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Social coercion of larval development in an ant species

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Abstract Ants provide one of the best examples of the division of labor in animal societies. While the queens reproduce, workers generally refrain from laying eggs and dedicate themselves exclusively to domestic tasks. In many species, the small diploid larvae are bipotent and can develop either into workers or queens depending mostly on environmental cues. This generates a conflicting situation between the adults that tend to rear a majority of larvae into workers and the larvae whose individual interest may be to develop into reproductive queens. We tested the social regulation of larval caste fate in the fission-performing ant *Aphaenogaster senilis*. We first observed interactions between resident workers and queen- and worker-destined larvae in presence/absence of the queen. The results show that workers tend to specifically eliminate queen-destined larvae when the queen is present but not when she is absent or imprisoned in a small cage allowing for volatile pheromone exchanges. In addition, we found that the presence of already developed queen-destined larvae does not inhibit the development of younger still bipotent larvae into queens. Finally, we analyzed the cuticular hydrocarbon profiles of queen- and worker-destined larvae and found no significant quantitative or qualitative difference. Interestingly, the total amount of hydrocarbons on both larval castes is extremely low, which lends credence on the chemical insignificance hypothesis of larval ants. Overall, our results suggest that workers control larval development and police larvae that would develop into queens instead of workers. Such policing behavior is similar in many aspects to what is known of worker policing among adults.

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Introduction

Colonies of eusocial insects demonstrate highly cooperative behavior and division of labor: a large number of workers forego reproducing themselves to carry out group domestic tasks, such as collecting food and rearing brood (Wilson 1971; Heinze and Holldobler 1994). However, this apparently harmonious arrangement is nonetheless often marred by conflicts over who will reproduce and who will not (Chapuisat and Keller 1999). For example, in some basal ant species, all females are born with spermathecae and thus have the potential to mate. Why then do most individuals commit themselves to the role of helper? It has been shown that only behaviorally dominant females reproduce whereas challengers are coerced to remain sterile (Peeters and Higashi 1989; Peeters et al. 1992; Ito 1993; Monnin and Peeters 1999; Tay and Crozier 2000; Monnin and Ratnieks 2001). In other species, the sexualization of diploid brood gives rise to morphologically distinct queen and worker castes, which limits reproductive conflicts among adults since workers have lost the ability to mate. However, conflicts remain when larvae are bipotent, meaning they can develop into either caste (Bourke and

Ratnieks 1999). Hence, diploid larvae could expect to have higher direct fitness by developing into queens rather than workers. Adults, in contrast, would derive more benefits from colony resources being allocated to worker production, which ensures colony growth and survival.

Conflicts over larval caste fate may be particularly exacerbated in species that disperse through colony fission (Pamilo 1991; Boulay et al. 2007). During fission, new colonies are formed when a young queen abandons the mother colony with a group of workers (Cronin et al. 2013). Thus, queens are protected by workers throughout their entire lives and, from the perspective of adults in the colony, only a very small proportion of diploid larvae need to develop into queens to ensure colony reproduction. Consequently, larvae compete to become queens, which may have resulted in the evolution of a complex, socially regulated system of queen production (Bourke and Ratnieks 1999). One way in which workers can regulate the production of new queens is by controlling larval food intake, thus limiting larval growth (Bonavita-Cougourdan and Passera 1978). Indeed, in the honey bee, queen-destined larvae receive specific proteins that are contained in the royal jelly (Kamakura 2011; Wolschin et al. 2011) and in ants, the food given to queen-destined larvae is richer in proteins than that given to worker-destined larvae (Smith et al. 2008; Smith and Suarez 2010; Caut et al. 2013). In addition to controlling food, workers may also physically aggress cheating larvae—that is, larvae that would selfishly develop into queens in a circumstance in which no queen should be produced—as in *Myrmica* species and *Harpegnathos saltator* (Brian 1973; Penick and Liebig 2012).

The role of the queen in larvae policing—defined as any behavior including biting that prevent larval development into queen—is controversial (Vargo and Passera 1991). However, her pheromones are known to help regulate the production of sexuals in several species of ants and bees, either by directly inhibiting the sexualization of larvae (Vargo and Passera 1991; Cnaani et al. 1997; Bourke and Ratnieks 1999) or by indirectly modifying worker behavior, thus provoking larvae policing (Lensky and Slabezki 1981; Boulay et al. 2007). Finally, the queen may lay down chemical signals using her Dufour's gland to mark challengers and encourage punishment by workers (Gilley 2001; Monnin et al. 2002; Smith et al. 2012a).

