

Production of sexuals in a fission-performing ant: dual effects of queen pheromones and colony size

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Abstract Models based on the kin selection theory predict that in social hymenopterans, queens may favor a lower investment in the production of sexuals than workers. However, in perennial colonies, this conflict may be tuned down by colony-level selection because of the trade off between colony survival and reproductive allocation. In this study, we present a survey of sexual production in colonies of *Aphaenogaster senilis*, a common species of ant in the Iberian Peninsula. Similar to most species that reproduce by fission, males were found in large excess compared to gynes (172:1). Sexuals were more likely to be found in queenless than in queenright (QR) field colonies. However, we also found a few gynes and numerous males in very

large QR colonies. We compared these data with those available in the literature for *A. rudis*, a congeneric species from North America that has independent colony founding. The sex ratio in this species was only five males for each female, and sexuals were mostly found in QR nests, irrespective of colony size. We confirmed queen inhibition of sexual production in *A. senilis* in laboratory experiments and provide evidence that this inhibition is mediated by a nonvolatile pheromone. To seek the potential source of such a queen pheromone, we analyzed the secretions of two conspicuous exocrine glands, the Dufour's and postpharyngeal glands (DG and PPG, respectively) in both queens and workers. Both secretions were composed of hydrocarbons, but that of DG also contained small quantities of tetradecanal and hexadecanal. The hydrocarbon profile of the DG and PPG showed notable caste specificity suggesting a role in caste-related behavior. The PPG secretions also differed between colonies suggesting its role in colony-level recognition. We suggest that in *A. senilis*, there are two modes of colony fission: First, in very large colonies, gynes are produced, probably because of the dilution of the queen pheromone, and consequently one or more gynes leave the mother colony with workers and brood to found a new nest. This is beneficial at the colony level because it avoids the production of costly sexuals in small colonies. However, because the queen and workers have different optima for sexual production, we hypothesize that queens tend to overproduce the pheromone to delay their production. This in turn may drive workers to leave the mother colony during nest relocation and to produce sexuals once they are away from the queen's influence, creating a second mode of colony fission.

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Introduction

Although colonies of social insects are characterized by a high level of cooperation between individuals, there are also multiple entangled conflicts of interests (Mehdiabadi et al. 2003). One such conflict is between workers and queens over the proportion of resources allocated into sexuals vs workers (reproductive allocation). Models based on the kin selection theory have suggested that workers are selected to capitalize on a relatively high investment in sexuals, while the queen may gain more by investing more in workers, which guarantee better colony survival (Pamilo 1991). However, the reproductive allocation conflict is also deeply affected by the colony social structure. It may be limited in species in which the workers' inclusive fitness relies exclusively on the queen offspring, or it may be extended if workers have an opportunity to replace the actual queen with a more fertile or more related one (Bourke and Chan 1999; Bourke and Ratnieks 1999; Herbers et al. 2001). In species that form perennial nests, the expression of caste conflict over reproductive allocation may be constrained by colony-level selection. Like solitary iteroparous organisms, colonies cannot allocate an excessively high proportion of resources to reproduction because this would jeopardize their survival. This explains why small nests generally allocate a lower proportion of resources than larger ones to reproduction (Oster and Wilson 1978; Brian 1979; Elmes and Wardlaw 1982). This is also in agreement with the observation that augmentation of sexual production parallels colony productivity (Sundström 1995) and resource availability (Herbers and Banschbach 1998; Aron et al. 2001), although other studies have failed to find such a relation (Backus 1995; Herbers and Banschbach 1999; Walin and Seppä 2001). The effect of colony size and resource availability may be further confounded because large colonies are also likely to dominate resources and to collect relatively higher quantities of food than smaller ones.

A particular aspect of reproductive allocation conflicts concerns the proportion of resources invested into gynes vs workers (caste allocation conflict). Studies conducted on various social hymenopterans have demonstrated that queens limit or determine the timing of gyne production. In the honeybee *Apis mellifera*, gyne (virgin queens) production in the presence of the queen is limited to the swarming period, but if the queen is removed, emergency queen cells are immediately constructed and several gynes are reared (Butler 1961; Winston et al. 1990). In the bumble bee *Bombus terrestris*, the timing of gyne production seems to be under queen influence (Alaux et al. 2005, 2006). Similar queen influence was also demonstrated in several ant species including *Solenopsis invicta* (Vargo 1988), *Linepithema humile* (Vargo and Passera 1991, 1992), *Cataglyphis iberica* (Cerdá et al. 2002), and *Monomorium pharaonis* (Lim and

Lee 2005). The queen may directly affect larval physiology, or she may inhibit gyne production indirectly by modifying worker behavior. Hence, apart from a few notable cases of maternal effect (de Menten et al. 2005) or genetic control (Julian et al. 2002; Volny and Gordon 2002), the differentiation between gynes and workers seems to occur at the larval stage and depend on the type of care provided by the nurses (Wilson 1971; Brian 1973; Vargo and Passera 1991; Wheeler 1994). In addition, while workers of many species have retained functional ovaries and could increase the colony reproductive allocation by laying their own male eggs, other constraints (worker–worker conflicts over male parentage and colony-level selection) generally prevent them from doing so under queenright (QR) conditions (Woyciechowski and Łomnicki 1987; Ratnieks 1988; Hammond and Keller 2004). Under queenless (QL) conditions, these constraints disappear, and workers produce male offspring.

