



Queen replacement in the monogynous ant *Aphaenogaster senilis*: supernumerary queens as life insurance

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In social groups, the replacement of the breeder may generate conflicts. In eusocial hymenoptera these can occur between queens, between queens and workers, or between workers. We investigated queen replacement in the Mediterranean ant *Aphaenogaster senilis*, a monogynous (one queen per colony) species that reproduces exclusively by colony fission. We first investigated the potential for worker nepotism by analysing the sociogenetic structure in 31 colonies using microsatellite markers. No polyandry was detected, which eliminates the possibility of worker nepotism. Second, 30 colonies were experimentally orphaned to analyse the production of gynes (unmated, hopeful queens). Workers reared on average two gynes from the previous queen's diploid brood, with the firstborn gyne emerging on average 17 days before the other, supernumerary, gyne(s). The production of only a few gynes severely limits the potential for gyne selection based on quality criteria. Furthermore, such selection would be biased by the asynchrony of gyne emergence which precludes comparing gynes of the same age and maturity. Behavioural observations showed that gynes interacted aggressively and that the firstborn gyne was always dominant. She usually survived while supernumerary gynes seldom did, even though they did not differ in weight. Workers also attacked gynes, and preferentially targeted supernumerary gynes. We propose that supernumerary gynes are produced as insurance in the eventuality of the death of the firstborn gyne, and that the delay between the emergence of gynes minimizes the risk that aggressive interactions between gynes will lead to all gynes being killed.

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In societies of cooperative breeders and of eusocial insects, helpers (workers) pass on their genes indirectly via the reproduction of related breeders (queens and males). Helpers are expected to maximize inclusive fitness, and this may give rise to conflicts of interest. In cooperative breeders and in primitively eusocial insects, helpers may attempt to become breeders (e.g. Hart & Monnin 2006). This is impossible in highly eusocial Hymenoptera, where workers are physically incapable of mating. Instead, reproductive conflicts may occur over sex allocation or male production (Bourke & Franks 1995; Ratnieks et al. 2006), but in most species workers are constrained to helping their queen because she is the only mated female. However, workers may have the opportunity to select the queen they work for in some species, and this opens up the possibility that workers may select the queen that would give them the highest inclusive fitness by producing more numerous, fitter or more related sexuals. Similarly, in cooperative breeders

helpers that are too subordinate to become breeders may nevertheless affect who will become the replacement breeder.

In eusocial Hymenoptera, queen selection may occur during queen replacement and/or during colony foundation. Queen replacement occurs when an old or dead queen is replaced by a young queen. Colonies replacing their queen routinely produce more queens than necessary, and thus most of the queens will not survive. Queen selection also occurs during pleometrotic colony foundation. Pleometrosis consists in several, usually unrelated, queens jointly starting a colony. Once workers begin foraging these queens compete for control of the colony, until only one queen remains (secondary monogyny). Finally, several queens may compete when colonies are produced by dependent colony foundation (i.e. fission, budding or swarming; Bourke & Franks 1995; Peeters & Molet, in press), that is, when queens depart together with workers to establish a new colony. Typically, queens are produced in excess, and the number of new colonies that can be produced is constrained by the number of workers available and not by the number of queens. Consequently, several new queens compete for the possession of each new colony, either before departing the mother colony or once in the new colony (Gotwald 1995; Tarpay et al. 2004).

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Queen selection is an important phenomenon in the many species where it occurs. It may profoundly affect social organization, for instance how queens cooperate and compete in pleometrosis. It also allows the study of the potential conflicts between queens, between queens and workers, and between workers (in polygynous or polyandrous species), as well as the trade-off between individual and collective interests.

Three main parameters may determine queen elimination. First, in genetically heterogeneous colonies, that is, polygynous or polyandrous colonies, selection could favour the evolution of nepotistic behaviours with workers selectively attacking queens that do not belong to their matriline or patriline (Hamilton 1987). In this context, nepotism would require workers to assess relatedness. However, to date, nepotism has not been conclusively demonstrated (e.g. Balas & Adams 1996; Adams & Balas 1999; Hannonen & Sundström 2003; Tapy et al. 2004; Holzer et al. 2006). Second, natural selection should favour behaviours that would result in the elimination of low-quality queens. This requires genetic or environmental (i.e. food supply or temperature) variation in the reproductive potential of queens. A sufficient number of queens needs to be produced for a choice to be possible, and queens need to be produced in relative synchrony, otherwise age differences may blur differences in quality. That is, other things being equal, early emerging queens would be advantaged relative to late emerging queens because they would have more time to harden their cuticle, develop their ovaries and start producing queen signals (Howard & Blomquist 2005; Monnin 2006; Le Conte & Hefetz 2008). Third, natural selection may favour a process that allows the colony to requeen rapidly. In this case, the first queen to emerge would be selected as the new queen. Natural selection would then favour colonies that produce only one or a few queens asynchronously, while the two hypotheses above predict the production of many queens in relative synchrony.

The individuals responsible for queen selection may differ depending on the evolutionary context. If the colony comprises several subfamilies, workers may be the main determinant of queen selection by helping queens that belong to their subfamily. In contrast, if either quality or age is determinant then both queens and workers may take part in the process. Queens may compete directly, and higher-quality queens or firstborn queens, respectively, may have an advantage. Fights between queens have indeed been observed in pleometrotic associations in ants (Sommer & Hölldobler 1995; Balas & Adams 1996; Bernasconi & Keller 1998) and during queen replacement in the honeybee, *Apis mellifera* (Tapy et al. 2004). However, such unrestrained competition puts the colony at risk of losing all its queens, which would result in the colony gradually dying out. Workers may reduce this risk by helping the most promising or the firstborn queen (Balas & Adams 1996; Bernasconi & Keller 1998).