In this study, we analyzed the social regulation of queen production in colonies of *Aphaenogaster senilis*, a common ant species that disperses by colony fission. Although diploid larvae are bipotent until they reach the second instar stage, most larvae nonetheless develop into workers under natural conditions (Boulay et al. 2009). Young queens are produced in very small numbers and only on rare occasions, when the colony is large enough to permit fission or when the mother queen dies (Ledoux 1981; Boulay et al. 2007, 2009). When a colony is queenless, there is a nonlinear relationship between the number of bipotent larvae and the production of new queens, which are never greater than 6–7 in number (Ruel et al. 2012; Villalta et al. 2015). To explain this pattern, it has been hypothesized that more developed queen-destined larvae signal their presence to compel less developed larvae to develop into workers. We conducted bioassays to determine if workers are able to prevent larvae from becoming queens via policing and if policing behavior was influenced by queen pheromones. We then tested if older queen-destined larvae had a negative effect on the sexualization of younger larvae. Finally, we performed chemical analyses to determine if queen-destined and worker-destined larvae exhibited distinct cuticular hydrocarbon (CHC) profiles. Many authors have suggested that CHCs act as the main signals of fertility in adults (Liebig et al. 2000; Cuvillier-Hot et al. 2001; Endler et al. 2004; Le Conte and Hefetz 2008; Smith et al. 2012b, 2013; van Oystaeyen et al. 2014), but their role in conveying larval caste fate remains unknown.

Materials and methods

Model system, colony collection, and maintenance of laboratory colonies

A. senilis is a monandrous and monogynous species that is distributed over most of the Iberian Peninsula. Most colonies contain between 1000 and 2000 monomorphic workers (range 200–3000). The colonies used in our experiments were collected in Doñana National Park (southwestern Spain) between June 2007 and September 2012. In the laboratory, they were housed in artificial nests composed of an open plastic box ($28 \times 18 \times 11$ cm), whose walls were coated with Fluon to prevent ants from escaping. As shelter, ants were given five 20-cm-long test tubes; one third of each tube was filled with water, which was kept in place with a cotton plug. Nests were maintained under controlled conditions (27 °C, 50 % humidity) and were given sliced mealworms (*Tenebrio molitor*) three times a week.

Experiment 1: worker and queen control of the sexualization of larvae

To obtain the requisite number of queen-destined larvae for this experiment, we created two "production" groups from 25 colonies. Each production group was composed of 200 workers and 20 first-instar larvae. In order to limit possible bias in the composition of the groups, one half of the workers were collected outside the nests or near the entrance (and were most likely foragers or guards) while the other half was collected inside the nests (and were most likely nurses). The production groups were checked daily. As soon as a queen-destined fourth-instar larva was detected, it was removed and immediately placed, along with a worker-destined fourth-

instar larva, in a group with 50 workers from the same source colony. Fourth-instar queen-developing larvae are about two times bigger and easily distinguishable from worker-developing larvae (Villalta et al. 2016). These "observation" groups were housed in Petri dishes (9 cm in diameter) and experienced one of the following treatment conditions: (1) the queen from the source colony was present and unhindered in her movements (queenright, QR; $n = 15$); (2) the queen from the source colony was present but imprisoned in a 2-mL tube, whose lid had been replaced by wire mesh allowing the exchange of volatile chemicals with the workers (queen imprisoned, Qimp; $n = 12$). The mesh was sufficiently fine to prevent antennal contacts between the workers and the queen; (3) the workers were in contact with the source-colony queen up until the beginning of the experiment but not during the observation period (queenless, QL; $n = 15$); or (4) the workers had already been queenless for 2 weeks when the observation period began (queenless for 2 weeks, QL2w; $n = 9$). Humidity was maintained in the Petri dishes by the addition of a moist cotton ball.

Behavioral observations began 30 min after the larvae were introduced and lasted 30 min. Each minute, we recorded the number of ants that interacted with each larva; we noted the number of these interactions that were aggressive in nature (i.e., bites and threats—opening the mandibles with the gaster bent below the thorax). We then monitored the mortality levels of both larva types over a 5-day period. At the end of a trial, all the adult ants, including the queen, were returned to their source colony. Observation groups from each of the 25 source colonies were exposed to all four treatments; trials were conducted at least 1 month apart.

Experiment 2: effect of larvae with established caste fates on the sexualization of younger larvae

As in the previous experiment, we created two production groups of 200 workers and 20 first-instar larvae for each of 20 recently collected source colonies. Larval development was monitored daily for 3 weeks. When the first queen-destined fourth-instar larva was detected in a production group, two experimental groups were immediately created using the group's source colony. These experimental groups were composed of 200 workers and 20 first-instar larvae. One experimental group received the queen-destined fourth-instar larva, while the other group received a worker-destined fourth-instar larva from one of the production groups from the same source colony. We continued to supply both experimental groups with the same numbers of queen-destined or worker-destined fourth-instar larvae as they appeared in the production groups over the next 7 days. The total number of larvae supplied varied from 1 to 6, depending on the availability of queen-destined larvae in the production groups (the availability of worker-destined larvae was not limiting). On the eighth day, all the fourth-instar larvae we had added were removed (some had already reached the pupal stage). We then monitored the development of the first-instar larvae over the next 6 weeks in each experimental group.

