

Fire Ant Queen Longevity and Age: Estimation by Sperm Depletion

WALTER R. TSCHINKEL

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

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ABSTRACT Because ant queens mate only at the beginning of their reproductive lives and draw on this stored sperm for every female produced, the sperm content of the queen's spermatheca declines with age. The potential reproductive life of queens of the fire ant, *Solenopsis invicta*, was estimated in the field by determining the spermathecal sperm content of queens from colonies of increasing ages, and extrapolating their declining sperm counts to zero. Estimated potential life span was 6.77 yr for Tallahassee, Fla., ants and 5.83 yr for Gainesville, Fla., ants. These methods may have general utility in studying the demography of social Hymenoptera.

KEY WORDS *Solenopsis invicta*, spermatheca, longevity

THE STUDY OF social-insect demography has been greatly hampered by the difficulty of determining the age and longevity of queens in perennial colonies. Wilson (1971) lists a few longevity estimates but nearly all are from the laboratory. Almost no field estimates of such ages exist. Two estimates of honeybee queen survival (Prost 1956, Bozina 1961) report 3-5 yr as normal; extremes exceed 9 yr. No mean longevity and confidence intervals were given. With patience, both age and longevity can be determined in the laboratory, but differences in temperature, growth conditions, and reproductive rates make transfer of laboratory estimates to the field practically meaningless. Problems of the repeated recovery of marked queens over long periods make ordinary methods of estimation very difficult in the field.

Most queens of the social bees, wasps, and ants mate only at the beginning of their reproductive lives (Page 1986). The queen stores the spermatozoa in a bladder called the spermatheca; this constitutes the total sperm supply for her entire life. A number of sperm-count studies on newly mated ant queens have been published. Most sought to determine the number of times queens mate (Kerr 1961, Moser 1967, Ball & Vinson 1983, Glancey & Lofgren 1985). The literature on honeybee sperm is substantial and is reviewed by Page (1986).

Every female egg that the queen lays must deplete the supply by at least one spermatozoan. 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 egg range from 20 to 30. Glancey & Lofgren (1985) showed that mother fire ant queens from colonies in the field contained a wide range of sperm numbers, implying sperm depletion.

If all newly mated queens start with a similar number of sperm, it should be possible to estimate both age and longevity by determining residual sperm counts. Using this procedure on the fire ant, *Solenopsis invicta*, I am able to report the first reliable field estimate (with confidence intervals) of potential queen longevity of any perennial social insect species.

Materials and Methods

Newly mated queens were collected on the day of mating from five widely separated localities in the southeastern United States (Table 1). The sperm count of newly mated queens was indirectly estimated by determination of DNA content using a diphenylamine assay slightly modified from Burton (1956): fire ant sperm, rather than purified DNA, was used as a standard, and 0.4 N HClO₄ was used instead of 0.5 N. The optical density was converted to sperm count through split samples subjected to both DNA assay and hemacytometer counts. As an additional check, the mean for each mating flight and locality was also determined by hemacytometry on a single pooled sample of 10 spermathecae from each. For hemacytometry, the spermatheca was ruptured and dispersed in 1.0 ml insect Ringer's saline, then diluted to 10 ml with 0.5 M NaCl. Sperm in four 0.1-mm³ fields of this dilution were counted and the counts averaged. This was repeated on a second droplet.

The decline of sperm in relation to queen age was determined by sampling colonies of known ages. Fire ants are rare to absent in late-succession communities such as broadleaf forests, but, after the forest is cleared, are among the first to colonize the new habitat (Tschinkel 1986), sometimes populating newly cleared areas at densities averaging greater than three queens per square meter in a

