

Female-biased sex ratios and unusually potent males in the social parasite *Anergates atratulus* (Hymenoptera: Formicidae)

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Abstract

Colony-level sex ratios of *Anergates atratulus* (SCHENCK, 1852) were found to be significantly more female-biased than expected from the socially parasitic life history of this inquiline ant. This might reflect either the sex ratio preferences of its *Tetramorium* host workers or local mate competition. Histological analyses of *A. atratulus* males showed that spermatogenesis is completed by the time they reach sexual maturity. Nevertheless, because their gasters are almost completely filled by the seminal vesicles with large masses of mature and immature sperm cells, it appears that they repeatedly replenish the sperm supply in their seminal vesicles after mating. Though we could observe a maximum of only three successful copulations per male in a small laboratory colony, males are therefore presumably much more potent than most other ant males.

Key words: Sex allocation, local mate competition, spermatogenesis, inquilinism, mating.

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Introduction

When mating takes place in small patches and involves mostly relatives, mothers are selected to maximize their fitness by producing just the number of males that guarantees all their daughters are inseminated (HAMILTON 1967). Such "local mate competition" is thought to underlie the highly female-biased sex ratios of many parasitoid and fig wasps (e.g., WERREN 1980, HERRE 1985). In contrast, it appears to be a rather unusual phenomenon in the social Hymenoptera (BOURKE & FRANKS 1995) in which synchronized mating flights normally promote outbreeding. In ants, strong local mate competition is expected only from a few monogynous taxa, in which mating occurs in or near the nest, as in several social parasites (WINTER & BUSCHINGER 1983, BUSCHINGER 1989).

In theory, the stable sex ratio under local mate competition approaches infinity when there is only one mother per patch (HAMILTON 1967), assuming that males have an unlimited sperm supply. The latter is usually not the case in the Hymenoptera, as in almost all species the testes degenerate before males reach sexual maturity and their sperm often suffices only for one or two copulations (HÖLLDOBLER & BARTZ 1985). Nevertheless, local mate competition is expected to select for extraordinarily high virility (BOURKE & FRANKS 1995). Indeed, males of those few species, for which regular sib-mating has been documented, appear to be capable of large numbers of copulations. For example, males of *Technomyrmex albipes* (F. SMITH, 1861) have been observed to mate with several of their sisters (YAMAMUCHI & al. 1991), and the wingless males of the genus *Cardiocondyla*, in which sib-mating is extremely common (SCHREMPF & al. 2005, LENOIR & al. 2007), have even evolved prolonged, life-long spermatogenesis (HEINZE & HÖLLDOBLER 1993).

Another ant in which mating supposedly involves close relatives is *Anergates atratulus* (SCHENCK, 1852), a rare workerless social parasite that lives with *Tetramorium* cf. *caespitum* (LINNAEUS, 1758). Colonies usually contain one or a few highly fertile physogastric queens (BUSCHINGER & al. 2003). *Anergates* males are wingless, lightly pigmented, and pupa-like, and they mate with female sexuals in the nest before the latter disperse on the wing to invade other *Tetramorium* societies. *Anergates atratulus* therefore shows all the prerequisites of local mate competition. According to anecdotal observations, sex ratios are highly female-biased (WHEELER 1908, WASMANN 1915, cited in STITZ 1939), and based on the apparent neoteny of pupoid *Anergates* males, BOURKE & FRANKS (1995) suggested that their testes might be fully functional. Results from earlier histological analyses were ambiguous: MEYER (1955) observed that the testes of mature males were to some extent degenerated, but nevertheless concluded that new sperm might mature between copulations. In contrast, HEINZE (2000) did not find any evidence of prolonged spermatogenesis in a single then available male.

Here, we investigate local mate competition in *A. atratulus* in more detail. In particular, we summarize collecting data on numerical sex ratios and present data from histological analyses of the gaster of males.

