

Unequal resource allocation among colonies produced by fission in the ant *Cataglyphis cursor*

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Abstract. How organisms allocate limited resources to reproduction is critical to their fitness. The size and number of offspring produced have been the focus of many studies. Offspring size affects survival and growth and determines offspring number in the many species where there is a trade-off between size and number. Many social insects reproduce by colony fission, whereby young queens and accompanying workers split off from a colony to form new colonies. The size of a new colony (number of workers) is set at the time of the split, and this may allow fine tuning size to local conditions. Despite the prevalence of colony fission and the ecological importance of social insects, little is known of colony fission except in honey bees. We studied colony fission in the ant *Cataglyphis cursor*. For clarity, “colony” and “nest” refer to colonies before and after colony fission, respectively (i.e., each colony fissions into several nests). The reproductive effort of colonies was highly variable: Colonies that fissioned varied markedly in size, and many colonies that did not fission were as large as some of the fissioning colonies. The mother queen was replaced in half of the fissioning colonies, which produced 4.0 ± 1.3 (mean \pm SD) nests of markedly varied size. Larger fissioning colonies produced larger nests but did not produce more nests, and resource allocation among nests was highly biased. When a colony produced several nests and the mother queen was not replaced, the nest containing the mother queen was larger than nests with a young queen. These results show that the pattern of resource allocation differs between *C. cursor* and honey bees. They also suggest that *C. cursor* may follow a bet-hedging strategy with regard to both the colony size at which fission occurs and the partitioning of resources among nests. In addition, colony fission may be influenced by the age and/or condition of the mother queen, and the fact that workers allocating resources among nests have incomplete knowledge of the size and number of nests produced. These results show that the process of colony fission is more diverse than currently acknowledged and that studies of additional species are needed.

Key words: bet-hedging; *Cataglyphis cursor*; colony fissioning; Dependent Colony Foundation; maternal investment; offspring number; offspring size; reproductive strategy.

INTRODUCTION

How organisms allocate limited resources to reproduction is critical to their fitness. The optimal allocation depends on the balance between costs on the one hand (resources lost) and benefits on the other (viable offspring produced). The latter can depend both on the size and number of offspring produced. Offspring size, in conjunction with environmental factors such as resource availability and density, determines offspring survival, growth rate, competitive ability, and fecundity. Offspring size also determines the number of offspring an individual can produce, because there is a trade-off in size and number in many species (Stearns 1992, Godfray 1995, Fox and Czesak 2000, Williams 2001, Parker et al. 2002, Brown 2003, Mappes and Koskela 2004, Hudson and Trillmich 2008, Shik 2008, Warne and Charnov

2008). Individuals may differ in reproductive effort and in how they allocate resource among offspring, and studying this variability is important to better understand evolutionary dynamics and how species may adapt to environmental uncertainty.

Individuals may differ in their pattern of resource allocation because of environmental factors. For instance, individuals with more resources may produce larger and/or more offspring (Fox and Czesak 2000, Crespi and Lessig 2004, Allen et al. 2008). Resource allocation may also differ because of genetic factors. For instance, coastal and inland populations of arctic foxes differ in litter size (Axenovich et al. 2007). In many species, individuals may be selected to produce offspring of varied sizes because the environment varies in unpredictable ways so that parents cannot produce offspring of a single optimal size (Koops et al. 2003, Dziminski and Alford 2005, Marshall et al. 2008, Olofsson et al. 2009). The advantages of such diversified bet-hedging strategy is that large offspring survive even

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if conditions are poor, thereby guaranteeing some success in bad years/localities, whereas small offspring are cheaper to produce and hence allow taking advantage of good years/localities.

Social insects have long been studied in evolutionary biology and ecological research. They are particular in that selection acts at both individual and colony levels. The individual level is most relevant to genetics, e.g., when several queens vie for reproduction, whereas the colony level is most relevant to resource acquisition, defense, and growth. Social insects are interesting systems to investigate reproductive allocation, and this is particularly so for the many species that produce new colonies by colony fission. In these species, a colony splits into two or more independent colonies (e.g., honey bees, army ants), and female offspring are the newly produced colonies. These female offspring may differ markedly in size because resource allocation is not constrained by individual limitations, as is the case in species where queens found new colonies alone. Social insects that reproduce by colony fission are convenient study organisms because resources allocated to new colonies can be measured readily, such as the number of workers.

However, life history theory for species employing colony fission can be complex. Quantifying sex allocation, for example, is problematic because workers accompanying a founding queen should be considered part of the investment in female reproductives, but were also part of the investment in colony maintenance and growth of the mother colony. Thus, while numerical sex ratios in many fissioning species are male biased (male:queen ratio for *Eciton burchelli* was 500:1 [Gotwald 1995]; for *Aphaenogaster senilis*, 172:1 [Boulay et al. 2007]; and for *Cataglyphis cursor*, 4:1 [Pearcy and Aron 2006]), this is unlikely to provide the most heuristic approach, and fissioning species are more useful for examinations of female investment (new colonies) at present.

In fissioning species where colonies have one queen mated to one male, modeling infers that when colony fission has been completed, the mother colony should be larger than each daughter colony, and that daughter colonies should be of equal size to one another (Bulmer 1983, Pamilo 1991, Crozier and Pamilo 1996). There are, however, few data to test these predictions. Some species usually produce a single daughter colony (honey bees [Seeley 1996]; stingless bees [Inoue et al. 1984, van Veen and Sommeijer 2000]; and ants [Gotwald 1995, Fernández-Escudero et al. 2001]), whereas others produce several (ants; Briese 1983, Lenoir et al. 1988), and in these species the mother colony allocates 10–50% of her resources to all daughter colonies. In addition, life history traits may constrain resource allocation during colony fission. For instance, new world army ants (Ecitoninae) rely on mass predation to forage so that their colonies require a minimum size to function, and fissioning colonies thus allocate roughly half of their

resources to a single new colony (Gotwald 1995). In ants, the restricted dispersal of colonies produced by fission (Peeters and Ito 2001) may result in some level of population genetic viscosity (*C. cursor*; Clémencet et al. 2005, Hardy et al. 2008) and competition between related colonies. Such Local Resource Competition could affect how colonies allocate resources to new colonies. For instance, they could allocate more resources to new colonies dispersing further away. However, the costs incurred by a higher dispersal (higher energetic costs, higher worker mortality from predation and becoming lost) may hinder such preferential allocation, particularly in smaller fissioning colonies. Overall, the paucity of information on colony fission is surprising, given that many ants, bees, and wasps reproduce by colony fission and that many of these species have important ecological impacts (e.g., honey bees, stingless bees, swarm-founding wasps, army ants), including many invasive ants (Holway et al. 2002, Suarez et al. 2005, Heinze et al. 2006).

