

Female Promiscuity Does Not Lead to Increased Fertility or Fecundity in an Arctiid Moth (*Utetheisa ornatrix*)

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Abstract Prior work has demonstrated significant phenotypic benefits to female promiscuity in the arctiid moth *Utetheisa ornatrix*. We were interested in determining whether *U. ornatrix* females also derive genetic benefits from mating multiply. We specifically tested whether, by mating with several males, females are able to exploit postcopulatory mechanisms that decrease the risk of fertilization by incompatible sperm. We show evidence that *U. ornatrix* females are not taking multiple mates as fertilization insurance because: (1) females that mate once are as fertile as those that mate three times; and (2) females that take three different mates are no more fertile than those that mate three times with the same male.

Keywords Female promiscuity · genetic benefits · Arctiidae · fertilization · polyandry

Introduction

The intricacies of mating systems of animals in a wide variety of taxa have been revealed in recent years through detailed studies using modern molecular techniques that were previously unavailable (Hughes 1998; Coltman et al. 1999; Wilmer et al. 2000). Many of these studies have produced a similar finding concerning female

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reproductive behavior and sexual selection. Namely, it has come to light in many species that females take more mates than previously realized (Arnqvist and Nilsson 2000; Birkhead and Moller 1998). The models and hypotheses presented to address this phenomenon can be broadly lumped into two categories. Some have argued that multiple matings may help females to accrue direct, material benefits such as an adequate sperm supply (Drnevich et al. 2001), defensive chemicals (González et al. 1999), or nutrient donations and/or oviposition stimulants (Wagner et al. 2001). Others have suggested that the benefits are more genetically based, allowing females to fertilize their eggs with more compatible sperm or with sperm from superior males (Fox and Rauter 2003; Jennions and Petrie 2000; Zeh and Zeh 1996, 1997). These hypotheses have gained traction in recent years with an increasing body of evidence suggesting that females may reap both material benefits (Schwartz and Peterson 2006) and/or genetic benefits (Ivy and Sakaluk 2005) via polyandry. Our primary goal with the current study is to address potential genetic benefits (specifically the increased likelihood of fertilization by compatible sperm) gained through promiscuity in an arctiid moth.

Female *Utetheisa ornatrix* (henceforth called *U. ornatrix*) are highly promiscuous, taking an average of 11 mates of the course of the short 3–4 week adult life (Bezzerides and Eisner 2002). Promiscuity is likely tied to the transmission of nuptial gifts by the male (Eisner and Meinwald 1995; Bezzerides et al. 2005). Spermatophore of *U. ornatrix* are known to contain three commodities: sperm, pyrrolizidine alkaloid (PA), and nutrient (Rossini et al. 2001). The PAs are sequestered by both sexes from their larval food plants (legumes of the genus *Crotalaria*) and protect the moth at all life stages (Eisner and Meinwald 1987; Eisner and Eisner 1991; Hare and Eisner 1993; Dussourd et al. 1988; Eisner et al. 2000). Females endow their eggs with the defensive chemicals and can replenish their PA supply by mating (González et al. 1999). Previous work has shown that the female can protect individual eggs with the alkaloidal donations of multiple males (Bezzerides and Eisner 2002). Multiple mating has also been shown to be valuable to females in terms of increased egg production, with the fecundity of a female increasing by up to 15% with each mating (LaMunyon 1997). An open question concerned the effect of multiple mating on the fertility of the female. Specifically, we wanted to know if one mating provides an *U. ornatrix* female with a sufficient amount of sperm to fertilize a lifetime's supply of eggs. In addition, we wanted to know whether or not multiple mating by females increases the probability that their eggs will be fertilized by compatible sperm. Lastly, we were interested in determining if *U. ornatrix* females ensure the receipt of a diversity of sperm via precopulatory discrimination against males with whom they have already mated.

In this study, we examined the benefits of multiple mating in *U. ornatrix* by dividing females into four classes and subjecting each class to a different series of mating treatments. One group was never exposed to a male. A second group of females mated with only one virgin male. The third group mated with the same male three times and the fourth group mated with three different males. By determining the fecundity, fertility, and longevity of all of the females in the study, we were able to better understand the benefits of promiscuity based on the number of copulations and the number of different partners taken by females in the four treatment groups.