Table 1. Sperm counts of newly mated *S. invicta* queens from throughout the species range in the southeastern United States

| Locality | Date (1985) | Mean sperm count (millions) ^a | ±SD (millions) | n |
|-----------------------|-------------|--|----------------|----|
| Tallahassee, Fla. | 9 May | 6.42 | 0.86 | 10 |
| | 21 May | 7.07 | 1.18 | 17 |
| | 10 June | 7.17 | 0.76 | 10 |
| | 13 June | 6.96 | 0.35 | 9 |
| | 28 June | 7.07 | 1.28 | 10 |
| Gainesville, Fla. | 4 July | 7.50 | 0.86 | 10 |
| | 21 May | 7.75 | 1.22 | 21 |
| College Station, Tex. | 13 June | 6.64 | 1.71 | 10 |
| | 9 June | 6.21 | 0.80 | 10 |
| Morgan Co., Ga. | 8 June | 7.07 | 1.18 | 10 |
| Miami, Fla. | 26 May | 6.75 | 1.28 | 10 |
| | 15 June | 6.60 | 0.42 | 3 |

^a There is no significant difference among the values (ANOVA).

single flight (Tschinkel & Howard 1983). Mating flights and colonization take place during a 3-mo period, peaking in June in the Tallahassee area; 8–10 flights per season are not unusual (Morrill 1974). Later mating flights are smaller and their colony-founding is ever less likely to succeed in the face of approaching winter and growing colonies already founded (Markin et al. 1972). It follows that, at least for several years after initial clearing, fire ant populations consist of relatively even-aged cohorts. Thus, by choosing sites cleared during the winter or early spring before mating flights began, I was able to sample populations aged 1.8, 3.7 and 4.7 yr, with an uncertainty of about 3 mo on the actual age. Colonies 0.5 yr old were reared in the laboratory from newly mated queens.

During the late winter and early spring, queens were captured from 31 colonies at these sites (Tschinkel & Howard 1978) and total worker population was estimated. These population estimates will be reported elsewhere. The spermathecae of the captured queens were prepared for sperm counts by hemacytometry.

Data were analyzed by analysis of variance (ANOVA) or linear regression, as appropriate. Regressions of sperm versus queen age squared were used for inverse prediction of potential life span. The 95% confidence intervals for these life-span estimates were computed according to Zar (1974).

Results and Discussion

All newly mated queens start with similar numbers of sperm (Table 1). ANOVA of the DNA estimates of sperm counts over all localities and mating flights showed that there was no significant difference among these ($F = 1.233$; $df = 13, 126$; $P > 0.5$).

The results show a clear decline in sperm count with increasing queen age (Fig. 1). Queen age ac-

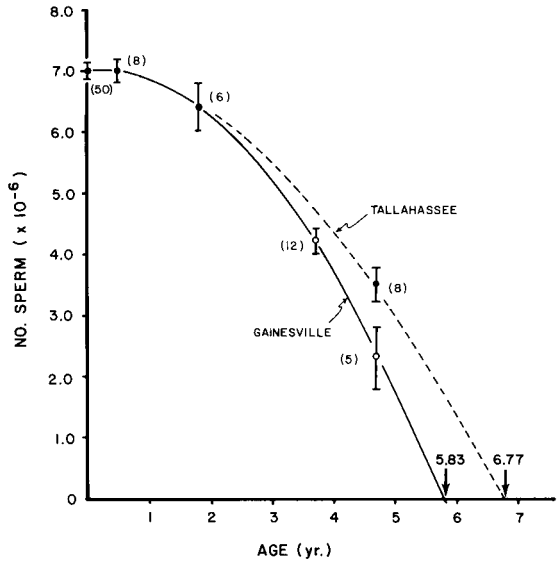


Fig. 1 The decline of the number of sperm in the spermatheca as a function of queen age. Populations from Tallahassee and Gainesville, Fla., are shown. Bars indicate SEM. Sample sizes are in parentheses. The relationship is linear when number of sperm is plotted against the square of the age, and the maximum potential age is estimated by extrapolation of these linear regressions to zero sperm. The 95% CI for the Tallahassee ant queens are 6.12–7.08 yr; and for Gainesville ant queens, 4.67–6.79 yr.

counts for about 85% of the variation in sperm count. A regression of sperm count in millions (y) versus the square of the age (x) gave the best fit, possibly because the colonies were in the logarithmic growth phase for most of the samples, making sperm depletion geometric. The regression for Tallahassee was $y = -0.16x + 7.00$, $R^2 = 0.85$; and for Gainesville, $y = -0.21x + 7.08$, $R^2 = 0.84$.

