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Poor odours, strength, and persistence give their rewards: the strategy of

*Mutilla europaea.*

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ABSTRACT

Social insect colonies are attractive for many arthropods. The rare velvet ant, *Mutilla europaea*, visit colonies of *Polistes* wasps, but to date it was unclear which resources it targeted therein. Our field observations and bioassays showed that velvet ants visit the colonies of the social wasp *Polistes biglumis* almost undisturbed and probably feed on larval wasp saliva. Chemical insignificance and resistance are the characteristics that allow velvet ants to visit unharmed wasp colonies and gain such a nutritious reward.

Key words: cleptoparasitism, *M. europaea, P. biglumis*, social insects, larval wasp saliva
INTRODUCTION

Social insect colonies are an incredibly valuable source of resources. They are composed by numerous, related individuals that endoparasites and pathogens can use as hosts and a worker caste that social parasites can exploit; they contain food storage and immature brood that other animals can prey upon and nests that they can use as shelters. It is not surprising, then, that social insect colonies are infested by arthropods which penetrate, depredate or exploit the colony resources at various extent (cleptoparasites, Evans and West-Eberhard 1970; for general reviews, Schmid-Hempel 1998; social predators, Nash and Boomsma 2008). Among other parasites, mutillid wasps visit social insect colonies (Brothers et al. 2000 and references therein). For example, the females of the velvet ant *Mutilla europaea* Linnaeus (Hymenoptera: Mutillidae) enter bumblebee colonies to reproduce as bumblebee parasitoids. Moreover, they have also been observed visiting *Polistes* spp. colonies. Although velvet ants possibly search wasp colonies for food (Brothers et al. 2000), what resource they target therein is unknown. *M. europaea* has a poorly known natural history because it is extremely rare (Brothers et al. 2000). Adults are sexually dimorphic; males are smaller than females and have wings, whereas females are wingless, brilliantly coloured and have a hardened cuticle (Evans and West-Eberhard 1970).

During our long-term study on populations of *Polistes biglumis* Linnaeus (Hymenoptera: Vespidae), we have noticed several times that females of *M. europaea* visit these wasps’ colonies (M.C.L. pers. obs.). Wasps from the family Vespidae are known for having very good recognition abilities (Lorenzi et al. 1997) and therefore it is reasonable to ask how female velvet ants can visit their colonies and how the residents respond to these intruders. We performed behavioural observations in field *P. biglumis* colonies to detect which resources velvet ants target and recognition bioassays to identify how resident wasps defend their colonies against velvet ants.

MATERIALS AND METHODS
Study sites

The study was performed over two summers in 2006 and 2007, at two different sites in the Western Alps (Montgenèvre, Hautes Alpes, France and Ferrere, Val Stura di Demonte, Cuneo, Italy), where two dense *P. biglumis* populations live.

Behavioural observations of naturally occurring visits of wasp nests by velvet ant females

A total of 117 *P. biglumis* colonies were located: 78 were in the pre-emergence period (i.e., founding phase, when the colony is composed of its single foundress and her immature brood) and 39 in the post-emergence period (i.e., the colony is composed of its single foundress and her adult and immature brood). We carried out 222 hours of field colony observation (about 2h per colony). We recorded the content of each nest cell (i.e. whether the cell was empty or contained eggs, larvae, pupae or honey) before and after each observation session.

During the field colony observations, only 3 velvet ants visited each a wasp colony. In the analysis of these observational data, we focused on the velvet ants and calculated the number of bites they received by the resident wasp(s), the time velvet ants were under attack, the time they spent walking on the nest or headfirst in each nest cell (the only behaviours velvet ants exhibited while on wasp nests). Additional behavioural data on velvet ants visiting wasp nests were collected by A.U. who observed 2 velvet ants females each visiting a wasp nest and noted their behaviour and the nest-cell contents. Thus, data on the behaviour of velvet ants on wasp nests come from 5 individuals.

Sample collection for recognition bioassays

We could not collect large numbers of samples because velvet ants are rare and difficult to collect. We rarely saw them in field (about 20 specimens were seen over the two seasons of study). Furthermore, velvet ant females are fast, armed with a sting, and difficult to capture. However, we
were able to collect 9 velvet ant females for behavioural tests by direct sightings and dry pitfall traps. Pitfall traps were largely unsuccessful (we trapped 3 females in 153 traps placed).

