Relationship Between Ovariole Number and Spermathecal Sperm Count in Ant Queens: A New Allometry

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ABSTRACT Spermathecae and ovaries were dissected from newly mated queens of 25 species of ants belonging to five subfamilies. The number of ovarioles in the ovaries and sperm in the spermathecae were counted. The log of the sperm count (y) showed a strong correlation ($R^2 = 0.79$) with the log of the ovariole number (x) as follows: $\log y = 1.59 \log x + 3.12$. The number of sperm stored per ovariole increases from about 2,000 for queens with only 6 ovarioles to about 30,000 for queens with about 200. These relationships constitute complex kinds of allometry. Ovariole number is an index of a queen's reproductive capacity, but queens with more ovarioles also have a higher capacity per ovariole. The data further indicated that sperm are stored in relation to the number needed. This implies that sperm are not cheap for queens to store or males to produce, even for species with very low reproductive potential and sperm need.

KEY WORDS Formicidae, reproduction, ovary, spermatheca, queens, ovariole number

EACH SPECIES of social insect has a characteristic mature colony size (Oster & Wilson 1978). Among monogynous ants, colonies may contain from a few dozen to 20 million workers, depending on the species (Wilson 1971). Because workers are much shorter-lived than queens, the egg-laying capacity of the queen ultimately limits colony size. In species with a single queen per colony, colonies evolve to larger sizes primarily through an increase in the queen's reproductive output (Wilson 1971). This is accomplished most obviously through the amplification of the number and length of the ovarioles, and results in a general correlation between the number of workers in a mature colony and the number of ovarioles in their queen's ovary (Wheeler 1910). Other, probably lesser, modes of increasing the reproductive rate include decrease in egg size (Hagan 1954) and increase in rate of egg maturation (Wilson 1971). Among the social insects, ovariole number ranges from the basic aculeate number of 6 to 320 in the honeybee (R. E. Page, personal communication) and to 3,000 in the termite, *Odontotermes badius* (Truckenbrodt 1973).

The queens of social Hymenoptera mate only at the beginning of their reproductive lives and store a lifetime supply of sperm in the spermatheca. Several authors have speculated that getting enough sperm may be a problem for queens with very high reproductive capacity (i.e., those with large colonies) (Cole 1983, Page 1986). This implies that queens with low reproductive capacity store more sperm than they need, and raises the issue of just how cheap sperm are to produce and store. Do queens store excess sperm, or just enough for their lifetime needs? There is only a modest literature on sperm counts in various species (Cole 1983, Page 1986), and there is little information on whether this supply limits the size or lifespan of a colony. Röseler (1973) states that the number of sperm in the bumblebees, *Bombus hypnorum* and *B. terrestris*, declines in the course of reproduction, but is not limiting. However, honeybee queens tend to be replaced when their sperm supply falls below 10% of its original 5 million (Taber 1977; S. Taber, personal communication). In this large-colonied species sperm supply may be limiting. In the fire ant, *Solenopsis invicta*, another species with large colonies, sperm depletion ends a queen's ability to maintain a colony (W.R.T., unpublished data).

If sperm are sufficiently cheap to produce, their production should not be "visible" to natural selection, and males might regularly produce more than females need. Such is apparently not the case, for in most species of ants and bees, males seem to produce just enough sperm to fill the female's spermatheca (Page 1986), implying that sperm are not cheap to produce. Similarly, if sperm are cheap for females to store, this function would also be "invisible" to selection, and excess sperm might be stored without regard for the female's reproductive needs. In this case, there should be no correlation between the amount of sperm stored and the number of ovarioles in the queen's ovaries (a measure of her reproductive capacity).

I report here that, on the contrary, there is a strong relationship between reproductive capacity and sperm count across an enormous range of reproductive capacities in a wide range of ant species. This relationship constitutes a complex kind of allometry.

