Spermatophore size as determinant of paternity in an arctiid moth (*Utetheisa ornatrix*)*

(sperm selection/sperm precedence/nuptial gift/parental investment/polyandry)

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ABSTRACT Female *Utetheisa ornatrix* exercise postcopulatory mate selection, favoring sperm of larger males. Larger males also produce larger spermatophores, raising the question whether males are selected on the basis of body size or the size of their spermatophore. We here present evidence, based on matings in which males were induced to deliver spermatophores disproportionate in size to body mass, that the determinant is spermatophore mass.

In the moth *Utetheisa ornatrix* (family Arctiidae), the female exercises postcopulatory sperm selection (1). The female mates on average with several males during her life span (2, 3). Rather than utilizing the sperm of all her mates, she uses predominantly those from larger partners (1). Each mating provides the female with nutrient and pyrrolizidine alkaloid, both transferred to her with the male's spermatophore. The nutrient enables her to increase her fecundity (with each mating she produces, on average, an extra 32 eggs, amounting to about 15% of her basic output) (3), while the alkaloid, which she bestows upon the eggs, provides her with the means for protecting these against predation (4).

There is relatively little sperm mixing in *Utetheisa*. In experiments using enzymatic markers to determine offspring paternity, we showed that in twice-mated females it was the sperm of the larger male that tended to "win out," irrespective of whether the larger male was the first or second of the female's partners (1). Moreover, we presented evidence that the female herself may control the sperm selection process (if the female is inactivated by anesthesia, by a procedure that fails to inhibit sperm motility, the sperm fail to reach their destination in the spermatheca in timely fashion) (1). Selection of larger males by the female may have genetic implications. Larger males provide greater quantities of nutrient and alkaloid, abilities that could both be indicators of male fitness (1, 5).

The question that remained unanswered was how the female assesses the determinant parameter of the postcopulatory selective process: male size. We knew that male mass correlated with spermatophore mass (3, 6), so it was possible that the female gauged male size either directly, by estimation of the physical magnitude of her partner, or indirectly by assessment of spermatophore size. We here present evidence that it is apparently spermatophore size that is gauged.

Our experiments were prompted by the preliminary finding that males deliver subsized spermatophores if they had mated recently beforehand. This made it possible for us to mate females to a pair of males—a recently mated large male and a smaller virginal male—whose body size bore inverse proportionality to the size of their spermatophores. We found that under these conditions it was the sperm of the smaller males (that is, those with the relatively large spermatophore)

that tended to be favored. We here describe these experiments and discuss their implications.

MATERIALS AND METHODS

***Utetheisa ornatrix*.** We raised the moths in the laboratory, from stock collected near Lake Placid, Highlands County, FL. The larval diet was of the type we have been designating as CS diet, based on pinto beans and supplemented with seeds of one of *Utetheisa*'s natural food plants (*Crotalaria spectabilis*) (7). To ensure the virginity of experimental adults, these were separated by sex on the day of pupal emergence. The moths were given access to water only.

Matings were effected by confining individual pairs in sealed, humidified, cylindrical containers (0.35-liter volume), overnight (2000 to 1200), without water. Copulation lasted 10–12 hr, as it does in nature. Individuals were weighed immediately before and after confinement. The same individuals were similarly confined for a comparable period but without partners, on either the day before or the day after mating, and were also weighed before and after confinement. The latter weighings provided a measure of spontaneous mass loss during confinement.

The value for adult body mass used in analyses of correlations with spermatophore mass was that obtained for virgin moths, at 2–4 days after their emergence from pupae. In many cases this was the mass determined just before confinement in a mating chamber. After initial discharge of the meconium, body mass of *Utetheisa* given access to water remains virtually constant in the first days after adult emergence.

Spermatophore Mass. This parameter could not be determined directly. Although the spermatophore can be excised from the female's bursa after mating, we ascertained by dissection that spermatophores begin losing their contents (including sperm) to other chambers of the female's reproductive system before copulation is terminated. We therefore determined spermatophore mass indirectly, by taking the average between the male's weight loss during the period of confinement with the female, and the female's weight gain during that period (both weights were corrected for spontaneous mass loss of the individuals).

Relationship of Spermatophore Mass to Mating History. Spermatophore mass was determined for a group of virgin males ($n = 43$) mated to virgin females. Some of these males were then mated a second time (also to virgin females) at intervals of 1 ($n = 14$), 2 ($n = 8$), 3 ($n = 7$), or 6–7 ($n = 8$) days after the first mating, and the mass of their second spermatophores was then also determined. A group of 10 of the females that had received first spermatophores from males were then mated a second time (again to virgin males) on the day after

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mating, and the mass of spermatophores transferred was determined. At their first mating the male and female moths in these tests were 2–7 days old. In all cases females were dissected after the tests to confirm that they had received the expected number of spermatophores. Only 2 of 82 females had to be excluded from the tests for failing to meet this criterion.

