

Increased fecundity, as a function of multiple mating, in an arctiid moth, *Utetheisa ornatrix*

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Abstract. 1. Female *Utetheisa ornatrix* mate multiply and may receive up to thirteen spermatophores. Spermatophores provide the female not only with sperm but also with a nuptial gift of pyrrolizidine alkaloid that she transmits to the eggs, protecting them against predation. Thus, through multiple mating the female accrues nuptial gifts that add to the defence of her offspring.

2. Multiple mating in female *U. ornatrix* brings about increases in fecundity, but not in longevity or egg mass. These increases occur through the third mating; the fourth and fifth matings, however, have no additional effect on fecundity.

3. These results, taken with the fact that spermatophores are sizeable and are digested within the female, suggest that they bear nutritive gifts that the female uses in egg construction. Multiple mating thus allows females to accrue the resources to build additional eggs.

4. These results are discussed in relation to the potential benefits accruing to donating such gifts and to male and female mating strategies.

Key words. Nuptial gift, spermatophore, multiple mating, fecundity, longevity, egg mass, pyrrolizidine alkaloid, *Utetheisa ornatrix*, Arctiidae.

Introduction

At mating, males of the arctiid moth, *Utetheisa ornatrix*, pass more than sperm to females. With the spermatophore, males transmit a nuptial gift of pyrrolizidine alkaloids, which the male sequestered as a larva from its food plant, legumes in the genus *Crotalaria* (Dussourd *et al.*, 1988). The female bestows this alkaloidal gift on her eggs, together with a fraction of her own sequestered alkaloids. All stages of development, including the eggs as a consequence of their biparental endowment, are protected by the alkaloid against predation (Eisner & Meinwald, 1987; Eisner & Eisner, 1991).

Males advertise the magnitude of their alkaloidal gift in courtship, by way of a pheromone that they derive from the alkaloid (Dussourd *et al.*, 1991). Females select males on the basis of this signal (Conner *et al.*, 1981), favouring those able to bestow larger gifts. Following mating the female again exercises choice, this time by selecting between sperm of different males (LaMunyon & Eisner, 1993). She favours the sperm of larger males, assessing male size indirectly by gauging the size of the spermatophore (LaMunyon & Eisner, 1994).

Male size in *Utetheisa* correlates with alkaloid content, which in turn correlates with the male's alkaloid-donating capacity (LaMunyon & Eisner, 1993). Thus, by favouring sperm of larger males after mating, females are essentially reinforcing the selection criterion they already exercised in courtship. The strategy could enable females to detect male 'cheaters', such as might provide exaggerated readings of their alkaloid content prior to mating by inflating their pheromonal message (LaMunyon & Eisner, 1993).

U. ornatrix females mate with four to five males on average. Field data indicate that they take up to thirteen partners (LaMunyon, 1994). Quite aside from providing choice of sperm, multiple mating enables the female to accrue multiple nuptial gifts. Alkaloids that a female obtains from each of her mates are transmitted to eggs, irrespective of the fate of the accompanying sperm (LaMunyon, 1992). In addition to alkaloids, matings may convey other non-genetic gifts. The spermatophore of *U. ornatrix* is sizeable, amounting on average to 11% of virgin male body mass (LaMunyon & Eisner, 1994). Spermatophores are almost entirely broken down in the female's bursa, suggesting that they are put to nutritional use. Such nutrients might be accrued by multiple mating and enhance female fitness. This paper presents data on a number of female fitness parameters that are affected by multiple mating, most notably female fecundity, which varies as a function of the incidence of mating.

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Materials and Methods

Experiment 1

This experiment involved mating 121 females with one or more males, and assessing the effect of certain variables (number of matings, mass and diet of male partners, female mass and age) on parameters of female fitness (fecundity, egg mass, longevity, ovipositional period).

