

Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator*

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Abstract

The recently discovered social parasite *Acromyrmex insinuator* (Schultz, Bekkevoold & Boomsma 1998) exploits colonies of the leafcutter ant *A. echinator*. We document that *A. insinuator* represents a rare early stage in the evolution of social parasitism, because a worker caste is still partially present and mating phenology has remained at least partially similar to that of the host. *A. insinuator* is tolerant of host queens, and sexual offspring produced in parasitized colonies can be either exclusively *A. insinuator* or a mix of *A. insinuator* and *A. echinator*. The remarkably high abundance of *A. insinuator* in nests of the investigated Panamanian host population and the fact that *A. insinuator* colonies readily reproduce under laboratory conditions allowed us to test evolutionary predictions on reproductive life history evolution that are not possible in most other socially parasitic ants. We show that (1) *A. insinuator* has a semelparous 'big bang' reproductive life history which exploits host colonies without leaving reserves for survival; (2) social parasite sexuals are significantly smaller than *A. echinator* host sexuals, but still large compared to host workers, confirming an evolutionary scenario of gradual size reduction and loss of the worker caste after transition towards a socially parasitic life history; (3) major changes in the life history of ants can evolve relatively quickly compared to adaptations in morphology, caste differentiation and mating phenology.

Introduction

The study of reproductive allocation in social Hymenoptera has been remarkably successful in testing general models of sex allocation (see Bourke & Franks, 1995; Crozier & Pamilo, 1996; for recent reviews). Social insects have been much less amenable, however, to explicit tests of the major predictions of current life history theory (Bourke & Franks, 1995). Reasons for this are that: (1) comparative data are scarce (see Keller & Genoud, 1997,

for an exception); (2) life history parameters such as longevity, rates of colony growth and survival, age and size at maturity and the trade-offs between them (e.g. costs of reproduction) are very difficult to measure in natural populations (Oster & Wilson, 1978; Tschinkel, 1991; Tsuji & Tsuji, 1995); (3) most social insects have long generation times and are (with the exception of the domesticated honey bee) difficult to maintain and reproduce in the laboratory for prolonged periods of time; (4) the very presence of kin-conflict over aspects of resource allocation relevant to life history evolution adds an extra level of complexity to testing life history theory in social Hymenoptera (Bourke & Franks, 1995, also see Bourke, 1999, for a discussion concerning the relative importance of kin structure vs. other colony characteristics shaping the evolution of social insect societies); and (5) some central life history traits are relatively constant within lineages (e.g. all ants, termites and honey bees are

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perennial, whereas other groups such as bumble bees, halictid bees and wasps almost invariably have an annual life cycle (Bourke & Franks, 1995).

Some exceptions to these inhibiting rules for testing life history theory in social insects are found in socially parasitic ants. Social parasites insinuate themselves into the societies of other species where they exploit the resources and social structure of the host for their own reproduction. Social parasitism has evolved independently in a variety of lineages of eusocial Hymenoptera, and is particularly common in ants (Wilson, 1971). Four distinct types of true social parasites have been described for the Hymenoptera: guest-ants, temporary social parasites, slave-makers and inquilines, the last of these being taxonomically most diverse and displaying a gradient of increasingly specialized adaptations to a parasitic life-history according to the scheme of Hölldobler & Wilson (1990, pp. 467–469). A number of mainly alpine and boreal social parasites and their hosts have been studied, but few of these studies have addressed life history evolution. In addition, socially parasitic ant species have so far rarely been reported from the tropics (Hölldobler & Wilson, 1990).

The single well-studied case concerns Leptothoracine ants, which have a variety of social parasites and can be successfully reared in the laboratory (see Buschinger, 1989, for a review). In this group a major shift in reproductive life history has been reported for workerless *Epimyrma* social parasites, who have evolved a semelparous 'big bang' reproductive strategy, whereas related slave-making species that do not destroy their host colony have retained the perennial reproductive life history (Buschinger, 1989; Bourke & Franks, 1995). Based on the observations from *Epimyrma* it was hypothesized that workerless social parasites can evolve directly from their hosts without the need for an intermediate mode of social parasitism (Buschinger, 1989; Bourke & Franks, 1995).

Bourke & Franks (1991) define ant inquilines as social parasites whose newly mated queens gain entrance to mature host nests where they establish and reproduce alongside the host queen(s). Inquilines can be either completely workerless, with all eggs turning into sexual offspring, or have some fraction of eggs developing into workers. A general characteristic of inquilines is their small size compared to the host (e.g. Aron *et al.*, 1999). Caste determination in ants is closely related to the amount of food received during larval development (Wheeler, 1986), and Bourke & Franks (1991) hypothesized that by having a smaller size, and hence lower individual feeding requirements for sexual development than their hosts, inquiline queens would be able to maximize the number of their eggs that develop into reproductive offspring instead of workers. The degree to which an inquiline worker caste is maintained is then, as suggested by Nonacs & Tobin (1992), expected to be associated with the extent of inquiline size reduction

relative to the worker–queen caste-developmental threshold in the host.