Chemical analyses

Twelve queen-destined larvae and twelve worker-destined larvae were selected from twelve different queenless colonies. First, the larvae were photographed using a stereomicroscope equipped with a digital camera. Then, we used ImageJ open-source software to measure the length (a) and width (c) of each larva. Both measurements were treated as the two semi axes of a prolate spheroid, the surface area of which was calculated as (Beyer 1987) follows:

$$S = 2\pi a^2(1 + a \times c/e \arcsin(e)), \text{ where } e = \sqrt{(1 - a^2/c^2)}$$

The larvae were killed and then immersed in 100 μL of hexane for 1 h to extract their CHCs. We subsequently removed the corpses and evaporated the samples under a nitrogen flow. The extracts were stored at 4 °C until the gas chromatography-mass spectrometry (GC-MS) analysis was run. First, the extracts were injected into a gas chromatograph (GC 2010 Shimadzu) equipped with a flame ionization detector. The temperature program ran from 130 to 240 °C at 15 °C min^{-1}, and then from 240 to 300 °C at 3 °C min^{-1}. Twenty nanograms of C20 was added to each sample as an internal standard. The samples were then pooled by caste and injected into a Perkin Elmer TurboMass GC-MS using similar chromatographic conditions. The eluting hydrocarbons were identified by their fragmentation patterns.

Data analysis

All statistical analyses were performed using R (v. 2.14.1; R Core Team 2012). The behavioral results from the first experiment (i.e., the average number of contacts and aggressive interactions displayed by the workers towards the introduced queen-destined or worker-destined fourth-instar larvae) were analyzed using linear mixed-effect models (Lmer; lme4 package) where the experimental treatment, the caste of the introduced larva, and the interaction of the two variables were fixed effects; the identity of the source colony was included as a random effect to account for the non-independence of observations and measurements made on groups coming from the same mother colony. Larva mortality in the different treatment groups was compared using a generalized linear model (GLM) with a binomial distribution. Although a binomial distribution better models binary data such as death versus survival, GLM models with binomial distributions do not accurately estimate main effects. As a consequence, only the differences between levels are given in the Results section.

For the results of the second experiment, a linear mixed-effects model was fitted to test the effects of the number and caste of the supplied larvae, and the interaction between the two variables, on the total number of female pupae produced from week 2 to week 7 of the experiment. The identity of the source colony was included in the model as a random effect. A similar model was fitted using the proportion of queens produced among the female pupae as a response variable; the same fixed and random effects were used. Also, employing Pearson's correlation test, we examined correlations in both the total numbers of female pupae produced and the proportions of queens produced in the experimental groups that received queen-destined versus worker-destined fourth-instar larvae from the same source colony.

The amount of each CHC present was calculated based on the relative area of the GC-MS peaks (i.e., compared to the internal standard), and the amounts present on the larvae's entire bodies were estimated. The Wilcoxon test was used to compare the amounts of each compound found on queen-destined versus worker-destined larvae. We controlled for familywise multiple comparisons using the Holm-Bonferroni correction. Given that ten null hypotheses were tested, the alpha level was set to 0.005 instead of to 0.05.

Results

Experiment 1: worker and queen control of the sexualization of larvae

Worker policing of undesired queen-destined larvae was contingent on the presence of the queen. The average number of contacts between workers and introduced larvae was affected by the treatment (Fig. 1a; lmer: $F_{3, 91} = 31.40$, $P < 0.0001$), larval caste ($F_{1, 78} = 38.10$, $P < 0.0001$), and the interaction between the two variables ($F_{3, 78} = 3.84$, $P = 0.012$). Hence, overall, workers interacted more with queen-destined than worker-destined larvae in the QR, Qimp, and QL groups, but not in the QL2w group. A similar pattern was observed for aggressive interactions (Fig. 1b; lmer: treatment: $F_{3, 87} = 2.41$, $P = 0.072$; larval caste: $F_{1, 64} = 25.01$, $P < 0.0001$; interaction: $F_{3, 64} = 2.99$, $P = 0.037$). Although QR, Qimp, and QL workers behaved more aggressively towards queen-destined larvae than towards worker-destined larvae, QL2w ants did not (Fig. 1b). There were significantly fewer contacts and aggressive interactions displayed by QL2w workers towards larvae, irrespective of larval caste fate (Fig. 1a, b).