The queen's presence is generally conveyed through pheromonal bouquets that she emits and that can be spread via the workers throughout the nest. These pheromones may act coercively to manipulate workers, or they may act as a signal that informs workers and directs them to behave so as to gain maximum fitness (queen vs worker control; Keller and Nonacs 1993). In the honeybee, the queen mandibular secretion affects both worker ovarian development (Butler 1959; Hoover et al. 2003) and the construction of royal cells in which gynes are reared (Winston et al. 1990; Pettis et al. 1995; Dor et al. 2005). However, in this species, the inhibitory effect of the queen mandibular secretions alone is not as efficient as the actual presence of a fertile queen suggesting that other glands are involved (Katzav-Gozansky et al. 2006). In particular, the Dufour gland (DG hereafter) secretions that inhibit the production of queen-like secretions in workers may also participate in the complex queen pheromonal bouquet (Katzav-Gozansky et al. 2004). In the red imported fire ant *S. invicta*, the queen's poison gland is a source of multiple pheromones (Vander Meer et al. 1980; Vargo 1997) that may be complemented by the secretions of the postpharyngeal gland (PPG hereafter; Vargo and Hulsey 2000). It is worth mentioning that the PPG in many ant species has the same hydrocarbon composition as the epicuticle, which may be the path via which these putative pheromones are externalized. Many studies indicate that cuticular hydrocarbons act as fertility signals that govern individual workers' reproductive decisions (Peeters et al. 1999; Liebig et al. 2000; Cuvillier-Hot et al. 2001, 2004; Heinze et al. 2002; Monnin et al. 2002; Dietemann et al. 2003). In a recent study on *Camponotus floridanus*, it was found that the queen marks her eggs with her own hydrocarbons and uses them as a vehicle to signal her presence in the giant nest (Endler et al. 2004). However, a large body of evidence also suggests that cuticular hydrocarbons serve as colony recognition cues

(Vander Meer and Morel 1998; Lahav et al. 1999; Lenoir et al. 1999; Thomas et al. 1999; Wagner et al. 2000). Whether both functions are compatible, however, has remained elusive because colony and caste specificities in hydrocarbon composition have rarely been analyzed together in the same study.

The present study investigates the reproductive strategy of the ant *Aphaenogaster senilis*, a species that reproduces by colony fission whereby workers participate in colony foundation. A consequence of fission is that the number of female propagules is limited by the size of the mother colony and the minimum number of workers necessary to initiate a new nest. Moreover, colony fission may provoke local resource competition compared to independent colony founding, especially in ants, because dispersal of female propagules is limited by the lack of wings in workers. Consequently, the payoff of producing many gynes may be reduced compared to the production of males, which disperse farther away from the mother colony (Craig 1980; Bulmer 1983; Bourke and Franks 1995; Percy and Aron 2006).

Our aim was to test some of the pertinent predictions of the reproductive allocation theory in light of the peculiar mode of colony fission of *A. senilis*. According to pioneering works, fission occurs after a nest relocation, when part of the worker force abandons the old colony and founds a new one by producing a new queen (Ledoux 1971, 1976a). This differs from other well-known species like the army ants or the honeybee in which new queens are produced before the fission event (Gotwald 1995; Seeley 1995). We analyzed the number of males and gynes produced in field-collected QR and QL colonies. If only the queen affects reproductive allocation, we expected to find more males and gynes in QL colonies. If colony-level selection also acts on reproductive allocation, we expected sexual production to covary with colony size. We compared our data with those available in the literature for *Aphenogaster rudis* (Headley 1949; Talbot 1951), a species that performs independent colony foundation (Heithaus and Lubertazzi, personal communication). Worker ovarian development was also assessed for *A. senilis* under natural QL and QR conditions. Finally, we tested the volatility of the *A. senilis* queen signal in the laboratory and analyzed caste specificity of the PPG and DG, as a prerequisite to their potential implication in queen inhibition of sexual production.

Materials and methods

Sexual production, colony size, social condition, and worker ovarian development in nature

Demographic data were obtained from 151 nests of *A. senilis* collected between December 2002 and August 2006

near the Doñana National Park (South Spain: 36°58'N, 6°28'E) and 17 nests collected in Cataluña (North East Spain: 41°35'N, 2°32'E) between April 1985 and October 1988 (total, 168 nests). Both regions are characterized by a typical Mediterranean climate with a long warm and dry season and a cool winter. Moreover, preliminary analyses indicate that both populations have enough behavioral (diets and foraging activity) and demographic (nest density) similarities to pool the data (Cerdá and Boulay, personal observations). A few hours after nest collection, adult workers and sexual adults and pupae of both sexes were counted in all nests. 248 and 232 workers from three QL and four QR field-collected colonies of *A. senilis*, respectively, were also dissected to assess their ovarian development. We discriminated between two levels of ovarian development: 0, no oocyte in formation and 1, at least one oocyte in formation in one ovariole.

Sexual production in *A. senilis* was compared with two data sets available for *A. rudis* that present the demography of 117 nests collected from two populations in Ohio and Missouri between 1944 and 1949 (Headley 1949; Talbot 1951).

Queen signal volatility and effect on sexual production

The effect of *A. senilis* queens on the production of sexuals was studied in the laboratory using 20 freshly collected medium-sized colonies. Each was maintained in a plastic box that was divided into three 10×7×5-cm-adjointing compartments each housing equal-sized colony fragments (between 300 and 400 workers and about 100 young larvae). Workers of the fragment situated in the middle remained QR (QR treatment); those of the second fragment (to the left of the QR compartment) were kept as QL but could exchange antennal contacts with their QR nestmates through a single mesh (QL-SM treatment). The third fragment (to the right of the QR compartment) was separated from the QR compartment through a double mesh, which allowed the passage of volatile compounds only (QL-DM treatment). A fourth fragment of equal size was maintained in a separate container completely isolated from the queen influence (QL treatment). All the fragments were fed twice a week with sliced mealworms. They were maintained in the same rearing room and submitted to the same variations of temperature (22–27°C) and photoperiod (10:14 light/dark). The fragments were inspected twice a week for 14 weeks for the presence of sexuals. The newly emerged males and gynes were left in their natal fragment but were marked with a dot of paint on the thorax to ensure that they were counted only once.

