Male indifference to female traits in an arctiid moth (*Utetheisa ornatrix*)

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Abstract. 1. Female *Utetheisa ornatrix* (Lepidoptera: Arctiidae) mate selectively with large males able to transmit sizeable quantities of nutrient and defensive pyrrolizidine alkaloid with the spermatophore. The female gauges male size indirectly by assessment of the male's courtship pheromone.

2. Male *Utetheisa* invest upward of 10% of body mass in spermatophore production, and could therefore have been expected to be choosy; however, when offered females differing in alkaloid content, body mass, or mating status, males showed disregard of these parameters, both in their choice of partner and in their allocation of resources to the spermatophore.

3. It is concluded that *Utetheisa* males do not have the option to select females by *comparison shopping*. Females broadcast their attractant pheromone for less than an hour per day. Given this time constraint and the potentially high cost of mate localisation, males may have no choice but to mate on an opportunistic basis.

Key words. Nuptial gift, pyrrolizidine alkaloid, sexual selection, spermatophore.

Introduction

Females of the arctiid moth *Utetheisa ornatrix* (L.) (henceforth called *Utetheisa*) are selective and promiscuous. Wild-caught females mate on average with 11, and with as many as 22 partners (Iyengar *et al.*, 2001). Females favour males that are both larger in size and richer in pyrrolizidine alkaloid, traits that females appraise indirectly by assessment of the male's pheromonal scent (Eisner & Meinwald, 1995; Iyengar *et al.*, 2001). By selecting larger males, females profit both genetically and phenotypically. Larger males bestow genes that encode for large size and therefore for the production of sons that are favoured in courtship and daughters that are more fecund (Iyengar & Eisner, 1999). Larger males also transfer larger spermatophores, enabling the female to invest more nutrients in egg production and more pyrrolizidine alkaloid in egg protection (Eisner & Meinwald, 1995).

Spermatophores in *Utetheisa* vary in size, and may amount to over 10% of male body mass (LaMunyon &

Eisner, 1994). Given the magnitude of this nuptial gift, one would expect males themselves to be judicious in their choice of mate. There is no question that males have females of varying quality from which to choose. Female *Utetheisa* differ markedly in pyrrolizidine alkaloid content, body mass, and mating status. Males could therefore reap any number of benefits from being choosy, including rewards beyond those accrued by females. By favouring larger partners, the male could, for instance, access females that are not only more fecund (LaMunyon, 1997), but able to invest greater quantities of pyrrolizidine alkaloid in egg protection (Dussourd *et al.*, 1988). By the same token, males could profit from coupling selectively with virgins. It is only by mating with virgins that males can be certain of fathering at least some offspring (LaMunyon & Eisner, 1993, 1994). There is evidence in *Utetheisa* that the female exercises post-copulatory selection, thereby introducing the possibility that a male, by mating with a previously mated female, may be genetically excluded. Males could evidently profit in any number of ways from mating selectively. The purpose of this study was to determine whether males do in fact exercise mate choice.

Two types of experiments were carried out: (1) *choice* tests, in which individual males were presented with a pair of females that differed in either body mass, pyrrolizidine

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alkaloid content, or mating status; and (2) *allocation* tests, in which it was determined whether the magnitude of the male's spermatophore is affected by the body mass, pyrrolizidine alkaloid content, or mating status of the receiving female.

Male choosiness is of widespread occurrence in the animal kingdom, and has been demonstrated for a broad range of taxa, including insects (Van Dongen *et al.*, 1998), amphibians (Verrell, 1989), fish (Sargent *et al.*, 1986), and birds (Amundsen *et al.*, 1997). Both the male and female of the same species may be selective (Shellman-Reeve, 1999), as was thought initially might also be the case in *Utetheisa*. In species where male mate choice occurs, at least one of three conditions holds: (1) the male bestows gifts upon the female and/or the offspring (Gwynne, 1981; Petrie, 1983); (2) the male is subject to constraints imposed on sperm or spermatophore production (Dewsbury, 1982; Simmons & Bailey, 1990); and (3) the female varies in quality (Parker, 1983; Pitafi *et al.*, 1995). Because all three of these conditions prevail in *Utetheisa*, it is expected that the male of this moth will exercise mate choice.