Queen selection has been studied in depth in the swarm-founding honeybee (reviewed in Tapy et al. 2004), but has been little studied in fissioning ants despite its importance. Fissioning species are particularly suitable for such studies as queen elimination occurs both during colony foundation (either in the mother or in the daughter colony) and during queen replacement. Most of our knowledge of colony fission in ants comes from the highly derived army ants, which form huge colonies, have a nomadic lifestyle and forage by group raiding (reviewed in Gotwald 1995). We studied the process of queen selection during queen replacement in the ant *Aphaenogaster senilis*, a monogynous species reproducing exclusively by colony fission (Boulay et al. 2007). For clarity, unmated queens are hereafter called 'gynes' to differentiate them from mated queens. The former are hopeful reproductives, while the latter are actual reproductives. *Aphaenogaster senilis* has a more 'classic' life history than army ants, with stable underground

nests and colonies with a mean \pm SE of 1260 ± 69 workers ($N = 168$, Boulay et al. 2007). Two issues were specifically addressed. First, we investigated which selection pressures are most likely in this species. The potential for nepotism was determined by assessing the sociogenetic structure of colonies, as the mating frequency of *A. senilis* queens is unknown. To assess whether gyne selection could be based on gyne intrinsic quality or age difference, we analysed the pattern of gyne production in colonies undergoing queen replacement caused by experimental orphanage. Second, we conducted behavioural observations in these experimentally orphaned groups to investigate how monogyny is restored. More specifically, we monitored aggressive interactions to determine whether gyne dominance correlated with gyne weight (considered as an indirect measure of gyne quality) or gyne birth rank. This also allowed us to investigate who the actors of gyne selection (gyne and/or workers) are.

METHODS

Model System and Sampling

Aphaenogaster senilis is a monogynous and monodomous (single nest) species from the western Mediterranean basin. It does not hibernate but produces most of its brood in spring (Boulay et al. 2009). Males and gynes may be produced throughout the year, with a peak of production in early summer (Boulay et al. 2009). Sexual production is clearly male biased with on average 172.0 males produced for each gyne. Indeed, colonies rarely contain gynes, with no more than six gynes found in field colonies (Boulay et al. 2007, 2009). The small number of field colonies containing gynes indicates that only a few colonies produce gynes and/or that monogyny is restored rapidly, so that one is unlikely to find a colony at the time when it contains gynes. Gynes have short wings but do not fly, while males can fly from their nest; hence mating occurs in or near the nest that contains the gyne. When a colony fissions it is not known whether the gyne mates before or after departing the mother colony.

We collected 46 complete colonies in Doñana National Park, Andalusia, Spain, in May 2005 and May 2006 (Doñana 2: $N = 10$; Doñana 3: $N = 7$) and near Aznalcázar, Andalusia, Spain, in February and October 2007 (Aznalcázar 1: $N = 5$; Aznalcázar 2: $N = 24$). Thirty of these colonies were used for behavioural study and 24 for genetic analysis (Table 1). To confirm the sociogenetic structure of *A. senilis* in a distant site, we genotyped foragers collected from seven additional nests in August 2008 near Banyuls-sur-mer, southern France (Table 1). Doñana 2 and 3 are 5 km apart, and both are ca. 37 km from Aznalcázar 2. These three Spanish sites are approximately 1000 km from Banyuls-sur-mer. To improve the estimate of the allelic frequencies for each site, one individual from 21 additional colonies was genotyped (Doñana 3: $N = 7$; Aznalcázar 2: $N = 7$; Banyuls-sur-mer: $N = 7$).

Genetic Analysis

The sociogenetic organization of *A. senilis* colonies was determined using 602 workers and 19 queens (Table 1). These individuals were sampled from colonies kept in the laboratory, several months after field collection. We analysed 16–21 workers per colony, using six microsatellite markers: ASen12, ASen15, ASen83, ASen94, ASen178 (Galarza et al. 2009) and Myr3 (Bourke et al. 1997 modified from Evans 1993). DNA was extracted using a Qiagen DNAeasy kit. Each polymerase chain reaction (PCR) was then carried out in a 10 μ l volume containing 1 μ l of DNA solution, 10 μ M of each primer, 0.9 μ l of dNTP 10 mM, 1 μ l Buffer 10x and 0.75 units of Taq polymerase (Qiagen). Amplified fluorescent fragments were visualized using an automated ABI Prism 310 sequencer (Applied

Table 1
Genetic characteristics of *A. senilis* colonies

Site	GPS position	N	Nw	Nq	Na	Hs	R±SE	t	P
Doñana 2	36°58.490'N 06°28.200'W	10	193	9	10.8 (5–16)	0.84 (0.64–0.94)	0.72±0.04	0.65	0.54
Doñana 3	37°01.090'N 06°26.950'W	6	120	3	11.5 (5–14)	0.85 (0.63–0.93)	0.80±0.02	1.08	0.33
Aznalcázar 2	37°16.450'N 06°13.830'W	8	162	7	10.5 (5–17)	0.77 (0.56–0.94)	0.74±0.03	0.15	0.89
Banyuls-sur-mer	42°28.265'N 03°06.450'E	7	127	0	6.7 (3–11)	0.75 (0.56–0.89)	0.77±0.04	0.31	0.77

N colonies totalling Nw workers and Nq queens were genotyped per site. Na denotes the mean number of alleles over the six microsatellite loci, with range in parentheses. The expected heterozygosity was computed as Nei estimator Hs (mean and range). The average within-colony genetic relatedness among workers (R) is given with SE obtained by jackknifing over colony, and was tested against the value of 0.75 expected in a monogynous and monandrous colony, using a t test.

Biosystems Inc., Foster City, CA, U.S.A.). Allele sizes were estimated using Genescan 3.2.1 (Applied Biosystems).

Linkage disequilibrium between all pairs of loci and deviation from Hardy–Weinberg equilibrium at each locus were examined for the four sites by exact tests using Genepop 1.2 (Raymond & Rousset 1995). These tests were performed with a reduced data set containing a single worker taken randomly from each colony. After a Bonferroni correction for multiple tests, none of the probability tests for Hardy–Weinberg equilibrium and linkage disequilibrium were significant. Hence, no inbreeding was detected in the sites studied, and the loci used can be considered independent.

