Efficiency of Sperm Use in Queens of the Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae)

WALTER R. TSCHINKEL AND SANFORD D. PORTER[1]

Department of Biological Science, Florida State University,
Tallahassee, Florida 32306

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ABSTRACT Like most social Hymenoptera, queens of the fire ant, *Solenopsis invicta* Buren, mate only at the beginning of their reproductive lives. They receive an initial supply of about 7 million sperm which they gradually parcel out over a period of almost 7 yr until the supply is exhausted and they can no longer produce female offspring. Using the monogynous form of the fire ant, efficiency of sperm use was determined directly by allowing four newly mated queens to rear approximately 0.5 million workers. The mean sperm count of these queens declined by 1.7 million from the starting value, yielding an efficiency of 3.2 sperm per adult female offspring. Sperm-use efficiency was also determined indirectly for field queens based on calculations that show these queens expend 7.0 million sperm to produce 2.6 million workers for an efficiency of about 2.6 sperm per adult female. Lifetime worker production was calculated from annual estimates of colony growth and worker turnover. These estimates of fire ant sperm-use efficiency are about 10 times higher than those reported for honey bee queens and astronomically higher than those of most nonsocial animals. Apparently, efficient use of sperm is an important reproductive capability for *S. invicta* and other social insects with high reproductive outputs.

KEY WORDS Insecta, reproduction, spermatozoa, spermatheca

QUEENS OF SOCIAL HYMENOPTERA mate on only one occasion at the beginning of their reproductive lives. Sperm from mating is stored in a spermatheca and parcelled out to fertilize eggs until the queen dies or runs out of sperm. The number of spermatozoa stored by ant queens varies from 11,000 to 137 million and is related to the reproductive potential of the queen (Tschinkel 1987a). In at least one ant, *Solenopsis invicta* Buren, a colony-founding queen's reproductive life ends with exhaustion of her sperm supply after about 6.8 yr (Tschinkel 1987b). In such a case, both the size of the sperm supply and the efficiency with which it is used determine the total number of fertilized eggs a queen can produce in her lifetime. Parsimony of sperm use would increase the queen's reproductive potential.

Information on efficiency of sperm use in social Hymenoptera is limited. Harbo (1979) showed that queen honeybees use more sperm per egg when the spermatheca is packed with sperm than when the sperm concentration is lower. His estimates of sperm per adult worker range from 20 to 30. Earlier estimates of sperm released per egg laid were 10–12 (Adams 1912) and 50–100 (Bresslau 1905). The number of sperm entering the egg was reported to be 3–10 by Nachtsheim (1913) and 1–8 by Woyke et al. (1966).

Using two independent methods, we now report for the first time the efficiency of sperm use for an ant queen, *S. invicta*.

Materials and Methods

All sperm counts were made by the method of Tschinkel (1987b), in which the contents of spermathecae are dissected from queens, dispersed in 10 ml of 0.5 *M* NaCl, then counted in a hemacytometer.

Direct Determination. Laboratory colonies were reared from newly mated founding queens collected after the mating flight of 20 May 1985 in Tallahassee, Fla. Sperm counts were made on 12 postclaustral queens from this group. Forty colonies were housed in nests of dental plaster in phototray arenas with Fluon-coated inner walls. They were daily fed an excess of insects, fresh beef liver, and sugar water to maximize the growth rate. Water was supplied as needed.

The 20 fastest growing colonies were selected after 4 mo, and four of these survived to produce 0.5 million workers. The time required varied from 12 to 15 mo. The number of workers produced was estimated as follows. The trash piles containing dead ants and food middens were collected at monthly intervals when the colonies were small, but at biweekly intervals later. This trash was dried, mixed well, weighed, and subsampled. Worker heads were then picked out of these weighed subsamples and counted. The estimate of the number of ants in the total trash pile was made by dividing

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[1] Current address: Brackenridge Field Laboratory and Department of Zoology, University of Texas, Austin, Tex. 78712.