Materials and methods

Utetheisa

All experimental *Utetheisa* were reared in the laboratory as previously described (Conner *et al.*, 1981). Two types of moths were used: (–) moths, free of pyrrolizidine alkaloid, raised on a pinto bean-based diet; and (+) moths, laden with pyrrolizidine alkaloid, reared on pinto bean-based diet supplemented with pyrrolizidine alkaloid-containing seeds of *Crotalaria spectabilis*, a major foodplant of *Utetheisa*. Individuals were weighed 7 days after pupation, and were judged to be *size-matched* if they differed by less than 5 mg and *different-sized* if they differed by at least 20 mg (equivalent to about 10% of body mass). The age in days given for the moths indicates time since emergence from the pupa (day *l* = day of emergence).

Matings

Moths of known mass were confined in pairs for 24 h in humidified cylindrical containers (0.35 l). Individuals were monitored at 6-h intervals to check on the occurrence of mating (copulation lasts 10–12 h). Spermatophore mass was calculated on the basis of the weight change of the mating partners as previously described (LaMunyon & Eisner, 1994).

Choice tests

Individual randomly selected males were introduced into mating containers with two females that differed with respect to one of the designated traits. To qualify for experi-

mental inclusion, a male had to have been seen courting both females during a period when they were concurrently exhibiting the abdominal pulsation that typically accompanies female pheromone emission (Conner *et al.*, 1980). A record was kept of the females that mated, and the results were analysed by chi-square tests (Snedecor & Cochran, 1989).

Experiment 1: female pyrrolizidine alkaloid content. Forty-two (+) males were placed individually in mating containers with two 3-day-old virgin females [size-matched, one (+) and the other (–)]. The same choice of females was offered to 41 (–) males, and differences in the mate choice of the two sets of males were analysed using a *G*-test (Snedecor & Cochran, 1989).

Experiment 2: female body mass. Forty-two (+) males were placed individually in mating containers with two 3-day-old virgin females [different-sized, both (–)]. By using (–) females only, any possible confounding effects on female receptivity arising from differences in female pyrrolizidine alkaloid content were avoided.

Experiment 3: female mating status. Forty (+) males were placed individually in mating containers with two 8-day-old females [size-matched, both (–)]. One female was virginal while the other had mated with two (+) males on days 2 and 4.

Allocation tests

In these experiments, pairs of males were used [brothers, size-matched, both (+)], and the members of the pair were separately offered one of the members of a pair of females that differed with respect to one of the designated parameters (*n* = 20 pairs of males, per parameter tested). The difference in mass of the spermatophores transferred by the two males of a pair to their respective females was analysed using a paired *t*-test (Snedecor & Cochran, 1989).

Experiment 4: female pyrrolizidine alkaloid content. The males of each pair were mated respectively, to a (+) female and a (–) female. The two females were 3-day-old, size-matched sisters, unrelated to their male partners.

Experiment 5: female body mass. The males of each pair were mated respectively, to a larger and a smaller female. The two females were 3-day-old (–) sisters, unrelated to their male partners.

Experiment 6: female mating status. The males of each pair were mated respectively, to a virgin and a twice-mated female (the mating history of the females was as in experiment 3). The two females were size-matched (–) sisters, unrelated to the males.

Results

Males did not differentiate between females on the basis of pyrrolizidine alkaloid content, body mass, or mating status (Table 1). Moreover, being in possession or lacking

Table 1. Mating incidence of females of differing quality (number that mated per total number of trials) presented as pairs to single males in choice tests.