We studied resource allocation during colony fission in a natural field population of the ant *Cataglyphis cursor*. We determined how many colonies were produced by fission and how resources were apportioned among them, in relation to the size of the mother colony. We also paid attention to the dynamics of resource allocation. Colony fission is highly flexible in that workers accompanying a young queen to found a new colony are committed to this colony (i.e., invested) only when they actually depart from the mother colony. Until then, they can be reallocated so as to adjust the size and number of colonies produced. Colony fission could even be aborted altogether with negligible costs: Workers that were preparing to split off can remain in the mother colony and only the young queens would become superfluous and would have to be culled. In addition, new colonies do not disperse far in *C. cursor* (Lenoir et al. 1990). Hence, they may remain in transitory contact with the mother colony and their size may be fine-tuned to local conditions (e.g., resource availability, degree of competition) by additional resource allocation. Such prolonged contact with the mother colony may also allow rescuing and reallocating the resources of a failing colony.

METHODS

Model system

Cataglyphis cursor is a thermophilic ant common in the northern Mediterranean basin. Colonies are composed of a single queen that has mated with about six males (5.6 ± 1.3 [mean \pm SD] in Pearcy et al. 2004a, and 5.8 ± 2.7 in our study population [Chéron et al., *in press*] and of about 700 workers of variable size [Lenoir et al. 1988]). Colonies inhabit a single nest (monodomy). New colonies are exclusively founded by colony fission, whereby young queens disperse on foot and found new colonies with nestmate workers (Lenoir et al. 1988, Clémencet et al. 2005).



FIG. 1. A worker *Cataglyphis cursor* ant transports another worker toward a new nest.

C. cursor is a good model to study colony fission in the field because colony reproduction is seasonal and occurs at a precise time of the year. In the south of France, colonies hibernate without brood from mid-October to mid-March. When colonies become active in spring they produce a first brood of sexuals before switching to worker production. Young queens mate near the entrance of the colony at the end of May or beginning of June and re-enter the colony, and colony fission occurs the following days (Lenoir et al. 1988). Colony fission is conspicuous and unmistakable as a few workers carry other workers, queens, males, and all brood stages to new nest(s) (Fig. 1; Lenoir et al. 1988). Transports can easily be monitored because *C. cursor* is sufficiently large to be conspicuous and our study site is sparsely vegetated.

A peculiarity of *C. cursor* is that most new queens are produced via thelytokous parthenogenesis (automictic parthenogenesis with central fusion; Pearcy et al. 2004a). These queens (hereafter parthenogenetic queens) are essentially clones of the mother queen and colony members are thus equally related to all these parthenogenetic queens (mother queen, $r \sim 1$; workers of all patriline, $r \sim 0.5$). From a relatedness viewpoint, colonies producing only parthenogenetic queens should therefore invest resources equally in all new colonies. In contrast, conflict of interest may arise in the relatively few colonies producing sexual queens (Pearcy et al. 2004a). Workers of each patriline may favor allocating more resources to full-sister queens ($r = 0.75$) than to mother and parthenogenetic queens ($r = 0.5$) or half-sister queens ($r = 0.375$). However, a caveat here is that it is unclear whether workers can discriminate among more or less related queens (Ratnieks 1991, Ratnieks

and Reeve 1991, Keller 1997). Overall, parthenogenesis should have little impact if any on resource allocation among offspring colonies.

Identification of fissioning colonies

We defined a study area of 9905 m² near Argelès-sur-Mer, in the south of France, in a sandy area with sparse low vegetation next to the beach (42.572250° N, 3.043670° E). Following exhaustive searching, we mapped 268 colonies within the site (Fig. 2); 249 colonies were located between 13 and 15 May, and a further 19 colonies were found subsequently.

In order to identify which colonies were likely to fission, we recorded the presence of young queens and/or foreign males (i.e., males harassed by workers) at the entrance of each colony. Such presence is indicative of mating, and hence, imminent queen replacement or colony fission. Males remained in the vicinity of the entrance all day and were thus a more reliable indicator than young queens, which often only appeared briefly before retreating into the colony. The presence of single males was not considered evidence of fission as they may be transient. Monitoring was undertaken once daily for each colony from 16 to 28 May, and once every other day from 28 May to 22 June. Of the 268 colonies monitored during the study, 73 (27%) were found to have 2 to 40 males and/or several young queens active at their entrance. This strongly suggests that colonies do not fission every year.

Once identified, the 73 candidate fissioning colonies were observed for at least two 5-min sessions daily, until a transport (i.e., workers carrying other workers, queens, or brood) was observed toward another nest. Observations were then increased to two to five 10-min observation sessions daily. Following transporting workers permitted us to locate new nests produced by fission. These new nests had not been spotted during the intensive survey to map all colonies of the study site and often showed digging activity and small mounds of freshly excavated soil. We monitored transports from the first 19 colonies that fissioned. While other colonies fissioned, it was not possible to follow more colonies.

Monitoring colony fission and collection of nests

One idiosyncrasy of social insects reproducing by colony fission is that the life span of the colony is not restricted to the life span of its queen. When the queen dies she is replaced by a daughter queen who inherits the colony (e.g., honey bees and army ants; Seeley 1996, Gotwald 1995). Therefore, when queen replacement occurs simultaneously with colony fission it is impossible to discriminate the mother colony, which has just replaced the queen, from daughter colonies. It is thus impossible to analyze resource allocation between mother and daughter colonies. For this reason, we adopt a specific terminology to refer to the various types of colonies: hereafter, “colony” and “nest” refer to colonies before and after the occurrence of colony