Paternity of Offspring of Twice-Mated Females. Thirty-two females were each mated with two males: a small virgin male (mean mass = 62.8 mg; range 46.6–71.1 mg), and a larger male that had mated 1 or 2 days earlier (mean mass = 79.0 mg; range 69.6–103.3 mg). The interval between matings was 2–6 days. The sequence of mating was 17 females with smaller male first, 15 females with larger male first. The females all met the following criteria: (i) they laid viable eggs in the interval between matings and (ii) upon dissection following death at the end of their life span they proved to contain remnants of two spermatophores. This provided reasonable assurance that both matings had been fertile. All eggs produced after the second mating were allowed to hatch. When the emergent larvae had grown to about 1 cm in length they were stored frozen (–80°C) for subsequent paternity analysis.

Paternity was assigned on the basis of variation at the glucose phosphate isomerase (GPI) allozyme locus. Allozyme phenotypes were determined by standard electrophoretic techniques (8–11). To ensure that paternity assignments could be made, the parental moths were chosen on the basis of allozyme makeup, which was checked beforehand (by analysis of the amputated left mesothoracic leg), as previously described (1). For each paternity analysis, the relevant samples (the female, her two males, and 45 of the offspring) were run electrophoretically on the same gel, to permit direct visual comparison of band patterns. The 45 offspring included individuals produced by the female shortly after the second mating and then in mid- and later life.

With 16 of the females, the two male partners shared no alleles, and paternity could be unambiguously assigned to the offspring. With the other 16 females, the two male partners shared one allele. Offspring which lacked that allele could be definitively assigned to a sire, but those that bore the allele could only be given a probabilistic paternal assignment, which we calculated as previously described (1).

Paternity was assigned to each of the 45 offspring of the 32 females, and expressed for each female as percent of 45 sired by one male or the other (the calculations were also made, separately, for the subset of females with probabilistically assigned progeny).

RESULTS

Relationship of Spermatophore Mass to Mating History. As is evident from Fig. 1, spermatophore mass varied as a function of male mating history. Spermatophores of virgin males were substantial, and they did not differ significantly in mass, whether delivered to virgin females or to nonvirgins mated on the previous day. Spermatophores from virgin males transferred to virgin females correlated significantly with the mass of the donating male, and they amounted on average to 11% of male mass (Fig. 2).

Males delivered subsize spermatophores if they had mated within 1–3 days beforehand. After an intermating interval of 6–7 days they were again able to deliver full-size spermatophores (Figs. 1 and 3). Subsize spermatophores delivered after delays of 1–3 days did not correlate with male body mass; those delivered at full size, following 6–7 days, did show such correlation (Fig. 4).

There was no correlation of female body mass and spermatophore mass, either for spermatophores received by the females as virgins (Fig. 2) or for those received by females the

FIG. 1. Relationship of spermatophore mass to mating history of male donor and female recipient. First spermatophores: virgin males mated with virgin females or with females mated on previous day. Second spermatophores: males remated with virgin females, after interval of given days following their first mating. Because the data are significantly heteroscedastic (Bartlett's test; $P < 0.001$), they were analyzed by Kruskal-Wallis nonparametric ANOVA ($P < 0.001$) (12). Bars showing the same letters are not significantly different at an experiment-wide $P = 0.05$ (Tukey-Kramer multiple comparison test) (13). Numbers above bars give sample sizes.

day after a previous mating (Fig. 5). Spermatophores delivered by males to the mated females also failed to correlate with the mass of the donating males (Fig. 5) [a multiple regression analysis performed with this sample to remove possible confounding effects introduced by the female body mass and the mass of the female's previously received spermatophore showed male mass to remain independent of spermatophore mass ($P = 0.097$) (12)].

Paternity of Offspring of Twice-Mated Females. As is evident from Fig. 6, the smaller (virgin) males sired, on average, a higher fraction of progeny than did the larger (mated) males (the percent distribution was essentially the same for the total sample as for the subsample in which paternities had been probabilistically assigned, lending confidence in the estimation technique).

In 5 of the 32 cases neither of the males could be assigned "principal sire" status (that is, the statistically valid distinction of having sired more than 50% of the progeny; G statistic) (1, 13). An analysis of the remaining 27 cases showed the small males to have been principal sires in 19 (or 70%) of these. In 14 of the 19 cases, the sperm precedence of the small male was virtually total (upward of 90% of progeny sired).

FIG. 2. Spermatophore mass, plotted as a function of male and female body mass, for matings in which both partners were virgins ($n = 43$; data from left column of Fig. 1). Spermatophore mass correlated ($P < 0.01$) with male body mass but not with female body mass.