The moths, all laboratory-reared, were of two types: (i) alkaloid-free, raised on a diet based on pinto beans (*Phaseolus vulgaris*); and (ii) alkaloid-containing, raised on pinto bean diet supplemented with *Crotalaria spectabilis* seeds. Individuals were taken from the colonies as pupae, segregated by sex, and, upon adult emergence, were maintained individually in chambers (7.5-cm diameter and height; 16D : 8L light cycle) on water alone (offered on cotton wad). In nature, female moths may imbibe nectar, puddle water, or even dew (S. Smedley, personal communication). Pupae were weighed (to 0.01 mg) and the values taken to be representative of adult body mass.

The 121 females were all raised on the *Crotalaria* seed diet and were therefore alkaloid-containing. Beginning at post-emergence age of 2–3 days, and repeated on average four to five times during the next 8–9 days, each female was provided a virgin male, left with her for the duration of the dark phase of the light cycle. *U. ornatrix* mate when thus paired (personal observation). Near the end of the dark phase each pair was checked for occurrence of mating [copulation can almost always be detected this way since it tends to last throughout the night (Conner *et al.*, 1980)]. After onset of light (and spontaneous disengagement of males who had mated) the male was removed. Fifty-nine of the females were given exclusively males raised on the *Crotalaria* seed diet; the other sixty-two were given males raised on the pinto bean diet. Moths that were witnessed in copula were scored as mated. During posthumous dissection, the number of colla within the bursa of each female was counted. The collum, a sclerotized portion of the spermatophore not cleared from the bursa, provides a reliable indication of spermatophore transfer (personal observation); one spermatophore is transferred per mating. Numbers of colla per female were: forty-five females (one spermatophore), thirty-five females (two spermatophores), thirty females (three spermatophores), eleven females (four or five spermatophores).

The technique of checking the mating chambers visually for occurrence of copulation was an accurate predictor of the frequency of spermatophore transfer. The experiment included 507 separate male/female pairings, and 260 matings were observed. The sum total of colla detected in females was 250. Prediction of whether or not a spermatophore was passed, based on occurrence or non-occurrence of mating, was accurate in 489 of the 507 cases (96.5% accuracy).

To permit collection of eggs, the chamber of each female was kept lined with a sheet of wax paper, which was removed and replaced each day. Daily egg production per female was tallied by counting the eggs on the sheets. The figures for daily output were summed for each female and, in order to estimate

better the total number of eggs each female constructed, yolked eggs detected posthumously in the reproductive system were added to give total lifetime fecundity (mean eggs at autopsy: 1.5). Female lifespan and oviposition period were measured, respectively, from the day of pupal emergence, and the first day of egg laying (females usually mated on the first day of male presentation and began laying eggs the next day; lifespan and oviposition period were therefore similar).

Egg mass was determined for a subset of sixty-five of the females. To obtain this figure, the eggs produced by each female each day were weighed together, and the mass was divided by the total number of eggs weighed.

To test for the effects of the number of spermatophores received on parameters of female fitness, the data were analysed by analysis of covariance (ANCOVA), with male pupal mass and diet and female pupal mass, age and lifespan included where appropriate as covariates to remove any confounding effects of these variables (SYSTAT, 1992). Data for pupal mass were not available for the mates of all 121 females. Therefore, analyses were performed on the eighty-two females for which male pupal mass was known, and when it failed to be a significant covariate, the ANCOVAs rerun with the data from all 121 females, with male pupal mass excluded. For females that received multiple spermatophores, the figure for male pupal mass was the sum total of pupal mass of her males.

Experiment 2

Because laboratory colonization inevitably results in some inbreeding, which may be detrimental to fertility (Wildt *et al.*, 1987), the effect of mating frequency on egg viability with experiment 1 females was not tested. Instead, this relationship was investigated in a set of forty-eight female *U. ornatrix* collected in the environs of a natural patch of *Crotalaria spectabilis*, in Manatee County, Florida.

The females were confined individually in chambers lined with wax paper as oviposition substrate, as were the females in experiment 1, and the total number of eggs laid per female over a 4-day period was tallied. Eggs were kept live for 3 days to check on viability (by the end of 3 days, if eggs are viable, the developing embryos within are clearly discernible). Viability was expressed as percentage of total number of eggs showing signs of development. After the 4 days, the females were killed by chilling, and checked for numbers of spermatophores received by counting the colla in their bursae.