Methods and materials

Anergates atratulus occurs locally in probably all of the range of its host species *T. cf. caespitum*, from the Iberian Peninsula and Southern England to Turkey, the Caucasus Mts., and Central Kazakhstan (e.g., COLLINGWOOD 1979, ARAKELIAN 1994). Together with its host it was also accidentally introduced to North America (e.g., CREIGH-

Tab. 1: Sites at which colonies of the inquiline ant *Anergates atratulus* were found and number of sexuals they produced. Sex ratios were significantly different from an equal sex ratio of 0.5 (χ^2 -test, ** $p < 0.01$, * $p < 0.05$). (Colony numbers refer to the ethanol-preserved collection of A.B.)

| Colony number | Collecting date | Locality and elevation | Males (incl. male pupae) | Females (incl. female pupae) | Sex ratio |
|---------------|-----------------|---|--------------------------|------------------------------|-----------|
| 2.489 | 28.VII.1966 | Switzerland, Valais, Saas Fee, 1800 m | 23 | 162 | 0.88** |
| 3.439 | 26.VIII.1969 | Switzerland, Valais, Vercorin, 1400 m | 5 | 420 | 0.99** |
| 3.733 | 18.VII.1970 | Switzerland, Valais, Visperterminen, 1400 m | 9 | 74 | 0.89** |
| 3.789 | 27.VII.1970 | Switzerland, Valais, Bellwald, 1600 m | 3 | 21 | 0.87** |
| 4.239 | 26.V.1971 | Italy, South Tyrol, Brixen, 800 m | 27 | 82 | 0.75** |
| 4.484a | 14.VIII.1971 | Germany, Bavaria, Wolfratshausen, 550 m | 15 | 31 | 0.67* |
| 8.068 | 22.VII.1978 | Switzerland, Valais, Moosalp, 2000 m | 9 | 42 | 0.82** |
| 11.819 | 26.VII.1984 | France, Hautes Alpes, Col de Granon, 2000 m | 1 | 62 | 0.98** |
| 13.166 | 6.VI.1987 | Germany, Bavaria, Homburg, 300 m | 5 | 23 | 0.82** |
| 16.218 | VIII.2005 | Switzerland, Valais, Moosalp, 2000 m | 11 | 46 | 0.81** |

TON 1950). Its rarity, the patchiness of its distribution within host populations, and the difficulty of detecting the one or sometimes few queens in the large, subterranean host nests make it impossible to locate or sample colonies in a systematic way.

Tetramorium colonies parasitized by *A. atratulus* were identified by one of us (A.B.) by inspecting host colonies in summer for *A. atratulus* sexuals and / or their grey larvae in several populations and over a period of 40 years. Sex ratios were determined by counting all adult and pupal *Anergates* in the field-collected samples that had been preserved in ethanol. Adults and pupae could easily be assigned to either gender because of lack of wings or wing buds in the males respectively male pupae. The colony fragment from which the specimens for this study were reared was kept alive until the last larva had pupated.

Males for histological studies came from parts of a colony with *A. atratulus* larvae collected by A.B. on 25 July 2005 at an elevation of approximately 2000 m at Törbel (Moosalp), canton of Wallis, Switzerland. The colony hibernated for 12 weeks at 5 - 10 °C and was thereafter kept at room temperature. Males started copulating with female sexuals within less than one day after emergence, and 16 of 21 dissected female sexuals were found to be inseminated.

Of the eight males eclosing from the brood, six were preserved in 96 % ethanol or Bouin's fixative, embedded in Durcopan and sectioned with a microtome at 2 μ m, the remaining two were dissected under a binocular microscope.