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the head count by the fraction of the total weight represented by the subsample. Five such estimates were made for each collection and averaged. The lifetime sum of these estimates for a colony, plus the workers and brood still alive at termination, yielded the total number of ants produced by that queen.

Determination by Turnover Rates. Sperm-use efficiency was also indirectly determined from estimates of life-time worker production based on annual worker turnover. All newly mated fire ant queens contain about 7 million sperm in their spermathecae and exhaust their supply on average in 6.8 yr (Tschinkel 1987b). Sperm-use efficiency can be calculated by dividing the initial supply by the total number of workers produced over the life of the queen. Total worker production can be determined if both colony size (number of workers) and worker turnover rates are known. When colony size is constant, total workers (T) is simply the product of annual turnover (A) times the queen's reproductive lifespan (n, yr) times colony size (P). For queens in growing colonies, the total lifetime production of ants can be approximated by summing the midyear colony size (P_i) over the reproductive life of the queen (n years) and multiplying by the annual turnover:

$$T = A(P_1 + P_2 + \ldots P_n)$$

Annual turnover was determined for a stable monogynous population of colonies at their maximum size (i.e., nongrowing colonies) near Tallahassee. Mean colony size was the same during both winters of this study (approximately 200,000) (Tschinkel 1988). The population had not been subjected to control measures or other disturbances in at least 20 yr. Every 2–4 wk, total workers, sexuals, larvae, and pupae were estimated for two haphazardly selected colonies by digging each entire colony into 5–8 bins. Each bin contained a different stratum of the nest. Each bin of dirt and ants was weighed, mixed well, then subsampled three times. Subsamples were taken to the laboratory where they were weighed and carefully inspected to determine the number and type of ant that each contained. Totals for a bin were estimated by dividing each subsample count by the fraction of total bin weight contained in the subsample. Total colony estimates were calculated by averaging subsample estimates and summing bin estimates. Standard errors for total colony worker number averaged about 3% with this procedure. Annual turnover was determined by two independent methods.

Method I. In colonies of stable size, all worker production represents turnover and serves only to replace workers that die. It takes an average of about 3 d for pupae that are just becoming pigmented to eclose as adult workers (S.D.P., unpublished data), although this figure is somewhat higher in winter and lower in summer. Therefore, dividing the number of pigmented pupae by the number of workers yields an estimate of the 3-d turnover of workers, and the sum of 121 3-d increments over the entire year yields the annual turnover. Winter production is somewhat overestimated and summer underestimated, but the effects approximately cancel each other out. Because the data were calculated monthly, the values to be summed were linear interpolations between data points. Because pigmented pupae immediately precede adult workers, this method is probably a good estimate of turnover.

Method II. Fire ant worker longevity in relation to temperature was determined by P.C. and S.D.P. (unpublished data). In conjunction with estimates of the mean temperature to which workers are exposed in the field, these longevity data can be used to estimate field longevity and, therefore, weekly percent mortality. Again, because colony size is constant, weekly mortality equals worker turnover. Longevity computations were made for workers of mean size (headwidth, 0.7 mm). From the longevity versus temperature data, a graph of percent weekly mortality versus temperature was constructed. The stratified samples of field colonies then yielded data on the percent of workers found at each depth and the temperature at that depth. These data were graphically converted to estimates of percent weekly mortality. For nonsampled weeks, the value(s) of the previous sample were used. The sum of 52 weekly estimates then gave the annual percent mortality, which, because colony size was constant, was equal to the annual worker turnover.

Contribution of Female Alates. Numerically, the female alates are a minor fraction of the colony. Their annual contribution to sperm depletion was found to be only about 8–9% that of worker turnover in full-sized colonies. For growing colonies, this is probably somewhat generous because queens do not produce many sexuals during the first third to half of their lives. Therefore, 5% was added to the turnover values. For convenience, we refer only to worker production in the remainder of this paper but include the 5% female sexuals within this figure. Voucher specimens have been deposited in the Museum of Comparative Zoology, Harvard University, Cambridge, Mass.