Results

Experiment 1

Analysis of covariance revealed that the number of spermatophores a female received significantly affected both her fecundity ($F_{3,115} = 7.28$, $P < 0.001$) and her oviposition period ($F_{3,115} = 3.30$, $P = 0.023$). Male pupal mass (mean 146.5 mg; range: 80.2–206.5 mg) was an insignificant covariate in the ANCOVAs for both parameters, and was excluded. A plot

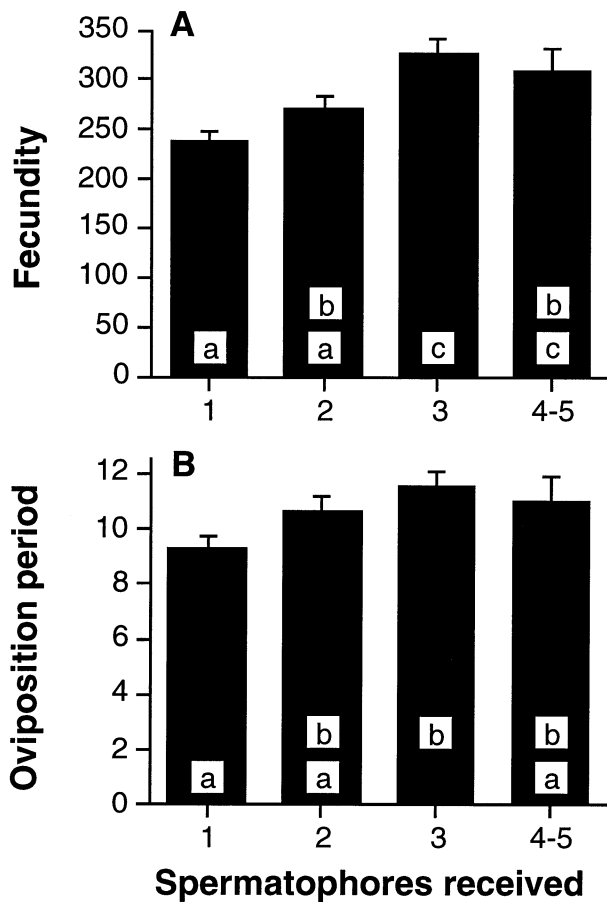


Fig. 1. Mean lifetime fecundity (A) and oviposition period (B) for experiment 1 female moths mated one, two, three or four to five times. Bars sharing letters are not significantly different at $P = 0.05$ (Tukey HSD multiple comparisons test)

of the means adjusted for the effects of the covariates (male diet, female pupal mass) shows that both fecundity and the period of oviposition generally increased with the numbers of successful matings (Fig. 1). With the second spermatophore, females laid on average thirty-four more eggs in an oviposition period that increased by 1.3 days (Fig. 1). Similarly, the third spermatophore corresponded to fifty-five additional eggs and 0.9 more days of oviposition (Fig. 1). Neither fecundity nor the oviposition period changed significantly with the fourth and fifth spermatophores received. In addition to mating activity, female pupal mass (mean 133.9 mg; range 72.2–200.9 mg) was also a significant determinant of both fecundity ($F_{1,115} = 106.84$, $P < 0.001$; Fig. 2) and oviposition period ($F_{1,115} = 27.50$, $P < 0.001$). Finally, no significant interactions were found between the covariates and fecundity or oviposition period, satisfying this assumption of ANCOVA.