Apparently, when perennial ants are secondarily forced to revert to a semelparous life history, an entire suite of characters changes in concert, leading to higher rates of brood development, intranidal mating, reduced dispersal and a highly patchy distribution of the social parasite, restricted to the densest host populations. Bourke & Franks (1995, p. 351) conclude that these deviant life histories have most likely evolved in direct response to the distribution of host colonies before parasitism, but independent data from other socially parasitic ants to test the generality of these trends have not been available. In the present paper we present such an independent test from a social parasite – host species pair of *Acromyrmex* leafcutter ants and confirm the interpretation of the basic trends shown for *Epimyrma* social parasites. We analyse the main characteristics of the reproductive life-history of the recently described queen-tolerant inquiline *Acromyrmex insinuator* Schultz, Bekkeveld & Boomsma 1998, parasitizing the closely related Panamanian leafcutter ant *A. echinator* Forel 1899 (recently elevated to species status by Schultz *et al.*, 1998). The host species *A. echinator* has long-lived facultatively polygynous (multiple queens) colonies headed by queens that have high mating frequencies (Bekkeveld *et al.*, 1999). Leafcutter ants belong to the subfamily Myrmicinae, tribe Attini, and are characterized by their mutualistic dependence on symbiotic fungi which are cultivated on a substrate of leaf fragments (Mueller *et al.*, 1998). Nonparasitic *Acromyrmex* species normally produce annual bouts of sexual offspring which engage in synchronized nuptial flights, and have three worker castes (majors, media and minima) with a highly complex division of labour (Weber, 1972). Colonies of *A. echinator* infected with *A. insinuator* are relatively common and highly amenable to rearing in the laboratory. We have exploited this by collecting comparative data on reproductive allocation, both under controlled laboratory conditions and in the field. These data allow us to document: (1) the dramatic shift in the use of colony resources by the social parasite compared to that of the host, leading to a 'big bang' reproductive life history, and (2) the incipient nature and incomplete adaptation of the socially parasitic life history of *A. insinuator*.

Materials and methods

Field collections and laboratory set-up

Thirty-nine *A. echinator* host colonies were excavated in Gamboa, Panama, over the years 1993, 1994, 1996 and 1998 (12, eight, six and 13 colonies, respectively). From 15 unparasitized *A. echinator* colonies and 14 *A. insinuator* infested colonies all queens, together with samples of workers and fungus, were collected alive and transferred

to the laboratory in Aarhus, Denmark. The remaining 10 colonies (seven unparasitized and three parasitized) and sexual offspring (males and gynes) in any of the 39 colonies were frozen immediately and later stored at -70°C . The laboratory colonies were set up in a rearing room at a constant temperature of 25°C and 60–70% relative humidity (RH), resulting in close to 100% RH in the fungus garden (see Boomsma *et al.*, 1999, for rearing details). Individual colony dynamics were studied in the laboratory during the period 1993–97. Colonies collected in 1998 were included solely to increase the sample size for the variables queen number and prevalence of social parasitism in the field.

Genetic analyses

In connection with a study of queen mating frequency in the host species *A. echinator* (Bekkevoold *et al.*, 1999), worker genotypes were investigated using the microsatellite DNA primer pair *Etta5-6TF* (developed by Fjerdingsstad *et al.*, 1998). From these data nestmate regression relatedness estimates (*b*) were produced for seven colonies parasitized by *A. insinuator* and 13 unparasitized *A. echinator* colonies (see Bekkevoold *et al.*, 1999, for molecular details and methods used for estimating nestmate relatedness values). *A. insinuator* sexual offspring could be distinguished morphologically from those of *A. echinator*, but minima caste workers of the two species were morphologically indistinguishable (Schultz *et al.*, 1998). The *Etta5-6TF* microsatellite locus was monomorphic for *A. insinuator*, but the single *A. insinuator* allele differed by 9–12 repeats from any of the *A. echinator* alleles, allowing unambiguous molecular identification of *A. insinuator* workers in parasitized *A. echinator* colonies.

