

Female choice increases offspring fitness in an arctiid moth (*Utetheisa ornatrix*)

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In *Utetheisa ornatrix* (Lepidoptera, Arctiidae), the female mates preferentially with larger males. Having a larger father results in the eggs being more richly endowed with defensive pyrrolizidine alkaloid (which the female receives from the male with the sperm package, in quantity proportional to the male's body mass, and passes on to the eggs); having a larger father also results in the sons and daughters themselves being larger (body mass is heritable in *Utetheisa*). We provide evidence herein that these consequences enhance the fitness of the offspring. Eggs sired by larger males are less vulnerable to predation (presumably because of their higher alkaloid content), whereas sons and daughters, by virtue of being larger, are, respectively, more successful in courtship and more fecund. The female *Utetheisa*, therefore, by being choosy, reaps both direct phenotypic and indirect genetic benefits.

Fisherian selection | good genes | nuptial gift | pyrrolizidine alkaloid

In the moth *Utetheisa ornatrix* (henceforth called *Utetheisa*), the female exercises mate choice. She mates preferentially with large males, thereby potentially deriving both direct phenotypic benefits and indirect genetic benefits.

The details of this reproductive strategy are complex (1). *Utetheisa*, as a larva, feeds on plants of the genus *Crotalaria* (family Fabaceae), containing poisonous pyrrolizidine alkaloids (henceforth called alkaloids). *Utetheisa* is insensitive to the alkaloids, and the larva stores the chemicals systemically, retaining them through metamorphosis into the adult stage. At mating, the male transfers a substantial fraction of his alkaloidal load to the female with the sperm package (spermatophore; ref. 2). The gift is transmitted by the female in part to the eggs, together with a supplement of her own alkaloidal supply (3). All developmental stages of *Utetheisa* are protected by the alkaloid. The larvae and adults are rejected by spiders (4, 5), and the eggs are avoided by ants (6) and coccinellid beetles (3). The spermatophore in *Utetheisa* is of substantial size, amounting on average to over 10% of male body mass (7). It also contains nutrient, which the female invests in egg production. Females mate on average with four to five males (8) over their lifespan of 3 to 4 weeks. With each mating, the female is able to increase her fecundity by 15% (9). Fecundity in *Utetheisa* is also a function of intrinsic female body mass: large females lay greater numbers of eggs (9).

Female *Utetheisa* do not mate randomly with males but do so selectively with males of higher alkaloid content. The female does not gauge male alkaloid content directly but does so indirectly, on the basis of a pheromone (hydroxydanaidal) that the male produces from alkaloid, in proportion to his alkaloid load, and airs during close-range precopulatory interaction with the female (2, 10). Males richest in alkaloid, having the strongest pheromonal scent, are also largest, and apt to bestow the largest alkaloidal (and presumably nutritive) gifts. In essence, by selecting males of high alkaloid content, the female is selecting males of large size.

We recently established that body mass is heritable in *Utetheisa* (11). This finding indicated that by favoring larger males, females obtain not only larger nuptial gifts but also larger sons and daughters. The offspring, as a consequence, could receive

direct phenotypic benefits (from the nuptial gifts) and indirect genetic benefits (from the expression of largeness in sons and daughters). We postulated that these benefits should be measurable and found that the offspring of preferred males do indeed fare better than the offspring of nonpreferred males. Specifically, we showed that (i) eggs sired by preferred males are less vulnerable to predation; (ii) sons of preferred males are more successful in courtship; and (iii) daughters of preferred males are more fecund.

Our basic protocol was as follows: (i) we confined a female with two males (one large, one small) until she chose to mate with one of them (preferred male, primary mating); (ii) we confined a second female with the nonpreferred male and allowed mating to take place (secondary mating); (iii) we allowed the first and second females to lay eggs and checked these for relative vulnerability to predation by a coccinellid beetle (experiment 1); or alternatively, (iv) we allowed the eggs of the first and second female to develop into adult sons and daughters and tested the sons for relative success in courtship trials (experiment 2) and the daughters for relative fecundity (experiment 3).

Materials and Methods

Utetheisa. All *Utetheisa* were raised in the laboratory from stock collected near Lake Placid, Highlands County, FL.