The average genetic relatedness among workers within colonies was estimated using Relatedness 5.0.1 (Queller & Goodnight 1989, 1997), with each site considered as a deme. The background allelic frequencies for each deme were estimated from the entire data set by weighting colonies equally. The Nei unbiased estimator of expected heterozygosity (Nei 1978) was computed for each locus and site from these background allelic frequencies. Inspection of worker genotypes allowed us to determine whether colonies were monogynous and monandrous. This was deemed the case when a maximum of three alleles and two genotypes could be observed per loci, given the probability of nondetection of males, that is, the probability that two males share the same haplotype (Boomsma & Ratnieks 1996).

Experimental Orphanage

Colonies of *A. senilis* are potentially long lived because orphan workers can rear gyne to replace their mother. For this purpose, they use young larvae produced by the former queen, and they generally rear more gynes than necessary (Ledoux 1971; Boulay et al. 2007). To study the potential for selection during elimination of supernumerary gynes, we induced gyne production by orphaning groups of workers. Thirty colonies were used to set up 30 orphaned groups of 500–800 workers and 200 first- and second-instar larvae (i.e. still undifferentiated), so that the production of gynes was not limited by the number of larvae available. Each orphaned group was reared in a plastic nest located in a foraging area of 16 × 26 cm. They were maintained in the laboratory under a controlled temperature of 25 ± 3 °C and a 12:12 h day:night cycle. They were fed three times a week with dead crickets and artificial food (for 1 litre: 125 g agar, two eggs, two crushed vitamin pills, 125 g honey) and provided with ad libitum sugar and water.

We checked the presence of queen larvae and queen pupae daily for 3 months, starting 2 weeks after the orphaned groups were set up. The aim of this census was to quantify the mortality of queen larvae and queen pupae, which could result from intrinsic mortality or from killing by workers or by already emerged gynes. In *A. senilis*, male larvae are unmistakably smaller than queen larvae. We checked gyne emergence daily to determine their age and birth rank. Gynes were numbered following their order of emergence, that is, gyne 1 for the firstborn, gyne 2 for the second born and so on. They were weighed and individually colour-marked, with a dot of paint on the thorax, 2 days after emergence. Morphological measurements and dissections of gynes were not possible because

they were rapidly dismembered and sometimes eaten by workers when they died. The life span of gynes was recorded for a maximum of 60 days following the emergence of the first gyne. Some males were produced in orphaned groups but none was observed flying in the room and no mating was observed. Hence the probability that gynes were inseminated in the laboratory is low. The potential effect of this is analysed in the Discussion.

Behavioural observations were carried out to determine how monogyny is restored. To determine whether gynes or workers are involved, we recorded the behavioural interactions between gynes and between gynes and workers. Observations started as soon as two gynes had emerged, when monogyny had to be restored, and lasted until only one gyne remained or for a maximum of 30 days when two gynes remained alive. We used the scan-sampling method (Lehner 1996) and performed simultaneous instantaneous observations of all the gynes of a given orphaned group approximately once an hour for 7 h daily. This allowed us to estimate the proportion of time that each gyne allocated to each behaviour. We defined six aggressive and six nonaggressive behaviours during preliminary experiments, as well as three locations of gynes within or outside the nest (Table 2). During each scan, one of the 12 behaviours and one of the three locations were recorded for each gyne. Gynes could bite/grab one another and display dominance towards one another. The dominance display consisted of the dominant gyne standing in front of the subordinate gyne with her abdomen bent towards the latter. Biting was a brief behaviour, while grabbing and dominance displays generally lasted several minutes. Gynes could also bite/grab a worker, and be bitten/grabbed by a worker. Nonaggressive behaviours described the activity of gynes and their social interactions with nestmates (see Table 2).

The dominance status of gynes was determined after the end of observations. In each orphaned group, the gyne that performed more and received fewer aggressive acts was considered dominant. Aggressive acts performed by workers towards gynes were not taken into account to determine which gyne was dominant.

All tests are two tailed.

RESULTS

Potential Factors Affecting Gyne Selection

The genetic data indicate that queens of *A. senilis* mate only once. The number of alleles per locus and site ranged from three to 17 and expected heterozygosity ranged from 0.56 to 0.94 (Table 1). Given this genetic variability, the probability of failing to detect a second father was low, and ranged from 0.00006 to 0.00028. The mean genetic relatedness within colonies over the four sites ± SE was 0.752 ± 0.011 (the SE was obtained by jackknifing over colony) and did not differ significantly from 0.75, the relatedness expected for monogynous and monandrous colonies (t test: $t_1 = 0.032$, $P = 0.924$). This was verified for each site studied (Table 1). Inspection of the genotypes confirmed that *A. senilis* was monandrous at the four sites. After we removed four of the 602 workers genotyped that clearly originated from other colonies (i.e. that differed from all

Table 2
Behaviours and spatial locations of gynes for the 13 groups where dominance status could be determined

	Total occurrences	Mean frequency \pm SD
Aggressive behaviours		
Bite/grab a gyne*	141	0.029 \pm 0.045
Display dominance towards a gyne*	81	0.014 \pm 0.030
Bitten/grabbed by a gyne†	141	0.037 \pm 0.049
Receive dominance from a gyne†	81	0.016 \pm 0.030
Bite/grab a worker	3	0.0005 \pm 0.0015
Bitten/grabbed by a worker	108	0.022 \pm 0.037
Nonaggressive behaviours		
Walk‡	617	0.129 \pm 0.075
Antennal contact with a gyne or a worker‡	1190	0.293 \pm 0.090
Feed‡	82	0.022 \pm 0.021
Self-grooming‡	124	0.031 \pm 0.020
Groomed by workers	502	0.116 \pm 0.064
Stand immobile	1160	0.293 \pm 0.086
Spatial localization		
In the nest, close to the brood	1600	0.400 \pm 0.181
In the nest, away from the brood	2044	0.484 \pm 0.143
In the foraging area	584	0.116 \pm 0.131

* Bite/grab a gyne and Display dominance towards a gyne were pooled in each orphaned group to quantify aggressive acts performed by gynes against gynes.