Results

Direct Determination. The sample of 11 queens drawn from the experimental group immediately after the claustral period contained a mean of 7.3 million sperm (SD 0.97 million; $n = 11$). After producing an average of 524,000 (SD 37,000) workers, the four surviving queens had a mean sperm count of 5.6 million (SD 1.4 million), a significant decrease of 1.7 million from the value for postclaustral queens (t test: $t = 2.46$; df = 13; $P < 0.05$). Dividing the number of sperm used by the number of workers produced yields a mean efficiency of 3.2 sperm per worker.

Determination of Turnover Rates. *Method I.* Fig. 1 shows the ratio of pigmented pupae to work-

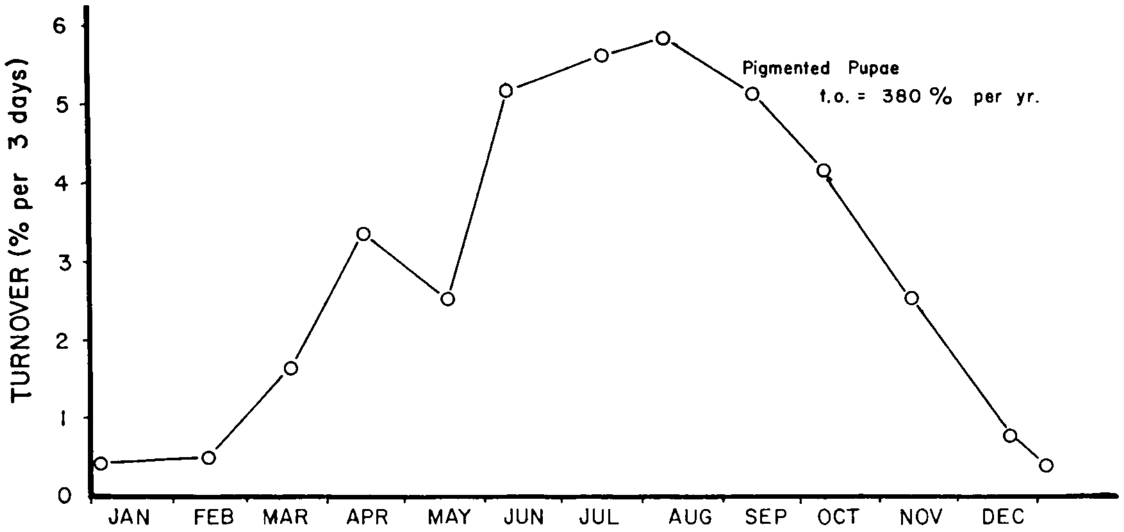

Fig. 1. The mean 3-d percent-turnover of workers (number of pigmented pupae/number of workers × 100) for each sampled month. The annual turnvover rate was estimated by summing the monthly values and the interpolated 3-d values over the year. The development time for pupae from first pigmentation to worker eclosion is about 3 d. t.o., turnover. Standard errors (SEM) for monthly values were (in order from January to December): 0.25, 0.17, 0.65, 0.77, 0.40, 0.37, 0.61, 0.28, 0.11, 1.13, 0.17, 0.42%. Number of colonies sampled was two for January, August, October, and December; three for February and March, and four for all other months.

ers × 100 (i.e., the 3-d turnover) for each monthly sample. This is a strongly seasonal function because brood production is temperature dependent. The annual sum was made with these monthly values and values graphically interpolated between them. Thus, 121 values were summed to give the annual turnover of 380%. Addition of 5% for female sexuals gave a final value of 385%.