FIG. 3. (Upper) Spermatophores dissected from female bursae shortly after termination of copulation: (left to right) from virgin male and from males mated 1, 2, and 6 days beforehand. (Lower Left) Excised bursa, containing two spermatophores received from virgin males on consecutive nights. (Lower Right) The two spermatophores dissected from the bursa shown in Lower Left.

The mass of the spermatophore delivered by the males in these tests can be inferred. Thus, we estimate the spermatophore

mass of the small males (mean body mass = 62.8 mg) to have been on average 6.9 mg (calculation from regression equation, Fig. 2 Left) and that of the larger males, which had mated either 1 or 2 days beforehand, to have been on the order of 2.0–3.2 mg (inference from Fig. 1).

DISCUSSION

Our results, taken in conjunction with earlier data (1), provide strong evidence that female *Utetheisa*, in exercising postcopulatory sperm selection, base their choice on spermatophore mass, not male body mass. In our previous experiments with doubly mated females, where the males were all virgin and body mass can be expected to have

FIG. 4. Spermatophore mass, plotted as a function of male body mass, for second matings of males, effected at given intervals after their first mating (data from four columns on right of Fig. 1). Spermatophore mass correlated ($P < 0.05$) with body mass only when between-mating interval was 6–7 days.

FIG. 5. Spermatophore mass, plotted as a function of male and female body mass, for matings in which males were virgins and females had mated on previous day with another virgin male ($n = 10$; data from second column of Fig. 1). Spermatophore mass correlated with neither male nor female body mass.

FIG. 6. Offspring paternity of twice-mated females, given as fraction of progeny sired by either male. Data are plotted separately for the whole sample and for the subsample in which offspring were given probabilistic paternity assignments. Columns give mean plus one SEM. The difference in offspring sired by the two males was significant (Wilcoxon signed ranks test, $P = 0.012$) (12).

correlated with spermatophore mass, the larger male was chosen (that is, became principal sire) in 70% of cases (1). In our current tests, where the proportionality between male body mass and spermatophore mass was experimentally reversed, it was the smaller male that "won out" with precisely that frequency.

We have no evidence on how the female gauges spermatophore mass, although it seems likely that she does so proprioceptively, with stretch receptors in the wall of the chamber (bursa) in which the spermatophore is deposited. Such receptors are known to occur in other Lepidoptera (14).

Our findings raise questions about the mating strategy of *Utetheisa*. Male *Utetheisa* produce full-size spermatophores only when they are virginal, or when they mate after several days of postcopulatory "abstinence." Males that mate within 1–3 days after having mated produce small, potentially non-competitive, spermatophores. Do *Utetheisa* males, in nature, ordinarily space their matings days apart to maximize their reproductive chances? Also, might males in nature recover their ability to produce full-size spermatophores more quickly than they did in the laboratory, where we maintained them on water alone? *Utetheisa* as adults ordinarily partake of nectar, but this resource in itself can hardly be expected to provide lipids and nitrogenous nutrients [such as lepidopteran spermatophores are known to contain (15)] in amounts sufficient for markedly accelerated spermatophore production. The spermatophores of *Utetheisa* are indeed sizable. The figure of 11% of body mass that we obtained for spermatophores of virgin males is among the highest on record. In males of other Lepidoptera, where spermatophore mass may also correlate with body mass, spermatophores have been reported in the range of 1.5–15.5% of male mass (16–19).

A further point relevant to male strategy stems from the finding that in matings of males with nonvirgins inseminated on the previous day, the proportionality of spermatophore mass to male body mass broke down. Males in such matings may simply be unable to transmit spermatophores at full size. Indeed, as we discovered by dissection, on the day following a mating the spermatophore in the female's bursa is still relatively undiminished in size (its contents have been mostly voided but its thick wall is still largely intact). It is not until the second day after mating that the bursa is largely unobstructed (by that time the spermatophore is essentially col-

lapsed and has partly disintegrated). Whether males in nature have the means to recognize recently inseminated females, and whether they discriminate against such females, remains unknown. [Interestingly, in our previous laboratory study with twice-mated females (1), the second mating took place, on average, several days after the first mating. Had the second mating been consistently on the day after the first, we might not have noted the correlation between mating success and male mass.]

Our data raise questions also concerning the behavior of female *Utetheisa*. Size of spermatophore received by the female bears no relationship to female mass. A female's ability to gauge male mass (and therefore male fitness) from spermatophore mass should therefore be unaffected by her own physical size. Only on the day after mating should a female be unable to accommodate another male's spermatophore in full. Do females in nature ordinarily abstain from courting on the day after mating?

Such data as we have provide no information on mating frequency of male *Utetheisa* in nature. Females, we suspect, may mate at intervals of several days. During their life span, which we judge from laboratory longevities to be in the order of 3–5 weeks, they mate on average with four or five males (2). The likelihood that they take these partners all in quick succession is low.

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