Research article

Are workers of *Atta* leafcutter ants capable of reproduction?

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**Summary.** Workers of most eusocial Hymenoptera can produce sons after queen loss, which (posthumously) benefits the queen and increases worker inclusive fitness. However, the evolutionary loss of worker ovaries has occurred in several lineages, while workers in other taxa may be infertile despite having ovaries. Workers of *Atta* leafcutter ants only lay trophic eggs in queenright colonies. Although *Atta* colonies are commonly kept at universities, museums, and zoos, no reports of worker sons in orphaned colonies exist, suggesting that *Atta* workers are infertile. To explicitly test this, we created eleven orphaned laboratory nests of *Atta cephalotes*, *A. sexdens*, and *A. colombica*, and maintained them for 3–6 months after queen loss. Eight colonies did not produce any brood, but three nests produced 1–4 worker-derived male larvae and pupae. Microsatellite genotyping indicated that these were worker sons. However, all males were tiny (3.5–9 mm long) compared to normal queen sons (16 mm long), and would almost certainly be unable to mate. We also found reproductive eggs, but most of these had no yolk and were thus inviable. We conclude that *Atta* workers are not completely infertile, but that worker fertility is low compared to the sister genus *Acromyrmex*, where workers routinely produce normally-size males after queen loss in the laboratory. We hypothesize that worker reproduction in orphaned *Atta* field colonies is almost never successful because the last workers die before their sons can be raised to adulthood, but that the importance of worker-laid trophic eggs for queen feeding has precluded the evolutionary loss of worker ovaries.

**Keywords:** Dwarf males, worker sons, orphaned colonies, worker sterility, worker infertility.

**Introduction**

In the majority of eusocial Hymenoptera with physical female castes, workers are unable to mate because they lack genitalia and a functional spermatheca, but typically possess ovaries and can lay unfertilized eggs that develop into males (Choe, 1988; Bourke, 1988). The contribution of the workers to the parentage of the males under queenright conditions (i.e., in the presence of a healthy mother queen) may range from zero to the majority of the males, depending on taxonomic group and species (Hammond and Keller, 2003; Wenseleers and Ratnieks, submitted). In species where worker sons are rarely produced in queenright colonies, a major proximate cause of the absence of worker sons is typically the low proportion of reproductive workers (Dijkstra et al., 2005; Wenseleers et al., 2004). The tendency of workers not to form reproductive eggs in the presence of the queen is most frequently explained as an adaptive response to an evolutionary history of efficient queen- or worker policing (Ratnieks, 1988; Wenseleers et al., 2004a, 2004b).

Worker reproduction is always detrimental to the fitness of the queen as long as she is alive, because the raising of worker sons reduces the amount of colony resources available to her own sexual offspring. However, the ability of workers to reproduce after the queen’s death is expected to benefit the deceased queen as well as the workers (Ratnieks, 1988; Franks et al., 1990), and to carry no real costs as the colony is normally doomed. However, obligate worker sterility (i.e., the complete absence of ovaries in workers) is reported to occur in at least one genus of stingless bees (*Friesomelitta*: Boleli et al., 1999) and nine genera of ants (*Anochetus, Ecton, Hypoponera, Leptogenys, Monomorium, Pheidole, Pheidologeton, Solenopsis, Tetramorium*: Wheeler, 1910; Oster and Wilson, 1978; Hölldobler and Wilson, 1990; Villet et al., 1991; but see Whelden, 1963 for *Ecton*), and it is likely that more examples will be discovered in the future.
Natural selection will only favor obligate worker sterility when four conditions are simultaneously meet: 1. The ontogeny and/or maintenance of ovaries is costly and reduces the ability of workers to work for the colony; 2. Workers do not use trophic eggs to feed the larvae or the queen; 3. Attempted worker reproduction is never successful in the presence of the queen; 4. Workers do not benefit from laying reproductive eggs after queen loss, either because the development time of the males is long compared to the life-span of the workers (so that the last workers in the colony are likely to die before any workers sons can be raised to adulthood), or because gynes are only present in the population during a short mating season (so that worker sons from orphaned colonies are unlikely to find a mate). Factors that are expected to further facilitate the evolution of obligate worker sterility are polygyny, a long life-span of queens compared to workers, and the presence of a mechanism for the supersede of senescent queens and/or “emergency replacement” of dead queens (cf. Hatch et al., 1999; Faustino et al., 2002), because these reduce the probability that a worker will become orphaned during her life-time. A high degree of queen-worker dimorphism is expected to be another facilitating factor, because this would tend to constrain worker fecundity and thus reduce the pay-off of for worker reproduction.

The occurrence of obligate worker sterility in a given species does not reveal much about the evolution of this trait, as in such a species it is impossible to test how unsuccessful worker reproduction would be after queen loss. A better understanding of the evolution of obligate worker sterility may be gained from species in which workers have retained their ovaries, but never succeed in reproducing after queen loss (hereafter: “infertile workers”). An example of infertile workers is found in the Argentine ant Linepithema humile, where workers can lay trophic eggs but apparently never reproduce in orphaned colonies (Aron et al., 2001; S. Aron, pers. comm.).