† Being bitten/grabbed by a gyne and Receiving dominance from a gyne were pooled in each group to measure aggressive acts received by gynes from other gynes. Some behaviours have the same number of occurrences (e.g. 141 bite/grab a gyne and bitten/grabbed by a gyne) yet differ in frequencies because the latter are calculated from a different number of scans.

‡ These behaviours were pooled to calculate an index of activity.

colony members by at least eight of 12 alleles), workers were full sisters in 26 of 31 colonies (84%). In the other five colonies the queen genotyped was either mother to some workers and sister to others (four colonies) or sister to all workers (one colony), indicating a queen turnover following colony fission or queen death.

Experimentally orphaned groups produced very few gynes, with subsequent delay between them. On average \pm SD, orphaned groups produced 2.0 ± 1.1 gynes ($N = 30$ groups), with 10 groups producing one gyne and 20 groups producing two to five gynes (see Appendix). Supernumerary gynes emerged a mean \pm SD of 17 ± 11 days after the first gyne ($N = 27$ gynes). A large variation was observed, with supernumerary gynes emerging as early as the same day as the first gyne, and up to 53 days later (Fig. 1). However, most supernumerary gynes emerged at least a week after the firstborn. Gynes emerging late did not necessarily emerge after selection had been carried out between older gynes. Among the 11 gynes that emerged in third position or later, six emerged in groups where only one of the preceding gynes remained and five emerged in groups where two of the preceding gynes remained (see Appendix).

Restoration of Monogyny

Queen elimination may occur at the larval instar stage. Overall, the 30 orphaned groups produced 79 queen larvae ($\bar{X} \pm \text{SD} = 2.6 \pm 1.5$ per group, $N = 30$ groups), of which 61 emerged as gynes. Thus, queen brood mortality was at least 23%. Queen brood disappeared in 10 groups where one to three gyne(s) had already emerged, as well as in five groups where no gyne had yet emerged. This suggests that the presence of a gyne has no significant effect on queen brood mortality (binomial test: $P = 0.092$).

Queen elimination during the adult stage could not be studied in two of the 20 groups that produced at least two gynes. In one case one of the two gynes produced was killed by workers following marking, and in the other case the first gyne died before the second gyne emerged so that gynes did not coexist in the nest (group 113; see

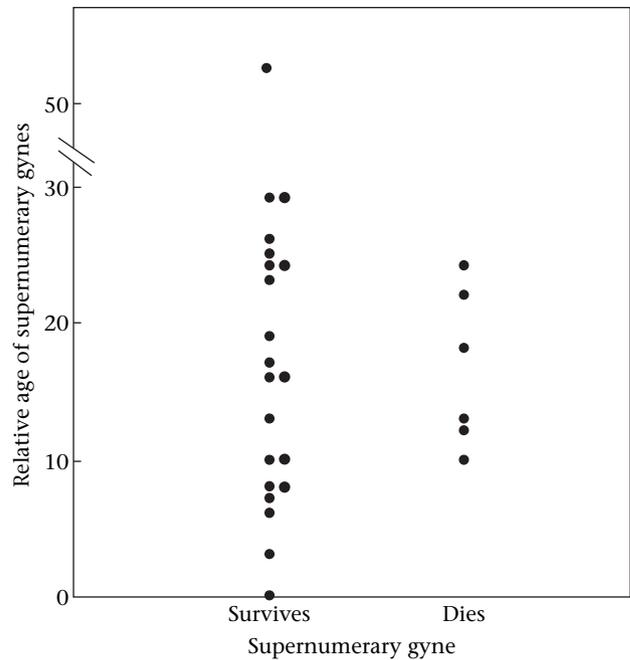


Figure 1. Relative age of supernumerary gynes, that is age difference between supernumerary and firstborn gynes, and survival or death of the former.

Appendix). In the 18 remaining groups, all but one gyne were eliminated in 14 groups (78%), whereas two gynes coexisted for 30 days in the other four groups. In the groups where monogyny was restored, it was restored after a mean \pm SD of 12 ± 10 days ($N = 23$ gynes). Gynes were eliminated from the day of emergence up to 28 days later.

Aggressive Interactions

Aggressive interactions between gynes were observed in 13 of the 18 groups. Indeed, three groups where the gynes coexisted for only 1 day provided no behavioural information, and in two other groups no aggression between gynes was recorded. Gynes were observed for a mean \pm SD of 136 ± 71 scans ($N = 31$ gynes, range 21–256), depending on the duration of gyne coexistence. One gyne was clearly dominant in all the groups where gyne aggression was observed, as she performed most aggressive acts and received none. Dominant gynes performed a total of 219 aggressive acts towards subordinate gynes ($\bar{X} \pm \text{SD} = 16.8 \pm 16.7$ acts/gyne, $N = 13$ gynes, range 1–62). In sharp contrast, subordinate gynes performed only three aggressive acts, all of which targeted another subordinate. Given that dominant gynes never received aggression, we are confident in our inference of dominance status even in colonies where few aggressive acts were observed (Fig. 2).