Multiple mating did not significantly affect egg mass (mean $1 \times$ mated \pm SEM = $132 \pm 1 \mu\text{g}$). Egg mass did, however, vary with the age of the female ($F_{1,266} = 4.64$, $P = 0.032$). An estimate of the partial regression coefficient for female age ($B = 0.8$) indicated that egg mass increased by $0.8 \mu\text{g}$ with

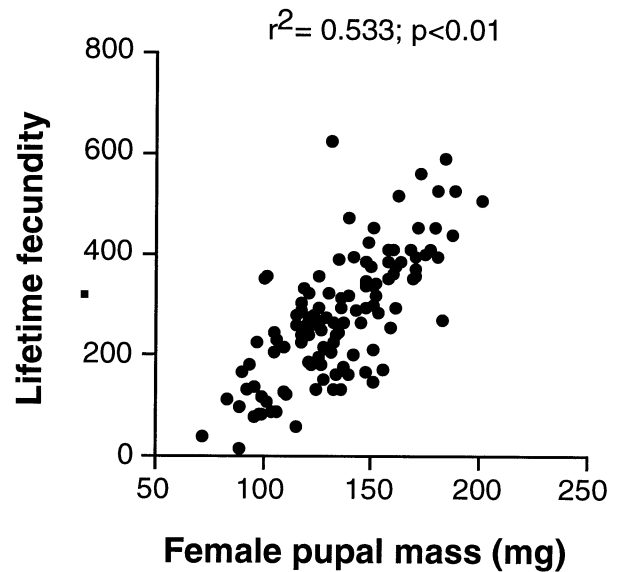


Fig. 2. Lifetime fecundity vs. female pupal mass for laboratory-reared females mated one to five times ($n = 121$).

each day of a female's life. Male pupal mass was again an insignificant covariate and was excluded from the ANCOVA. Of all the covariates (female of origin, female pupal mass, female age, and male diet), male diet was the only covariate found to interact significantly with the number of spermatophores received, thus failing to meet the assumption of ANCOVA ($F_{1,276} = 3.46$, $P = 0.017$); this covariate did not have a significant effect on egg mass, and was omitted from the final ANCOVA analysis.

Female longevity did not vary with the number of spermatophores received or with the covariates male diet and male pupal mass, but it did vary significantly with the covariate female pupal mass ($F_{1,115} = 34.82$, $P < 0.001$). An increase of 14 mg in female pupal mass corresponded to a one day increase in female lifespan (based on the partial regression coefficient of 0.07). Once-mated females lived an average of 14.0 days (± 0.6 SEM). In a separate analysis, female longevity correlated significantly with lifetime fecundity ($r = 0.508$, $P < 0.01$), but this variable was not included as a covariate in the ANCOVA because it had a significant interaction with the number of spermatophores received, and thus inclusion of fecundity would have reduced the precision of the analysis.

These results could be confounded by the fact that some females that were given the opportunity to mate multiply did not. In essence, these moths placed themselves in the once-mated category, and something associated with the reluctance to mate may have contributed to the lower reproductive output observed in once-mated females. To test this possibility, the fecundity of these reluctant females (with three to six mating opportunities; $n = 31$) was compared with that of once-mated moths that had only one to two mating opportunities ($n = 14$) and therefore not given the same opportunity to be reluctant. ANCOVA with male diet and female pupal mass as covariates did not identify a significant difference in the fecundity of these two groups of moths. Thus, the fecundity of once-mated

Table 1. Fertility of eggs laid by field-collected females as a function of the number of spermatophores they received.

	Number of spermatophores					
	1	2	3	4	5	≥ 6
% eggs fertile	99.0	97.7	96.6	98.0	97.9	87.2
SEM	<0.01	<0.01	0.02	<0.01	<0.01	0.09
Sample size	6	11	10	4	5	12

females was not affected by any factors introduced by the reluctance to mate multiply.

Experiment 2

Eggs laid by most field-collected females were fertile: no significant differences in egg fertility were found among the mating frequency categories of females (Kruskal–Wallis test, $P = 0.691$, Table 1). However, the category with the highest incidence of mating included two females with markedly reduced fecundity: one female was completely sterile, and 40% of the eggs laid by the other female were infertile.

Discussion

The results of this study show that several parameters of female fitness, particularly fecundity, increase significantly with the number of ejaculates received. Thrice-mated females laid eighty-nine more eggs than did once-mated females, an increase in reproductive output of 37%. Not surprisingly, the period over which oviposition took place also increased with multiple mating; more eggs require a longer interval during which to oviposit. On the other hand, neither female longevity nor egg mass was affected by multiple mating. Because increased egg mass has not been found to boost offspring fitness in other Lepidoptera (Wiklund & Karlsson, 1984), there may be no selective advantage for female *U. ornatix*, regardless of the number of ejaculates received, to make larger eggs.