We also collected 3 *P. biglumis* foundresses from their pre-emergence colonies.

Wasps and velvet ants were freeze-killed and stored in freezer until they were used as dead insects in recognition bioassays.

**Recognition bioassays**

Interactions between velvet ant and *P. biglumis* occurred rarely in the field. Therefore, we simulated the visit of wasp nests by velvet ants and tested the response of *P. biglumis* wasps to velvet ant by performing recognition bioassays in the field. Several studies have used recognition bioassays in the past to simulate visits of insects on wasp nests (e.g., Bonavita-Cougourdan et al. 1991; Lorenzi et al. 1997; Ruther et al. 2002; Cristina Lorenzi et al. 2011).

We performed the bioassays on a total of 90 *P. biglumis* colonies in the two study sites. Of the 90 colonies, 71 were in the pre- and 19 in the post-emergence period. We artificially introduced into the 71 pre-emergence colonies each of the following: 1) a dead velvet ant female (n = 6); 2) a dead velvet ant female that had previously been washed in 1 ml pentane for 1 h to remove the epicuticular lipids (n = 3); and 3) a *P. biglumis* foundress (i.e. foundresses of alien colonies, potential usurpers) (n = 3). For logistic reasons (e.g., change of weather, wasp(s) leaving the nest), we could not perform the three tests in all the nests; in the results, the largest sample size for each comparison is reported. Additionally, we tested the reaction of post-emergence colonies to velvet ants by introducing one of two dead velvet ant females into the 19 post-emergence colonies.

The bioassays were performed in undisturbed, non-manipulated wasp colonies during sunny days, within the activity period of the wasps, by presenting colonies with one dead insect at a time. Dead insects were presented in random order, at intervals > 10 min. We checked for potential effects of the rank of presentation of *Mutilla* females on wasps’ responses by means of r. Dead
insects were held with forceps, placed 1 cm from the anterior face of the nest, and kept there for 1 min from the first unambiguous reactions by the resident wasp(s). We counted how many attacks (bites, grasping, darts; see Table 1 for a description) resident wasp(s) exhibited towards the presented insect. In pre-emergence colonies the number of foundresses was always 1. In post-emergence colonies, we divided the total number of attacks by the number of resident wasps which were on the anterior face of the nest during the bioassay. In this way, each tested colony supplied a single datum. Subsequently, we compared the number of attacks and the number of grasps (the most aggressive behaviour) that each dead insect received in each test, using the Wilcoxon test. We tested whether wasp responses were affected by the rank of presentation of dead insects using Spearman r, test.

Tests were not blind because of obvious morphological differences between species. However, reactions by resident wasps were sufficiently clearly cut to avoid subjective interpretation.

Statistical analyses were carried out using the statistical program SPSS 17.0.
RESULTS

Behavioural observations of naturally occurring nest visits by velvet ant females

During the 222 hours of field observations of *P. biglumis* colonies, we recorded only 3 visits by velvet ant females, of which 1 occurred in a pre-emergence colony and 2 in post-emergence colonies. Visits lasted respectively 31, 90 and 27 min.

In the three visits, velvet ants were rarely attacked by resident wasps. Indeed, they were under attack on average for only 3.79% of the time they spent on the wasp nests and were ignored by the wasps for the remaining time. Attacks were more frequent in the first 10 min of each visit than later (although the difference is not significant: Wilcoxon test, N = 3, Z = -1.604, P = 0.109). The two additional visits by velvet ants which we happened to see occurred in pre-emergence colonies and lasted 6 and 15 min respectively (from the moment we started the observation).

Overall, the 5 velvet ant females spent on average 69.87% of the time (range 50.7 - 83.3%) headfirst in the cells of *P. biglumis* nests, with only the tips of their abdomens protruding. Velvet ants never visited cells with honey stores. Instead, they spent significantly more time (up to 12 min) headfirst in cells containing *P. biglumis* larvae than in any other cells, i.e. empty, with eggs, or with pupae (Wilcoxon test, N = 5, Z = -2.023, P = 0.043). Wasp larvae had no visible damages after velvet ants left the cells. Velvet ants spent the rest of the time on nests walking. They had no liquid food exchange with adult wasps.

Recognition bioassays

During our bioassays, wasp responses were not affected by the rank of presentation of dead insects ($r_s$, P > 0.4, all comparisons).