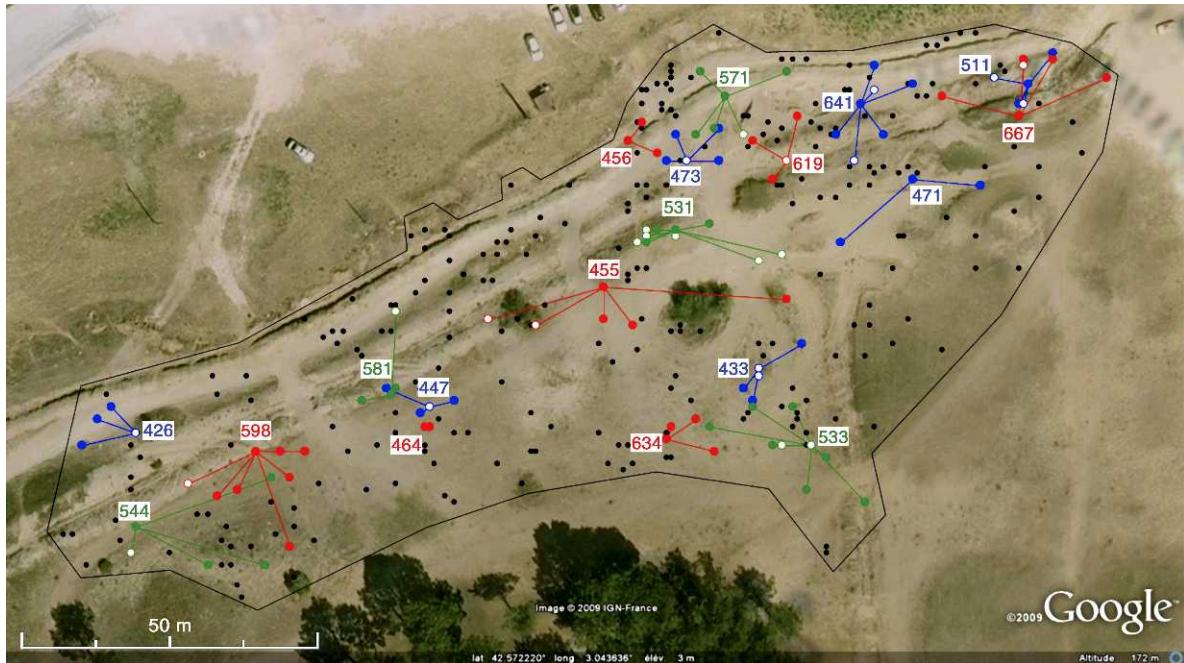


FIG. 2. Map of the study site in Argelès-sur-Mer, southern France. Black dots indicate *C. cursor* colonies, and colored dots indicate the monitored fissioning colonies, with lines joining the initial nest (central point) to the new nests (outer points). Nests that were empty or contained a foreign colony are in white. Numbers indicate the colony study number.

fission, respectively. That is, each “colony” fissioned into several “nests.” Depending on the topic of interest we further distinguish (1) the nest with the mother queen from nests with young queens and (2) the initial nest (which the colony inhabited before fission) from new nests (founded after dispersal to a new nesting site). These two classifications may be intertwined as the mother queen may remain in the initial nest or relocate to a new nest.

For each monitored fissioning colony, we recorded transports from the initial nest to new nest(s). Each new nest was collected seven days after its foundation, and the initial nest was collected together with the last new nest founded by the colony. Transports from the initial nest toward new nests lasted 3.4 ± 1.9 days, with the number of transports decreasing daily and approaching zero on the seventh day, indicating that resource allocation to new nests was complete when they were collected. In addition, excavations revealed that almost all new nests had restored monogyny and contained a single queen (see *Results: Pattern of nest production*).

During excavation care was taken to ensure all inhabitants were recovered. Workers, worker cocoons, and sexual cocoons were counted (sexual cocoons are larger than worker cocoons). The number of larvae was not recorded, as it was not possible to collect all larvae during excavation. All queens, sexual cocoons, and ~ 100 workers per nest were kept in 90% alcohol with 10% Tris-EDTA for dissection and further genetic analyses. The first new nest was founded on 21 May and the last on 7 June. For comparison, 17 colonies that

did not fission were also collected on 12 and 14 June. Males and gynes had never been observed at the entrance of those colonies, and excavation confirmed that they contained no gyne or queen pupae.

Reproductive effort and resource allocation

We quantified the size and number of nests produced by each colony, permitting us to examine patterns of resource investment. Colonies may allocate more resources to more valuable nests; for instance, to the nest with the mother queen (Pamilo 1991) or to the initial nest, which may represent a valuable investment as it is already excavated. We also determined if each colony invested resources equally or unequally among (1) all its nests, (2) its nests with young queens, and (3) its new nests, by computing the binomial skew index B (see *Statistics* below; Nonacs 2000). We studied the dynamics of resource allocation to new nests by comparing, within colonies which produced several new nests over several days, the mean size of new nests produced on the first day of colony fission with the means size of new nests produced on subsequent days.

Colony mapping

The position of all *C. cursor* colonies in the study area was recorded using a GPS with a precision of 4 m. The location of new nests produced by colony fission was recorded when they were discovered. For higher accuracy, the position of new nests relative to their initial nest was also recorded by measuring the distance and bearing with a 50-m tape measure and compass.

One new nest was overlooked in this process. Tape measure distances were used to calculate the dispersal distance of new nests, while GPS coordinates were used to calculate the distance to the nearest neighbor and the aggregation index R of Clark and Evans (1954).

Dissections and genetic analyses of queens

Sexual cocoons were opened in the laboratory to determine the sex of the pupae. All were male. Queens were dissected, and the presence of yellow bodies was used to identify the mother queen. In addition, queens were genotyped at 12 microsatellite loci to confirm that the queens from the various nests produced by each colony were related, and to deduce the mode of production of young queens (parthenogenetic vs. sexual). We assessed whether young queens were most likely to have been sexually or parthenogenetically produced assuming thelytokous parthenogenesis with central fusion, as demonstrated in another population of *C. cursor* (Pearcy et al. 2004a, Pearcy et al. 2006). This mode of parthenogenesis allows some recombination to occur so that a queen heterozygous at one locus can produce young queens that are either heterozygous or homozygous at this locus. In colonies where the mother queen remained, young queens were considered to have been sexually produced when they had at least one allele not present in the mother queen. In colonies where the mother queen had been replaced, young queens were considered to have been sexually produced when they differed from one another by at least one allele at a locus heterozygote in the two gynes: otherwise they were considered to have been parthenogenetically produced.

Microsatellite loci used were Ccur 26, Ccur 46, Ccur 76 (first set of loci), Ccur 11, Ccur 63, Ccur 89 (second set), Ccur 51, Ccur 58, Ccur 65, Ccur 99 (third set), Ccur 61 and Ccur 100 (fourth set; Pearcy et al. 2004b, Chéron et al., *in press*). DNA was extracted from queen heads using a Qiagen DNAeasy kit (Qiagen, Venlo, The Netherlands). Each polymerase chain reaction (PCR) was then carried out in a 10- μ L volume containing (for the first and second set of loci) 1 μ L of DNA solution, 0.15 μ L of dNTP 40 mmol/L, 1 μ L buffer 10 \times , 0.75 unit of *Taq* polymerase (Qiagen), and 0.10 μ mol/L of each primer; or (for the third and fourth set of loci) 1 μ L of DNA solution, 0.20 μ L of dNTP 40 mmol/L, 1 μ L buffer 10 \times , 1 unit of *Taq* polymerase and 0.10 μ mol/L of Ccur 51, 0.10 μ mol/L of Ccur 58, 0.25 μ mol/L of Ccur 65, and 0.15 μ mol/L of Ccur 99 (third set), or 0.75 μ mol/L of Ccur 61 and 0.20 μ mol/L of Ccur 100 (fourth set). Amplified fluorescent fragments were visualized using an automated ABI Prism 310 sequencer (Applied Biosystems, Foster City, California, USA). Allele sizes were estimated using Genescan 3.2.1 (Applied Biosystems).