Gynes and workers performed a total of 222 and 108 aggressive acts towards gynes, respectively (Table 2). Thus, 67% of the aggressive acts received by gynes came from another gyne. Workers appeared to reinforce the dominance hierarchy established by gynes. They were seen attacking gynes in seven of 13 groups, where they performed 1–27 aggressive acts against each gyne attacked (Fig. 2). They exclusively attacked subordinate gynes in five groups. These subordinates did not survive, and workers were observed immobilizing and finally dismembering two of them (groups 75 and 109; Fig. 2). Workers attacked both the dominant and the subordinate in two other groups (groups 83 and 97; Fig. 2). The dominant survived in both groups while the subordinate survived in one group only (Fig. 3). The hypothesis that workers reinforce the dominance hierarchy established by gynes is further supported by the fact that five of the seven subordinate

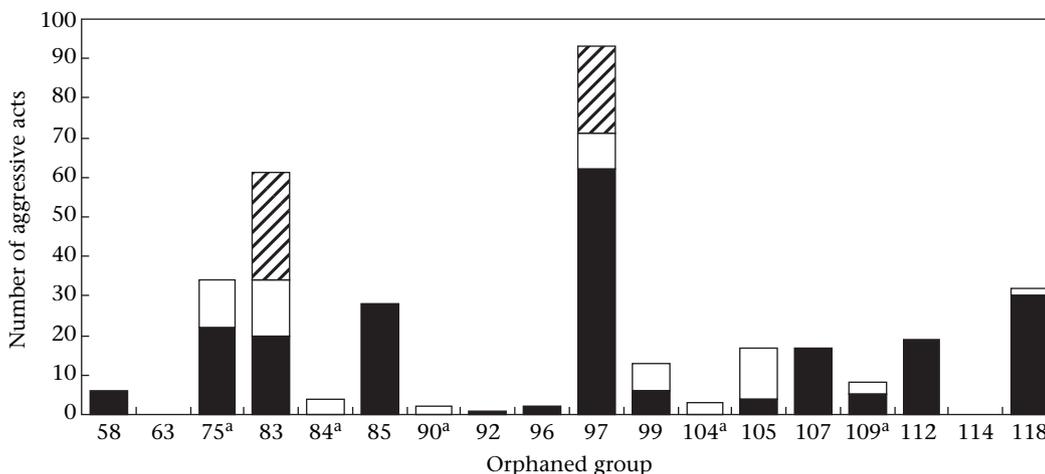


Figure 2. Number of aggressive acts received by gynes in the 18 orphaned groups where at least two gynes coexisted. Black bars: aggressive acts performed by dominant gynes towards subordinates; white bars: aggressive acts performed by workers towards subordinates; hatched bars: aggressive acts performed by workers towards dominant gynes. The dominance status of gynes could not be determined in five groups where gynes performed no aggression. A superscript 'a' indicates that workers were seen killing a gyne.

gynes attacked by workers were first attacked by the dominant gyne. Finally, workers were seen attacking and killing a gyne in three other groups where dominance status was unknown (groups 84, 90 and 104; Fig. 2).

Correlates of Dominance

In the 13 groups where dominance was determined the dominant gyne was the firstborn and she survived (Fig. 3). In contrast, only four of the 22 subordinates survived (Fisher's exact test: $N = 35$ gynes, $P < 0.0001$), so that in four of these 13 groups monogyny was not restored. These subordinates may have died later on, as monogyny must eventually be restored. Hence we analysed the characteristics of gynes relative to dominance status rather than to survival (see below), because dominance is unambiguously related to gyne selection while survival might have other origins such as accidental death, lack of water, starvation or disease. This is further supported by the fact that in the six groups with unknown dominance status, the firstborn gyne survived in half the groups (Fig. 3 plus colony 113, see Appendix).

Given the very strong effect of birth rank, one might expect that the smaller the age difference between a supernumerary gyne and the firstborn gyne the higher the probability of survival of that

supernumerary gyne. This is not supported by the data. The supernumerary gynes that survived did not differ less in age from the firstborn gyne than the supernumerary gynes that did not survive (Mann-Whitney test: $U = 62.50$, $N_1 = 6$, $N_2 = 21$, $P = 0.977$; Fig. 1).

There was no phenotypic difference between dominant and subordinate gynes (Fig. 4). Dominant gynes were neither heavier nor more active than subordinates (Wilcoxon signed-ranks tests: $N = 13$ groups; $Z = 0.63$, $P = 0.529$ and $Z = 1.50$, $P = 0.133$, respectively). Dominant gynes received less allogrooming from workers and stayed less in the vicinity of the brood (Wilcoxon signed-ranks tests: $N = 13$ groups; $Z = 2.20$, $P = 0.028$ and $Z = 3.18$, $P = 0.001$, respectively). However, these results could stem from the fact that dominant gynes were older than subordinates when behavioural observations were performed. Indeed, older gynes may have received less allogrooming and spent relatively less time close to the brood because they began to go outside of the nest at a mean \pm SD age of 15 ± 5 days ($N = 24$ gynes), possibly to seek mating.

DISCUSSION

Queen replacement is a critical event. It allows the colony to survive its queen and thus significantly increases colony life

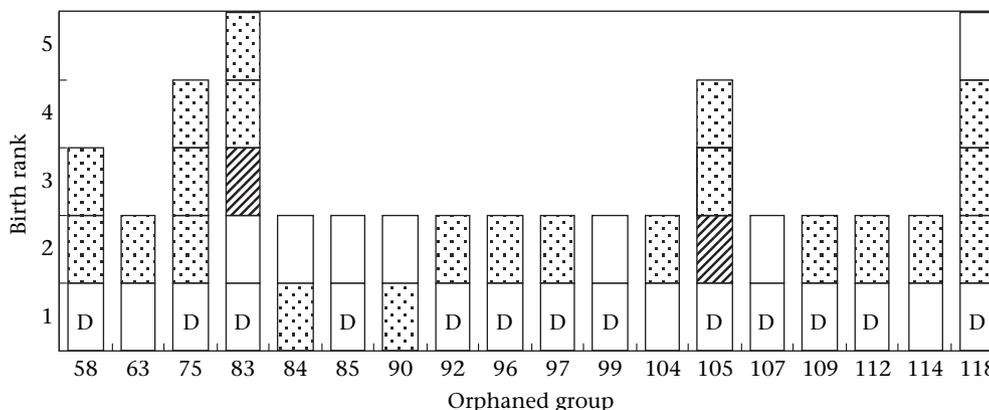


Figure 3. Survival (white boxes) or death (dotted boxes) of gynes depending on their birth rank in the 18 orphaned groups where at least two gynes coexisted. D denotes the dominant gyne in the 13 colonies where dominance could be determined, and hatched boxes represent gynes that were killed by workers following marking.