Experiment no.	Male	Female quality Female 1	Female 2	No. of trials	Mating incidence Female 1/Female 2	χ^2 (d.f. = 1)	*P*-value
1	(+)	(+)	(−)	42	18/24	0.86	0.35
	(−)	(+)	(−)	41	22/19	0.22	0.64
2	(+)	(− large)	(− small)	42	20/22	0.10	0.75
3	(+)	(− virgin)	(− mated)	40	18/22	0.40	0.53

(+) individuals were reared on an alkaloid-containing diet;
(−) individuals were alkaloid-free.

pyrrolizidine alkaloid did not affect the male's indiscriminate selection of mating partners (*G*-test, $G = 0.97$, $P = 0.32$). There were also no differences in the size of spermatophores transferred by size-matched males to females that differed in pyrrolizidine alkaloid content, body mass, or mating status (Table 2).

Discussion

Male *Utetheisa* are evidently non-selective in courtship. They differ from the females in this regard. The latter, in tests comparable to the ones used here with males, did prove to be selective in their choice of mate (Iyengar *et al.*, 2001). The male, however, appears to be oblivious to all three female parameters chosen for analysis. How is one to interpret such lack of selectivity? Could the female have evolved the capacity not to disclose her traits? With regards to one parameter, mating status, this could have been expected, given that by disclosure of non-virginity females could risk rejection. With respect to body mass and pyrrolizidine alkaloid content, however, one could argue that the females could capitalise from disclosure, just as the males do by advertising these traits to the female, and that reciprocal proclamation of bodily size and pyrrolizidine alkaloid content could be a part of the courtship dialogue in *Utetheisa*. Yet these parameters serve as merit badges in the male only. The female either fails to reveal these characteristics or does so in a manner that the male ignores or is unable to decipher.

There could be a simple explanation for why the male *Utetheisa* is seemingly indiscriminate in his choice of mate. Male *Utetheisa* lack the time to search for females by *comparison shopping*. As is typical for moths, it is the female *Utetheisa* that initiates courtship, and she does so by emitting an attractant pheromone (Eisner & Meinwald, 1995). She restricts the release of the attractant to a period of less than an hour at dusk (Conner *et al.*, 1980). By doing so she may in fact prevent males from locating more than one female per calling period, which could be to her advantage, in that it would, potentially, reduce the level of competition she faced. By imposing limits on the length of the calling period, the female could in fact have forced the male into adoption of a strategy whereby he foregoes mate assessment and accepts females on a *come-as-they-may* basis. Selectivity, given his proclivity for gift-giving, could have been to the male's advantage, but the female's strategy essentially prevents him from exercising choice. The evolution of the male strategy could also have been influenced by the cost of mate finding in many lepidopterans (Watt, 1968). Locating more than one female per daily calling period could, quite simply, be energetically too expensive. The argument has previously been made, that when search costs are high and the encounter rate low, one would expect indiscriminate acceptance of partners to be the norm (Reeve, 1989).

The data show further that the *Utetheisa* male is indifferent to female quality, even after copulation has been initiated. The magnitude of the spermatophore bestowed by the male upon the female varies as a function of male mass, but apparently not in accord to female mass, pyrrolizidine

Table 2. Spermatophore mass of size-matched males presented separately to females of differing quality ($n = 20$ pairs of males per test). Values are given as means ± SE. A paired *t*-test was used.

Experiment. no.	Male	Female quality	Spermatophore mass (mg)	*t*	*P*-value
4	(+)	(+)	11.72 ± 1.09	0.60	0.56
	(+)	(−)	11.88 ± 1.18		
5	(+)	(− large)	14.12 ± 1.09	0.21	0.84
	(+)	(− small)	14.04 ± 0.98		
6	(+)	(− virgin)	11.22 ± 0.67	1.15	0.26
	(+)	(− mated)	10.93 ± 0.66		

(+) individuals were reared on an alkaloid-containing diet;
(−) individuals were alkaloid-free.

alkaloid content, or mating history. The male therefore is programmed to deliver nutrient and pyrrolizidine alkaloid in amounts reflective of his maximal giving capacity, rather than female quality. Because sperm from larger spermatophores tend to win out in post-copulatory selection in *Utetheisa* (LaMunyon & Eisner, 1994), this strategy makes sense. The strategy is different from that of some other insects, in which the males do have the capacity to control the size of their spermatophores (Gage, 1998).

Acknowledgements

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