The higher level of aggression displayed towards queen-destined larvae in the QR group meant they had a higher level of mortality compared to worker-destined larvae (Fig. 2; glmer: $z = 3.513$, $P = 0.0004$). However, in the Qimp and QL groups, queen-destined and worker-destined larvae had equal levels of mortality. The mortality of queen-destined larvae in
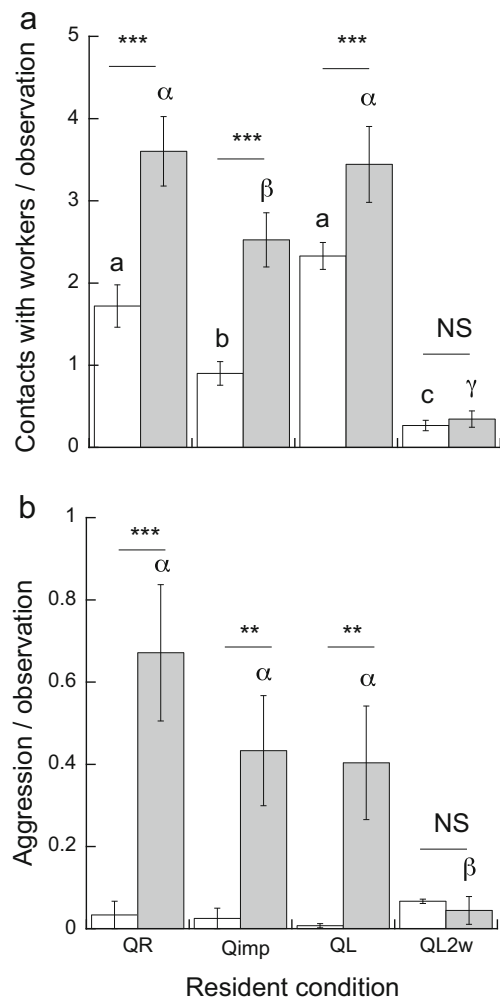

Fig. 1 Number of contacts (**a**) and proportion of aggressive interactions (**b**) (mean ± SE) displayed by workers towards larvae in the different treatment groups during the 30-min observation period. Differences between worker-destined larvae and queen-destined larvae across the treatment groups are denoted with *a* and *b* versus *α* and *β*, respectively. The *asterisks* indicate differences between larval castes within the treatment group. The color of the bars shows which groups received queen-destined (*gray*) versus worker-destined (*white*) fourth-instar larvae

the QL2w group was similar to that of larvae in the Qimp and QL groups (Fig. 2).

Experiment 2: effect of larvae with established caste fates on the sexualization of younger larvae

The results of the second experiment provided no evidence that the presence of queen-destined larvae negatively affected the sexualization of younger larvae. Over the 7 weeks of the experiment, the number of first-instar larvae that developed into worker or queen pupae was unaffected by caste or the number of fourth-instar larvae supplied during week 1 (Fig. 3a; lmer: caste: $F_{1, 18} = 0.04$, $P = 0.85$; number: $F_{1, 18} = 0.003$, $P = 0.96$). The interaction between caste and the number of supplied larvae was also insignificant (lmer:
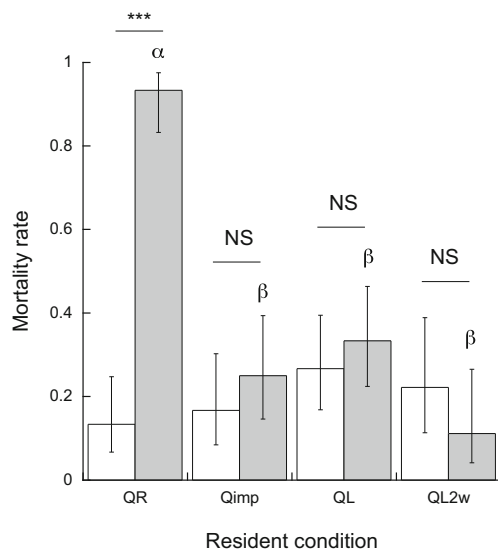
Fig 2 Mortality of worker-destined (*white bars*) and queen-destined (*gray bars*) larvae (mean ± SE) after five days in each of the treatment groups. Significant differences across treatment groups for the queen-destined larvae are denoted by α and β. The asterisks indicate significant differences between worker-destined and queen-destined larvae among the treatment groups

caste × number: $F_{1, 18} = 0.05$, $P = 0.83$). Only seven male pupae were produced over the whole experiment. The proportion of queen-destined pupae produced decreased significantly as greater numbers of fourth-instar larvae were supplied (Fig. 3b; lmer: number: $F_{1, 18} = 25.92$, $P > 0.0001$). Neither larval caste nor the interaction between caste and the number of supplied larvae had a significant effect (lmer: caste: $F_{1, 18} = 4.38$, P = 0.051; lmer: caste × number: $F_{1, 18} = 1.79$, $P = 0.20$).

The identity of the source colony accounted for a large amount of the total variance in the number of female pupae produced and in the proportion of queen-destined pupae produced (70.2 and 60.0 %, respectively). Furthermore, there was a significant correlation between the numbers of female pupae produced as well as between the proportions of queen-destined pupae produced in the two experimental groups created from each source colony (Fig. S1; Pearson's correlation: $t_{18} = 4.25$, $P = 0.0004$ and $t_{18} = 5.95$, $P < 0.0001$, respectively).