Materials and Methods

Laboratory-Reared *U. ornatix*

For years we have maintained an *U. ornatix* colony descended from individuals collected from the Archbold Biological Station in Lake Placid, Highlands County, Florida. The moths are reared on two types of pinto-bean based diets. The diets are identical, except that in one, [(+) diet], 10% of the pinto beans are replaced by seeds of the plant *Crotalaria spectabilis*, a natural food plant of *U. ornatix* (Bogner and Eisner 1991). Moths reared on this diet contain monocrotaline, the principal PA found in *C. spectabilis*, at levels [0.6 mg (Bogner and Eisner 1992)] closely approximating those found in animals collected from the field [0.7 mg (Conner et al. 1990)]. Moths raised on the diet that is not supplemented with *C. spectabilis* seeds [(-) diet] are consequently PA-free (Conner et al. 1981).

Matings

Two- to four-day old virgin males and females were randomly selected from the colony and weighed. Individuals were considered size-matched if they differed by less than 7 mg. One group of females consisted of four size-matched individuals. Each group consisted of a female that was never exposed to a male (NM, for non-mated), a female that mated one time only (OM, for once-mated), a female that mated with the same male three times (3TS, for three-times-same), and a female that mated with three separate males (3TD, for three-times-different). Each of the first males for groups OM, 3TS, and 3TD was a size-matched virgin male. The second male for the 3TD group had already mated once to control for mating status. Likewise, the third male for the 3TD group had mated twice. 26 groups were set up in this manner.

Individuals were paired in small humidified cylindrical containers (0.35 l) in the late afternoon and checked at 6 h intervals for verification of mating [mating lasts 10–12 h in *U. ornatix* (LaMunyon and Eisner 1994)]. The containers were lined with wax paper, a substrate on which females readily oviposit. After uncoupling, the male was removed and the female was allowed to remain in the container. Males and females that were slated to mate more than once were given one night between matings.

Fecundity

Once a day eggs were removed from the containers. Eggs laid on the wax paper were set aside in large petri dishes for subsequent determination of fertility. Eggs found elsewhere fell into one of three categories. Some were found loose on the container floor, others were stuck to the cotton wick used to humidify the containers, others were stuck in the netting that served as the lid of the container. Fecundity was defined as the total of all eggs laid by a female over her lifetime, both on and off the wax paper.

Fertility

All eggs laid off the wax paper were included in the analysis of fecundity but excluded from the analysis of fertility. Eggs hatch most reliably from the wax paper.

Thus, it was decided that they should be included in the determination of fertility only when laid on that surface. Eggs laid on the wax paper accounted for 83.4% ($\pm 2.0\%$) of the eggs for the females that took at least one mate. In the case of the NM females, eggs laid on the wax paper accounted for 57.1% ($\pm 4.8\%$) of the total eggs laid.

Total fertility was defined as the percent of eggs laid on the wax paper that hatched within 1 week. Seven days after eggs were laid, counted, and set aside, they were assessed for hatching success. Unhatched eggs fell into one of two categories. They were either yellow and wrinkled with no sign of a developing larva inside, or black with the remnants of a larva still present in the capsule. For determination of the percentage of eggs fertilized, the number of eggs that had a developing larva (regardless of whether or not it eventually emerged) was divided by the number of eggs laid. The percentage of eggs hatched was calculated by dividing the number of emerged larvae by the number of eggs laid. Consequently, the difference between the two categories can be interpreted as the percentage of eggs that were fertilized but in which larval development arrested before emergence.

Longevity

Longevity was defined as the number of days the female moth was alive after the first mating or, for NM females, it was defined as the number of days they were alive after being confined alone. NM females were confined alone at the same time females in the other groups were paired with their first mate.

Mate Choice Trials

Two- to four-day old (+) virgin females were mated with 2–4 day old (+) virgin males. After the mating, the male was removed from the mating chamber. Two days later the females were confined with the male they had mated with previously and a size-matched second male. The “new” male had also mated once prior [to a separate (+) female] so that the two males were as phenotypically similar as possible. A portion of the forewings of the two males was colored with a marker in order to identify the mating male. The same experiment was replicated using all (–) individuals.

Statistics

The only groups included in the statistical analyses were those in which an accurate measure could be made of fecundity, fertility, and longevity. One 3TS female laid only a single egg on the wax paper over the course of her lifetime. Given that we based fertility solely upon eggs laid on the wax paper, this female, along with the three other females in her group, was eliminated from the study. Consequently, 25 groups of four females were included in the statistical analyses. Results from the mate choice trials were analyzed with a chi-square test. When data was normally distributed (Shapiro–Wilk’s test, $P > 0.05$), with equal variances (Levene’s test, $P > 0.05$), comparisons were made with a randomized-blocks ANOVA. All other data was analyzed using a randomized-blocks Kruskal–Wallis ANOVA (henceforth known as

KW ANOVA). In the case of the analysis of longevity, post-hoc comparisons were necessary and the alpha level for significance was adjusted to 0.0083 following standard Bonferroni adjustments. All reported P -values are two-tailed. All values of fecundity, fertility, and longevity are expressed as mean \pm SE.