Queen–worker chemical polymorphism

For chemical analyses, DG and PPG were excised from workers and queens of freshly collected colonies. Less than

14 h after collection, the ants were gently placed in Petri dishes at 9°C for half an hour to calm down before being frozen at -18°C for about an hour. The glands were dissected under a stereomicroscope and immersed in 50 µl of hexane. For workers, glands from three individuals were pooled per sample to obtain enough material for proper analysis. Queen secretions were copious enough to allow individual analyses. The samples were stored at -18°C until analysis. Glandular content was identified by combined gas chromatography/mass spectrometry using a DB-5 fused silica capillary column that was temperature programmed from 150 to 300°C at 5°C min⁻¹ for PPG and from 60 to 210°C at 5°C min⁻¹ then 15°C min⁻¹ to 300°C for DG. Quantification was achieved by gas chromatography using the above chromatographic conditions.

Statistics

Statistical analyses were performed using SAS 9.1 (for generalized linear models [GLZs]) or Statistica 6.0 (for all other statistics). The proportions of QL and QR colonies and the proportions of single and multiple queen colonies among the QR colonies were compared between *A. senilis* and *A. rudis* using the Pearson Chi-square test. We also checked the homogeneity of the male-to-gyne numerical ratio between Doñana and Cataluña by means of the Yate-corrected Chi-square (the total number of gynes was too small to be compared between years). A factorial analysis of variance (ANOVA) was conducted to test the effect of the social condition (QL vs QR) and species (*A. senilis* vs *A. rudis*) on colony size. The proportion of nests producing at least one gyne was determined using a GLZ with the binomial error distribution and the Logit link function. For *A. senilis*, the predictors were “social condition” and “colony size” nested within “social condition.” This permitted testing the effect of colony size independently for QL and QR colonies. In *A. rudis*, no gyne was found in QL nests (see “Results”); therefore, the GLZ for this species could only test the colony-size effect. The effects of social condition and colony size nested within social condition were also tested on the numbers of males per colony using the Poisson error distribution and Log link function. Main effects were tested with type 3 likelihood ratio (LR) test. The Wald Chi-square test was used to test whether the colony size was positively or negatively associated with gyne and male production in QL and QR colonies. Finally, the percentage of workers with level 1 ovarian development was compared between QL and QR colonies with a mixed model, with the binomial error distribution and Logit link function in which the colony was treated as a random factor. Models-adjusted means and standard errors were calculated from the parameter estimates (Littell et al. 1996).

The results of the laboratory experiments were analyzed with nonparametric statistics. The absolute numbers of gynes and males were compared between treatments (QR, QL-SM, QL-DM, and QL) with the Friedman ANOVA by ranks. Pairwise differences were estimated using a Statistica Visual Basic macro available at <http://www.statsoft.nl/>, which consists of a post-hoc test based on the differences of sums of ranks (DSR hereafter; Zar 1984).

Analysis of the hydrocarbon profiles was performed using peaks that were identified in all the samples and whose averages across all the samples (including workers and queens) were higher than 1%. To eliminate colinearity and reduce the impact of high percentages, chemical variables were standardized as follows:

$$y_{i,j}' = \frac{y_{i,j} - \hat{y}_i}{\sigma_i}$$

where $y_{i,j}$ is the percentage value for peak i in the sample j , and \hat{y}_i and σ_i are the mean percentage and standard deviation of peak i in all samples. $Y_{i,j}'$ varied between -1 and +1 and had a mean of 0 (Quinn and Keough 2002). Differences in profiles were first explored using the principal components analysis (PCA). Eigenvectors (ev hereafter) were used to estimate the contribution of the chemical variables to each axis. Multiple ANOVAs were then conducted on the scores of five main components extracted from the PCA to estimate profile differences between castes and colonies. Main effects “caste” and “colony” were tested using Pillai’s trace. ANOVA gave differences between castes and colonies for each component independently.

Results

Sexual production, colony size, social condition and worker ovarian development in nature

Of the 168 excavated nests of *A. senilis*, 154 (91.7%) were QR and 14 (8.3%) were QL. These proportions did not differ significantly from those of *A. rudis* in which 108 nests (92.3%) were QR and nine nests (7.7%) were QL (Pearson Chi-square, $\chi_1^2 = 0.04$, $P = 0.845$). However, among the QR nests, the proportion of multiple-queen colonies was higher in *A. rudis* (17.6%) than in *A. senilis* (less than 1%; Pearson Chi-square, $\chi_2^2 = 25.84$, $P < 0.001$). Field-collected colonies of *A. senilis* contained an average of 1,260±69 workers (mean±SE; $n=168$; range, 121–3,906), whereas those of *A. rudis* were significantly smaller and contained only 301±26 workers (mean±SE; $n=117$; range, 11–2,079; ANOVA_{Species}, $F_{1, 264}=78.61$, $P<0.01$). In both species, the number of workers did not differ significantly between the

QL and the QR nests (ANOVA_{Social condition}, $F_{1, 264}=0.05$, $P=0.829$; ANOVA_{Social condition×Species}, $F_{1, 264}=0.02$, $P=0.890$).