Fungus garden dynamics in *A. insinuator* colonies

Larvae of leafcutter ants are raised entirely on a diet of symbiont fungus (Quinlan & Cherrett, 1979) and the size of the single fungus garden was therefore assumed to be a good estimator of the overall resource-level of individual *Acromyrmex* colonies. Each colony transferred to the laboratory was set up with its fungus garden (the initial volume of fungus transferred from the field was 100–300 mL) placed in an inverted, transparent measuring beaker (initially 600 mL and, where appropriate, increased to 800 or 1000 mL later on). All colonies were given equal and ample opportunities to forage for fresh leaf material (mainly *Rubus fruticosus*). In the years 1993–97 the volume of fungus garden was estimated by eye for each colony every 3–6 days. From these estimates the average volume of fungus maintained by each colony could be calculated on a monthly and annual basis. The estimated volume of fungus garden correlated well with the fresh weight of the actual fungus mass in a single comparative test performed in July 1996 ($r = 0.940$, $n = 12$, $P < 0.0001$).

Reproductive output and phenology

Four of the 17 parasitized and six of the 22 unparasitized colonies contained males and gynes (winged prospective queens) when collected in the field. Between 3 and 26 months after transfer to the laboratory, four parasitized and nine unparasitized colonies produced sexual offspring. None of the colonies collected in 1998 reproduced during the period included in this study and estimates of reproductive investment were thus based on colonies collected in 1993–96 only. In the years 1994–96, the number of sexual brood eclosing was recorded on a monthly basis for each laboratory colony ($n = 10$ reproducing colonies during this period). Using random samples of mature males and females from four *A. insinuator* colonies and four unparasitized *A. echinator* colonies the average dry weights (\pm SE) of males and gynes were estimated for the two species (*A. insinuator*: males: 5.16 ± 0.24 mg, $n = 17$; gynes: 7.14 ± 0.54 mg, $n = 20$; *A. echinator*: males: 5.61 ± 0.12 mg, $n = 25$; gynes: 15.55 ± 0.25 mg, $n = 18$). For each colony the total reproductive investment was estimated by multiplying the total number of males and females produced by the relative sex-specific average dry weights.

Results

Queen numbers and host colony kin structure

From the 17 parasitized colonies 1–12 *A. insinuator* queens were collected together with 0–4 host queens. The remaining 22 unparasitized *A. echinator* colonies contained 0–2 queens (Fig. 1). As all colonies were dug up without prior notice of the presence of social parasites,

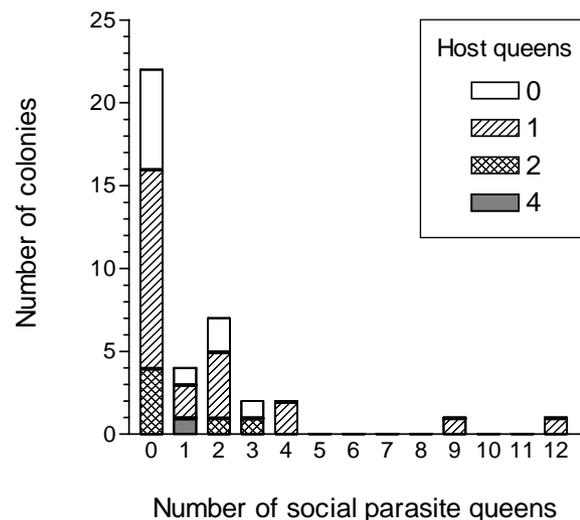


Fig. 1 Frequency diagram showing the number of *A. insinuator* queens in 39 *A. echinator* host colonies and the number of host queens in these colonies indicated by different bar shades.

the parasitisation ratio of 17 out of 39 (44%) of *A. echinator* colonies in the Gamboa population is likely to be unbiased, which implies that the social parasite is at least locally common. All *A. insinuator* colonies transferred to the laboratory had *A. echinator* host queens but aggression between host and parasite queens was never observed, and there was no correlation between the number of parasite queens per colony and the number of host queens ($r_s = 0.035$, $P = 0.835$, $n = 39$, Fig. 1). There was no significant difference in relatedness among host workers in colonies with *A. insinuator* queens present compared to unparasitized colonies ($b \pm SE = 0.337 \pm 0.078$ in seven colonies containing *A. insinuator*; $b \pm SE = 0.339 \pm 0.037$ in 13 unparasitized colonies, two-tailed $t = 0.025$, d.f. = 18, $P = 0.981$) showing that the kin structure of host colonies is unlikely to be relevant for the invasion and reproductive success of the social parasites. Within the class of parasitized colonies, however, the 22 colonies with a single host queen had a total of 39 parasite queens. This number is on average about twice as high as the mean number of parasite queens in the colonies with zero or >1 host queen (Fig. 1), both colony types in which a larger susceptibility to invasion of parasite queens might have been expected, suggesting that neither queenlessness nor reduced distinctness of kin structure has a positive effect on the potential load due to social parasites.