Statistics

We used parametric statistics for the complete data set ($n = 19$ colonies) and nonparametric statistics for

analyzing subsamples (colonies where the mother queen was not replaced, or where the initial nest was not absconded). Summary statistics are quoted as mean \pm standard deviation for the former and as median and quartiles for the latter. Tests were carried out with Statistica version 8.0 (StatSoft 2008), except for testing bias in resource allocation which was carried out using the binomial skew index B (Nonacs 2000) with the program "Skew calculator 2003" (P. Nonacs, *unpublished software*). B was computed using the number of workers present in each nest as the "benefits." B equals zero when the skew is as expected from the binomial distribution (i.e., resource allocation varies among nests by chance), it is positive when the skew is higher (i.e., allocation is more skewed than by chance), and it is negative when it is lower (i.e., allocation is more even than by chance). The observed values of B were tested against zero, the expected value by chance, with the program "Skew calculator 2003."

RESULTS

Pattern of nest production

Colonies produced all their new nests over a period of 3.3 ± 2.5 days on average, ranging from one colony founding all of four new nests on the same day to two colonies founding five and nine new nests over nine days. When colony fission was complete each colony occupied 4.0 ± 1.3 nests (range 2–7 nests, total = 76 nests for the 19 colonies), each containing 183 ± 214 workers (range 30–1284 workers; Table 1, Fig. 3). Most nests had restored monogyny when collected: 72 were monogynous, one contained no queen, and three (from three different colonies) were polygynous (Table 1). These polygynous nests contained the mother queen plus 4 to 13 young queens (Table 1). One of these nests and two additional nests from other colonies also contained dead queens, suggesting that several young queens initially co-occur in nests and that monogyny is restored within a few days. This possibility is supported by preliminary field experiments conducted in May–June 2008 with individually color-marked queens, in which we observed two queens transported to the same new nest ($n = 3$), workers removing a dead queen from a new nest that contained one queen when collected ($n = 6$), and marked queens being carried to a new nest where they were not found when the nest was excavated ($n = 4$).

A further 25 nests had been abandoned (1.3 ± 1.3 nest per colony; Fig. 2): 21 were empty when collected, and four were occupied by foreign colonies, as revealed by microsatellite analysis (Table 1). Nineteen workers from each of these four nests were genotyped and found to be daughters of the foreign queen, except three workers with genotypes compatible with the queen of the initial nest. This rules out the possibility of intraspecific social parasitism and suggests that the foreign colonies either re-occupied vacated nests, or expelled the resident colony. We did not observe a foreign colony expelling

TABLE 1. Outcome of the 19 monitored colony fissions in the ant *Cataglyphis cursor* from the study site in Argelès-sur-Mer, southern France.

Colony number	Colony size before fission (no. workers)	Number of nests after fission			Size of occupied nests after fission (no. workers)	Location of the mother queen	Investment in reproduction (%)	Mode of production of young queens	Number of supernumerary queens
		Occupied	Empty	Foreign					
426	294	3	0	1 (i)	62–153	P	0
433	549	4	2 (i)	0	102–181	new nest	67	P	0
447	290	3	1 (i)	0	30–148	S	0
455	722	4 (i)	1	1	47–497	initial nest	31	S	0
456	1628	3 (i)	0	0	152–1150	P	7†
464	252	2 (i)	0	0	72–180	P	0
471	789	3 (i)	0	0	137–492	initial nest	38	P	5 (d)
473	278	4	1 (i)	0	42–102	new nest	72	S	0
511	1328	3 (i)	2	0	44–890	new nest	33	S	0
531	531	4 (i)	5	1	45–218	P	0
533	999	7	1	1 (i)	65–206	P	0
544	1040	4 (i)	1	0	213–305	initial nest	77	P	4 (d) + 1†
571	495	5 (i)	1	0	60–152	P	0
581	291	3 (i)	1	0	48–158	new nest	46	P	3†
598	780	7 (i)	1	0	56–234	new nest	88	P	0
619	452	3	1 (i)	0	88–197	P	0
634	577	4 (i)	0	0	69–293	P	0
641	791	5 (i)	2	0	58–257	P	0
667	1808	5 (i)	1	0	49–1284	new nest	29	P + S	13 (a)‡

Notes: Colony size before fission is the sum of workers found in all nests from that colony after fission. Number of nests after fission gives the status (occupied by the colony, empty, or occupied by a foreign colony) of all nests that were initiated during colony fission, with “(i)” indicating the initial nest. Investment in reproduction is calculated for colonies that retained the mother queen (identified in boldface) and is the percentage of workers that were found in all nests headed by a young queen, i.e., workers that had left the mother queen. Young queens were produced by parthenogenesis (P) or sexual reproduction (S). The number of supernumerary queens in a colony is the number of young queens in excess of the number of nests. They were alate (a), dealated (d), or recently deceased (†) and were found in only five nests from five colonies. Ellipses indicate that data are not available, as in these colonies the mother queen did not survive colony fission.
 ‡ The 13 supernumerary queens were sexually produced.

the resident colony. However, we cannot rule out this possibility as our protocol allocated little time to the observation of new nests. Indeed, we observed new nests only when a transporting worker was arriving; hence, nest usurpation could have gone unnoticed if it occurred

when transports had ceased. The fate of the occupants of the 25 abandoned nests (six initial and 19 new nests) is unclear. They may have failed to establish a new colony and died. Alternatively, they may have relocated to a new nesting site, returned to their initial nest, or merged

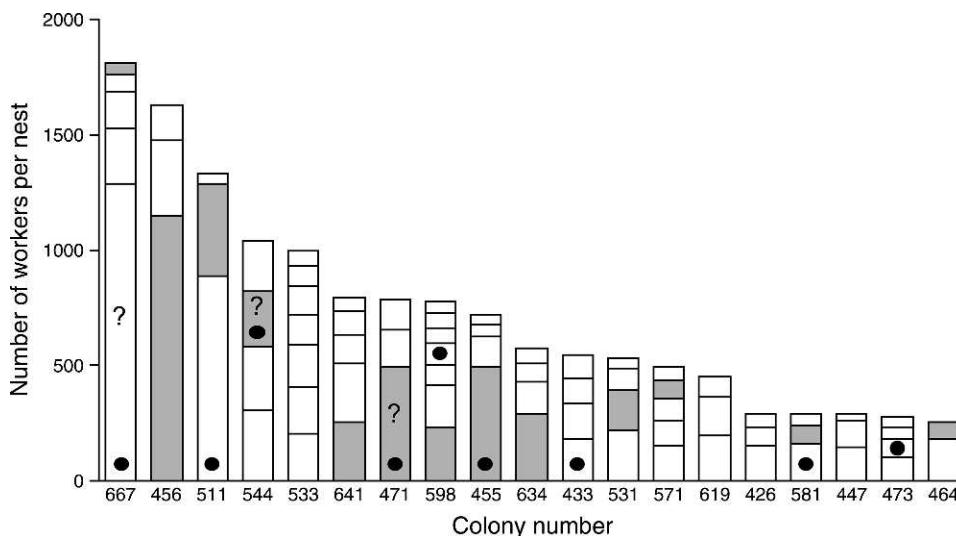


FIG. 3. Number of *C. cursor* workers per nest, by colony. Colonies are ordered by decreasing size from left to right. Within colonies, nests are ordered by decreasing size from bottom to top. Black dots indicate nests with the mother queen, initial nests are shaded gray, and question marks indicate polygynous nests that may have not completed colony fission (Table 1). All colonies shown had a significant ($P < 0.001$) index of skew among all nests, i.e., including the nest with the mother queen and the initial nest.