Cuticular hydrocarbon analyses

Both queen-destined and worker-destined larvae were relatively depauperate in CHCs compared to adults and eggs (Ruel et al. 2013b). GC-MS analysis revealed only ten peaks that corresponded to 11 CHCs, whose chain lengths ranged from 25 to 30 carbons (Table 1). Methyl-branched alkanes accounted for 37 ± 4 % and 29 ± 5 % of the total CHC amounts observed on queen-destined versus worker-destined larvae, respectively. The remaining CHCs were linear alkanes. Dimethyl alkanes were completely absent. Overall, CHC amounts were significantly greater on queen-destined larvae
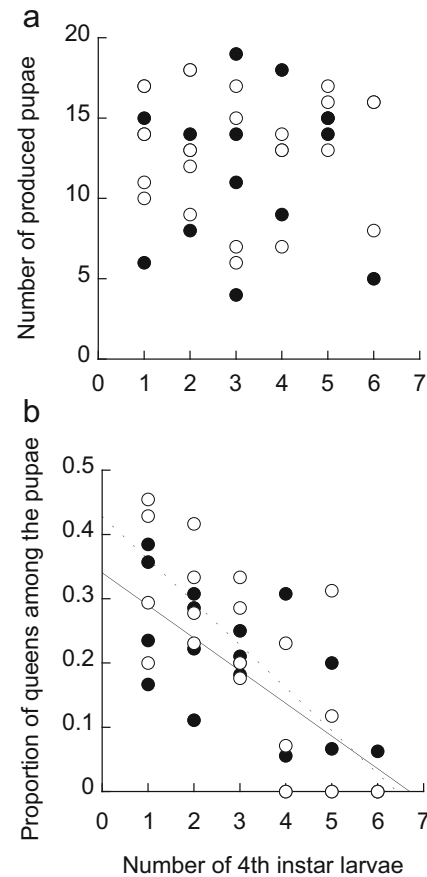

Fig. 3 Relationship between the number of fourth-instar larvae supplied during the first week of the experiment and the total number of female pupae that developed from first-instar larvae (**a**) as well as the proportion of queens produced from among the female pupae (**b**). The *filled circles/solid line* and the *empty circles/dotted line* represent the groups that received queen-destined versus worker-destined fourth-instar larvae, respectively

Table 1 Amounts of CHCs found on queen-destined and worker-destined larvae. Compounds are ordered by retention time. Quantities are expressed in nanograms per square millimeter (mean ± SE). The alpha level was set to 0.005 after the Holm-Bonferroni correction

Compounds	Queens	Workers	*P* values
C25	0.30 ± 0.06	0.57 ± 0.14	0.14
C26	0.25 ± 0.07	0.26 ± 0.07	0.98
C27	1.09 ± 0.31	1.45 ± 0.42	0.04
3MeC27	0.72 ± 021	0.88 ± 0.26	0.71
C28	0.12 ± 0.03	0.02 ± 0.01	0.02
10 + 12MeC28	0.17 ± 0.05	0.20 ± 0.06	0.86
C29	2.09 ± 0.60	2.54 ± 0.73	0.18
11MeC29	0.25 ± 0.07	0.14 ± 0.04	0.09
5MeC29	0.36 ± 0.10	0.31 ± 0.09	0.44
C30	0.24 ± 0.07	0.22 ± 0.06	1.00
Sum	5.58 ± 1.61	6.59 ± 1.90	0.41

than on worker-destined larvae (21.7 ± 1.4-ng larva^{-1} versus 12.6 ± 0.8-ng larva^{-1}, respectively; the Wilcoxon test: $W = 78$, $P < 0.0001$). However, when cuticular surface area was taken into account, CHC amounts per square millimeter did not differ significantly between queen-destined and worker-destined larvae (5.58 ng mm^{-2} versus 6.59 ng mm^{-2}; the Wilcoxon test: $W = 57$, $P = 0.41$). Following the Holm-Bonferroni correction, castes did not differ in their surface area corrected amounts of any of the CHCs (Table 1).

Discussion

As is probably the case in many species of social Hymenoptera, in *A. senilis*, young larvae are bipotent and can develop into workers or queens depending on environmental conditions. Theory predicts that an individual larva may experience greater direct fitness if it develops into a queen (Bourke and Ratnieks 1999). The following question therefore arises: why do most larvae forgo developing into queens to become helpers? Previous studies have shown that workers may control larval development by controlling food quality and quantity (Ichinose et al. 2009). This study shows that workers may also police cheating larvae and that this behavior is likely to depend on exposure to the queen's contact pheromones. These pheromones have not been identified but they can include CHC like in other ant species (van Oystaeyen et al. 2014) or other secretions (Lensky and Slabezki 1981). However, in contrast to what was hypothesized, queen-destined larvae do not inhibit the sexualization of younger larvae. Furthermore, according to our chemical analyses, queen-destined larvae do not display a specific CHC.