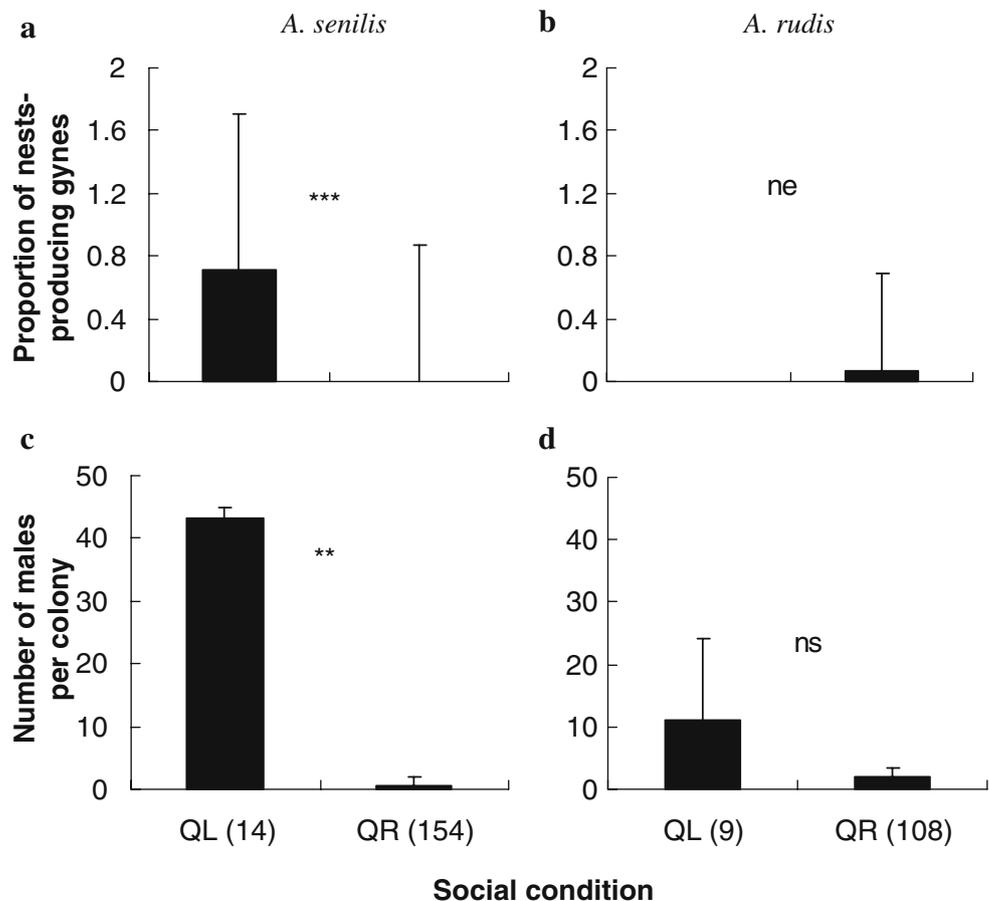
The pattern of sexual production in the field-collected colonies showed large differences between species. Overall, colonies of *A. rudis* produced 5.5 males for each gyne while those of *A. senilis* produced 172.0 males for each gyne. The proportion of male to gyne in *A. senilis* did not differ between Cataluña and Doñana (Yate Chi-square, $\chi^2_1 = 1.40$, $P = 0.238$). In *A. senilis*, gyne number never exceeded three individuals per colony, and they were more likely to be produced in QL than in QR nests (Fig. 1a; LR test_{Social condition}, $\chi^2_1 = 13.57$, $P < 0.001$). Moreover, the probability of gyne presence depended on colony size in QR (Fig. 2a; LR test_{Colony size (Social condition)}, $\chi^2_1 = 14.37$, $P < 0.001$; Wald Chi-square_{Colony size/QR}, $\chi^2_1 = 9.19$, $P < 0.01$) but not in QL nests (Wald Chi-square_{Colony size/QL}, $\chi^2_1 = 1.06$, $P = 0.303$). The logistic regression indicated that the probability of gyne presence in a QR colony increases more rapidly as a function of colony size for colonies larger than half the range (e.g., larger than 1,892 workers), which represented only 12.5% of the colonies. In *A. rudis*, up to 33 gynes were found per colony, all of which

were in QR nests (Fig. 1b) and independently of colony size (LR test_{Colony size}, $\chi^2_2 = 1.50$, $P = 0.473$).

The social condition and colony size also affected male production differently according to the species. In *A. senilis*, more males were produced in QL than in QR colonies (Fig. 1b; LR test_{Social condition}, LR test, $\chi^2_1 = 27.33$, $P < 0.001$). Moreover, the significant interaction between social condition and colony size (LR test_{Colony size (Social condition)}, $\chi^2_2 = 94.03$, $P < 0.01$) revealed that the number of males increased significantly with colony size in QR (Fig. 2b; Wald Chi-square_{Colony size/QR}, $\chi^2_1 = 124.26$, $P < 0.001$) but not QL colonies (Wald Chi-square_{Colony size / QL}, $\chi^2_1 = < 0.001$, $P = 0.951$). Again, the number of males per QR colony increased rapidly as a function of the number of workers only for extremely large nests. By contrast, in *A. rudis*, neither social condition nor colony size nested in social condition significantly affected the number of males per colony (Fig. 1d; LR tests, $\chi^2_1 = 2.39$, $P = 0.303$ and $\chi^2_1 = 0.60$, $P = 0.439$, respectively).

The number of workers with developed ovaries was evenly high under QL and QR conditions (77.02 and 78.45%, respectively; $F_{1, 472}=2.32$, $P=0.1284$). This percentage did not vary between colonies ($Z=1.42$, $P=0.0774$).

Fig. 1 Proportion of *Aphaenogaster senilis* and *A. rudis* nests that had at least one gyne (a, b) and number of males per colony (c, d; model-adjusted means±SE) in field-collected nests as a function of social condition. Data for *A. senilis* were obtained from two populations in the Iberian Peninsula. Estimates for *A. rudis* were recalculated from raw data available in Headley (1949) and Talbot (1951). Double asterisks, $P < 0.01$; triple asterisks, $P < 0.001$; ns, $P > 0.05$; ne, nonestimable difference. Values between parentheses denote sample size