Larval Diets. These were of two types (10): one based on pinto beans and lacking alkaloid [(-) diet]; the other [(+) diet] also based on pinto beans but containing a supplement of seeds of *Crotalaria spectabilis*, a major food plant of *Utetheisa*. *Utetheisa* reared on (+) diet [herein called (+) *Utetheisa*] contain the principal alkaloid in *C. spectabilis*, monocrotaline, at a level (0.6 mg per adult; ref. 12) comparable to that in *Utetheisa* reared on *C. spectabilis* plants in nature (0.7 mg per adult; ref. 13). *Utetheisa* raised on (-) diet [herein called (-) *Utetheisa*] contain no detectable amount of alkaloid (10).

Adult Body Mass. This parameter is subject to unpredictable variation, because adult *Utetheisa* differ as to when, after emergence, they discharge their meconial waste. We knew from previous studies that pupal mass on day 7 after pupation (pupal duration is 9–11 days in *Utetheisa*) is a reliable correlate of adult body mass (11), and we use this measure herein to express adult body mass. In the current study, adults that are said to be “size-matched” differed by less than 5 mg in pupal mass, whereas those said to be “different-sized” differed by at least 20 mg (or about 10%) in pupal mass. In males, a difference of 20 mg ensured that the individuals differed substantially in alkaloid and hydroxydanaidal content (2, 13).

Matings. All matings (experiments 1–3) were carried out in small, humidified, cylindrical containers (0.35 liter). Events were

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monitored visually (under red light) at intervals of at most 6 h, to check on mating success [copulation lasts 10–12 h in *Utetheisa* (7)].

Oviposition. Mated females (experiments 1–3) were individually placed in humidified, cylindrical containers (0.35 liter), lined with wax paper, on which they readily oviposited. For determination of lifetime fecundity, females were allowed to oviposit in the chambers until they died.

Larval Rearing. For purposes of larval rearing (experiments 2 and 3), eggs from the first 3 days of a female's output were transferred to a small humidified chamber while still affixed to pieces of their wax paper backing. After 7 days and after the eggs hatched, four subsets of 8–10 larvae were confined in four separate, cylindrical containers (0.1 liter) for separate parallel raising (this separation provided a measure of control for random environmental factors exerting a determinant effect on the larvae). Larval food supply in the chambers was renovated every 4 days until pupation, after which, at pupal age of 7 days, the pupae were weighed.

Pupal masses provided the basis for determining average offspring body mass. Masses were calculated separately for sons and daughters for the offspring from each mating category (primary and secondary mating) in experiment 2. For each set of progeny, we first calculated the mean body mass of sons and daughters from the four larval containers and then, from these four values, derived the son and daughter means for that progeny. The individual progeny means, in turn, provided the basis for calculating the overall son and daughter means for the mating category.

Sampling of Adult Offspring. For each set of progeny slated for fitness evaluation (experiments 2 and 3), two subsets of individuals were selected for actual assessment: a group of three sons and three daughters randomly selected from the sample (randomly chosen sons and daughters) and a group of three sons and three daughters (mean-sized sons and daughters) selected to be of average body mass (these individuals differed by no more than 5 mg from the mean mass of their siblings). The double sampling procedure provided a dual basis for evaluation of relative offspring fitness.

Experiment 1: Vulnerability of Eggs. We confined 30 virgin, 3-day-old (–) *Utetheisa* females individually in mating chambers with two different-sized, 3-day-old, virgin (+) *Utetheisa* males. Courtship was monitored visually for the first hour to ensure that both males made fluttering advances to the female, as they typically do during precopulatory interaction, when they also evert the glandular brushes bearing hydroxydanaidal (10). When a mating took place (primary mating), the partners were allowed to remain *in copula* until they spontaneously disengaged, at which time the male was euthanized (after recording whether he was the larger or the smaller of the pair; males were identified by wing marks) and the female was transferred to an oviposition chamber.

The nonpreferred male was then transferred to a second mating chamber and paired with a size-matched sister of the first female. After mating took place, the male was euthanized, and the female was transferred to another oviposition chamber.

The eggs from the two females were then tested for vulnerability to predation. We could be certain that we would be testing for the defensive effectiveness of the father's nuptial gift, because both mothers were (–) *Utetheisa* females and only the fathers bore alkaloid.