Results

Fecundity

There were no significant differences in the fecundity of the females among the three groups that took at least one mate (ANOVA: $F_{2,24}=0.61$, $P=0.55$, Fig. 1).

Fertility

Fertility was high (>89%) in all groups that took at least one mate. Comparisons between the OM, 3TS, and 3TD groups yielded no significant differences when analyzing either the fraction of eggs that were fertilized (KW ANOVA: $H_{2,24}=0.03$, $P=0.99$) or the fraction of eggs that eventually hatched (KW ANOVA: $H_{2,24}=2.99$, $P=0.22$, Fig. 2).

Longevity

There were significant differences in the longevity of females in the four groups (KW ANOVA: $H_{3,24}=26.29$, $P<0.0001$). Multiple comparisons showed the NM group to differ from every other group ($P<0.0001$ for each comparison, Fig. 3).

Mate Choice Trials

Females failed to differentiate between a previous partner and a new male. Out of 50 trials, (+) females mated 29 times with their previous (+) partner and 21 times with the new (+) male. This trend was not significant ($\chi^2=1.28$, $df=1$, $P=0.26$). Also, out of 50

Fig. 1 Total number of eggs laid by females in each of the four categories. *NM* Non-mated, *OM* once mated, *3TS* mated three times with the same male, *3TD* mated three times with three different males.

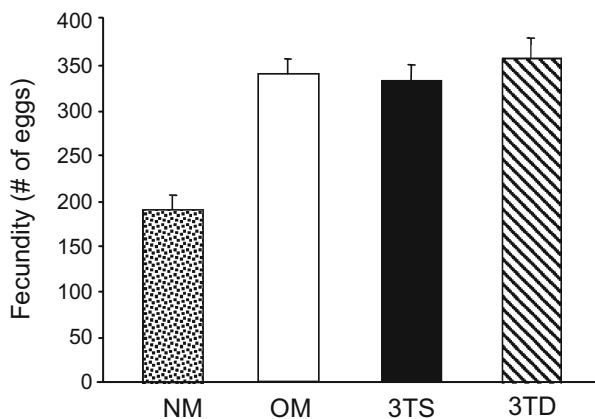
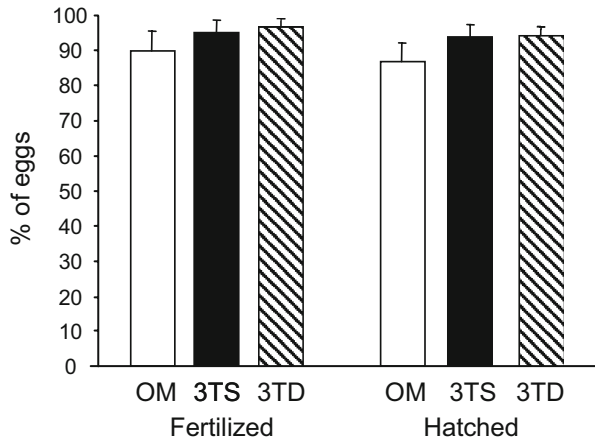


Fig. 2 Fate of eggs laid by females in each of the categories where the female took at least one mate. Eggs were scored as fertilized if a developing larva was seen inside the egg, regardless of whether or not it eventually hatched. Eggs from which an *U. ornatrix* larva successfully emerged were scored as hatched.

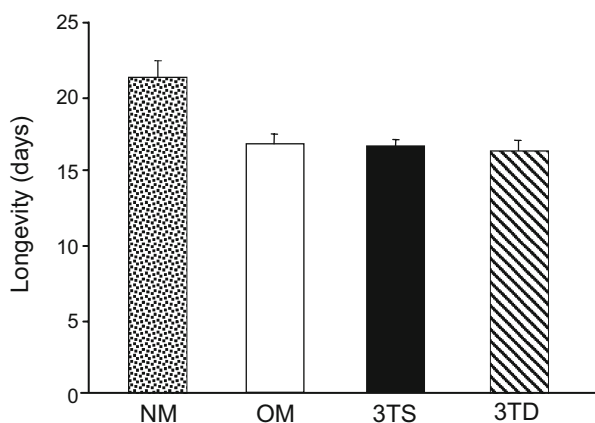


trials, (-) females mated 20 times with their previous (-) partner and 30 times with the new (-) partner. Again, this trend was not significant ($\chi^2=2.0$, $df=1$, $P=0.16$).