Molecular analyses of 98 major caste, 28 medium caste and 31 minor caste workers from seven *A. insinuator* colonies revealed that minima *A. insinuator* workers were present in all colonies, whereas no media or major inquiline workers were detected.

Fungus garden dynamics in relation to parasite presence

Parasite presence had a highly detrimental effect on the colony fungus garden in all laboratory colonies (Fig. 2). Fungus gardens in parasitized colonies that were transferred to the laboratory did not achieve significant growth and were dead or seriously decimated 12 months after establishment in the laboratory in four out of five documented cases (Fig. 2). Comparable unparasitized colonies normally increased their fungus garden volume rapidly and were usually prevented from further growth by the laboratory set-up after their fungus garden had reached a volume of 1 L (Fig. 2). One year after establishment in the laboratory the mean fungus volume ($\pm SE$) in unparasitized colonies was 0.87 ± 0.05 L ($n = 9$), whereas parasitized colonies had a much lower average fungus volume of 0.10 ± 0.06 L ($n = 5$), a difference which was highly significant ($t = 9.868$, d.f. = 12, $P < 0.0001$). However, in one of the parasitized colonies (colony # 22 in Fig. 2), the fungus garden prospered for 26 months after transfer to the laboratory, although the growth rate of the fungus garden was low. A maximal fungus garden volume of 1 L was reached

after 20 months during which no reproduction took place. Hereafter, a large number of *A. insinuator* sexuals were produced followed by the deterioration and death of the colony 39 months (c. 1200 days) after transfer to the laboratory. Such a course of events was not observed in unparasitized *A. echinator* colonies, as reproduction in these laboratory colonies was never detrimental to colony survival.

Reproductive output

A. insinuator colonies produced between two and 326 sexual offspring per year, with sexuals in individual colonies eclosing over a 2–4-month period, after which production of sexual brood ceased, seemingly due to declining colony resources. In three such deteriorating *A. insinuator* colonies workers were repeatedly observed removing live larvae from the brood piles and leaving them to perish outside the nest. Four of these larvae from two colonies were genotyped and identified as *A. insinuator*, suggesting that host workers had detected these larvae as alien or in some other way jeopardising colony survival. Sexuals reared in colonies with *A. insinuator* queens were either completely parasite produced (six colonies) or a mixture of parasite and host sexuals (two colonies, one which produced nine parasite sexuals and four host sexuals, and another which produced one parasite sexual and 197 host sexuals).

The field samples showed that the average annual reproductive investment in *A. insinuator* colonies was about 50% of that in unparasitized *A. echinator* colonies, but this difference was not statistically significant (average dry weight investment $\pm SE$: *A. insinuator*: 1.02 ± 0.43 g, $n = 4$ colonies, *A. echinator*: 1.94 ± 0.99 g, $n = 6$ colonies, Mann–Whitney U -test: $U = 10.0$, $P = 0.762$). After transfer to the laboratory, *A. insinuator* colonies had their first sexual brood eclosing after on average ($\pm SE$) 8.3 ± 4.6 months, whereas *A. echinator* colonies did not produce sexuals until 19.8 ± 3.4 months following transfer (Mann–Whitney U -test: $U = 4.0$, $P = 0.034$). Like in the field, the annual reproductive investment in *A. insinuator* laboratory colonies was about half, but not significantly different from, the annual reproductive investment of *A. echinator* colonies at their first attempt at reproduction in the laboratory (average dry weight investment $\pm SE$: *A. insinuator*: 0.63 ± 0.42 g, $n = 4$ colonies, *A. echinator*: 1.08 ± 0.19 g, $n = 9$ colonies, Mann–Whitney U -test: $U = 10.0$, $P = 0.260$). Unparasitized *A. echinator* colonies kept in the laboratory normally reproduced annually, and there was no significant difference in the reproductive investment over reproductive bouts (years) (repeated measures ANOVA: $F_{[5,2]} = 1.274$, $P = 0.347$, based on six colonies with data for three consecutive years of reproduction). The three laboratory colonies collected in 1996 reproduced only once (two colonies) or twice (one colony) during the study period. Combining the reproductive output in field