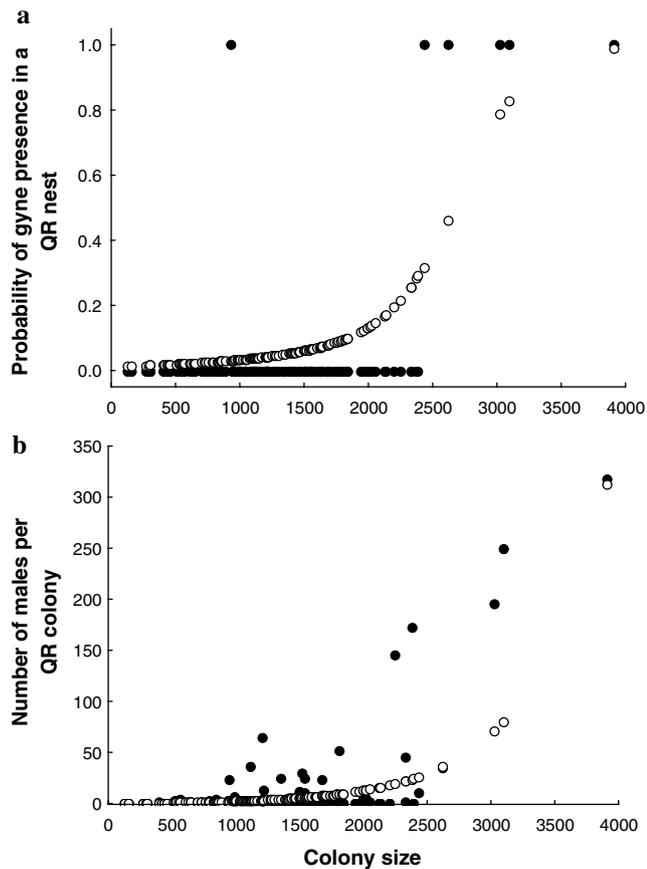


Fig. 2 Relation between colony size (number of workers) and gyne (a) and male (b) production in QR colonies of *A. senilis*. Black symbols are the observed data and open symbols are predicted data from the logistic and Poisson regressions, respectively

Queen signal effect on sexual production

In the laboratory, sexuals were produced from the fifth week on after the fragments separation. In total, 13 out of the 20 QL fragments, 12 out of the 20 QL-DM fragments, and 8 out of the 20 QL-SM fragments, but none of the QR fragments, produced gynes. The number of gynes produced per fragment was also clearly affected by queen presence (Fig. 3a; Friedman ANOVA, $\chi^2_3 = 24.12$, $P < 0.001$). The number of gynes produced in QL and QL-DM fragments was significantly higher than in the QR fragments (DSR=30.5 and 22.0, respectively, $P < 0.05$), whereas the number of gynes in QL-SM fragments was intermediate and not significantly different from the QR, the QL-DM, or the QL fragments (DSR=11.5, 10.5, and 19, respectively, $P > 0.05$). The number of males produced per fragment also differed among treatments (Fig. 3b; Friedman ANOVA, $\chi^2_3 = 17.39$, $P < 0.001$). More males were produced in the QL or QL-DM than in QR fragments (DSR=25, $P < 0.05$ and DSR=26.5, $P < 0.01$, respectively). The number of males in the QL-SM fragments was intermediate and did not significantly differ from the QR (DSR=16.5, $P > 0.05$), the

QL-DM (DSR=10, $P > 0.05$), or the QL (DSR=8.5, $P > 0.05$) fragments.

Caste specificity of DG and PPG secretions

Both queen and worker DG contained large quantities of hydrocarbons and small amounts of tetradecanal and hexadecanal. The latter aldehydes were relatively more abundant in workers than in queens (Fig. 4, Table 1). Hydrocarbons constituted a complex mixture of linear and methyl-branched alkanes, alkenes, and alkadienes containing between 13 and 20 carbons. The PCA conducted on 17 major hydrocarbon peaks from the DG gave ev's higher than 1 for the first five components, which explained 81% of the total variance (these components explained 32.48, 24.16, 12.33, 6.99, and 5.64% of the total variance, respectively). Projections of the individuals on the first

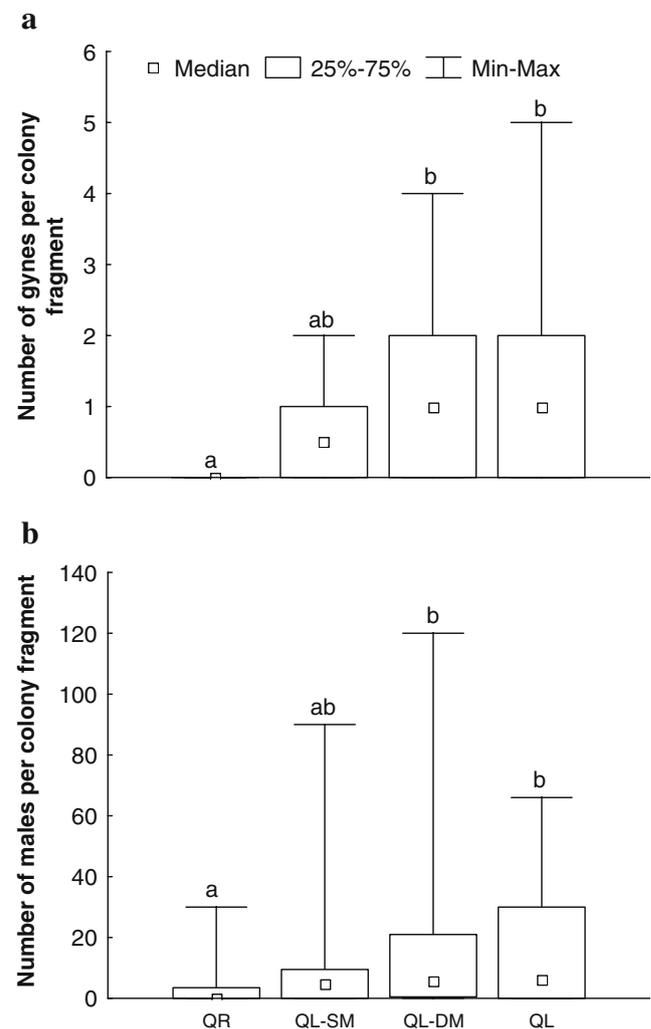
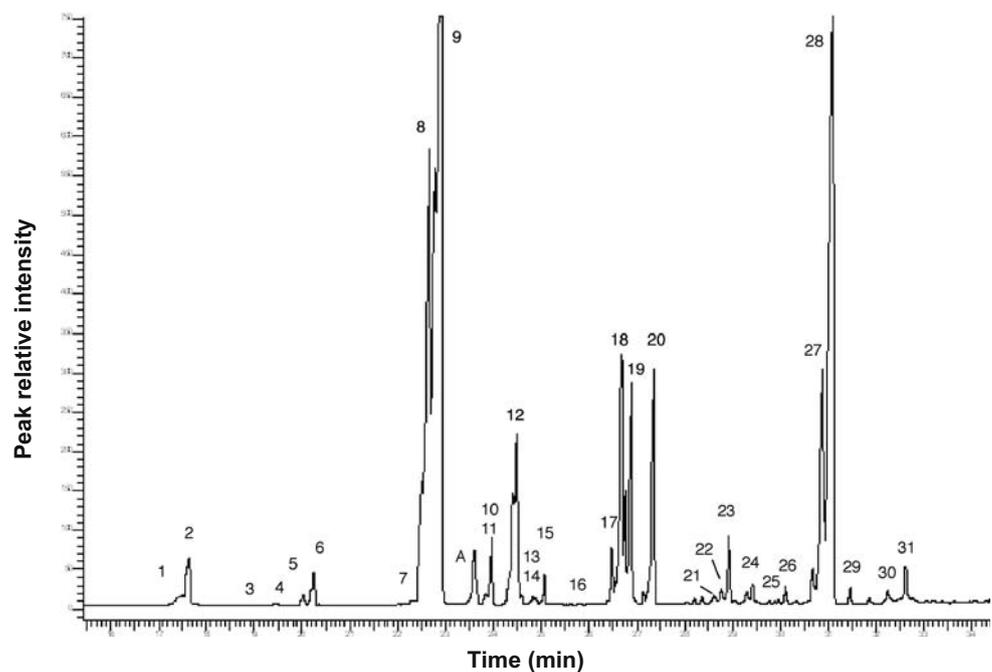