Discussion

It does not appear as though *U. ornatrix* females are taking multiple mates as a means of acquiring adequate quantities of sperm. There were no significant differences in the hatching success of eggs laid by once-mated and thrice-mated females. Given the overall high degree of fertility seen in the study, it seems most unlikely that a simple lack of sperm provides the explanation behind the average 11 mates taken by female *U. ornatrix*. In addition, females mated to the same male three times were equally fertile as those that acquired a diversity of sperm from three different mates. Consequently, it comes as no surprise that *U. ornatrix* females have not evolved mechanisms that allow for recognition of and discrimination against previous partners. This stands in contrast to examples of previous-partner

Fig. 3 Longevity of females in each category. Longevity was defined as the number of days the female was alive after exposure to the first male. Females in the NM group were confined individually at the same time members of the other groups were presented with their first mate. Their longevity was measured as the number of days they lived after individual confinement.



discrimination that have been demonstrated in other insect such as crickets (Bateman 1998) and flies (Hosken et al. 2003).

Interestingly, we also found no differences in the fecundity of females in each of the experimental categories where they took at least one mate. This contradicts previous results in which fecundity was found to increase by 15% with each mating (LaMunyon 1997). These prior experiments, however, were subject to the common caveat that in each case the successive males presented to the female were virginal. Thus, with each mating, the female could be expected to receive a spermatophore that represented a significant investment on the part of the male. Males require 6–7 days between matings in order to pass a full-size spermatophore to a second mate (LaMunyon and Eisner 1994). The explanation for the discrepancy between the previous and current results lies in the fact that in this study the second and third males presented to a female had mated previously. Males in this study were given only a single night between matings. Consequently, they were undoubtedly passing sub-size spermatophores to their second and third mates. While it remains untested if sub-size spermatophores contain a smaller quantity of sperm than spermatophores from virgin males (which could potentially affect female fertility), they undoubtedly contain less nutrient. For the female, the small boost of nutrient received at the second and third mating was not enough to elevate her fecundity beyond that of a female receiving just one large spermatophore. This finding suggests that females may have to accept many small nuptial gifts in order to receive enough nutrient to experience a significant increase in fecundity. In addition, in terms of phenotypic benefits, these results emphasize the importance of mating with either virgin or well-rested males. Whether or not females discriminate against previously mated males is an issue worthy of investigation.

We believe that this experimental design is a closer approximation to natural conditions than the repeated presentation of virgin males to a female. While we do not have data on male mating history in the field, given that the sex ratio is equal (unpublished data, based on collections from five locations in Florida), and that females mate on average 11 times (equivalent to mating every other day during their adult life), it seems highly unlikely that a female would encounter a virgin male for every mating. Years of laboratory observations have shown males to be willing to take a mate on consecutive nights. In one recent experiment, when given the opportunity, males were shown to mate on as many as ten consecutive nights (Sarver 2002).

There were two infertile females in the group that took just one mate. They should not be overlooked, in that their complete infertility may explain at least a degree of the multiple mating seen in *U. ornatrix*. Females that take multiple mates may avoid inbreeding, which has been presented as a possible genetic benefit to polyandry (Stockley et al. 1993; Brooker et al. 1990). Females may, on occasion, encounter and mate with a close relative and, given no other choice of sperm, lay infertile eggs due to the negative effects of homozygosity for deleterious recessive alleles. Given that the pairings in our experiments were conducted between randomly chosen individuals, it is possible that some of the matings were between close relatives. Thus, while it seems females are not remating simply to ensure an adequate quantity of sperm, they may be taking multiple mates as a means of avoiding the costs of inbreeding. This type of benefit to polyandry has been shown to occur in the field cricket *Gryllus bimaculatus* (Tregenza and Wedell 2002) and is an issue worthy of further attention.

Also of note is the fact that there were significant differences found by comparing females that never mated with those that took at least one mate. For example, females that never mated lived significantly longer than females in all other groups. This suggests there may be some type of cost incurred via mating, although its exact mechanism remains unknown. It is possible that simply being exposed to males may shorten the lifespan of the female, as is the case in *Drosophila* (Partridge et al. 1987; Partridge and Fowler 1990). Other explanations include the physical energetic costs in terms of egg production (Partridge et al. 1987) and the potential negative effects of male derived proteins (Chapman et al. 1995; Wolfner 1997).

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