Table 1. List of species, collection localities, and dates

Identification no.	Species of queen	*n*	Collection locality[a]
	Ponerinae		
1	*Odontomachus clarus* Roger	2	COZ
2	*Hypoponera opaciceps* (Mayr)	1	TLH
3	*Hypoponera* sp.[b]	1	TLH
	Dolichoderinae		
4	*Conomyrma flavopecta* M. R. Smith	1	TLH
	Myrmicinae		
5	*Solenopsis* (Diplorhoptrum) n.s.	2	TLH
6	*Solenopsis geminata* (Fabr.)	3	TLH
7	*Solenopsis invicta* Buren	24	TLH
8	*Pogonomyrmex badius* (Latreille)	1	TLH
9	*Pheidole dentata* Mayr	1	TLH
10	*Pheidole* sp. A	2	COZ
11	*Pheidole* sp. B[b]	1	COZ
12	*Pheidole* sp. C	2	COZ
13	*Aphaenogaster fulva* Roger	1	TLH
14	*Aphaenogaster rudis* Emery	1	CT
	Myrmicinae/Attini		
15	*Cyphomyrmex rimosus* Spinola	2	TLH
16	*Trachymyrmex septentrionalis* McCook[b]	1	TLH
17	*Acromyrmex versicolor* Pergande	3	AZ
18	*Atta texana* (Buckley)[c]	16	TX
	Formicinae		
19	*Brachymyrmex depilis* Emery	2	TLH
20	*Prenolepis imparis* Say	3	TLH
21	*Camponotus castaneus* (Latreille)	1	TLH
22	*Camponotus* sp. A (*fumidus* group?)	1	COZ
23	*Camponotus* sp. B	1	COZ
24	*Camponotus* sp. C	1	COZ
25	*Lasius alienus* (Foerster)[b]	2	CT

[a] COZ, Isla Cozumel, Quintana Roo, Mexico, July 1985; CT, South Glastonbury, Conn., August 1985; TLH, Tallahassee, Fla., June 1985; TX, College Station, Tex., collection date unknown; AZ, Phoenix, Ariz., October 1985.

[b] From young colonies.

[c] The sperm count is the mean of 16 queens from Moser (1967). Ovariole count is from a single preserved specimen.

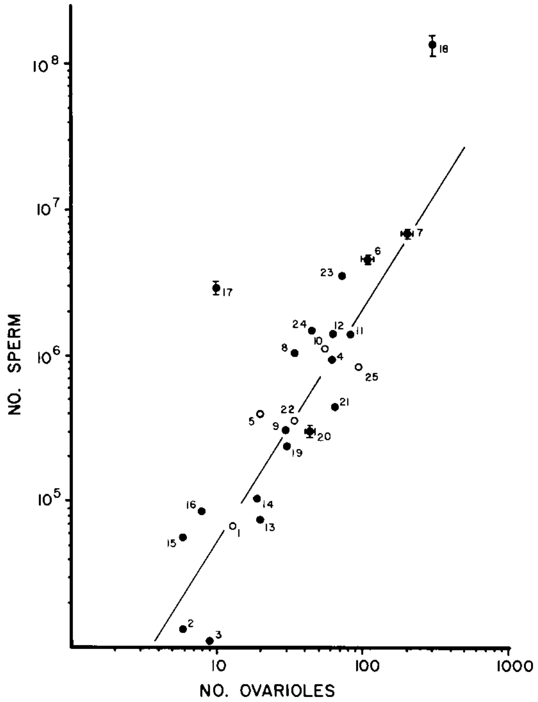

Fig. 1. The number of spermatozoa in the spermatheca of ant queens as a function of ovariole number (log scale). Each point represents a different species of ant queen. When more than two individuals were available, $\bar{x} \pm 1$ SD is shown. Means of two individuals are shown as open circles. The number next to each datum is that of the species identification number in Table 1. Nos. 17 and 18 were outliers and were not used in the regression.

Materials and Methods

Newly mated queens of 25 species of ants were collected after mating flights in Tallahassee, Fla.; Isla Cozumel, Mexico; College Station, Tex.; Phoenix, Ariz.; and Glastonbury, Conn. If it was not possible to dissect them immediately, they were kept in test tubes with water and cotton plugs. A list of species, collection localities, and dates is given in Table 1.