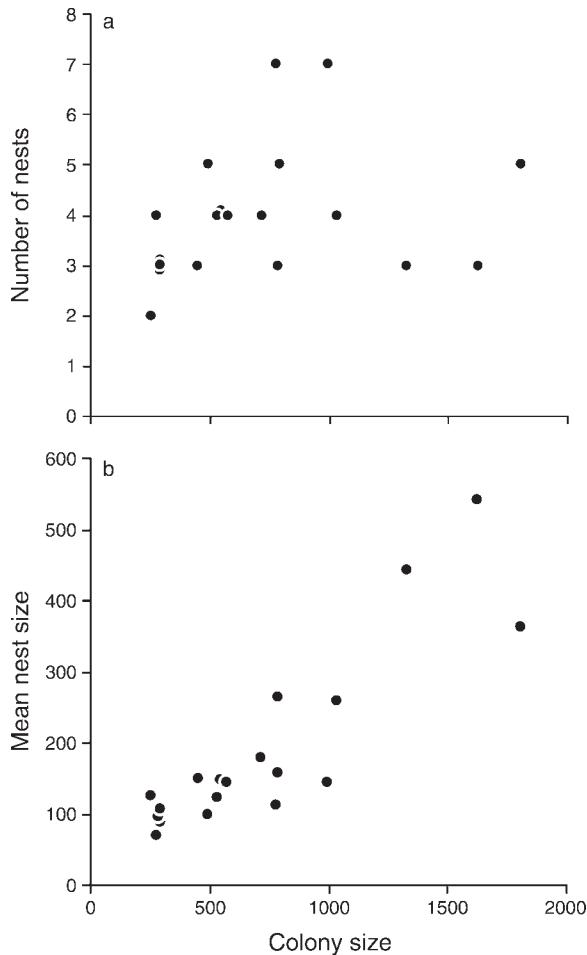


FIG. 4. (a) Number and (b) mean size of nests produced according to the size of the colony before fission. For clarity, overlapping dots have been slightly moved over the y -axis.

with another new nest. Time limitations meant that we could not observe transports departing from new nests. However, transports were observed arriving at the initial nest of four colonies, and opportunistic observations of the new nests of two of these colonies showed some transports from four new nests toward the initial nest. Three of these four new nests were empty when collected, suggesting reallocation of workers can occur during or immediately after colony fission.

Origin of young queens

The microsatellite analysis shows that young queens were produced by thelytoky (14 colonies), by sexual reproduction (4 colonies), or by a combination of both (1 colony; Table 1). In the single colony that combined thelytokous and sexual reproduction evidence suggests that parthenogenetic queens were produced before sexual queens. Indeed, each of the four parthenogenetic queens had lost their wings and headed a monogynous nest, whereas the 13 sexual queens were still winged and had not fissioned as they were found in the same nest as

the mother queen. Colonies that produced young queens by sexual reproduction do not seem to differ in any other respect from colonies that produced young queens by parthenogenesis, but the data are inconclusive at this time.

Size of fissioning colonies and relationship with size and number of nests produced

We inferred the size of each colony before fission as the number of workers present in all its nests after fission (i.e., initial nest plus new nests). Colonies that fissioned varied markedly in size, ranging from 252 to 1808 workers (731 ± 456 workers, $n = 19$; Table 1 and Fig. 3). The 17 non-fissioning colonies collected on 12–14 June contained 684 ± 290 workers (range 190–1302 workers) and were not different in size from fissioning colonies (Mann-Whitney U , $P = 0.962$). Non-fissioning colonies were collected approximately two weeks later than fissioning colonies, and thus may have grown in size during this time. However, even when taking this into account, it is clear that the sizes of fissioning and non-fissioning colonies largely overlap.

The size of a colony before fission was not correlated to the number of nests it occupied after fission (Pearson correlation, $n = 19$, $r = 0.261$, $P = 0.281$), and was thus correlated to their mean size ($r = 0.876$, $P < 0.001$). That is, larger colonies did not produce more nests, they produced larger nests (Fig. 4). Colonies did not allocate an equal number of workers to each of the nests they occupied when fission was complete. All colonies showed a significant bias among nest size, as measured with Nonacs' binomial skew index B (Fig. 3). This biased allocation was more pronounced in larger colonies (Pearson correlation, $r = 0.58$, $P = 0.009$).

We further tested whether colonies allocated more resources to the nest with the mother queen or to the initial nest, as these may be more valuable than other nests. These two factors may interact, but owing to the limited sample for each of the possible situations we considered presence/absence of the mother queen and continued use/vacation of the initial nest separately (seven colonies retained the mother queen and the initial nest, including four colonies where the mother queen relocated to a new nest; two colonies retained the mother queen and vacated the initial nest; six colonies replaced the mother queen and retained the initial nest; and four colonies replaced the mother queen and vacated the initial nest; Fig. 3).

This paragraph focuses on the nine colonies where the mother queen survived colony fission. These colonies invested $53\% \pm 23\%$ of their workers in nests with young queens (Table 1, Fig. 3), and the nest with the mother queen (median and quartiles of 243, 158, and 497 workers) was larger than the mean of nests with young queens (median and quartiles of 123, 74, and 149 workers; Wilcoxon paired test, $n = 9$, $P = 0.038$). Modeling predicts that nests with young queens should be of the same size (see *Introduction*). We tested this

prediction by computing the skew index B for each colony when excluding the nest with the mother queen. This index revealed a significant skew among nests with young queens in eight colonies ($P < 0.003$) and no skew in colony 471 ($P = 0.184$; Fig. 3). Queen replacement may be more frequent in colonies producing parthenogenetic queens than in those producing sexual queens (9 replacements in 15 colonies vs. 1 replacement in 5 colonies, respectively; Table 1), but because of the limited number of colonies producing sexual queens this is inconclusive (Fisher exact test, $P = 0.3034$).

We will now focus on the 13 colonies where the initial nest remained occupied (Fig. 3): Within colonies, the initial nest (median and quartiles of 243, 85, and 394 workers) did not contain more workers than the mean of new nests (median and quartiles of 134, 103, and 239; Wilcoxon paired test, $n = 13$, $P = 0.221$). The skew index B shows that worker allocation among new nests (i.e., when excluding the initial nest) was biased in 11 colonies having several new nests ($P < 0.001$) and was not biased in colony 471 ($P = 0.186$).