Fig. 3 Number (medians, quartiles and ranges) of gynes (a) and males (b) produced in the *A. senilis* QR and the various QL fragments after 14 weeks. Different letters denote significant differences ($P < 0.05$)

Fig. 4 Secretions of *A. senilis* worker DG. Compound names are given in Table 2 (*peak A* is anabasine contamination from the poison sac)



two components clearly separated both castes (Fig. 5a). This was mostly due to queens containing relatively more 5- and 3-methylpentadecane, hexadecane, heptadecane, nonadecane, and nonadecene (all positive ev) and less pentadecene, heptadecene, and nonadecadiene (all negative ev). Multiple analysis of variance conducted on the first five components revealed both significant between-caste and between-colony differences (Pillai's trace, $P < 0.001$ and $P < 0.002$, respectively). Univariate ANOVA revealed significant between-caste differences on components 1 ($F_{1, 48} = 17.76$, $P < 0.001$), 2 ($F_{1, 48} = 119.71$, $P < 0.001$), and 3 ($F_{1, 48} = 6.95$, $P = 0.014$) and between-colony difference on component 3 ($F_{11, 25} = 4.25$, $P = 0.001$). This suggests that caste specificity is very pronounced and may obscure colony specificity.

Worker PPG content has already been described elsewhere (Lenoir et al. 2001). It is copiously filled with long-chain linear and methyl-branched alkanes with a carbon length ranging between 25 and 31. Queen PPG contained the same compounds as workers albeit in different proportions (Table 2). PCA of the relative quantities of the 22 major PPG peaks revealed that workers and queens differed in components 1 and 3, which explained 27.15 and 13.10% of the total variance, respectively (Fig. 5b). Individual profile projection on component 2, which explained 15.87% of the variance, did not reveal any clear-cut separation between castes or colonies. Components 4–7, which also had ev's higher than 1, explained 11.53, 8.79, 6.16, and 4.78% of the total variance, respectively. Queens were characterized by relatively high proportions of 3-methylheptacosane, 3,7-+3,9-dimethylheptacosane, 4,8-+4,10-dimethyloctacosane, 5-methylnonacosane, triacontane, and 10-+12-methyltriacontane. As in DG,

multivariate ANOVA conducted on the first five components confirmed significant differences between castes and colonies (Pillai's trace: $P < 0.001$ and $P < 0.002$, respectively). Castes differed significantly with respect to the first and third components (ANOVA, $F_{1, 58} = 254.09$, $P < 0.001$ and $F_{1, 58} = 143.78$, $P < 0.001$, respectively) while colonies differed with respect to the first five components (ANOVA, $F_{1, 58} = 18.96$, $P < 0.001$; $F_{1, 58} = 4.53$, $P < 0.001$; $F_{1, 58} = 13.50$, $P < 0.001$; $F_{1, 58} = 8.44$, $P < 0.001$; $F_{1, 58} = 9.64$, $P < 0.001$).

Discussion

Colony fission permits the formation of propagules composed of a queen accompanied by an already efficient worker force. The small number of gynes produced in *A. senilis* nests is consistent with the hypothesis that in cases of colony fission, excess gyne production is disadvantageous because only one will eventually head each propagule, and that the number of gynes is limited by the size of the mother colony (Bulmer 1983; Bourke and Franks 1995). Males, on the other hand, are the dispersing sex, and therefore their mass production is beneficial. In the field, 172 *A. senilis* males were produced for each gyne, in accordance with the theory. This is in contrast to *A. rudis*, in which male production was only fivefold greater than that of gynes, again as predicted from this species' mode of reproduction. Another difference between the two species is that in nature, the *A. senilis* queen seems to inhibit sexual production, at least in most of the range of colony size, while in *A. rudis*, gynes are produced only in QR colonies, and males are produced both in QL and QR colonies.