Most of the queens were newly mated, but a few were from very young colonies, as indicated by the small number of workers or the presence of nanitic workers. Such queens were unlikely to have undergone significant sperm depletion.

Queens were dissected in insect Ringer's saline. The number of ovarioles in each ovary was counted. Species with fewer than 8–10 ovarioles per ovary usually showed no variation in this number. Those with more were somewhat variable, but individuals within a species rarely differed by more than 15%. The large sample of *S. invicta* ($n = 24$) allowed estimation of variability for species with large ovaries ($\bar{x} = 206$ ovarioles; SD = 23; CV = 11%).

The spermatheca was placed in a small volume of Ringer's saline where it was ruptured and the sperm dispersed. This dispersion was diluted to a fixed volume with 0.5 *M* NaCl. A final volume was chosen such that the sperm count was between ca. 100,000–1,000,000 per ml. A droplet of the final dilution was withdrawn with a broad-bore pipette (to reduce adsorption of sperm to glass) and placed on a hemacytometer. The average of four 0.1-mm^3 fields is reported. The standard deviations were usually less than 20% of the mean values, reflecting an adequate compromise between precision and effort. Thirteen of the 25 species are represented by a single queen. For those represented by three or more queens, the counts showed good agreement among individuals of a species. The values were: *S. invicta*, $\bar{x} = 6.96 \times 10^6$, SD = 0.34×10^6, CV = 5%, $n = 50$; *Atta texana*, $\bar{x} = 1.37 \times 10^8$, SD = 0.24×10^8, CV = 18%, $n = 17$ (Moser 1967); *Acromyrmex versicolor*, $\bar{x} = 2.91 \times 10^6$, SD = 0.34×10^6, CV = 12%,

$n = 3$; *Prenolepis imparis*, $\bar{x} = 3.0 \times 10^5$, SD $= 0.25 \times 10^5$, CV = 8%, $n = 3$.

Data were log transformed and analyzed by linear regression and analysis of variance (ANOVA). Sample sizes are given in Table 1.

Results and Discussion

The logarithm$_{10}$ of the number of spermatozoa in the spermatheca and the number of ovarioles are related in a linear fashion (Fig. 1). Thus, $\log y = 3.12 + 1.59 \log x$; $R^2 = 0.79$; where y is the number of sperm in the spermatheca and x is the number of ovarioles in the ovaries. Almost 80% of the variation in the logarithms is explained by this relationship ($F = 83.5$; df = 1,22; $P < 0.001$; ANOVA), and the correlation is present across the full range of reproductive capacities. Queens store sperm in relation to their reproductive needs even when these needs are low. At the lower extreme, queens of *Hypoponera* sp. store a mere 11,000 spermatozoa, but near the upper extreme, queens of *S. invicta* can pack a hefty 7 million. Mature colonies of the former consist of fewer than 100 workers, while the latter range up to 200,000.

The relationship is an exponential one, similar to that of two body dimensions to one another (Huxley 1932). Just as ordinary allometries result from different growth rates of body parts, so must the sperm/ovariole allometry result from a constant relationship between the increase in the capacity to store sperm and the increase in ovariole number during the evolution of ant life histories. Ovariole number increases more slowly than sperm count, resulting in an allometric rather than isometric function. Consequently, the number of sperm stored per ovariole (y/x) increases allometrically with the number of ovarioles (Fig. 2) according to the equation $\log (y/x) = 0.59 \log x + 3.12$; $R^2 = 0.34$; ($F = 11.2$; df = 1,22; $P < 0.01$; ANOVA). For example, *Hypoponera* sp. stores 2,200 sperm for each of its 6 ovarioles, and *S. invicta* stores about 30,000 for each of its 200. If, as seems reasonable, the number of sperm is proportional to the reproductive capacity of the ovary, reproductive output can be increased by increasing the capacity of each ovariole, as well as by increasing the number of ovarioles. The former is probably what previous investigators have identified as an increase in ovariole length. But if the number of potential oocytes in each ovariole were constant, a *S. invicta* queen could produce only 33-fold as many oocytes as a *Hypoponera* queen, not the millions she produces in her lifetime.