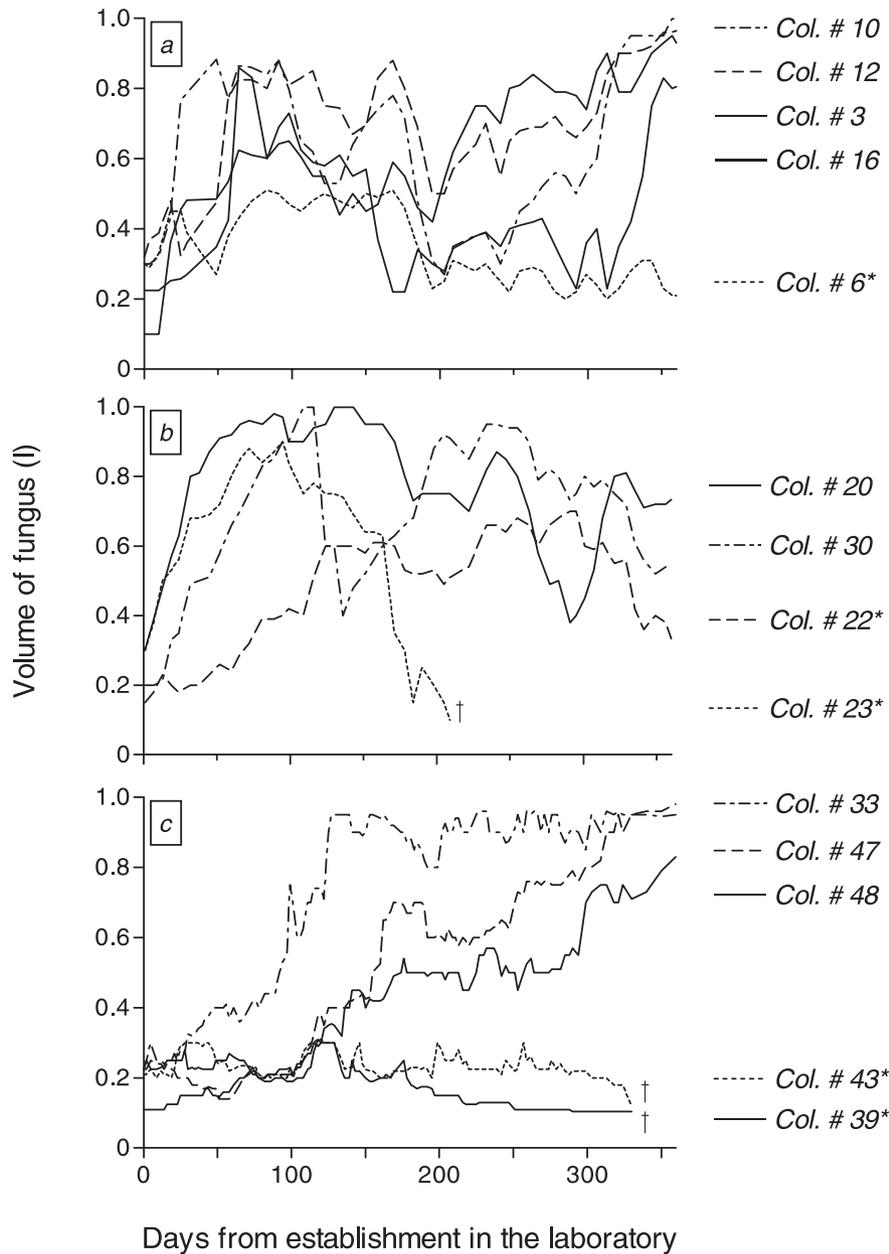


Fig. 2 Development of fungus garden volume (litres) in the first year after establishment in the laboratory for nine unparasitized *A. echinator* colonies and five colonies with *A. insinuator* parasite queens collected in the years 1993 (a), 1994 (b) and 1996 (c). Parasitized colonies are indicated by an asterisk, and crosses indicate that colonies were sampled and frozen after all foragers (major caste workers) had died. Data are presented per year as overall growth rates differed across years, partially due to feeding conditions being not quite optimal in 1993 and 1994.

and laboratory colonies did not reveal a significant difference between sexual investment in the two species either (Mann–Whitney U -test: $U = 43.0$, $P = 0.287$).

Four *A. insinuator* and nine *A. echinator* colonies reproduced (four and 17 times, respectively) in the years in which development in fungus volume was recorded in the laboratory (between 1993 and 1997). Parasitized and unparasitized colonies showed clear differences in repro-

ductive investment in relation to fungus volume. Unparasitized colonies brought into the laboratory did not initiate reproduction at fungus volumes below ≈ 0.75 L (mean \pm SE fungus volume = 0.80 ± 0.03 L, $n = 9$), whereas sexuals were produced at much lower fungus volume in *A. insinuator* colonies (mean \pm SE fungus volume = 0.37 ± 0.09 L, $n = 4$) with higher reproductive investments per volume of fungus than in