Table 1 Relative concentration (means±SE) of the main compounds identified in the DG of *A. senilis* workers ($N=37$) and queens ($N=12$)

Peak number	Compound names	Queens	Workers
1	C13: 1	0.06±0.07	0.75±0.58
2	<i>C13</i>	<i>0.74±0.51</i>	<i>1.95±0.52</i>
3	5MeC13	0.04±0.04	0.30±0.22
4	3MeC13	0.99±0.61	0.30±0.20
5	C14: 1	0.07±0.04	0.65±0.23
6	<i>C14</i>	<i>1.46±0.70</i>	<i>2.71±1.66</i>
7	C15: 2	0.37±0.40	0.33±0.15
8	<i>C15: 1</i>	<i>1.15±0.55</i>	<i>12.83±5.71</i>
9	<i>C15</i>	<i>24.61±8.08</i>	<i>20.91±0.89</i>
10	7MeC15	0.09±0.19	0.61±0.52
11	<i>5MeC15</i>	<i>2.08±5.19</i>	<i>1.20±1.02</i>
12	<i>3MeC15</i>	<i>11.69±6.38</i>	<i>2.15±1.49</i>
13	<i>C16: 1</i>	<i>8.62±4.22</i>	<i>1.82±1.30</i>
14	Tetradecanal	1.29±1.22	0.40±0.26
15	<i>C16</i>	<i>2.90±1.74</i>	<i>1.80±0.94</i>
16	4MeC16	0.30±0.38	5.29±4.36
17	<i>C17: 2+MeC16</i>	<i>0.35±0.37</i>	<i>2.84±1.76</i>
18	<i>C17: 2</i>	<i>4.54±2.28</i>	<i>6.11±3.31</i>
19	<i>C17: 1</i>	<i>2.25±1.11</i>	<i>4.71±1.96</i>
20	<i>C17</i>	<i>8.91±1.31</i>	<i>4.36±1.63</i>
21	9+7+5MeC17	1.34±0.72	0.70±0.72
22	C18: 2	0.28±0.10	0.97±0.63
23	C18: 1	1.03±0.34	1.07±0.39
24	<i>C18</i>	<i>1.13±0.72</i>	<i>1.07±0.52</i>
25	4MeC18	0.34±0.14	0.39±0.16
26	Hexadecanal	0.08±0.06	1.08±1.00
27	<i>C19: 2</i>	<i>5.53±2.36</i>	<i>8.95±4.64</i>
28	<i>C19: 1</i>	<i>15.04±6.60</i>	<i>12.72±7.65</i>
29	<i>C19</i>	<i>1.94±1.17</i>	<i>0.59±0.34</i>
30	9MeC19	0.65±2.12	0.19±0.13
31	C20	0.13±0.27	0.22±0.18

Peaks in italics were used in PCA. See the text for peak selection strategy. Compounds are ordered by retention time. Peak numbers refer to Fig. 4.

Most field-collected *A. senilis* QL colonies had a brood that in all likelihood had originated from the former queen before her disappearance, and from which, gynes could be raised. This possibility was simulated in the lab by splitting the brood between the different nest fragments and showing that QL workers were able to rear a limited number of gynes, which reinforces some of Ledoux's findings (Ledoux 1976b). We can exclude the possibility of worker thelytoky, which has been suggested as a possible mode of gyne production in QL colonies of *A. senilis* (Ledoux 1984), because this required a thermal shock to which our lab colonies were not submitted. This suggests that under QR conditions, the queen inhibits diploid brood determination to gynes either directly or by affecting worker care behavior towards these larvae (Wilson 1971; Brian 1973; Vargo and Passera 1991; Wheeler 1994).

Males produced in QL colonies could derive either from eggs laid by the former queen or from worker-laid eggs



Fig. 5 PCA projections of *A. senilis* workers (*W*) and queens (*Q*) DG (**a**—components 1 and 2) and PPG (**b**—components 1 and 3) hydrocarbon profiles. Analyses were done on the 17 and 22 major peaks for DG and PPG, respectively (see Tables 1 and 2)

through arrhenotoky. Moreover, because the percentage of workers having at least one developed oocyte was evenly high under QL and QR conditions, it is unlikely that policing induces workers to refrain from laying eggs in the presence of the queen like in *A. cockerelli* (Hölldobler and Carlin 1989) and *A. smythiesi* (Iwanishi et al. 2003). Therefore, under QR conditions, the huge amount of worker-derived haploid eggs might be selectively destroyed and recycled to feed worker larvae, whereas under QL conditions, some of them would be allowed to develop.

The inhibition of sexual production is apparently mediated by contact cues released by the queen. The separation of a colony fragment from the queen through a double mesh, which prevented the transfer of contact cues but allowed volatiles circulation, was sufficient to launch sexual production. By contrast, using a rather conservative statistical test, the augmentation of sexual production by single mesh separation was not significant. In the congeneric species *A. smythiesi japonica*, queen cues that inhibit male production are also transmitted by contact (Iwanishi and Ohkawara 2005),

Table 2 Relative concentration (means±SE) of the main compounds identified in the PPG of *A. senilis* workers ($N=37$) and queens ($N=12$)

