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SPERMATOZOON COUNTS IN MALES AND INSEMINATED QUEENS OF THE IMPORTED FIRE ANTS, *SOLENOPSIS INVICTA* AND *SOLENOPSIS RICHTERI* (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

The Coulter Counter IIA, an automatic particle counter, was a fast, accurate device for counting spermatozoa in fire ants. Queens of *Solenopsis invicta*, the red imported fire ant (RIFA) had an average 5,669,683 ± 48,148 spermatozoa in the spermatheca two weeks after mating. RIFA males, ready for a nuptual flight, had an average of 8,761,637 spermatozoa. Males and females of *S. richteri*, the black imported fire ant, had spermatozoon counts extremely close to those of *S. invicta*. Spermatozoon counts from field collected physogastric *S. invicta* queens ranged from 551,594 to 5,634,883. A previous study reported counts (determined by a hemacytometer) of only 377,000 spermatozoa in mated RIFA females. These new figures appear to more accurately reflect the reproductive potential of the fire ant.

RESUMEN

La Coulter Counter II A, una contadora automatica de partícula, fue un aparato rápido y preciso para contar espermatozoas en las hormigas "fire ants". Reinas de *Solenopsis invicta*, la "red imported fire ant" (RIFA, tuvo un promedio de 5,669,683 ± 48,148 espermatozoas en la espermacica 2 semanas después del apareamiento. Los machos RIFA, listos para el vuelo nupcial, tuvieron un promedio de 8,761,637 espermatozoas. Machos y hembras de *S. richteri*, la hormiga "black imported fire ant" tenía el conteo de espermatozoas extremadamente similar a los de *S. invicta*. Conteos de espermatozoas de reinas de *S. invicta* colectadas en el campo, variaron de 551,594 a 5,634,883. Un previo estudio reportó conteos (determinado por un "hemacytometer") de solo 377,000 espermatozoas en hembras RIFA apareadas. Estos nuevos datos aparentemente reflejan más exacto el potencial reproductivo de la hormiga "fire ant".

The construction of a reliable computer model of an insect's life cycle is dependent upon accurate life table parameters including a knowledge of the reproductive potential and longevity of the female. Since all available evidence shows that a red imported fire ant, *Solenopsis invicta* (RIFA) queen mates only once, a primary factor in her reproduction is the number of spermatozoa transferred to the spermatheca during mating. Ball and Vinson (1983) provide the only report of sperm production and insemination rates in RIFA. They measured sperm volume with a hemacytometer and determined that the seminal vesicles of males contained ca. 1,150,000 spermatozoa and that an average of ca. 370,000 spermatozoa were transferred to the spermatheca during mating. The entire reproductive tract of males after a mating flight was devoid of spermatozoa.

These spermatozoon counts seem low in the face of data that mature field colonies may contain 250,000 workers and survive several years (Markin et al. 1974). Also, we have maintained colonies in the laboratory for over 5 years that contained from 100,000-200,000 workers at any one time. Because of the apparent discrepancy between numbers of spermatozoa, worker longevity and colony size, we decided to determine spermatozoon numbers using an automatic particle counting Coulter Counter (model TA II). We were able to utilize it to determine the numbers of spermatozoons in newly mated RIFA queens, aged RIFA males, physogastric RIFA queens from field colonies, RIFA queens from laboratory colonies of known ages, mated RIFA queens from multiple (polygynous) colonies, virgin RIFA alate females, queens of *S. richteri*, the black imported ant (BIFA), collected from the field, and males of *S. richteri* from field colonies.

Methods

The Coulter Counter® is maintained at our laboratory for aerosol droplet size measurements (Haile et al. 1978). Originally developed as a high speed blood cell counter and cell size analyzer, the Coulter Counter has been found to be useful also in the counting of human spermatozoa (Gordon et al. 1965). Particles or cells are suspended in an electrolyte solution and are drawn through a small aperture, across which passes an electric current. As each cell or particle passes through the aperture it changes the resistance to the current which results in a voltage pulse that is proportional to the particle volume within a certain size range depending on the apterture size. For our study, a 100μ diameter aperture was used and monosized polystyrene microspheres 19.0μ in diameter were used for calibration.