Results and Discussion

Though numerical sex ratio data are from different populations and years and therefore must be considered as an-

ecdotal, they all showed female bias. Colony-level sex ratios (female sexuals / total sexuals) ranged from 0.67 to 0.98 (Tab. 1), matching previous reports (WASMANN 1915 in STITZ 1939: 40 females, 12 males; WHEELER 1908: a few hundred males, more than 1000 female sexuals). Numerical sex ratios and, because male and female sexuals of *A. atratulus* are approximately of the same size, presumably also investment sex ratios of all colonies, were significantly more female-biased than expected from the hypothesis that in social parasites mothers invest equally in male and female offspring (TRIVERS & HARE 1976) (χ^2 -test, $p < 0.01$ in nine of ten colonies, $p < 0.018$ in colony 4484a). However, in six of ten colonies sex ratios were not significantly different from the 0.75 investment ratio generally expected for monogynous, monandrous ants. Only four colonies produced sex ratios that were extremely female-biased and reminiscent of those in ants with local mate competition, such as *Cardiocondyla batesii* FOREL, 1894 and *C. elegans* EMERY, 1869 (SCHREMPF & al. 2005, LENOIR & al. 2007). This may reflect sperm limitation in males, as corroborated by the cessation of spermatogenesis in mature males (see below), the influence of host workers, or competition among mothers in polygynous colonies (e.g., CREMER & HEINZE 2002). Unfortunately, the number of mothers could not be determined because of the large size of host nests.

As already shown by ADLERZ (1886), testes of *A. atratulus* consist of 2 \times 3 follicles and the accessory glands are very large. Accessory gland secretions have many functions, for example in sperm competition or the production of a spermatophore (e.g., GILLOT 2003). In a 4 days old male, which had not copulated, histology suggested remnants of ongoing spermatogenesis in parts of the testes,