What is the factor responsible for the increase in fecundity associated with multiple mating? It is not sperm replenishment. Fecundity of the experiment 1 females that mated once was not limited by sperm; these moths laid nearly every egg they constructed, and all were fertilized. As with all others in experiment 1, these females not only depleted their supply of eggs but also their fat body reserves (observed during autopsies). Thus, fecundity was limited by the resources required for egg construction. Based on these observations, it is more likely that mating boosts fecundity as a result of nutrient resources passed with the spermatophore. These spermatophores are large, comprising 11% of male body mass (LaMunyon & Eisner, 1994), and are digested within the female reproductive tract, consistent with the hypothesis that they are the source of a nutrient gift. Similar nutritional gifts occur widely in the Lepidoptera (Boggs & Gilbert, 1979; Oberhauser, 1989, 1992; Wells *et al.*, 1990a; Wiklund *et al.*,

1993; Ward & Landolt, 1995). In *U. ornatix*, the effect of this gift dropped off with matings in excess of three. Perhaps the female can produce a maximum number of eggs and cannot make use of the gifts received after three to four matings. Conversely, the male might 'hold back' on his gift when mating with an older, already mated female.

While multiple mating clearly affects fecundity, it does not seem to improve the quality of the sperm available, as measured by egg fertility in the field-captured females. Egg fertility was generally high, even for once-mated females, which should be more susceptible to abnormal or poor-quality sperm than females that can potentially dilute such abnormal sperm with inseminations from multiple partners. Interestingly, the only two females with low egg fertility had received a great number of spermatophores (more than twelve). Perhaps females risk contraction either of a sexually transmitted disease (Hurst *et al.*, 1995) or of some other sterility-inducing factor, and multiple mating increases this risk. Alternatively, because the females with the greatest number of spermatophores were probably also the oldest, lowered fertility could be a function of old age.

These results raise questions concerning the mating strategy of female *U. ornatix*. Frequent mating must increase substantially both the number and the alkaloidal protection of the eggs. Do females mate as frequently as possible to take advantage of this benefit? Mating too frequently may have costs (LaMunyon & Eisner, 1994). By mating on consecutive days, females lose the ability to gauge accurately the size of the last spermatophore, the criterion by which they opt either for or against the last male's sperm. Thus, daily matings disrupt post-copulatory choice of sire. Data directly addressing female mating strategy are lacking. However, the most promiscuous females in the field study contained the remnants of twelve to thirteen spermatophores. If these are taken over the course of a 4–5 week lifespan (the maximum observed in the laboratory), females in nature mate on average every 2–3 days.

The correlation of female size (pupal mass) with fecundity calls into question male mating strategy. While no data are available on male choice of females, opting to mate only with larger, more fecund females would increase the potential number of eggs available to sire with each mating. However, this strategy would also have the cost of missing potential matings, thus reducing reproductive success.

While ejaculate-derived nutrients clearly improve female reproduction, does the male also benefit by increasing his mate's reproductive capacity? The answer appears to be no. A male's sperm will generally be used only if they are transferred in a spermatophore larger than the others received by the female (LaMunyon & Eisner, 1994). Even if the sperm are used, they may be displaced by rival sperm any time the female remates. Males whose sperm are opted against are cuckolded for their nuptial gifts (LaMunyon, 1992; LaMunyon & Eisner, 1993). Thus, there is little certainty that a male will benefit by siring the offspring that receive his nutrient investment. The advantage to males is in securing paternity. In a manner analogous to that of orthopterans (Wedell, 1993), large ejaculates increase the likelihood of siring offspring (LaMunyon & Eisner, 1994). That females digest and use the nutrients

contained within the ejaculate is of little benefit to the male. Therefore, effort invested in construction of large spermatophores is best viewed as effort to sire offspring, or fertilization effort (Quinn & Sakaluk, 1986).

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