Extrapolation of these regressions shows that Tallahassee queens run out of sperm in a mean of 6.77 yr (95% CL, 6.12–7.08 yr) of age. Gainesville queens run out in 5.83 yr (95% CL, 4.67–6.79 yr). Because a queen can no longer produce workers after she runs out of sperm, the colony can live only one worker life-span beyond this point, unless the queen is replaced with one containing sperm (Tschinkel & Howard 1978).

Sperm depletion is not linear because the reproductive rate of the queen increases dramatically as the colony grows. However, sperm depletion should approach a constant rate as the colony approaches maximum size. Both of these expectations seem borne out in Fig. 1.

Among queens of equal age, variation in sperm count probably results primarily from differences in initial sperm content and differences in colony growth rate. Estimated longevity of queens in Gainesville was almost a year shorter than in Tallahassee (Fig. 1). This probably resulted from Gainesville's mean annual temperature for 1981–

85 being 1.44°C higher than Tallahassee's. Higher temperatures lead to higher colony growth rates and higher rates of sperm depletion. Other site characteristics, especially the abundance of prey, probably also affect growth rate and thus sperm depletion rate.

The method described here has general applicability to the determination of potential queen longevity in any ant or other social hymenopteran species, provided the amount of sperm taken on during the mating flight is reasonably similar, and monogynous colonies of known age are available for calibration of the depletion rate. Colonies in the laboratory are inadequate for this purpose because they rarely grow at the same rates as colonies in the field. I have found fairly similar numbers of sperm in newly mated queens of at least five ant species (Tschinkel 1987).

A number of factors can lead to complications. In polygynous colonies, queens may deplete their sperm at different rates, depending on reproductive dominance. Grossly variable colony growth rates may make queen age estimates difficult. However, in both cases, the method still gives information on the fraction of potential life span remaining, even if the correlation of this measure with age is weak. In small polygynous colonies, queens may not deplete sperm to a detectable extent. The method cannot be applied to termite queens because these mate throughout life.

Once the relation between sperm count and queen age has been established for a locality, the relationship can be used to determine the age of queens from colonies of unknown age. Using the sperm counts reported by Glancey & Lofgren (1985), I estimated the ages of their queens captured in the field. Glancey & Lofgren determined the sperm content of 13 queens captured in the field (their Table 3). Their Coulter counter method gave somewhat lower counts for newly mated queens (\bar{x} = 5.67 million, compared with my estimates of 6.96 million). Because hemacytometry makes fewer assumptions and is not subject to calibration errors, my values are probably closer to the actual values.

To estimate the ages of Glancey & Lofgren's queens using my Fig. 1, both sets of data were converted to percentage of original sperm remaining, overcoming the disagreement among the absolute values. Estimated ages ranged from less than 0.5 to 5.3 yr, with a mean of 3.4 yr (SD = 1.5; n = 13). Nine of the 13 colonies had lived out more than half their average life expectancy. Two were <1 yr old, indicating a turnover of 15% per year. This compares well with an expected turnover of 17–18% for queen longevity of about 5.5–6 yr.

What happens to the queen after she runs out of sperm? Available evidence indicates that in many cases she may be replaced by a reserve inseminated queen already present in the nest, but not laying eggs (Tschinkel & Howard 1978). Such inseminated nonlaying queens were first noted by

Glancey et al. (1972) and were probably the source of the replacement queens found in orphaned nests by Tschinkel & Howard (1978). This means that the colonies are probably much longer-lived than the queens, and that succeeding queens start their reproductive lives in colonies of unrelated workers. It also implies a second potential avenue toward reproductive success for newly mated fire ant queens—the penetration of an already successful colony. Once accepted, the queen need only outlive the functioning queen and possibly other contenders for “the throne.” Data from the Pharaoh's ant indicate that nonreproducing females (virgin, in this case) live about twice as long as reproducing females (Berndt & Nitschmann 1979).

The method reported here is the first technique capable of giving basic demographic information on social hymenopteran queens, including age, age structure, and potential longevity. Mark/recapture methods can provide some of these data but the long life span of queens of many social insect species makes this very slow, painstaking and risky. Studies of demography and population biology of social Hymenoptera have lagged for want of such information, which the present method may help to provide.

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