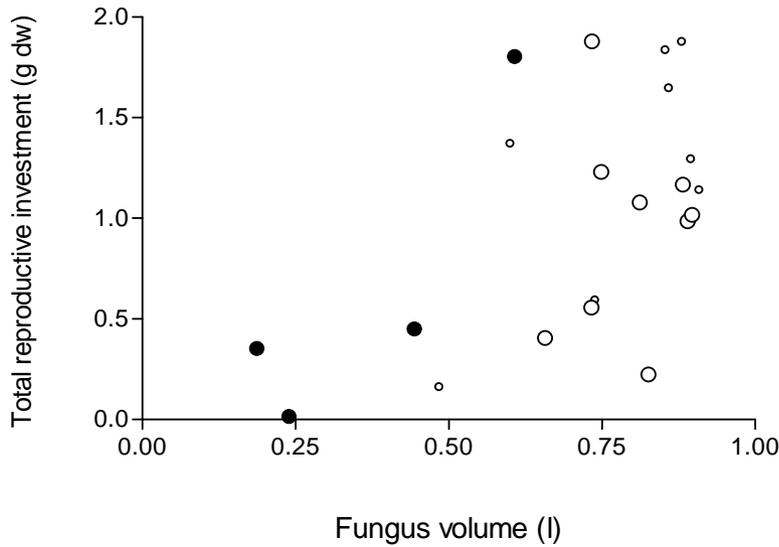


Fig. 3 The total investment in sexual offspring (g dry weight) in relation to the average volume of the fungus garden (litres) 3 months before the eclosion of sexual brood. *A. insinuator* colonies are indicated by filled circles and unparasitized *A. echinator* colonies by open circles, with first reproductive bouts indicated by large circles and successive bouts by smaller circles.

A. echinator colonies (Fig. 3). The fungus volumes at first reproduction and successive reproductive bouts in *A. echinator* laboratory colonies were not significantly different (average fungus volume during reproduction: 0.79 ± 0.05 L, Fig. 3).

Phenology of reproduction and mating in *A. insinuator*

All *A. insinuator* winged sexuals sampled in the field were collected in April/May, shortly before the onset of the rainy season when nonparasitic *Acromyrmex* species have their nuptial flights. However, in the laboratory, sexual offspring of *A. insinuator* were often produced at different times of the year than *A. echinator* sexuals (Fig. 4). In the laboratory 86% of all *A. echinator* sexuals eclosed in

June–August, whereas only 4% of all *A. insinuator* sexuals eclosed during these months. Instead, 95% of all *A. insinuator* sexuals eclosed during either the two preceding (April/May) or the two following months (September/October).

In two laboratory colonies one and two *A. insinuator* gynes, respectively, dealated and became established in their natal colonies alongside their mother queen and the host queen. Male sibs had been produced simultaneously with these gynes, and later dissections of the dealated daughters revealed dark yellow bodies (corpora lutea) with developed ovaries and enlarged spermathecae, implying that the females were sib-mated egg-layers. Dissections of three *A. insinuator* queens from one additional colony showed that all were mated egg-layers, signifying that multiple-queen *A. insinuator* colonies at

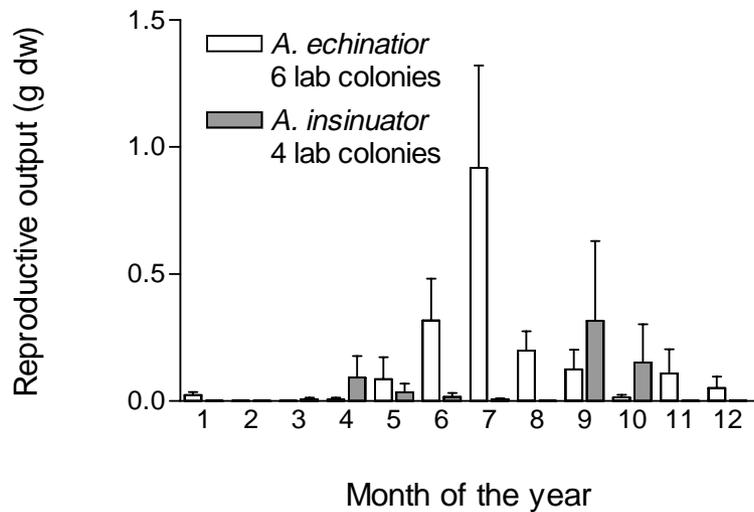


Fig. 4 The reproductive investment (average g dry weight + SE over colonies) in *A. insinuator* and *A. echinator* laboratory colonies partitioned over the months of the year.

least sometimes are truly polygynous. The daughter queens persisted in the colonies for at least 6 and 10 months, respectively, until all large host workers had died and colonies were frozen. No dealated daughter gynes were ever encountered in laboratory colonies of *A. echinator*.