Worker policing has been described as an efficient means of controlling cheaters in insect societies (Wenseleers et al. 2004). The workers of several species have been found to act aggressively towards individuals that could potentially challenge the queen's reproductive monopoly (Kikuta and Tsuji 1999; Monnin and Ratnieks 2001; Ruel et al. 2013a). The results of our first experiment show that worker policing also serves to control larval development. When the queen was present and had unhindered movement, adult workers demonstrated aggression towards queen-destined but not worker-destined larvae. Although we had to stop behavioral observations after 1 h; queen-destined larvae were likely aggressed during a much longer period. We may, therefore, hypothesize that prolonged aggression may have provoked the high level of mortality of the queen-destined larvae in under 5 days while the worker-destined larvae survived. In contrast, when adult workers had been queenless for 2 weeks prior to the beginning of the experiment (QL2w treatment), they had fewer overall interactions and fewer aggressive interactions with the larvae. As a result, both types of larvae showed higher levels of survivorship. When the queen was

removed or imprisoned (QL and Qimp treatments, respectively), there were no short-term effects on worker aggressive behavior: workers remained highly aggressive towards queen-destined larvae during the first hour of the experiment. However, 5 days into the experiment, queen-destined larvae experienced low levels of mortality that did not significantly differ from those of worker-destined larvae. The mortality rates of the fourth-instar larvae in the Qimp and QL treatments were similar to those in the QL2w treatment and were independent of larval caste fate. One possible explanation for this result is that the effects of queen's pheromones on workers' behavior remain a few hours even after the queen has disappeared: in this case, the workers' aggression towards larvae may remain high during 1 h after the queen has been removed but may decrease sufficiently rapidly to prevent larval death. Another possible (and non-mutually exclusive) explanation is that the queen actively participates in policing behavior, although we did not observe aggressive interactions between the queen and the queen-destined larvae.

Interestingly, the number of contacts between the workers and both types of larvae was very low in the QL2w treatment. One explanation of this relative disinterest of the workers towards the larvae after 2 weeks in queenless condition may be that workers start laying their own haploid eggs. As a consequence, they may spend less time caring for the larvae than when they are in queenright condition (Iwanishi et al. 2003; Villalta et al. 2015). Irrespective of larval type, the number of contacts in the Qimp treatment was higher than in the QL2w treatment but did not reach the level of the QR and QL treatments. This might be due to the fact that the imprisoned queen attracted several workers that intended to release her. These workers were, therefore, less in contact with the larvae.

Under both natural and laboratory conditions, queenlessness in *A. senilis* colonies stimulates the production of a few new queens (Ruel et al. 2013a; Villalta et al. 2015). The production of a small number of young queens is adaptive at the colony level because only one of them is likely to succeed her mother while the others will be rapidly eliminated by workers. Therefore, allocating a lot of resources to produce many queens would be counterproductive for a colony. What mechanisms limit queen production to a few individuals? Our results do not support the hypothesis according to which older queen-destined larvae inhibit the sexualization of younger bipotent larvae. However, our findings do highlight the colony-level costs of producing queens: while the total production of females (workers + queens) was unaffected when more fourth-instar larvae were added during the first week of the second experiment, smaller proportions of queen-destined pupae were produced during the following weeks. In addition, source colonies demonstrated large differences in their abilities to rear larvae and produce new queens. This variability may result from colony-level behavioral syndromes—in some

colonies, workers may be more efficient at nursing larvae than in others (Blight et al. 2015; Villalta et al. 2016).

Several studies have highlighted the role of CHCs in ant communication both within and among colonies. Within colonies, the queen and her eggs have specific HC profiles that differ from those of workers and worker-laid eggs (Endler et al. 2004; Ruel et al. 2013a; b see also van Oystaeyen et al. 2014). Surprisingly, we found that the larvae possessed very few CHCs, and no caste-related qualitative or quantitative differences were detected. Only ten peaks were found on the larvae while 18 peaks were found on eggs and 44 peaks were found on adults (Ruel et al. 2013a, b). In particular, the specific dimethylalkanes that abound on adult queens and their eggs were completely absent from the larvae. In addition, total amounts of larval HCs (21.7 ± 1.4 ng versus 12.6 ± 0.8 ng for queen-destined and worker-destined larvae, respectively) were lower than total amounts of egg HCs (87 ± 9 ng versus 73 ± 13 ng, for queen-laid and worker-laid eggs, respectively), even though eggs are approximately ten times smaller in volume (Ruel et al. 2013b). CHCs also play a role in nestmate recognition, and the fact that they are present in very low concentrations on larval cuticles fits with the idea that larvae are "chemically insignificant;" indeed, they can easily be exchanged among colonies without becoming the targets of aggression (Signorotti et al. 2014). This finding suggests that workers are able to discriminate between queen-destined and worker-destined larvae using other chemical cues. These cues could be composed of volatile compounds such as aliphatic esters identified in honeybee larvae (Le Conte et al. 1989; Trouiller et al. 1992) or polar compounds, which may not have been extracted with our procedure. Finally, non-chemical signal cues like behavior or morphology may identify larval caste.