We knew from previous work that coccinellid beetles are sensitive to pyrrolizidine alkaloid and prone to discriminate against *Utetheisa* eggs on the basis of alkaloid content (3). We

therefore chose a coccinellid beetle, *Harmonia axyridis*, for our assay (14). Individual *H. axyridis* (prestarved for 24 h) were placed in Petri dishes (5.0-cm diameter) and offered two egg clusters of 10 eggs each, sired respectively by the preferred and nonpreferred males. The clusters were from the third oviposition night of the females, and they were placed in opposite quadrants of the dishes, still attached to pieces of their wax paper backing. At 15-min intervals for the next 3 h, a visual count was taken of the number of eggs of each cluster that had been eaten by the beetle. The test was replicated 30 times (once for each set of matings) with 30 separate beetles. We used a Wilcoxon signed rank test to compare egg loss from the two clusters (15).

Two other values were obtained as part of this experiment, both pertaining to the females from the primary and secondary matings: lifetime fecundity (total egg output over the lifespan) and egg mass (20 eggs from the third oviposition night from each female were weighed). Comparisons were made by using paired *t* tests (15).

Experiment 2: Mating Success of Sons. A mating protocol identical to that in experiment 1 was followed, except that the mothers and fathers were all (+) individuals. Sample size again was 30. Offspring of the preferred and nonpreferred males were raised separately to adulthood on (+) diet (see *Larval Rearing*, above), whereupon two subsets of sons from each mating (see *Sampling of Adult Offspring*, above) were put to the test in mating assays. The assays consisted of placing one son of the preferred male and one of the nonpreferred male together with a 3-day-old, virgin (+) female in a mating chamber and keeping a record of which male succeeded in mating in the next 24 h (the males were wing marked for recognition purposes).

For each trial, we determined the relative mating success of the two types of sons, and from these scores, we calculated the overall mating success of the sons of the two categories by using a Sign test (15). The calculations were done separately for the randomly chosen sons and the mean-sized sons.

Experiment 3: Fecundity of Daughters. This experiment made use of the daughters produced in the 30 trials of experiment 2 and involved testing for the relative fecundity of daughters of the preferred and nonpreferred males. Mean fecundities were first determined separately for each trial. To this end, subsets of daughters of each category from a trial were chosen (see *Sampling of Adult Offspring*, above) and assigned in pairs (one of each category) to mate with size-matched (–) brothers. Lifetime egg output was then tallied for each daughter, providing a basis for calculation of the mean fecundity per category of daughter per trial. The overall mean fecundities for the entire sample of trials were then calculated from these means. Comparison of the overall means for the two sets of daughters was done with a paired *t* test (15). The calculations were carried out separately for the randomly chosen daughters and the mean-sized daughters.

Results

Body Size of Preferred and Nonpreferred Males. Larger males had a higher chance than smaller males of achieving “preferred” status in the primary matings of experiments 1 and 2. In experiment 1, 22 of 30 larger males met with mating success ($\chi^2 = 6.50$; $df = 1$; $P < 0.05$), whereas, for experiment 2, that ratio was 21 of 30 ($\chi^2 = 4.80$; $df = 1$; $P < 0.05$).

Body Size of Offspring of Preferred and Nonpreferred Males. Offspring sired by preferred males (data from experiment 2 and 3) were of significantly higher body mass than those of nonpreferred males; sons were larger by 7.6% and daughters by 5.7% (Table 1, first two rows).

Table 1. Comparisons of offspring from preferred and nonpreferred males ($n = 30$)

Comparison	Statistical test	Preferred male	Nonpreferred Male	P value
Son body mass, mg	Paired t	166.34 \pm 3.98	157.18 \pm 3.99	<0.05
Daughter body mass, mg	Paired t	164.29 \pm 3.87	154.37 \pm 3.87	<0.05
Eggs lost to predation	Wilcoxon signed rank	5.37 \pm 0.26	6.13 \pm 0.28	<0.01
Mating success of randomly chosen sons	Sign	22 of 30*	8 of 30*	<0.05
Mating success of mean-sized sons	Sign	23 of 30*	7 of 30*	<0.01
Fecundity of randomly chosen daughters	Paired t	392.01 \pm 5.44	370.21 \pm 7.08	<0.05
Fecundity of mean-sized daughters	Paired t	372.21 \pm 7.20	350.71 \pm 5.56	<0.005

Values are means \pm SEM.

*The unit value represents the mean value for offspring from a particular mating.

Experiment 1: Vulnerability of Eggs. Eggs sired by preferred males proved less vulnerable to predation. On average, by the end of 3 h, the coccinellid had eaten 13.0% more of the eggs sired by the nonpreferred male (Table 1, third row).

