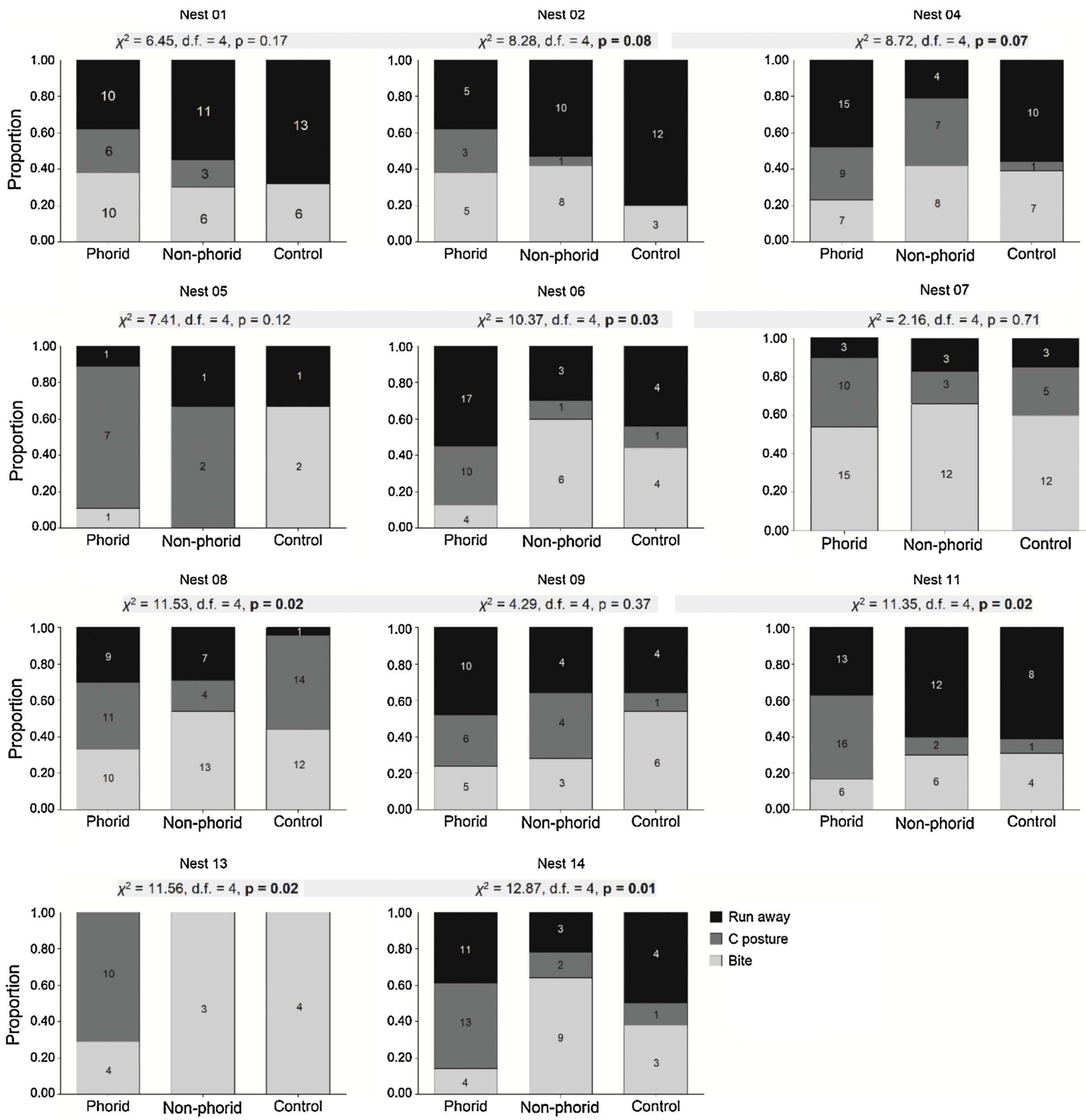

Fig. A1. Proportion of ant responses (C posture, bite and run away) against phorids, non-phorid flies and control for each nest. Numbers inside bar indicate the number of events. Statistical results are shown below nest label. P-value < 0.05 are in bold. Most nests (7 from 11) showed the same pattern found considering all together.