In conclusion, the results of this study clearly show that, in *A. senilis*, workers are able to detect and eliminate larvae that would selfishly develop into queens in small queenright colonies. This behavior, which depends on the presence of and contact with a queen, shares similarities with worker policing behavior observed among adults in other species of ants and other social insects. Interestingly, the life history of *A. senilis* is comparable to that of the honeybee *Apis mellifera* and comparing both systems may help understanding the evolution of communication between adults and larvae. A major challenge for further studies will be to identify the signals that allow adult workers to regulate the production of queen-destined larvae.

References

Beyer WH (1987) CRC standard mathematical tables, 28th edn. CRC Press, Boca Raton

Blight O, Díaz-Mariblanca GA, Cerdá X, Boulay R (2015). A proactive–reactive syndrome affects group success in an ant species. Behav Ecol: (in press)

Bonavita-Cougourdan A, Passera L (1978) Étude comparative au moyen d'or radio-actif de l'alimentation des larves d'ouvrières et des larves de reine chez la fourmi *Plagiolepis* pygmaea Latr. Insect Soc 25: 275–287

Boulay R, Hefetz A, Cerdá X, Devers S, Francke W, Twele R, Lenoir A (2007) Production of sexuals in a fission-performing ant: dual effects of queen pheromones and colony size. Behav Ecol Sociobiol 61:1531–1541

Boulay R, Cerda X, Fertin A, Ichinose K, Lenoir A (2009) Brood development into sexual females depends on the presence of a queen but not on temperature in an ant dispersing by colony fission, *Aphaenogaster senilis*. Ecol Entomol 34:595–602

Bourke AF, Ratnieks FL (1999) Kin conflict over caste determination in social Hymenoptera. Behav Ecol Sociobiol 46:287–297

Brian M (1973) Caste control through worker attack in the ant *Myrmica*. Insect Soc 20:87–102

Caut S, Jowers MJ, Cerda X, Boulay RR (2013) Questioning the mutual benefits of myrmecochory: a stable isotope-based experimental approach. Ecol Entomol 38:390–399

Chapuisat M, Keller L (1999) Extended family structure in the ant *Formica paralugubris*: the role of the breeding system. Behav Ecol Sociobiol 46:405–412

Cnaani J, Borst DW, Huang Z-Y, Robinson GE, Hefetz A (1997) Caste determination in Bombus terrestris: differences in development and rates of JH biosynthesis between queen and worker larvae. J Insect Physiol 43:373–381

Cronin AL, Molet M, Doums C, Monnin T, Peeters C (2013) Recurrent evolution of dependent colony foundation across eusocial insects. Annu Rev Entomol 58:37–55

Cuvillier-Hot V, Cobb M, Malosse C, Peeters C (2001) Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. J Insect Physiol 47:485–493

Endler A, Liebig J, Schmitt T, Parker JE, Jones GR, Schreier P, Hölldobler B (2004) Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. Proc Natl Acad Sci U S A 101:2945–2950

Gilley DC (2001) The behavior of honey bees (Apis mellifera ligustica) during queen duels. Ethology 107:601–622

Heinze J, Holldobler B, Peeters C (1994) Conflict and cooperation in ant societies. Naturwissenschaften 81:489–497

Ichinose K, Boulay R, Cerdá X, Lenoir A (2009) Influence of queen and diet on nestmate recognition and cuticular hydrocarbon differentiation in a fission-dispersing ant, *Aphaenogaster senilis*. Zool Sci 26: 681–685

Ito F (1993) Social organization in a primitive ponerine ant: queenless reproduction, dominance hierarchy and functional polygyny in *Amblyopone* sp. (*reclinata* group)(Hymenoptera: Formicidae: Ponerinae). J Nat Hist 27:1315–1324

Iwanishi S, Hasegawa E, Ohkawara K (2003) Worker oviposition and policing behaviour in the myrmicine ant *Aphaenogaster smythiesi japonica* Forel. Anim Behav 66:513–519

Kamakura M (2011) Royalactin induces queen differentiation in honeybees. Nature 473:478–483

Kikuta N, Tsuji K (1999) Queen and worker policing in the monogynous and monandrous ant, *Diacamma* sp. Behav Ecol Sociobiol 46:180–189

Le Conte Y, Hefetz A (2008) Primer pheromones in social hymenoptera. Annu Rev Entomol 53:523–542

Le Conte Y, Arnold G, Trouiller J, Masson C, Chappe B, Ourisson G (1989) Attraction of the parasitic mite Varroa to the drone larvae of honey bees by simple aliphatic esters. Science 245:638–639