Discussion

The evolution of social parasitism in leafcutter ants

Social parasitism is a rare phenomenon in leafcutter ants. Apart from *A. insinuator*, only two species of workerless inquiline that parasitize *Acromyrmex* leafcutter ants have been described. However, these two species are morphologically so different from their host species that they were assigned to a separate genus *Pseudoatta* (Gallardo, 1916; Delabie *et al.*, 1993). The evolution of social parasites of leafcutter ants is likely to be constrained by the extremely specialized fungal diet and the predominant monogyny (a single queen per colony) found in most species (Mintzer, 1990). No social parasites have been described for any of the 14 known species of the leafcutter ant genus *Atta*, nor for the eight (less exclusively tropical) genera of 'lower' attines.

An important hypothesized precondition for the evolution of socially parasitic behaviour in ants is facultative polygyny (e.g. Buschinger, 1970). If adoption of additional queens becomes an established behavioural trait, the efficiency of kin discriminating is likely to be reduced, thereby facilitating the establishment in host colonies of unrelated individuals showing incipient characteristics of socially parasitic behaviour (Bourke & Franks, 1991). This contention is corroborated by our current study showing that *A. insinuator* parasitizes the facultatively polygynous host *A. echinator*. However, the prevalence of queens in polygynous host colonies (Fig. 1) was lower than in monogynous colonies, indicating that polygyny no longer favours parasitism after the syndrome of social parasitic traits has evolved. *A. insinuator* itself is also facultatively polygynous and our data show that polygyny in this species may originate either via multiple invasions or, at least facultatively, via adoption of (sib)mated daughter queens. Both features are in agreement with observations from other inquilines (Buschinger, 1989). Multiple queen mating, as occurs in *A. echinator* (Bekkevold *et al.*, 1999), does not seem to be a factor promoting the evolution of social parasitism as this mating system seems to be general for both *Acromyrmex* and *Atta* (Ejerdingstad *et al.*, 1998; Boomsma *et al.*, 1999).

Semelparous reproduction in *A. insinuator*

In the laboratory, *A. insinuator* was able to manipulate host workers into raising inquiline sexuals even when no host reproduction took place and with host queens present in

the colonies. *A. insinuator* reproduction ultimately had a fatal effect on laboratory host colonies (Fig. 2), apparently due to suppression of the production of larger host workers, so that foragers (major and media castes) gradually died out. This indicates that inquilines were either able to suppress egg laying in host queens totally (if minima workers generally live longer or take longer to develop than media and major workers), or that they manipulated host workers into predominantly raising *A. insinuator* larvae at the expense of the larger, but not smaller brood of the host. The approximately 50% reduction in reproductive investment in *A. insinuator* colonies in comparison to *A. echinator* colonies corresponds with both species raising approximately equal numbers of sexuals (since the investment in raising *A. insinuator* sexuals was roughly half of that in *A. echinator* sexuals). This implies that *A. echinator* host workers were raising sexuals according to an unchanged within-colony demography, although they were manipulated into raising *A. insinuator* brood instead of their own.

In unparasitized *A. echinator* colonies, reproduction was never initiated in the laboratory unless the fungus garden had been maintained at a relatively large and constant volume for a considerable period of time (Fig. 3), almost certainly to ensure colony survival after reproduction and a, for ants normal, iteroparous life cycle. In contrast to this, most parasitized laboratory colonies started producing sexuals soon after transfer to the laboratory, irrespective of the size of the fungus garden. Parasitized colonies invested comparatively more in reproduction per volume fungus and seemingly reproduced in direct proportionality to the available fungus volume at take-over (Fig. 3). This parasitic life history ultimately resulted in the death of all *A. insinuator* laboratory colonies after a single reproductive bout.

At present, it remains unclear whether *A. insinuator* reproduction under natural conditions invariably follows a 'big bang' reproduction, resulting in the swift demise of the host colony, or whether inquilines are sometimes able to coexist perennially with host queens, in cases where the detrimental effects of inquiline reproduction can be compensated by continued worker production by the host queen. During our transfer of colonies to the laboratory, fungus gardens and numbers of major and media workers were significantly reduced. These losses were quickly overcome by renewed production of workers in unparasitized *A. echinator* colonies, but these 'bottlenecks' may have provided unusually favourable conditions for *A. insinuator* queens to become dominant egg layers and to start inhibiting the production of host workers to a larger extent than would normally occur in the field. One of the parasitized laboratory colonies did not reproduce at all until 26 months after transfer from the field, showing that *A. insinuator* queens at least sometimes coexist with host queens for a number of years before they are able to initiate reproduction. General life history theory (cf. Stearns, 1992) would

predict that a long-lived species constrained to having a semelparous life cycle would be selected to postpone reproduction as long as the total reproductive value of future offspring exceeds that of current offspring. This would imply that *A. insinuator* queens may occasionally increase their reproductive success by remaining subordinate when the growth perspectives of their host colony are particularly good. The smaller size of parasite queens compared to host queens may indicate a shorter lifespan, and hence a more ephemeral life history. Nonetheless, even if parasite queens have an overall shorter longevity than host queens (nonparasitic *Acromyrmex* laboratory colonies have been kept alive for more than 10 years, Weber, 1972) they may still live for a number of years before reproduction is initiated. Further detailed work will be necessary to assess to what extent parasite queens postpone reproduction or share reproduction with host queens in the field.