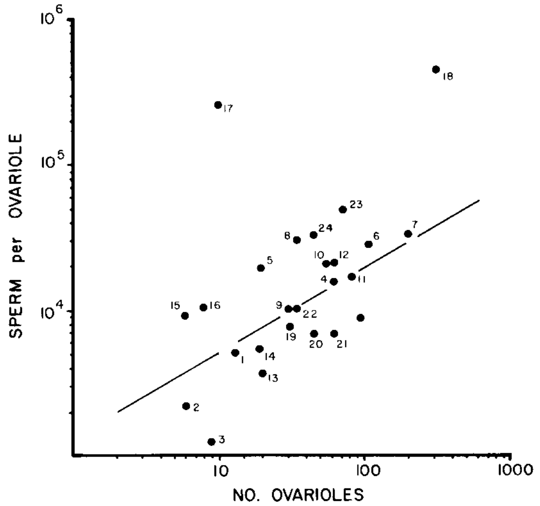

Fig. 2. The number of sperm per ovariole as a function of the number of ovarioles (log scale). Spermathecal sperm count increases much more rapidly than does the number of ovarioles. The numbers next to the points refer to the species identification numbers in Table 1. Nos. 17 and 18 were outliers and were not used in the regression.

Thus, sperm seem to be stored in proportion to potential egg production, although the two are almost certainly not in a one-to-one ratio. In her 7 yr of life, a queen *S. invicta* uses approximately 7 million sperm to produce about 2 million workers (estimated from colony size and worker lifespan). This suggests that 3.5 sperm are released for every egg fertilized. However, the worker count fails to include eggs that do not develop to adulthood. The actual number of eggs produced and fertilized by the queen could be much higher than 2 million, and therefore the sperm released per egg lower than 3.5. Similar estimates for the honeybee are usually 5–10 sperm per egg, but Harbo (1979) found 20–30 per egg. No data are available for species with small colonies.

My data and those from the literature (Page 1986) argue that sperm are neither cheap for males to produce nor for females to store, and that overproduction and overstorage have been counterselected. Ant sperm have mitochondria (Thompson & Blum 1967) and probably require energy during storage. Alternatively, the correspondence between male sperm production and female need and storage could also arise if the mechanisms that determine the number of cell divisions leading to gametes are the same for both sexes. Because females discard polar bodies, such a mechanism would result in 4-fold as many sperm as ova. If this is correct, the use of 7 million sperm by fire ant queens to produce 2 million workers and ca. 20,000 female alates would require 100% efficiency in sperm use and little preadult mortality. Whether ants have achieved this degree of parsimony remains to be seen.

Atta and *Acromyrmex* both (Fig. 1 and 2), store far more sperm than their ovariole count would indicate. Both genera are attines with large colonies. *Atta* queens require several males to fill the spermatheca (Kerr 1961, Moser 1967, Corso & Serzedello 1981). Perhaps the rules for packaging ova into ovarioles are different in these attines, or they store a great excess of sperm. Judging from the large size and extended longevity of *Atta* colonies, the latter seems unlikely. *Trachymyrmex* and *Cyphomyrmex,* both attines with small colonies, follow the same pattern as other ants (Fig. 1 and 2).

A number of rather simple "allometric" relationships ought to exist among several life-history characters of ant colonies. In monogynous species, mature colony size can be increased in several additive ways: an increase in the number of ovarioles, ovariole length, and rate of movement of eggs down the ovariole, or a decrease in egg size. Colony size can also be increased by increasing worker longevity, which in turn is related to large body size. The size dichotomy between queen and workers is probably also affected. It seems likely that many life-history characteristics of ants are linked to one another, rather than independently selected for. The elucidation of such "allometries" ought to contribute in important ways to our understanding of the evolution of ant life-history characteristics.

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