Ledoux A (1981) Conséquences de l'orphelinage sur la biologie des colonies d'*Aphaenogaster senilis* (Mayr.) (Hymenoptera Formicoidea). CR Acad Sci III-vie 293:237–240

Lensky Y, Slabezki (1981) The inhibiting effect of the queen bee (*Apis mellifera* L.) foot-print pheromone on the construction of swarming queen cups. J Ins Physiol 27:313–323

Liebig J, Peeters C, Oldham NJ, Markstädter C, Hölldobler B (2000) Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*? Proc Natl Acad Sci U S A 97:4124–4131

Monnin T, Peeters C (1999) Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav Ecol 10:323–332

Monnin T, Ratnieks FL (2001) Policing in queenless ponerine ants. Behav Ecol Sociobiol 50:97–108

Monnin T, Ratnieks FL, Jones GR, Beard R (2002) Pretender punishment induced by chemical signalling in a queenless ant. Nature 419:61–65

Pamilo P (1991) Evolution of colony characteristics in social insects. I. Sex allocation. Am Nat 137:83–107

Peeters C, Higashi S (1989) Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe*. Naturwissenschaften 76:177–180

Peeters C, Billen J, Hölldobler B (1992) Alternative dominance mechanisms regulating monogyny in the queenless ant genus *Diacamma*. Naturwissenschaften 79:572–573

Penick CA, Liebig J (2012) Regulation of queen development through worker aggression in a predatory ant. Behav Ecol 23:992–998

R Core Team (2012). R: A language and environment for statistical computing.

Ruel C, Cerdá X, Boulay R (2012) Behaviour-mediated group size effect constrains reproductive decisions in a social insect. Anim Behav 84:853–860

Ruel C, Hefetz A, Cerdá X, Boulay R (2013a) Recognition of caste and mating status maintains monogyny in the ant *Aphaenogaster senilis*. Behav Ecol Sociobiol 67:1295–1305

Ruel C, Lenoir A, Cerdá X, Boulay R (2013b) Surface lipids of queen-laid eggs do not regulate queen production in a fission-performing ant. Naturwissenschaften 100:91–100

Signorotti L, Jaisson P, d'Ettorre P (2014) Larval memory affects adult nest-mate recognition in the ant *Aphaenogaster senilis*. Proc R Soc B 281:20132579

Smith CR, Suarez AV (2010) The trophic ecology of castes in harvester ant colonies. Funct Ecol 24:122–130

Smith CR, Anderson KE, Tillberg CV, Gadau J, Suarez AV (2008) Caste determination in a polymorphic social insect: nutritional, social, and genetic factors. Am Nat 172:497–507

Smith AA, Hölldobler B, Liebig J (2012a) Queen-specific signals and worker punishment in the ant Aphaenogaster cockerelli: the role of the Dufour's gland. Anim Behav 83:587–593

Smith AA, Millar JL, Hanks L, Suarez AV (2012b) Experimental evidence that workers recognize reproductives through cuticular hydrocarbons in the ant *Odontomachus brunneus*. Behav Ecol Sociobiol 66:1267–1276

Smith AA, Millar JL, Hanks L, Suarez AV (2013) A conserved fertility signal despite population variation in the cuticular hydrocarbon phenotype of the trap-jaw ant *Odontomachus brunneus*. J Exp Biol 216:3917–3924

Tay WT, Crozier RH (2000) Nestmate interactions and egg-laying behaviour in the queenless ponerine ant *Rhytidoponera* sp. 12. Insectes Soc 47:133–140

Trouiller J, Arnold G, Chappe B, Le Conte Y, Masson C (1992) Semiochmical basis of infestation of honeybee brood by Varroa Jacobsoni. J Chem Ecol 18:2041–2053

van Oystaeyen A, Oliveira RC, Holman L, van Zweden JS, Romero C, Oi CA, d'Ettorre P, Khalesi M, Billen J, Wäckers F, Millar JG, Wenseleers T (2014) Conserved class of queen pheromones stops social insect workers from reproducing. Science 287:287–290

Vargo EL, Passera L (1991) Pheromonal and behavioral queen control over the production of gynes in the Argentine ant *Iridomyrmex humilis* (Mayr). Behav Ecol Sociobiol 28:161–169

Villalta I, Angulo E, Devers S, Cerdá X, Boulay R (2015) Regulation of worker egg laying by larvae in a fission-performing ant. Anim Behav 106:149–156

Villalta I, Blight O, Angulo E, Cerdá X, Boulay R (2016) Early developmental processes limit socially mediated phenotypic plasticity in an ant Behav Ecol Sociobiol in press.

Wenseleers T, Helanterä H, Hart A, Ratnieks FL (2004) Worker reproduction and policing in insect societies: an ESS analysis. J Evol Biol 17:1035–1047

Wilson EO (1971) The Insect Societies. Harvard.

Wolschin F, Mutti NS, Amdam GV (2011) Insulin receptor substrate influences female caste development in honeybees. Biol Lett 7:112–115