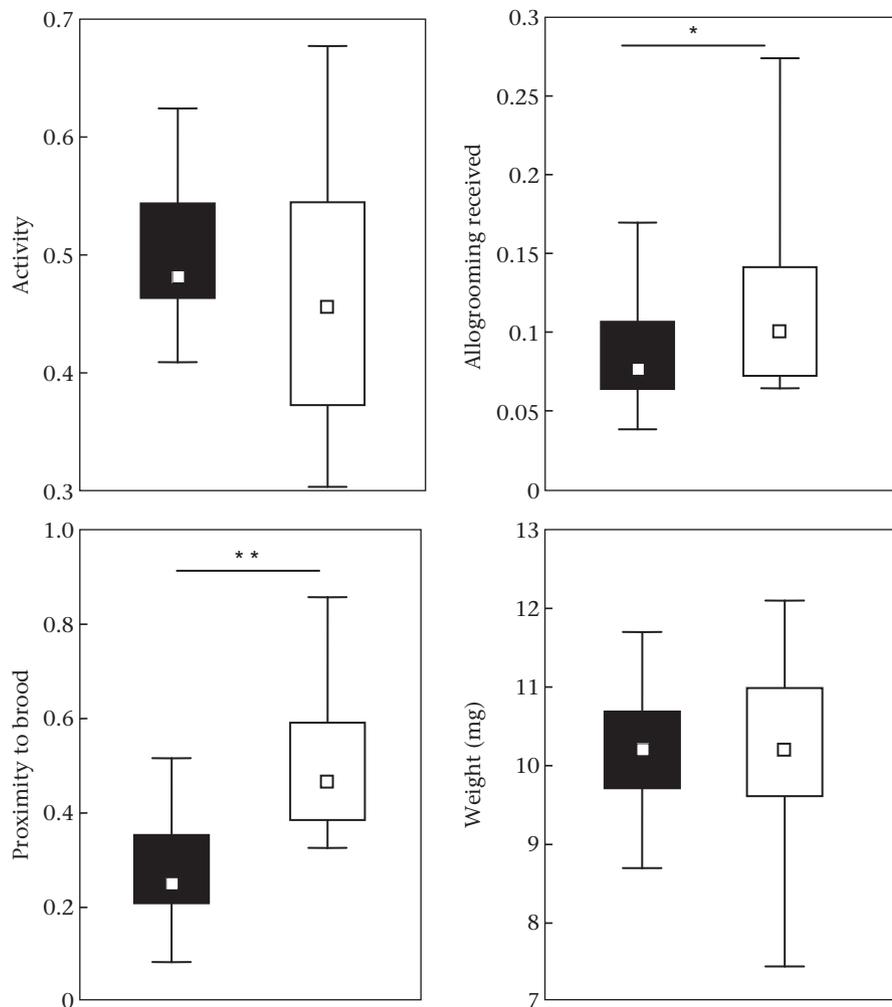


Figure 4. Correlates of dominance in the 13 groups where dominance status could be determined. Percentage of scans (median with quartiles and range) where dominant (black) and subordinate (white) gynes were recorded performing a particular behaviour. Wilcoxon tests: * $P < 0.05$; ** $P < 0.01$.

expectancy. Repeated queen replacement may allow a colony to survive several decades or more. Such extended colony life span increases the inclusive fitness of all colony members. The mother queen benefits from the production of grandoffspring, the daughter queen benefits from inheriting the colony, and the workers benefit from the production of nieces and nephews (Crozier & Pamilo 1996). Queen replacement by a daughter queen is apparently rare in monogynous species where queens are capable of flying (Hölldobler & Wilson 1990; but see Foitzik & Herbers 2001; Sanetra & Crozier 2002). In contrast, it may occur in all species reproducing by colony fission (honeybee: Tarpay et al. 2004; army ants: Gotwald 1995; queenless ants: André et al. 2001, 2006).

Conflicts may occur over the timing of queen replacement and over the identity of the replacement queen, with individuals potentially favouring more related, more fertile or longer-lived replacement queens (e.g. Tarpay et al. 2004; Hart & Monnin 2006). Our results show that the first gyne to emerge in orphaned *A. senilis* colonies is dominant and survives, while gynes emerging later almost always die. The firstborn gyne is thus likely to become the next queen. Gynes could not mate in our laboratory experiment, but this is unlikely to affect the result that firstborn gynes are favoured. When gynes become sexually mature and how they attract mates are unknown in *A. senilis*, but it is reasonable to assume that the firstborn gyne would mate before the supernumerary gynes, given that the former emerges on average 17

days before the latter. This may translate into an additional advantage to the firstborn as workers may recognize that she is mated and may cull the now useless supernumerary gynes. In addition, mating may change the behaviour of the firstborn, which may become more intolerant of other gynes. Therefore, if mating were to affect gyne selection it would probably be in favour of the firstborn gyne.