Method II. The estimated weekly percent worker mortality summed over 52 wk gave an annual turnover of 320% after addition of 5% for female sexuals (Fig. 2). This estimate is probably conservative because it is based on laboratory mortality rates; actual mortality rates in the field are likely to be somewhat higher because of predation, food limitations, etc. However, this estimate (Fig. 2) agrees well with the previous one (Fig. 1), suggesting that other factors do not add appreciably to overall mortality. Furthermore, higher mortality rates in field colonies would result in an even higher sperm-use efficiency than that calculated below.

Computation of Efficiency of Sperm Use from Turnover. The mean of the two annual turnovers (Fig. 1 and 2) was 353 ± 80% (SD); this was used for calculating sperm used per worker produced. Colony size (number of workers) was estimated for the midpoint of each year between colony founding and total sperm depletion (6.8 yr in Tallahassee; Tschinkel 1987b), using the logistic function for colony growth in Tschinkel (1988). The total workers (T) produced by a queen in her reproductive lifetime in Tallahassee was $T = 3.53\ (6000 + 19{,}000 + 54{,}000 + 118{,}000 + 176{,}000 + 205{,}000) + 2(215{,}000) = 2.64$ million workers. Because Tallahassee queens begin with 7.0 million sperm, (Tschinkel 1987b) this means that they deplete their sperm supply by 7.0/2.64, or 2.64 sperm for every worker or sexual female produced. This value agrees quite well with the 3.2 derived from direct determination in the laboratory. The mean of these two values is 2.9.

Discussion

Our two independent determinations have shown that a queen expends approximately three sperm in order to produce one adult worker. This value is much lower than that for the honey bee queen, which ranges from 10 to 100, with the best estimates being between 20 and 30 sperm per adult worker (Harbo 1979). Why honey bee queens are so much more wasteful of sperm is not known. Perhaps the ability of bees to replace queens with related queens mitigates selection for maximum efficiency of sperm use. It is also possible that queens normally die or are replaced before they exhaust their sperm supply. Efficiency differences between fire ants and honey bees may extend to the sequestration in the spermatheca of sperm deposited in the genital tract during mating. In multiple-mated honey bees, about 10% of each male's contribution is so sequestered (Page & Metcalf 1982). Fire ants mate only once (Ross & Fletcher 1985). Male fire ants contain about 150% as much sperm as is found in the spermatheca of newly mated queens (Glancey & Lofgren 1985), suggesting a minimum efficiency of sequestration of 66%. Ruttner (1985) suggested that high sperm-use efficiency in the honey bee is linked to low sequestration efficiency because of a narrow sperm duct. In the