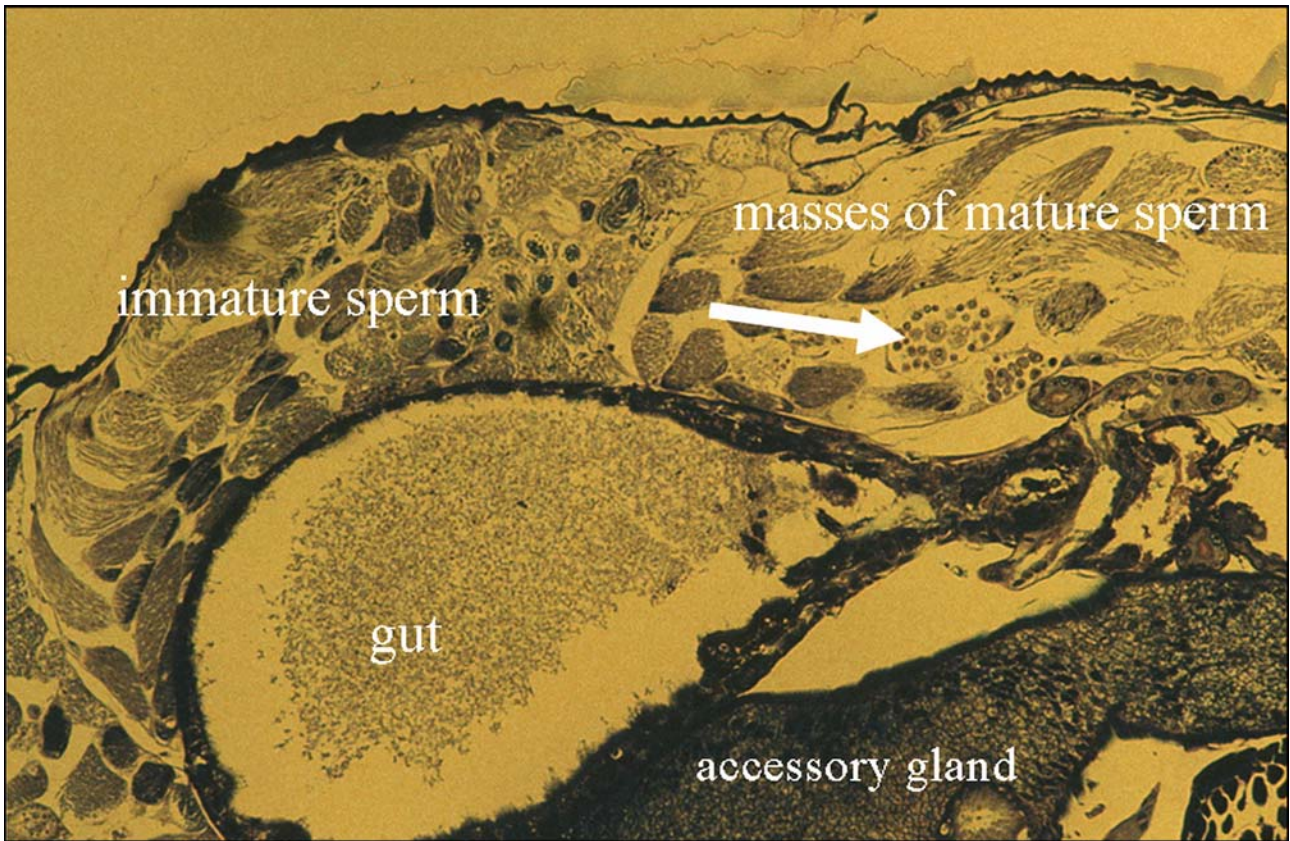


Fig. 1: Histological section of the gaster of a 4 days old male of *Anergates atratulus*, with masses of mature sperm, immature sperm bundles, and some clusters of cells still indicating ongoing spermatogenesis (arrow) (gaster tip to the right).



Fig. 2: Histological section of the gaster of a 5 days old male of *Anergates atratulus*, showing that it is almost completely filled with mature and immature sperm cells. Spermatogenesis appears to be completed.

with spermatocysts containing cells with large nuclei (Fig. 1). In contrast, spermatogenesis appeared to be completed in the other five males, which were all less than 5 days old but had copulated once or repeatedly. Their gasters were almost completely filled by the seminal vesicles with large masses of mature sperm cells and immature spermatid bundles in spermatocysts, suggesting that a depleted sperm supply can to some extent be replenished (Fig. 2). Males were observed copulating with female sexuals already on the first day after emergence and throughout all of their adult lives, which appeared to last no longer than five days. Short longevity was already suspected by MEYER (1955) based on the degeneration of the gut epithelium of *Anergates* males. Copulations were very long, up to 1 h and more, and thus among the longest copulations recorded for ants, with exception of species with suicidal mating, in which males die during copulation and in which either their genitalia or the whole body remains attached to the abdomen of their mates (e.g., army ants, GOTWALD 1995; *Diacamma*, FUKUMOTO & al. 1989). In our experimental colony, in which the number of simultaneously available female sexuals was limited, some of the males were observed copulating with at least three different female sexuals. This is similar to the observed mating frequencies of males of other ant species with limited sperm supplies, e.g., *Leptothorax gredleri* MAYR, 1855 (OBERSTADT & HEINZE 2003), and much below that of *Cardiocondyla* males with continuous spermatogenesis (e.g., HEINZE & al. 1998). However, the enormous size of the sperm masses and the fact that the accessory glands almost completely fill the gaster of the males indicate that *Anergates* males might copulate with many more female sexuals during the few days of their life.

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Zusammenfassung

Kolonien der inquilinen Ameise *Anergates atratulus* (SCHENCK, 1852) produzieren ein signifikant stärker weibchenlastiges Geschlechterverhältnis als aufgrund ihrer parasitischen Lebensweise zu erwarten ist. Dies könnte auf den Einfluss ihrer *Tetramorium*-Wirtsarbeiterinnen oder auf lokale Paarungskonkurrenz zurückzuführen sein. Histologische Untersuchungen zeigen, dass die Spermatogenese bei adulten *A. atratulus*-Männchen abgeschlossen ist. Da der Hinterleib aber fast komplett mit Massen reifer und reifender Spermien angefüllt ist, scheinen sie den Spermenvorrat in ihren Vesiculae seminales nach der Paarung mehrfach wieder auffüllen zu können. Obwohl in einer kleinen Laborkolonie nur drei erfolgreiche Kopulationen pro Männchen beobachtet werden konnten, sind sie vermutlich sehr viel "potenter" als normale Ameisenmännchen.

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