Colonies replacing their queen apparently favour birth rank over other criteria. Why is this so? First, variation in relatedness among workers cannot affect gyne selection in *A. senilis*. Our genetic analysis revealed that the queen mates only once so that workers are equally related to all gynes (full sisters), which precludes potential nepotistic behaviours and differential viability. Monandry appears to be the rule in the four sites studied and it might therefore be a general characteristic of the species. Second, the pattern of gyne production did not allow for selecting based on quality. On average, orphaned groups produced only two gynes, and they did not produce them simultaneously but after an interval of several days or even weeks. This effectively reduces the potential for selecting gynes based on size, aggressiveness, activity rate or fertility, as the firstborn gyne has more time to mature than subsequent gynes. In our experiment, the firstborn and supernumerary gynes did not differ in weight, suggesting that workers invest the same amount of resources in all gynes. Similarly, honeybee gynes show a limited variation in quality (Hatch et al. 1999; Tarpay et al. 2000). Third, firstborn gynes were clearly

dominant, and they often benefited from workers attacking supernumerary gynes. Indeed, gynes attacked by workers usually died in the following days. Overall, these results suggest that the potential for selection based on quality or relatedness criteria is low, whereas the potential for selection based on age difference is high. Our results also show that birth order determines which gyne will become the queen, as occurs in army ants (Gotwald 1995). The few supernumerary gynes, when they occur, are probably produced to ensure that the colony will requeen successfully, and they can be viewed as a form of life insurance. Thus, orphaned *A. senilis* colonies prioritize the rapidity of queen replacement, so that brood production may resume rapidly, as has also been suggested in the honeybee (Tarpy et al. 2000).

The scarcity of supernumerary, insurance, gynes produced by *A. senilis* is surprising given that other species produce more gynes when replacing their queen: ca. six in the army ant *Eciton hamatum* and up to 56 in driver ants (Gotwald 1995), one to two dozen in the honeybee (Tarpy et al. 2004) and up to 40 in the ant *Cataglyphis cursor* (C. Doums, unpublished data). The cost of producing a few additional gynes is presumably small, owing to the weak queen/worker dimorphism in *A. senilis*, while the cost of failing to replace the queen is extreme. The low number of gynes produced may result in part from selection during brood development. Indeed, queen brood destruction could result from a cryptic choice by workers, which may kill unhealthy queen brood or prevent supernumerary gynes from emerging too early. It could also stem from firstborn gynes killing other gynes before they emerge, as occurs in the honeybee where 30% of queens are killed in their cells by already emerged queens (Gilley et al. 2003). However, only 23% of queen brood was culled at the larval or pupal stage, and this cannot account for the scarcity of gynes produced.

A possible explanation for the low number of gynes reared is that the production of gynes asynchronously minimizes the risk of losing all gynes. Supernumerary gynes emerged 17 ± 11 days after the first gyne, with all but two emerging at least 5 days after the firstborn (Fig. 1). This gives a decisive advantage to the first gyne, which has a head start in hardening her cuticle, developing her ovaries and producing putative pheromones that may advertise her fertility to workers (Howard & Blomquist 2005; Monnin 2006; Le Conte & Hefetz 2008) or that may elicit workers to attack another gyne (Monnin et al. 2002). Thus, unless she is somehow unfit the firstborn gyne should win, and this limits the risk of all gynes killing one another. This interpretation is supported by the fact that workers were observed siding with the dominant gyne, which was always the firstborn. In addition, the age difference between the first and supernumerary gynes seems too large to stem merely from differential development times of larvae. Rather, it is likely to result from an active strategy to produce gynes in sequence. Workers may hasten the development of the first gyne by preferential feeding while slowing down the development of supernumerary gynes. This would both give a head start to the firstborn and allow the colony to have replacement gynes ready in the eventuality that the first gyne is unhealthy, dies from infection, or is killed by predators when mating outside of the nest. How workers identify the various queen larvae and their relative development is not known. Tactile and chemical cues or signals are likely to be involved (e.g. den Boer & Duchateau 2006), and larvae may also perform begging behaviours (Kaptein et al. 2005). However, the behaviour of larvae is difficult to study in *A. senilis* because they are stocked in large piles.

Our results on gyne selection during the process of queen replacement are also relevant to gyne selection when new colonies are produced by colony fission. Two modes of colony fission have been hypothesized in *A. senilis* (Boulay et al. 2007). First, groups of workers may split from the mother colony with diploid brood, either purposely or by accident during frequent colony emigration. Requeening would then take place in a group of orphan workers with brood,

which is basically the same situation as in our study. Second, queenright colonies may produce gynes that would leave with a group of workers to start a new colony. Gyne selection may then be slightly different. Gynes may compete aggressively with one another, and they may also compete to attract workers to join and found a new colony. The pattern would depend on the number of gynes and of new colonies produced, the timing of mating of gynes and the timing of restoration of monogyny (before or after leaving the mother colony). Thus, more data are needed on the process of colony fission to understand the unfolding of queen selection in *A. senilis*.

All colony members gain inclusive fitness benefits from replacing an old queen by a daughter queen (i.e. 'serial polygyny'). However, queen replacement is, as far as we know, rare in monogynous ant species that reproduce by independent colony foundation (Hölldobler & Wilson 1990). This may be because queens are incapable of returning to their natal nest after mating in a swarm (Bourke & Franks 1995, although it has been suggested in a slavemaking ant, Foitzik & Herbers 2001), or because they lack the ability to perform sexual calling as an alternative to mating in a swarm. However, queen replacement does occur in some species where queens normally found colonies independently (Sanetra & Crozier 2002) and more studies are needed to determine whether this alternative strategy is exceptional or more frequent than currently assumed, and how it affects conflicts of interest.

Our study shows that *A. senilis* colonies minimize conflict over colony inheritance by lowering the number of gynes competing to become the queen and by generating, or at least allowing the build up of, a strong power asymmetry between gynes. In cooperative breeders and primitively eusocial insects, hopeful reproductives are not produced after the death of the breeder, as in *A. senilis*, but instead queue for reproduction or attempt to overthrow the breeder. Consequently helpers may have less power to manipulate the outcome of the conflict. Another difference is that most or all adults may become breeders, so that no particular life insurance mechanisms are necessary to guarantee that the group will have a replacement breeder.