Spermatozoa from the female spermatheca were obtained by placing the queen in a Syracuse watch gass containing 8ml of Isoton II (an electrolyte saline solution). The abdomen was opened, the reproductive system carefully removed, and the spermatheca separated and transferred to a clean solution of Isoton II. The spermatheca was opened, the sperm teased out, and the empty spermatheca removed. Some spermatozoa were found in packets and these were broken up until no visible units could be seen. The solution was then poured into a 50ml beaker, stirred, and the watch glass rinsed with 42ml of the Isoton II. The 50ml of fluid was then placed in the Coulter Counter and ten 0.5ml samples were drawn through the counter.

The procedures used for the males were similar. The seminal vesicles were removed, transferred to a clean Isoton II solution, macerated, the packets broken up and fluid volume brought up to 50ml for analysis as described for the queens.

The 94 newly mated queens used in this study were collected from a mating flight on September 23, 1983 and examined on September 30, 1983 and the 20 virgin queens were taken directly from our laboratory colonies and examined immediately. Nine physogastric RIFA queens were collected from field colonies in the Gainesville area while 11 BIFA queens were collected near Tupelo, Mississippi. The 9 RIFA queens from laboratory colonies were originally collected after mating flights in the Gainesville area. They and their subsequent colonies were maintained in the laboratory using normal rearing procedures (Banks et al. 1981). Queens from polygynous colonies (40) were taken from colonies maintained in the laboratory,

but which had only recently been collected from College Station, TX, Ocala, FL, Monroe, GA and Hurley, MS. RIFA males (94) were collected from our laboratory colonies and held for 4 weeks before being examined. BIFA males (51) were collected in North Mississippi and examined 4 weeks after collection.

RESULTS

The spermathecae of the 94 newly-mated queens of RIFA held an average 5,669,683 ± 468,961 spermatozoa (Table 1) while no spermatozoa were detected in 20 virgin RIFA queens. An average of 8,761,637 ± 351,565 spermatozoa was obtained from 93 RIFA males (Table 2). Physogastric RIFA queens from field colonies contained from 551,594 to 5,634,883 spermatozoa (Table 3). BIFA queens from field colonies contained from 3,836,002 to 5,963,819 spermatozoa (Table 4) while the BIFA males av-

TABLE 1. NUMBER OF SPERMATOZOA FOUND IN SPERMATHECA OF 94 MATED RIFA QUEENS.

Spermatozoa (in millions)	Number of queens	Percentage
0-1	0	0.0
1-2	3	3.2
2-3	3	3.2
3-4	2	2.1
4-5	6	6.4
5-6	40	43.0
6-7	39	31.5
7-8	1	1.0

$\bar{x}$ of 94 queens = 5,669,683 ± SD468,961
Range: 1,149,300-7,228,936

TABLE 2. NUMBER OF SPERMATOZOA FOUND IN SEMINAL VESICLES OF AGED LABORATORY REARED RIFA MALES.

Spermatozoa (in millions)	Number of males	Percent
0-6	0	0.0
6-7	3	3.2
7-8	19	20.4
8-9	38	40.9
9-10	24	25.8
10-11	7	7.5
11-12	1	1.1
12-13	0	0.0
13-14	1	1.1

$\bar{x}$ of 93 males = 8,761,637 ± SD351,565
Range: 6,155,018-13,299,525

TABLE 3. NUMBERS OF SPERMATOZOA FOUND IN SPERMATHECAE OF 13 PHYSOGASTRIC RIFA QUEENS COLLECTED FROM THE FIELD.