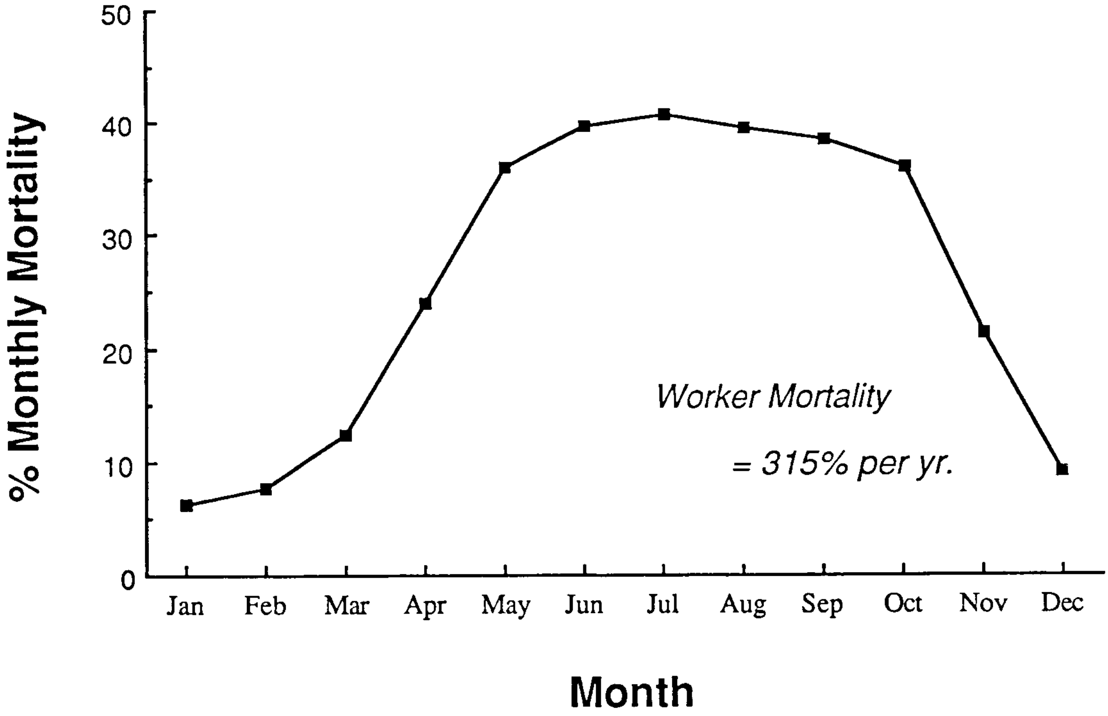

Fig. 2. The monthly percent worker mortality in field colonies. In colonies of constant size, this represents turnover (see text for calculations).

fire ant, both forms of efficiency are more than five times as great, suggesting that Ruttner's argument does not apply. Furthermore, honey bee queens can sequester up to 90% of sperm when mated with a single male (Page & Metcalf 1982).

The sperm expended per egg fertilized is probably lower than our estimate, because preadult mortality is not included in our turnover estimates, but the sperm used are included in the numerator of the efficiency estimate. Field estimates of preadult mortality are not available. Laboratory estimates on queenless colony fragments showed survival rates from egg to pupa of 30–80% (Porter & Tschinkel 1985). Survival in undisturbed queenright colonies is almost certainly higher, perhaps greater than 90%. The similarity of our two estimates of sperm efficiency suggests that preadult mortality in the field and in our queenright lab colonies was not very different.

These data suggest several other potential sources of sperm loss. Some sperm may not survive for the full 7 yr and may die in the spermatheca. Other sperm may fertilize eggs which are nonviable because of lethal mutations. The spermatheca may contain a residue of inaccessible sperm. None of these alone is likely to lead to large losses, but it is possible that together they may have a substantial effect; if so, it is possible that fire ant queens use only one or two sperm per egg.

While it is not known how fire ants achieve the observed high level of sperm parsimony, it must involve, at least in part, the anatomy and function of the spermathecal duct with its muscular valve, the chamber in which egg and sperm are brought into contact, and perhaps the egg itself. During spring and summer, queens in large colonies may lay 100–150 eggs per h (Tschinkel in press). Therefore, such queens are metering out approximately 300–500 sperm per h from a supply of several million. If we divide the number of hours it takes the queen to empty her spermatheca (6.8 yr = 60,000 h) into the number of sperm she starts with (7 million), we find that she expends a mean of about 118 sperm per h during her lifetime. Of course, this rate is substantially lower during the winter and higher during the summer. The annual average rises from about 60,000 during the first year of life to about 2 million during the sixth year (calculated from colony size, turnover rate, and sperm per worker). This translates into an annualized rate of about 6.8 sperm per h during the first year to about 235 per h during the sixth. Mean annual worker production rises in parallel from about 19,000 during the first year to 640,000 during the sixth year.

When the founding queen has exhausted her sperm supply, her colony is frequently able to replace her with another fully inseminated queen (Tschinkel & Howard 1978). The annual rate of sperm use in full-sized colonies (2 million per year) implies that the mean time to total sperm depletion would be only 3.5 yr for the replacement queen as opposed to 6.8 yr for the founding queen. In either case, queens would have only about three seasons in which to produce reproductive offspring. Conversely, the queens in a polygyne colony could be much longer-lived because oviposition rates are much lower.

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