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Appendix

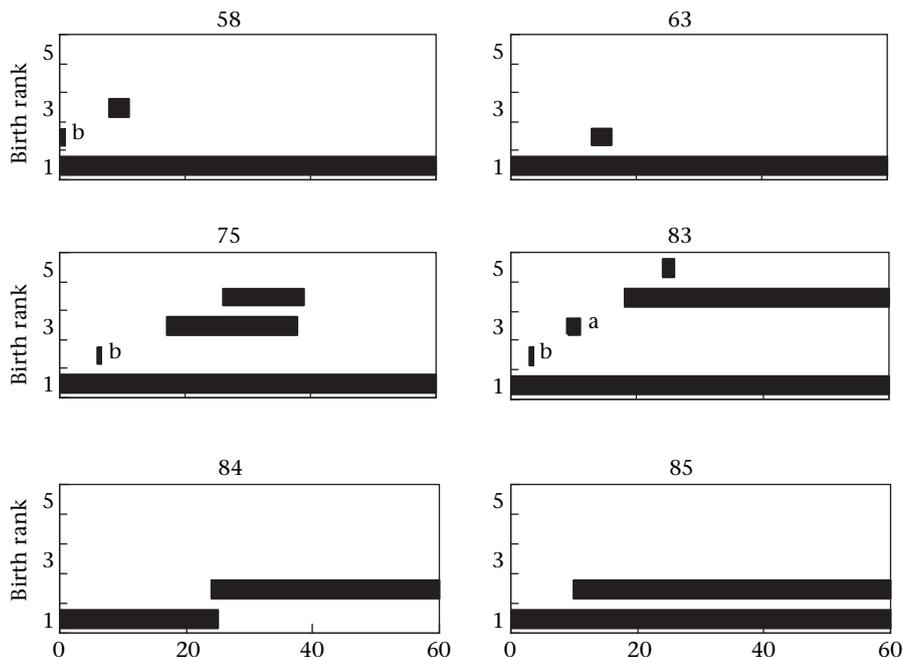


Figure A1. Age difference between gynes and survival in the 20 orphaned groups where at least two gynes were produced. Black bars represent the survival of gynes. Day zero corresponds to the emergence of the first gyne. The firstborn gyne was killed by us to terminate the experiment in group 114. 'a' indicates that the gyne was killed by workers following marking, while 'b' indicates that the gyne died the day she emerged.

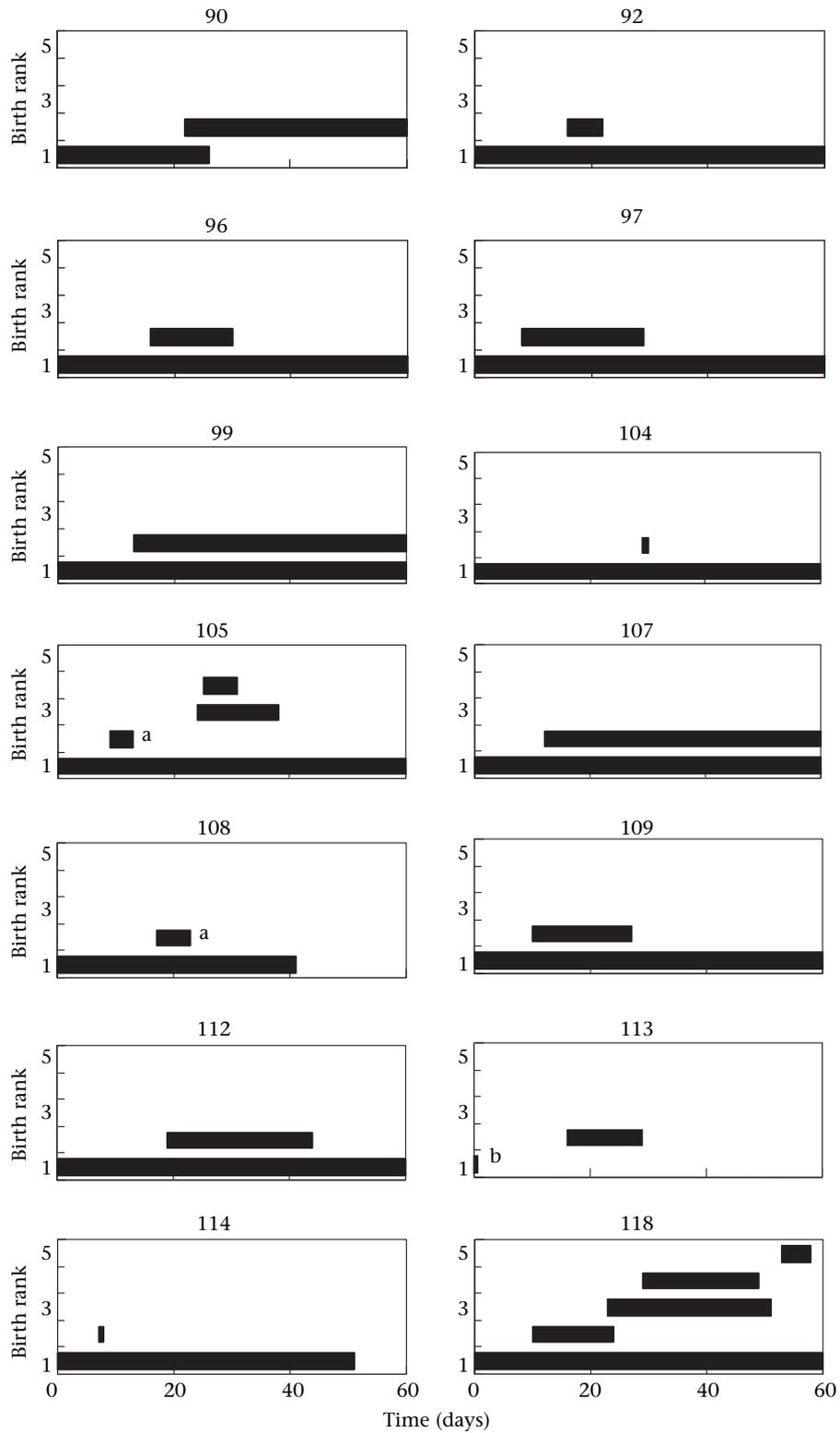


Figure A1. (Continued)