Another candidate taxon for infertile workers are Atta leafcutter ants, which are characterized by a high degree of queen-worker dimorphism and long-lived queens (up to 20 years: Weber, 1972; Hölldobler and Wilson, 1990). While most Atta workers never develop their ovaries in queenright colonies, a minority of the workers have very specialized ovaries which they use to produce trophic eggs to feed to the queen (Dijkstra et al., 2005). Although Atta display colonies are commonly kept at universities, museums, and zoos, to our knowledge only a single dubious report (Tanner, 1892, cited in Wheeler, 1903; see Discussion) of worker sons from orphaned Atta colonies exists. However, this lack of evidence is inconclusive, as queenright Atta colonies likewise reproduce only very rarely in the laboratory.

The aim of this study was to determine if Atta workers are indeed infertile, as anecdotal evidence suggested (Bazire-Bénazet, 1957; Hernandez, 1998; Dijkstra et al., 2005). We compare our results with those obtained from its sister genus Acromyrmex (Wetterer et al., 1998), where worker sons are routinely produced in orphaned colonies in the laboratory. Identifying the constraints on worker reproduction in Atta may help understand the evolution of obligate worker sterility in other taxa.

Materials and methods

Studying worker reproduction in leafcutter ants

Leafcutter ants are obligately dependent on cultivating a clonally propagated fungus inside their nest as food. Larval and adult ants eat fungal mycelium, as well as clusters (“staphylae”) of modified hyphal tips (“gongylidia”) (Hölldobler and Wilson, 1990). A problem with studying worker reproduction in Atta is the enormous size (millions of workers and hundreds of fungus gardens) of reproducing colonies in the field. Because our laboratory Atta colonies were far smaller than mature field colonies, our criterion for successful worker reproduction was the presence of worker-derived eggs and first or second instar larvae (hereafter: “young larvae”), rather than final instar larvae, pupae, or adults.

In Acromyrmex leafcutter ants (which have much smaller colonies than Atta), queenright laboratory colonies only produce sexuals when the total volume of the fungus garden is above a certain threshold (Bekkevold and Boomsma, 2000), but orphaned laboratory colonies can contain worker-derived male eggs and young larvae at any fungus volume (Dijkstra, 2005). Orphaned Acromyrmex workers typically raise a large proportion of the worker-derived brood into adult males with normal size and morphology between 14–40 weeks after queen loss, irrespective of time of year, even if the fungus garden volume is well below the threshold for reproduction by queenright colonies (Dijkstra, 2005). However, orphaned laboratory colonies of Acromyrmex leafcutter ants may also fail to produce any adult worker sons if the fungus garden is extremely small, resulting in the starvation of the larvae in the final instar. Starving Acromyrmex larvae are not cannibalized by the workers, but dumped intact on the rubbish heap outside the main nest, where they can be easily observed and counted.

Maintenance of orphaned nests

We divided a total of 10 queenright laboratory colonies of Atta sexdens (n = 2), A. cephalotes (n = 3), and A. colombica (n = 6) into orphaned and queenright nests, by moving 1–2 fungus gardens with a median total volume of 1000 ml (range 800–1900 ml) into separate nest boxes. Before division, the queenright colonies consisted of 1–4 fungus gardens with a median total volume of 2900 ml (range 1750–4000 ml). All colonies had been collected in Panama, except for two A. cephalotes colonies (CE-T1 and CE-T2) from Trinidad. We maintained the orphaned and queenright nests as separate colonies in the dark, at 25 °C and 70% RH. Further details of the colony set-up and feeding regime are given in Dijkstra et al. (2005). We regularly inspected the surface of the fungus garden and the rubbish heap for adult sexuals and discarded final instar larvae, respectively. Six months after colony division, we killed the orphaned nests by freezing the fungus at −20 °C. An exception was the orphaned nest of CE-T2, which was killed three months after colony division. We also froze an additional A. cephalotes nest from Panama (CE-P3), in which the queen had suddenly died four months earlier. We completely dissolved the fungus in 70% ethanol by gentle stirring. Workers, fungus, and fungus substrate tended to remain suspended, while brood tended to settle on the bottom. We took ca. 200 ml aliquots of the fungus suspension, and carefully searched the bottom layer for any eggs, larva, or pupae under 6.4x magnification against a dark background. We repeated this procedure until the entire fungus suspension had been searched, which typically took 1–1.5 h per nest. The sex and caste of Atta brood can be easily distinguished from the final larval instar onwards, and their pupae are not enclosed in cocoons.

Microsatellite genotyping

We found brood in two orphaned A. colombica nests (see Results). For these two nests, we genotyped all larvae and pupae (n = 6) with two microsatellite loci (Etta5-6TF and Etta7-8TF: Fjerdingstad et al., 1998), and reconstructed the genotype of the queen from 30 workers (15 small