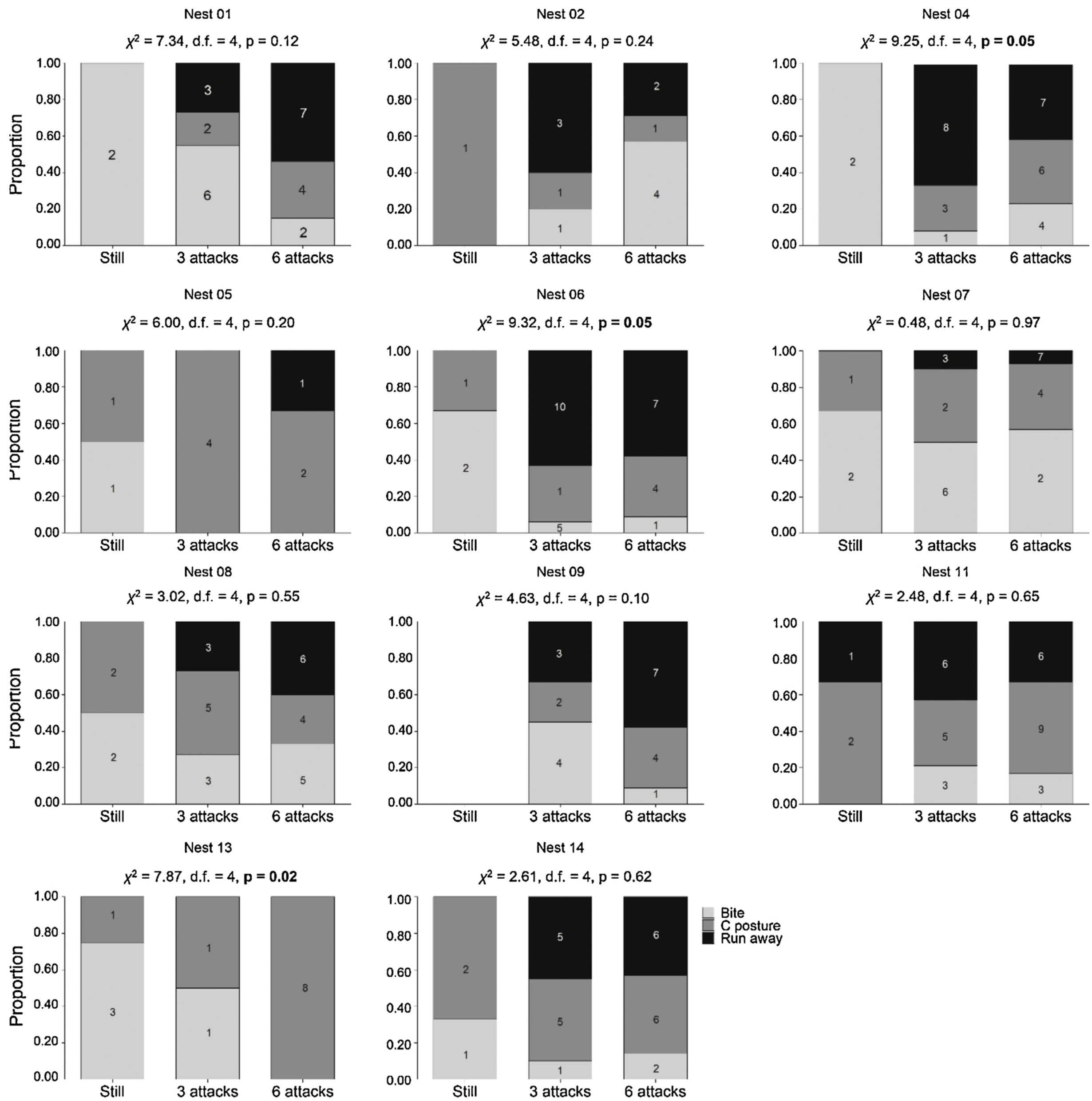

Fig. A2. Proportion of ant responses (C posture, bite and run away) against phorids attacking 3 and 6 times for each nest. Statistical results are shown below nest label. P-value < 0.05 are in bold. Most nests show the same pattern found considering all together.

Appendix B. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.beproc.2019.06.005.

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