When placed in front of a wasp nest (i.e. simulating a visit of the wasp colony), velvet ants were moderately attacked by resident foundresses, but attacks were significantly less than those to foundresses from alien colonies (Wilcoxon test, N = 41, Z = -2.710, P = 0.007) (fig. 1). Grasps were

**DISCUSSION**

Our field observations suggest that velvet ants may be cleptoparasites of the social wasp *P. biglumis*. During naturally occurring visits of *P. biglumis* nests, velvet ants ignored adult wasps as well as cells containing honey stores. Instead, velvet ants spent long periods of time headfirst in the cells which contained the host larvae (up to 12 min/cell). Wasp larvae had no visible damage after they were visited by velvet ants. The larvae of social wasps have huge salivary glands which open near their mouth. Larvae saliva is particularly nutritious, i.e. an important source of free amino-acids (reviewed in Hunt 2007), contains antibiotics (Turillazzi et al. 2004), and influences fecundity (Børgesen and Jensen 1995). Adult wasps often imbibe it after tactile stimulation as a food supplement (Cummings et al. 1999), performing the so-called trophallaxis (Wheeler 1918). Savoyard et al. (1998) also hypothesize that wasps perform special behaviors to communicate larvae to stop producing saliva when the nest is unattended (but see Jeanne and Suryanarayanan 2011). We hypothesize that velvet ants visit *P. biglumis* colonies to obtain saliva from the host larvae.

Unexpectedly, wasp colonies seem relatively undefended against velvet ants. During our observations, resident wasps attacked the velvet ants for less than 4% of the time the intruder spent on the nests. During the remaining time, resident wasps were involved in their routine colony activity. When wasps patrolled their nests and occasionally met velvet ants, wasps mostly ignored.
them. These observations suggest that velvet ants do not use propaganda or appeasement substances or repellents, since these substances should alter the behaviour of resident insects (e.g., D'Ettorre et al. 2000; Mori et al. 2000; Ruano et al. 2005). Our recognition bioassays also showed that M. europaea is usually attacked less than an alien P. biglumis foundress. Moreover, the reaction to a M. europaea is usually less aggressive than to a conspecific from another colony. These results also suggest that wasps estimate the potential intrusion of an alien foundress into their nests as riskier than that of a velvet ant. Indeed, conspecific foundresses cause a dramatic loss in fitness to foundresses, as they behave as social parasites in alien colonies. They displace the legitimate foundress, mark the host colony with their odour, destroy part of its brood and enslave the other to rear their own brood (Lorenzi and Cervo 1995; Lorenzi et al. in press). In comparison, velvet ants cause relatively small damages to the target colonies, as our data show that no larva had visible injuries after velvet ant visited them.

Chemical analyses of the epicuticular lipids have recently shown that velvet ants have less recognition cues than their P. biglumis hosts (Uboni et al., 2012). Therefore, chemical insignificance may allow velvet ants to visit wasp nests without being significantly attacked. Nevertheless, a few attacks happened during our field observations. These attacks were probably provoked by the few lipids that covered the velvet ant epicuticle, as demonstrated by our bioassays where washed M. europaea females were attacked less than intact velvet ants, but they were not enough to stop velvet ants from visiting the host colonies. During our field studies, we also observed some M. europaea males trying to approach P. biglumis nests and failing. Males and females of M. europaea do not differ in their chemical profile (A.U. M.C.L., and A.-G. Bagnères, unpublished). We hypothesise that the hardened cuticle of M. europaea females help them resist to the few attacks they receive from the resident wasps when they visit their colonies.

We conclude that a hard cuticle and a poor chemical profile are essential features that let a cleptoparasite enter wasp colonies almost unharmed and gain nutritious food rewards.
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REFERENCES


Table 1. Description of the aggressive behaviours recorded during our recognition bioassays.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
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<tbody>
<tr>
<td>Bite</td>
<td>A resident wasp stays on the nest with at least four legs and bites the dead insect with its mandibles.</td>
</tr>
<tr>
<td>Grasp</td>
<td>A resident wasp leaves the nest, flies to the dead insect, and grasps it.</td>
</tr>
<tr>
<td>Dart</td>
<td>A resident wasp performs a thrust against the dead insect, without touching it.</td>
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Fig 1. Results of recognition bioassays. Box plots show the number of attacks exhibited by resident wasp(s) against velvet ant (grey), either intact or pentane-washed, and alien *P. biglumis* foundresses (white) (* = P < 0.05; ** = P < 0.01).