Phenology and mating of the social parasite

In the field, sexual reproduction in *A. insinuator* colonies seemed to coincide with that in unparasitized *A. echinator* colonies, and incidental field observations suggest that *A. insinuator* males and females may well disperse on the wing and engage in nuptial flights similar to other free-living *Acromyrmex* species. No *A. insinuator* preflight sexuals were encountered in 14 parasitized *A. echinator* field colonies collected in January/February 1996, i.e. outside the 'normal' *Acromyrmex* reproductive season of April/May. Although the peak of host reproduction in the laboratory has shifted several months compared to the normal reproductive period in the field, the relative difference in phenology between parasite and host (Fig. 4) and the facultative gyne-dealation observed in two *A. insinuator* laboratory colonies suggest that a behavioural polyethism may exist, with a fraction of sexuals mating near or even in the nest whereas another fraction may still engage in mating flights and dispersal. Such a mixed mating strategy was suggested for the ant *Leptothorax acervorum*, a myrmecine ant in which intraspecific social parasitism occurs (Douwes *et al.*, 1987) and could represent a first step towards reproductive isolation of a socially parasitic morph. The observed dealation could likewise be interpreted to indicate that the species is under evolutionary transition from complete outbreeding, as found in its host (Bekkevold *et al.*, 1999), to intranidal mating, inbreeding and dispersal of young queens on foot, an evolutionary scenario suggested and documented for species in the socially parasitic genus *Epimyrma* (Buschinger, 1989).

The *A. insinuator* worker caste

Inquiline workers were detected in all *A. insinuator* colonies where minima workers were analysed genetic-

ally. Whereas inquilines are the most commonly described social parasites in ants, inquilines retaining a worker caste are rare. According to Hölldobler & Wilson (1990, Table 12-1) only six species of inquilines with a worker caste are currently known (not including *A. insinuator*). Inquiline species with workers have been inferred to be in an evolutionary transition towards losing the worker caste. Workers have been observed to occur even in otherwise workerless inquilines under stressed conditions (Elmes, 1976; Douwes *et al.*, 1988), suggesting that their occurrence in some inquiline species may be an atavistic trait expressed under unusual circumstances. *A. insinuator* minima workers were found both in mature field- and laboratory colonies, showing that their presence was not laboratory induced. Nonacs & Tobin (1992) have predicted that the degree to which an inquiline has lost its worker caste should be correlated with the degree of miniaturization of the queens relative to the caste-specific developmental threshold for sexual offspring in the host. The workerless inquiline ant *Plagiolepis xene* was recently found to have sexual pupae much smaller than those of its host *P. pygmaea*, but remarkably similar in size to pupae of host workers (Aron *et al.*, 1999). The fact that *A. insinuator* queens are about half the mass (g dry weight) of their host queens and are $\approx 40\%$ heavier than the major worker caste of the host (average weight ≈ 5 mg) seems in keeping with the retention of a minima worker caste as a transitional phase. However, it is not known whether *A. insinuator* minima workers perform selective brood rearing or bias colony production in any specific way, so that it cannot be excluded at present that their presence is currently still adaptive for the social parasite. An interesting detail of general importance emerging from our current study is that independent checking with genetic markers may be required to assure that a social parasite is truly workerless, as no morphological differences could be detected between the minima workers of *A. echinator* and *A. insinuator* (Schultz *et al.*, 1998). Such molecular work would be particularly interesting in *Pseudoatta*, as the possible presence of morphologically indistinguishable minima workers in these advanced social parasites of *Acromyrmex* ants would prove that the special condition of fungus growing constrains evolution towards a complete loss of the worker caste as observed in other social parasites (Wilson, 1971). On the other hand, genetic evidence for the absence of any worker caste in *Pseudoatta* would be important evidence for the generality of the evolutionary scenarios for social parasitism suggested by Buschinger (1989) and discussed and supplemented by Bourke & Franks (1991). The very close morphological similarity between *A. insinuator* and *A. echinator* suggests an evolutionary origin of the parasite by direct sympatric inquiline speciation from the host as hypothesized by Elmes (1973), Buschinger (1990) and Bourke & Franks (1991), but this is yet another contention to be verified by future phylogenetic work with genetic markers.

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