The analysis of the dynamics of resource allocation show that the size of new nests depended on their relative timing of foundation. Within colonies, the new nest(s) founded on the first day of colony fission were larger than those founded on subsequent days (Wilcoxon paired test, $n = 15$ colonies founding new nests over several days, $P = 0.0038$; Fig. 5).

Pattern of nest dispersal

Before the onset of colony fission, the 268 colonies identified within the site area were distributed randomly, with an aggregation index $R = 0.92$ (Fig. 2; Clark and Evans 1954). This confirms previous results by Lenoir et al. (1990) for other populations. The distance to the nearest neighboring colonies was 2.8 ± 1.8 m ($n = 268$, range 0.20–9.90 m).

After colony fission, new nests that were occupied ($n = 63$) had dispersed, on average, 7.1 ± 5.3 m (range 0.9–31.2 m). The mean dispersal distance of new nests from a given colony was not correlated to colony size before fission (Pearson correlation, $n = 19$ colonies, $r = 0.37$, $P = 0.118$). Nineteen new nests from 11 colonies (and six initial nests) had been abandoned by the time they were collected. This abandonment was not due to a short dispersal distance as these nests had dispersed as far as the new nests that were occupied ($n = 42$) (comparison within colonies of the mean dispersal distance of abandoned vs. occupied new nests: median of 6.6 and 6.9 m, respectively; Wilcoxon paired test, $n = 11$ colonies, $P = 0.657$).

DISCUSSION

Reproductive allocation is of paramount importance, but it has rarely been studied in social insects reproducing by colony fission. We studied colony fission in the ant *Cataglyphis cursor* under natural conditions and found reproductive effort of colonies and allocation

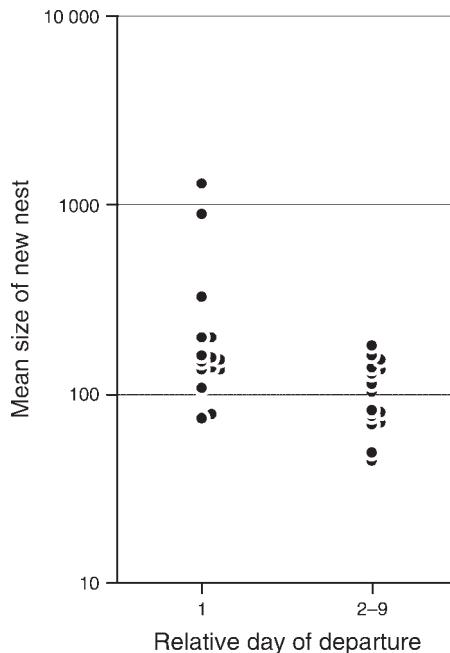


FIG. 5. Size of new nests according to their timing of departure from the initial nest. The graph shows new nests for 15 colonies that produced several (2–7) new nests over several (2–9) days. For each colony, the day of departure is relative to the departure of the first new nest on day 1. When colonies produced several new nests either on day 1 or on days 2–9, the mean size of new nests was used. For clarity, overlapping dots have been slightly moved over the x-axis. Note the log scale of the y-axis.

of resources among nests to be highly variable. Our results show that (1) most colonies did not fission; (2) colonies that fissioned showed a broad range of size, from 252 to 1808 workers, and the sizes of fissioning and non-fissioning colonies overlap; (3) fissioning colonies produced 4.0 ± 1.3 nests, with larger colonies producing larger nests but not more nests; (4) the mother queen was replaced in half of the fissioning colonies; and (5) resource allocation among nests produced was biased. Within a colony, the nest with the mother queen was larger than those with young queens in agreement with Pamilo's prediction (Pamilo 1991). In addition, nests with young queens were of markedly varied size, and the first nest(s) to be founded were larger than nests founded later. These differences cannot be attributed to differences in colony kin structure, as most colonies produced parthenogenetic queens only.

The finding that even small colonies fissioned was unexpected and suggests that selection against small colonies may be low in *C. cursor*. This is supported by the fact that the efficiency of foragers is independent of colony size because they forage solitarily and do not rely on pheromone trails for orientation (Lenoir et al. 2009). In addition, foragers from small colonies are not at a disadvantage compared with foragers from larger colonies in regards to the foraging area they can exploit

because *C. cursor* is not territorial (Lenoir et al. 1990). Indeed, colonies were randomly distributed in our study site, in contrast with the ant *Aphaenogaster senilis*, where colonies are overdispersed because of inter-colony competition (Boulay et al. 2010). Finally, inter-colony aggression is of little importance in *C. cursor* (Lenoir et al. 1990), whereas it is a major cause of colony death in strongly territorial species where adult colonies seek out and destroy incipient colonies (e.g., Tschinkel 2006).

Only 27% of colonies in our study area showed signs of sexual activity, demonstrating that fission is not an annual occurrence. Fissioning colonies differed markedly in size and the size of fissioning and non-fissioning colonies largely overlapped. This suggests a diversified bet-hedging strategy (Olofsson et al. 2009), with risk-averse colonies fissioning at larger size than risk-prone colonies. Another explanation for this high variability in the size of fissioning colonies is that they have incomplete control over fission. Colonies produce several young queens when they need to replace the ageing or dead mother queen, and it could be that some of these queens sometimes trigger fission, which would then occur at various colony sizes. This supposition is supported to some extent by the fact that half of the mother queens were replaced during colony fission. However, this does not explain why some of the fissioning colonies were small since colonies that fissioned and re-queened were not smaller than colonies that fissioned without re-queening (Mann-Whitney *U* test, $P = 0.307$).

Our observations also show that large *C. cursor* colonies did not produce more nests than smaller colonies, and therefore produced larger nests on average. Nevertheless, they strongly biased allocation among nests. This suggests a diversified bet-hedging strategy of producing offspring of varied size, the large ones having a high survival rate and the small ones yielding a high fitness when the environment is good. Overall, this suggests that *C. cursor* follows a complex bet-hedging strategy with regard to both the timing of reproduction (in terms of colony size at which to reproduce) and the allocation strategy (in terms of size and number of nests). Bet-hedging was suggested in the ant *Cardicondyla elegans*, in an altogether different context. In this species, nest mortality is very high during winter (~40%) and workers transport young female sexuals to other nests, possibly to avoid that they all die should their natal nest not survive the winter (Lenoir et al. 2007).

However, proximate factors could also contribute to the observed bias in nest size, such as incomplete knowledge of resource allocation. Indeed, brood, workers, and queens are transported to new nests by few carrying workers, and these transporters would need information on the size of all nests in order to be able to fine-tune allocation among them. It is likely that this is not the case. Because of the lack of chemical trails in *C. cursor*, for transporters to be aware of all new nests they

would either need to independently discover them or to be transported by an informed transporter. It seems more likely that each transporter contributes to the build up of one new nest at a time, and has information on the size of this new nest and of the initial nest only. Consequently, new nests may be in scramble competition to secure workers from the initial nest. Evidence for this is that new nests founded the first day of colony fission were larger than new nests founded later (Fig. 5), which suggests that transporters exhaust the initial nest as a source of individuals.