Compound names	Queens	Workers
C25	0.38±0.08	1.40±0.21
<i>11+13MeC25</i>	<i>0.87±0.24</i>	<i>2.57±0.55</i>
7MeC25	0.44±0.06	0.88±0.09
7,9DMC25	1.07±0.18	1.78±0.39
3MeC25	1.18±0.23	1.15±0.10
5,9DMC25	0.21±0.03	0.43±0.07
C26	2.24±0.34	0.63±0.06
<i>10+12MeC26</i>	<i>1.59±0.20</i>	<i>3.72±0.29</i>
8,12DMC26	2.24±0.32	2.82±0.21
<i>6,12+6,10DMC26</i>	<i>2.18±0.24</i>	<i>3.34±0.24</i>
4,8DMC26	1.62±0.29	3.11±0.42
C27	2.27±0.47	4.75±0.62
<i>9+11+13MeC27</i>	<i>7.46±0.84</i>	<i>15.84±1.08</i>
3MeC27	13.19±1.06	8.45±0.47
5,9DMC27	1.34±0.08	1.19±0.21
<i>3,7+3,9DMC27</i>	<i>12.04±0.85</i>	<i>5.44±0.70</i>
<i>10+12MeC28</i>	<i>10.65±0.79</i>	<i>15.66±0.81</i>
<i>4MeC28+8,12+10,12DMC28</i>	<i>11.05±0.88</i>	<i>11.22±0.77</i>
<i>4,8+4,10DMC28</i>	<i>4.34±0.39</i>	<i>2.65±0.30</i>
<i>4,8,12TMC28</i>	<i>0.96±0.17</i>	<i>1.71±0.25</i>
<i>11MeC29</i>	<i>4.56±0.38</i>	<i>5.67±0.25</i>
5MeC29	3.73±0.52	1.25±0.10
7,11DMC29	0.83±0.09	0.96±0.05
C30	3.77±0.98	1.29±0.11
<i>10+12MeC30</i>	<i>8.23±0.43</i>	<i>0.88±0.10</i>
DMC30	1.55±0.08	1.21±0.09

Peaks in italics were used in PCA. See the text for peak selection strategy. Compounds are ordered by retention time.

although in this species, the queen was not reported to inhibit gyne production. The transmission of such contact cues by messenger workers is very likely and would explain why limited antennal contacts through a single mesh did not completely suppress sexual production. However, we cannot exclude the possibility that egg marking with the queen pheromone signals the workers of the queen's presence. At the onset of our experiment, each fragment received the same amount of brood, including queen-laid eggs that were most likely to hatch within 2 weeks. Because sexuals mostly appeared from the fifth week on, this lag in sexual occurrence may be due to the putative egg-marking pheromone that persisted in the colony until all the eggs had hatched. The use of queen eggs as a medium to prevent workers from producing males was demonstrated in *C. floridanus* (Endler et al. 2004). By contrast, adding queen eggs daily to *L. humile* QL fragments did not inhibit gyne production, which suggests that eggs do not transmit the queen pheromone in this species (Vargo and Passera 1991).

The origin of queen pheromones inhibiting the production of sexuals is poorly known in ants. Some authors have suggested that these complex signals are emitted by several glands (Vargo and Hulsey 2000). In this study, we have

shown that the secretion of two prominent exocrine glands, the DG and the PPG, possess caste-specific compositions, which makes them potential candidates for the production of the queen pheromone. Careful analysis of the variations in compositions of *A. senilis* PPG shows that different subsets of hydrocarbons reflect caste and, although to a lower extent, colony specificity, raising the possibility that these subsets act independently for caste communication and nestmate recognition, respectively. The lack of trophallaxis in *A. senilis* reduces the circulation of hydrocarbons between nestmates (Lenoir et al. 2001), which may allow the queen to maintain her own identity within the colony.

The analysis of the DG also indicated important quantitative differences between castes but limited differences between colonies. To the best of our knowledge, this constitutes the first identification of DG constituents in a species of the genus *Aphaenogaster*. As in many other ant species, it is mostly composed of hydrocarbons but also has small quantities of aldehydes. Queens contain high amounts of pentadecane, 3-methylpentadecane, hexadecane, heptadecane, and nonadecadiene that were only found in small quantities in workers. The role of this gland in caste communication has not yet been studied in detail in ants (but see Dietemann et al. 2003). In the honeybee, queen DG secretion may be part of a complex pheromonal bouquet that signals her fertility as well as regulates the production of queen-like secretion in workers (Dor et al. 2005; Katzav-Gozansky et al. 2006). Further studies are now needed to determine whether the compounds that dominate *A. senilis* queen PPG and DG secretions are part of the queen signal.

The intensive nest survey revealed that a few QR colonies with an unusually large number of workers contained gynes. We can thus refute the hypothesis that gyne production is solely limited by resource availability, which may covary with colony size, because queens are only threefold heavier than workers; hence, the production of one or two gynes requires only a small amount of energy (unpublished data). Moreover, if sexual production were completely dependent on colony size, we would not expect it to occur also in small- to medium-sized QL colonies, which it did. It is more probable that when an *A. senilis* QR colony reaches a very large size, the queen pheromone dilutes within the nest, which allows sexual production. It is also possible that the queen stops producing the pheromone when the colony is large, although this hypothesis, which implies she obtains information on colony size, is less likely. Irrespective of the mechanism, the production of a future queen and many males would be followed by colony fission, as in the army ant and the honeybee swarm (Gotwald 1995; Seeley 1995). This phenomenon could be selected at the colony level because it avoids jeopardizing small colony survival by a large reproductive allocation (which includes the departure of an important worker

force). However, the high proportion of gynes produced in QL nests lends credence to the existence of an alternative mode of reproduction by which gynes are produced after fission as was initially suggested by Ledoux (1971).

It thus appears that in *A. senilis*, there is a dual mode of colony fission: One may be queen controlled, whereas the other is worker controlled. In the “swarm-type” mode of colony-fission, queens seem to be successful in delaying sexual production by releasing more inhibiting pheromone than is optimal for workers. As a countermeasure, workers could split during colony migration before the mother colony reaches the queen optimum size, to build an independent QL nest in which sexuals can be reared (QL-fission mode). The hypothetical occurrence of two such reproductive strategies may be possible in a species like *A. senilis* that performs colony fission and in which worker fitness does not depend exclusively on the queen. Moreover, changing ecological constraints like the availability of nesting sites may prevent natural selection from eliminating one strategy in favor of the other. By contrast, there is no evidence for such worker–queen conflict in *A. rudis*, which may reflect Bourke and Chan’s (1999) claims that the evolution of caste conflict for reproductive allocation should not necessarily occur in all types of social organization.

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