Experiment 2: Mating Success of Sons. The sons of preferred males showed a higher incidence of acceptance in the mating trials than did the sons of nonpreferred males. This higher incidence was true both for the randomly chosen sons (which won out in 73% of trials) and the mean-sized sons (which won out in 77% of trials; Table 1, fourth and fifth rows).

Experiment 3: Fecundity of Daughters. Daughters of preferred males had a significantly higher lifetime fecundity than the daughters of nonpreferred males. On average, randomly chosen daughters of preferred males laid an extra 5.9% eggs, whereas the mean-sized daughters of such males laid an extra 6.1% (Table 1, sixth and seventh rows).

Additional Data. Data that we obtained with the two types of mated females from experiment 1—the females from the primary mating, which had a choice of males, and those from the secondary mating, which had no such choice—showed that these females did not differ with respect to lifetime fecundity (paired t test; $P = 0.80$) and mean egg mass (paired t test; $P = 0.41$). Moreover, from mother–offspring data from experiment 2, we established that there is no difference for the two types of females in the maternal heritability of body mass (calculated as previously described; ref. 11; analysis of covariance: mother–son, $P = 0.96$; mother–daughter, $P = 0.87$). The act of choosing simultaneously between two males evidently has no effect on these female reproductive parameters.

Discussion

Some of our findings lend additional support to conclusions derived from previous work. The males chosen by the females in the primary matings were larger on average than the nonpreferred males. This means that the females, under the cramped quarters of our experimental mating chambers, exercised the same criterion of mate choice that they are known to exercise under more natural conditions (10).

The second point concerns the body size of the progeny. The offspring of preferred males were larger on average than those of nonpreferred males. This finding was only to be expected, given that the preferred males were the larger of the two fathers, and that body mass is heritable in *Utetheisa* (11).

But more important was the demonstration that the offspring of preferred males are indeed “superior.” They are superior in the phenotypic sense, in that, as eggs, they profit defensively from receipt of increased quantity of paternal alkaloid; they are superior in the genetic sense, in that, as larger sons, they are apt to be more acceptable in courtship and, as larger daughters, likely to be more fecund. It should be noted that these results

held true irrespective of the adult sampling procedure: our randomly chosen offspring and mean-sized offspring fared comparably in the assessments.

We did not prove directly that it is the increased quantity of paternal alkaloid that renders the eggs of preferred males less vulnerable. However, we do know that larger males transmit increased quantities of alkaloid to females at mating (2) and that females bestow increased amounts of alkaloid on eggs if the females are alkaloid-rich (16). One could therefore expect the eggs sired by preferred males to be of higher alkaloid content. We attribute the greater vulnerability of eggs sired by nonpreferred males to their being underendowed with alkaloid.

Previous studies tell us that a greater quantity of alkaloid is not the only phenotypic benefit that the *Utetheisa* female receives by choosing a larger mate. She also obtains nutrient with the spermatophore (9); because spermatophore size varies in accordance to male body mass (7), large males can be expected to bestow more nutrient at mating. By accessing larger males, therefore, the female gains extra nutrient for potential investment in egg production.

Earlier work had also shown that the female herself profits from receipt of the male’s alkaloidal gift. She does not transmit the entire gift to the eggs but retains some of the alkaloid systemically for her own protection (16). It has been shown experimentally that *Utetheisa* females devoid of alkaloid, and therefore defenseless vis à vis spiders, are rendered invulnerable to such predators from the very moment they uncouple from their alkaloid-donating mating partner (17).

In discussions of sexual selection in animals, it is customary not only to recognize the two primary benefits accrued by the choosing mate—the direct phenotypic benefits and the indirect genetic benefits—but also to distinguish between genetic benefits of two kinds (18, 19). One type, Fisherian benefits, involves the expression in the sons of the trait that, in the father, provided the key to success in courtship. The other type, “good genes” benefits, also a consequence of female mate choice, involves expression in both sons and daughters of general improvements in quality (increased fecundity, viability).

In *Utetheisa*, the genetic benefits are essentially a combination of the two types. Size being heritable in this moth means that by selecting appropriately in courtship, the female is able to bestow on her sons the very quality of largeness that accounted for the success of the father and on her daughters the largeness, which in the female is linked to fecundity. To “top it all,” the female receives phenotypic benefits as well. The reproductive stakes at issue for the female *Utetheisa* as she appraises her suitor are evidently multiple and high.

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