$\bar{x}$ Spermatozoa ± SD	$\bar{x}$ Spermatozoa ± SD
551,594 (3,265)	
1,321,101 (17,956)	4,084,284 (23,979)
1,689,687 (9,374)	4,143,897 (20,408)
2,919,345 (26,740)	4,557,337 (36,712)
2,470,140 (11,869)	4,903,575 (20,242)
3,029,344 (10,270)	5,473,825 (16,754)
3,335,200 (25,275)	5,634,883 (22,086)

TABLE 4. NUMBER OF SPERMATOZOA FROM SPERMATHECAE OF 11 FIELD-COLLECTED BIFA QUEENS.

$\bar{x}$ Spermatozoa ± SD	$\bar{x}$ Spermatozoa ± SD
3,836,002 (15,615)	5,602,880 (26,433)
5,129,388 (35,052)	5,636,312 (21,126)
5,218,666 (28,475)	5,698,044 (28,898)
5,230,338 (40,503)	5,762,428 (33,017)
5,416,181 (19,991)	5,963,819 (13,372)
5,423,265 (29,274)	

eraged 8,428,896 spermatozoa (Table 5). The counts of aged laboratory-reared queens ranged from 5 to 6 million spermatozoa (Table 6). Queens from polygynous colonies held between 5 and 6 million with the Florida and Texas queens showing the greatest variation in counts (Table 7).

DISCUSSION

Ball and Vinson (1983) reported finding about 1,150,000 spermatozoa in the 2 seminal vesicles of each of 5 males. Our data for 94 males (Table 2)

TABLE 5. NUMBER OF SPERMATOZOA FOUND IN SEMINAL VESICLES OF 51 AGED FIELD-COLLECTED BIFA MALES.

Spermatozoa (in millions)	Number of males	Percent
0- 5	0	0.0
5- 6	2	3.9
6- 7	4	7.8
7- 8	3	5.9
8- 9	29	56.9
9-10	13	25.5

$\bar{x}$ of 51 males = 8,428,986 ± SD95,625
Range: 5,429,793-9,732,615

TABLE 6. NUMBER OF SPERMATOZOA FROM SPERMATHECAE OF COLONY QUEENS OF RIFA THAT WERE COLLECTED IN THE FIELD AND REARED WITH THEIR COLONY IN THE LABORATORY FOR VARYING PERIODS OF TIME.

Age of queen[1]	$\bar{x}$ Spermatozoa ± SD
5.25 yr.	5,067,090 (21,724)
4.25 yr.	5,807,450 (22,492)
3.33 yr.	5,045,703 (14,208)
2.42 yr.	5,217,794 (28,876)
2.42 yr.	5,218,638 (23,299)
1.42 yr.	6,105,850 (21,379)
1.42 yr.	6,219,795 (20,752)
.42 yr.	5,101,074 (20,752)
.42 yr.	5,577,768 (21,497)

[1]All queens examined 10/6/83; age determined from time of capture.

TABLE 7. TOTAL SPERMATOZOA FROM SPERMATHECAE OF FIELD-COLLECTED RIFA QUEENS FROM MULTIPLE-QUEEN COLONIES IN FLORIDA, GEORGIA, MISSISSIPPI AND TEXAS.

Location	$\bar{x}$ Spermatozoa ± SD
Florida	5,428,136 (1,643,276)
Georgia	5,633,101 (156,697)
Mississippi	6,000,188 (227,328)
Texas	5,186,387 (1,889,974)

were 7.6 times higher, with a much lower standard deviation. Likewise, their spermatozoon counts from mated queens (370,000) were much lower than our counts (5,669,683). We surmise that inadequate dispersion of the spermatozoa and measurement of sperm volume may account for the discrepancies. The high standard deviations reported by Ball and Vinson (1983) supports this argument. It is also possible that the males they sampled might have had some sort of problem in spermatogenesis. Our lowest value for a male was 6,155,018 and this particular male had a deformed, empty right seminal vesicle.

In only 2 instances did we find spermatozoon counts below 1,000,000 in queens. The lowest counts were obtained from a physogastric queen collected from a colony in a field near Gainesville, FL (551,594), and a queen from a polygynous colony collected near College Station, TX (720,680). However, other multiple queens collected from Texas contained between 5 and 6 million spermatozoa.