One limitation of this study is that we only monitored transports departing from the initial nests. At the end of colony fission some new nests had been abandoned, as also occurs in *Proformica longiseta* (Fernández-Escudero et al. 2001), and a few of these abandoned nests had been reoccupied by foreign colonies, as already observed by Lenoir et al. (1988). Since we did not monitor transports departing from new nests, it is possible that some abandoned new nests had relocated unnoticed to another unidentified nesting site, or that some new nests had fissioned again. This would result in us underestimating the number of new nests produced and the inferred size of colonies before fission. However, while following transports arriving at new nests we did observe a few transports departing from those new nests, and all went back to the initial nest. This suggests that nest size may be adjusted in the few days following colony fission. Scramble competition for workers may lead to some new nests being too small to be viable, and the merging of failing new nests with the initial nest would allow rescuing these resources. Readjustment of worker numbers may also allow fine-tuning nest size to local conditions, which can change rapidly over a few weeks as many colonies produce new nests. Experimental reduction of colony density in the ant *A. senilis* showed that colonies indeed detect such changes and can adapt their reproductive behavior accordingly (Boulay et al. 2010). A second concern is the presence of several queens in some nests, which therefore may not have finished fissioning at the time of collection. However, this concerns only three of 76 nests in three of 19 colonies and should thus have little effect on our findings. Finally, the present study does not take into account the quality of workers allocated to nests, in terms of age, size, and lineage. Transporters may seek to optimize survival of new nests by maximizing the morphological and genetic diversity of workers as this would ensure a more efficient division of labor and better resistance to pathogens (Crozier and Fjerdingstad 2001). Alternatively, transporters may preferentially transport more valuable workers if this allows minimizing the number of transports required and associated costs. For instance, a new nest may require fewer workers if they are younger and larger as they are then likely to survive longer and to be more efficient at most tasks. This possibility remains to be investigated in *C. cursor*.

In ants, colony fission entails restricted dispersal on foot (*C. cursor*; Clémencet et al. 2005, Hardy et al. 2008). Related colonies may thus compete with one another. Our study provides suggestive evidence for Local Resource Competition (LRC) in that the mean distance of foraging and dispersal were similar (5.8 ± 2.7 m and 7.1 ± 5.3 m, respectively, $n = 14$ and 19 colonies; Lenoir et al. 1990 and our results). Hence, the foraging areas of new nests largely overlapped with that of their initial colony and often overlapped with one another, leading to some level of scramble competition for food. However, the distance to the nearest neighbor was smaller (2.5 ± 1.7 m) than both foraging and dispersal distance, so that after fission, the 76 nests had, on average, 5.5 ± 3.2 nests within the mean foraging distance (i.e., within a 5.8 m radius). This is an underestimation, as we only mapped the new nests produced by 19 out of 73 candidate fissioning colonies (26%). Thus, LRC could occur, but most competition does not occur between mother and daughter or between sister colonies. Furthermore, the fact that small colonies fission and that even large colonies produce some small nests suggests that LRC is unlikely to be severe.

Nest spacing may be affected by interspecific competition, a factor that we could not take into account in the present study. However, interspecific competition does not necessarily affect nest distribution, and indeed it has little effect in litter ant communities (Theunis et al. 2005, Herbers 2011). In our study site, the other abundant species were *Aphaenogaster senilis*, three species of *Messor*, and *Tapinoma nigerrimum*. *C. cursor* forages at the hottest hours of the day (Lenoir et al. 2009), and there was little evidence for direct competition with *Messor* and *Tapinoma*. *Aphaenogaster senilis* occasionally competed aggressively for dead insects, but only a thorough study could measure the impact of these interactions.

This work shows that the strategy of colony fission of *C. cursor* differs markedly from that employed by honey bees and army ants. We suggest that the patterns of colony fission in social insects are more diverse than currently acknowledged and that studies of other species are needed to facilitate comparative studies. Indeed, at this stage, our understanding of resource allocation during colony fission is poor and much remains to be investigated. Models comparing the success of alternative strategies (e.g., production of one vs. several daughter colonies, production of daughters of fixed vs. varied size) under different environmental conditions and/or with different life history traits (e.g., solitary vs. social foraging) would be useful and would likely yield testable predictions. Empirical data are also needed. For instance, in *C. cursor*, quantifying the survival and growth of new nests according to their size and to environmental conditions (abiotic conditions, colony density at the scale of the foraging area, food availability) would be a first step in determining whether this species follows a diversified bet-hedging strategy, as

we suggest. Also, studies of transports would clarify whether transporters contribute to the build up of one or several nests at a time, and hence, whether biased allocation among nests stems from incomplete knowledge of allocation.

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LITERATURE CITED

- Allen, R., Y. M. Buckeley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *American Naturalist* 171:225–237.
- Axenovich, T. I., I. V. Zorkoltseva, I. R. Akberdin, S. V. Beketov, S. N. Kashtanov, I. A. Zakharov, and P. M. Borodin. 2007. Inheritance of litter size at birth in farmed arctic foxes (*Alopex lagopus*, Canidae, Carnivora). *Heredity* 98:99–105.
- Boulay, R., J. Galarza, B. Chéron, A. Hefetz, A. Lenoir, L. van Oudenhove, and X. Cerdá. 2010. Competition among philopatric females regulates population size and affects resource allocation in an ant species dispersing by colony fission. *Ecology* 91:3312–3321.
- Boulay, R., A. Hefetz, X. Cerdá, S. Devers, W. Francke, R. Twele, and A. Lenoir. 2007. Production of sexuals in a fission-performing ant: dual effects of queen pheromones and colony size. *Behavioral Ecology and Sociobiology* 61:1531–1541.
- Briese, D. T. 1983. Different modes of reproductive behaviour (including a description of colony fission) in a species of *Chelaner* (Hymenoptera: Formicidae). *Insectes Sociaux* 30:308–316.
- Brown, C. A. 2003. Offspring size-number trade-offs in scorpions: an empirical test of the van Noordwijk and de Jong model. *Evolution* 57:2184–2190.
- Bulmer, M. G. 1983. Sex ratio theory in social insects with swarming. *Journal of Theoretical Biology* 100:329–339.
- Chéron, B., T. Monnin, P. Fédérici, and C. Doums. 2011. Variation in patriline reproductive success during queen production in orphaned colonies of the thelytokous ant *Cataglyphis cursor*. *Molecular Ecology*, in press.
- Clark, P. J., and F. C. Evans. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. *Ecology* 35:445–453.
- Clémencet, J., B. Viginier, and C. Doums. 2005. Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers. *Molecular Ecology* 14:3735–3744.
- Crespi, E. J., and H. Lessig. 2004. Mothers influence offspring body size through post-oviposition maternal effects in the redbacked salamander, *Plethodon cinereus*. *Oecologia* 138:306–311.
- Crozier, R. H., and E. J. Fjerdingstad. 2001. Polyandry in social Hymenoptera: disunity in diversity? *Annales Zoologici Fennici* 38:267–285.
- Crozier, R. H., and P. Pamilo. 1996. Evolution of social insect colonies. Sex allocation and kin selection. Oxford University Press, Oxford, UK.
- Dziminski, M. A., and R. A. Alford. 2005. Patterns and fitness consequences of intraclutch variation in egg provisioning in tropical Australian frogs. *Oecologia* 146:98–109.
- Fernández-Escudero, I., P. Seppä, and P. Pamilo. 2001. Dependent colony founding in the ant *Proformica longiseta*. *Insectes Sociaux* 48:80–82.

- Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology* 45:341–369.
- Godfray, H. C. J. 1995. Evolutionary theory of parent-offspring conflict. *Nature* 376:133–138.
- Gotwald, W. H. J. 1995. *Army ants: the biology of social predation*. Cornell University Press, Ithaca, New York, USA.
- Hardy, O. J., M. Pearcy, and S. Aron. 2008. Small-scale spatial genetic structure in an ant species with sex-biased dispersal. *Biological Journal of the Linnean Society* 93:465–473.
- Heinze, J., S. Cremer, N. Eckl, and A. Schrempf. 2006. Stealthy invaders: the biology of *Cardiocondyla* tramp ants. *Insectes Sociaux* 53:1–7.
- Herbers, J. M. 2011. Nineteen years of field data on ant communities (Hymenoptera: Formicidae): What can we learn? *Myrmecological News* 15:43–52.
- Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33:181–233.
- Hudson, R., and F. Trillmich. 2008. Sibling competition and cooperation in mammals: challenges, developments and prospects. *Behavioral Ecology and Sociobiology* 62:299–307.
- Inoue, T., S. F. Sakagami, S. Salmah, and S. Yamane. 1984. The process of colony multiplication in the Sumatran stingless bee *Trigona (Tetragonula) laeviceps*. *Biotropica* 16:100–111.
- Keller, L. 1997. Indiscriminate altruism: unduly nice parents and siblings. *Trends in Ecology and Evolution* 12:99–103.
- Koops, M. A., J. A. Hutchings, and B. K. Adams. 2003. Environmental predictability and the cost of imperfect information: influences on offspring size variability. *Evolutionary Ecology Research* 5:29–42.
- Lenoir, A., S. Aron, X. Cerdá, and A. Hefetz. 2009. *Cataglyphis* desert ants: a good model for evolutionary biology in Darwin's anniversary year: a review. *Israel Journal of Entomology* 39:1–32.
- Lenoir, A., E. Nowbahari, L. Quérard, N. Pondicq, and C. Delalande. 1990. Habitat exploitation and intercolonial relationships in the ant *Cataglyphis cursor* (Hymenoptera Formicidae). *Acta Oecologica* 11:3–18.
- Lenoir, A., L. Quérard, N. Pondicq, and F. Berton. 1988. Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). *Psyche* 95:21–44.
- Lenoir, J. C., A. Schrempf, A. Lenoir, J. Heinze, and J. L. Mercier. 2007. Genetic structure and reproductive strategy of the ant *Cardiocondyla elegans*: strictly monogynous nests invaded by unrelated sexuals. *Molecular Ecology* 16:345–354.
- Mappes, T., and E. Koskela. 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution* 58:645–650.
- Marshall, D. J., R. Bonduriansky, and L. F. Bussière. 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology* 89:2506–2517.
- Nonacs, P. 2000. Measuring and using skew in the study of social behavior and evolution. *American Naturalist* 156:577–589.
- Olofsson, H., J. Ripa, and N. Jonzén. 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society B* 276:2963–2969.
- Pamilo, P. 1991. Evolution of colony characteristics in social insects. I. Sex allocation. *American Naturalist* 137:83–107.
- Parker, G. A., N. J. Royle, and I. R. Hartley. 2002. Intrafamilial conflict and parental investment: a synthesis. *Philosophical Transactions of the Royal Society B* 357:295–307.
- Pearcy, M., and S. Aron. 2006. Local resource competition and sex ratio in the ant *Cataglyphis cursor*. *Behavioral Ecology* 17:569–574.
- Pearcy, M., S. Aron, C. Doums, and L. Keller. 2004a. Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science* 306:1780–1783.
- Pearcy, M., J. Clémencet, S. Chameron, S. Aron, and C. Doums. 2004b. Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. *Molecular Ecology Notes* 4:642–644.
- Pearcy, M., O. Hardy, and S. Aron. 2006. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity* 96:377–382.
- Peeters, C., and F. Ito. 2001. Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annual Review of Entomology* 48:601–630.
- Ratnieks, F. L. W. 1991. The evolution of genetic odor-cue diversity in social Hymenoptera. *American Naturalist* 137:202–226.
- Ratnieks, F. L. W., and H. K. Reeve. 1991. The evolution of queen-rearing nepotism in social Hymenoptera: Effects of discrimination costs in swarming species. *Journal of Evolutionary Biology* 4:93–115.
- Seeley, T. D. 1996. *The wisdom of the hive: the social physiology of honey bee colonies*. Harvard University Press, Cambridge, Massachusetts, USA.
- Shik, J. Z. 2008. Ant colony size and the scaling of reproductive effort. *Functional Ecology* 22:674–681.
- StatSoft. 2008. *Statistica*. Version 8.0. StatSoft, Tulsa, Oklahoma, USA.
- Stearns, S. C. 1992. *The evolution of life histories*. Oxford University Press, Oxford, UK.
- Suarez, A. V., D. A. Holway, and P. S. Ward. 2005. The role of opportunity in the unintentional introduction of nonnative ants. *Proceedings of the National Academy of Sciences USA* 102:17032–17035.
- Theunis, L., M. Gilbert, Y. Roisin, and M. Leponce. 2005. Spatial structure of litter-dwelling ant distribution in a subtropical dry forest. *Insectes Sociaux* 52:366–377.
- Tschinkel, W. R. 2006. *The fire ants*. Harvard University Press, Cambridge, Massachusetts, USA.
- van Veen, J. W., and M. J. Sommeijer. 2000. Colony reproduction in *Tetragonisca angustula* (Apidae, Meliponini). *Insectes Sociaux* 47:70–75.
- Warne, R. W., and E. L. Charnov. 2008. Reproductive allometry and the size-number trade-off for lizards. *American Naturalist* 172:E80–E98.
- Williams, T. D. 2001. Experimental manipulation of female reproduction reveals an intraspecific egg size clutch size trade-off. *Proceedings of the Royal Society B* 268:423–428.