Ball and Vinson (1983) speculated that if a queen laid 500 eggs per day, her reproductive life span would be about 2 years. However, Fletcher et al. (1980) reported that a physogastric queen can lay in excess of 60 eggs per hr (>1400 eggs per day). Moreover, an oviposition rate of 500 eggs per day is too low and, in addition, this rate can not account for the number of ants in a mature colony (100,000 to 200,000). For example, if we assume

the adult worker population in a mature colony averages 100,000 per day and the average life span of an adult worker is 3 months, then there would be 4 complete turnovers of the worker population each year which would require 400,000 eggs (or spermatozoa) each year. This would require an oviposition rate of about 1100 eggs per day, twice that suggested by Ball and Vinson (1983); a rate that would deplete their estimated number of spermatozoa in less than 1 year.

Similarly, Saliwancik and Vander Meer (personal communication USDA, Gainesville, FL) have shown, via computer simulations, that an egg production rate of over 1500 per day is needed to maintain a stable ant colony of 150,000 workers. Their estimates assume a worker survival rate of 5 months and normal seasonal temperature changes. Based on the average number of spermatozoa we found in newly-mated queens (ca. 5.5 million), the queens in their simulations would utilize about 550,000 spermatozoa per year. This converts to a minimum reproductive life span for the queen of 10 years.

The preceding estimates assume all sperm are viable for the life of the queen and do not take into account the possibility of polyspermy which occurs in some insect species. Also, the estimates of spermatozoa (eggs) from the prior calculations are not consistent with our data from aged laboratory queens (Table 6); however, this is possibly an artifact attributable to low oviposition rates caused by confined rearing conditions.

The number of spermatozoa found in *S. invicta* males and females is considerably less than reported by Kerr for the leaf-cutting ant species, *Atta sexdens rubropilosa* (in Wilson, 1971). He found 44 to 80 million spermatozoa in males and 206 to 319 million in the female spermatheca and concluded that the females mated at least 3 times. Our data suggest a single mating; however, as Crozier (1977) states, "equivalence of male-carried and spermatheca amounts may indicate that the males do not deliver the same load to any one female, which might happen if they also mate multiply".

Ball and Vinson (1983) noted that males which completed the act of insemination had empty seminal vesicles. Thus, comparing the mean counts of the males and the newly-mated females in our studies, there are about 3 million excess spermatozoa which appear to be lost by the females. Ball and Vinson (1983) reported that since ant queens lack a mucous plug, these excess spermatozoa are expelled or absorbed.

Newly mated queens and queens maintained for less than three years in the laboratory contained bundles of spermatozoa that were easily dispersed in the dissecting medium. However, queens older than three years had bundles that tended to remain in packets and had to be teased apart in order to free the spermatozoa; it is possible that the age of a queen can be partially determined from this characteristic.

Gross examination of a sperm-filled spermatheca often gives the visual impression of a coiled rope within the structure. This same coiled rope appearance applied to the spermatozoa when they are forced from a seminal vesicle after removal from the male. Some of the queens we dissected showed little or none of this rope, yet all had spermatozoa. For instance, two queens from a polygynous colony from the Florida area lacked the rope yet one queen had over 2 million spermatozoa while the other had over 4 million. Conversely, a Texas polygynous queen with no rope had only 700,000

spermatozoa and one physogastric queen (Gainesville, FL area) which lacked the rope had only 500,000. Also, her spermatheca was filled with a multitude of brown scaley flecks. Two of our newly mated queens lacked the rope condition and their spermatozoa counts ranged between 1 and 3 million. We do not know if the absence of the rope is due to failure of the male to transfer certain physiological fluids or to a cause such as failure to complete the insemination. Finally, both the red and the black imported fire ants sometimes have only one seminal vesicle. However, the spermatozoon count in this single vesicle runs from 5 to 6 million spermatozoa, enough to fill the spermatheca of a female.

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