

# Experience counts: females favor multiply mated males over chemically endowed virgins in a moth (*Utetheisa ornatrix*)

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**Abstract** Determining the factors that affect male mating success is essential to understanding how sexual selection operates, including explanations of the adaptive value of female preferences and how variation in male traits is maintained in a population. Although females may appear to choose males based on a single parameter, female mate choice is often a complex series of assessments of male quality that can only be revealed through manipulation of multiple male traits. In the moth *Utetheisa ornatrix* (Lepidoptera: Arctiidae), females have been shown to judge males primarily on their production of a courtship pheromone, hydroxydanaidal, derived from defensive chemicals acquired as larvae. Recent work, however, suggested that other factors, including prior mating experience by males, may also influence the outcome of precopulatory interactions with females. I ran mating trials with one female and two males to determine whether there were any differences in male mating success based on their prior exposure to females, mating experience, and time between matings. Previously mated males were favored over virgins when both males lacked the pheromone, but courting experience and mating interval did not explain these differences in male mating success. Furthermore, multiply mated males lacking the pheromone were favored over virgin males that produced the pheromone, thus reversing the commonly observed trend of female precopulatory bias towards males with higher levels of the pheromone. These results demonstrate that males with mating experience can secure

copulations despite deficiencies in the pheromone, and I provide possible mechanisms and discuss their implications regarding sexual selection.

**Keywords** Sexual selection · Courtship · Pheromone · Promiscuity · Female choice

## Introduction

Classical sexual selection theory holds that males usually maximize their reproductive success by fertilizing as many mates as possible, whereas females typically achieve greater fitness by being selective and choosing fewer mates of high quality (Bateman 1948; Trivers 1972; Thornhill and Alcock 1983; Arnold and Duvall 1994). The prevalence of female mating preferences based on particular male traits are often explained by substantial direct and/or indirect benefits to mating with a high-quality male (Andersson 1994; Andersson and Simmons 2006; Kokko et al. 2006) and significant costs to mating frequently, particularly with low-quality males (Daly 1978; Arnqvist 1989; Chapman et al. 1995, 2003; Hurst et al. 1995). A male's phenotypic quality may vary as a result of his mating history, which may in turn affect female reproductive success when males provide valuable resources to females. For example, in species with a substantial precopulatory mating investment, previously mated males may have depleted resources that reduce the overall size and quantity of nutrients that can be transferred at mating (Choe and Crespi 1997; Vahed 1998), and, in species with postcopulatory parental investment, females mating with polygynous males usually receive less food for their young due to energy spent by those males in providing assistance to other females and their offspring (e.g., Slagsvold and Lifjeld 1994; Czapka and Johnson 2000).

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Since the mating history of males can affect their ability to provide phenotypic benefits, there may be strong selection for females to discriminate among males based on their mating history. At mating, many male insects transfer a substantial spermatophore containing sperm and nutrient, both of which can enhance female reproductive output (reviewed in Arnqvist and Nilsson 2000). The size of this male reproductive investment has been shown to decrease with multiple mating in many lepidopterans (e.g., Svård and Wiklund 1986; Carroll 1994; Hughes et al. 2000; Ryne et al. 2001). As a result, females that copulate with mated males risk receiving smaller spermatophores with fewer resources and suffering from reduced fecundity (e.g., Royer and McNeil 1993; Rutowski et al. 1987). This substantial cost may explain why many lepidopteran females mate preferentially with virgins (reviewed in Torres-Vila and Jennions 2005).

Across many taxa, however, many studies have demonstrated that mated males have an advantage over their inexperienced counterparts in securing matings (e.g., Clutton-Brock et al. 1989; Weatherhead and Boag 1995; Sakata et al. 2002; Goulet and Goulet 2006; Krupke et al. 2008). Although explanations have focused on female preferences for traits indicative of high genetic quality, mating behavior is not usually the result of the choosiness of one sex but instead the outcome of conflict between and within both sexes (Davies 1989; Chapman et al. 2003; Parker 2006). For example, the mating success of experienced males may be due to intersexual selection for symmetrical males consistently favored by females (Thornhill 1992; Liggett et al. 1993; Schlaepfer and McNeil 2000) or intrasexual competition among males for high-quality territories where the occupants reap the rewards of mating with multiple females (Weatherhead and Boag 1995; Cacho et al. 2006). Mated males may also profit from their prior experience by increasing the efficiency and effectiveness of their courtship (Hosken et al. 2003; Dukas 2005).

*Utetheisa ornatrix* (Lepidoptera: Arctiidae) is a moth that relies on chemistry for both defense and communication, and the complexity of the reproductive strategies of both sexes have made it an ideal organism for studying sexual selection. Larvae feed on plants of the genus *Crotalaria* (family Fabaceae) that contain poisonous pyrrolizidine alkaloids (PAs) that are stored systemically and retained through metamorphosis (Eisner and Meinwald 2003). While adult females use these larvally acquired PAs for defensive purposes only, males use a portion of their PAs to produce a courtship pheromone, hydroxydanaidal (HD), that they derive chemically from PAs. When a male encounters a female (located at dusk via a long-range female pheromone unrelated to PAs; Conner et al. 1980), the male airs his short-range pheromone from coremata, two brush-like structures everted during close precopulatory interaction with the female (Conner et al. 1981). In *U. ornatrix*, there is no

sexual coercion or forced copulation as seen in other organisms (Arnqvist and Rowe 2005; Birkhead and Møller 1998), and males can only copulate after the female presents her abdomen to the male (Conner et al. 1981).

Females strongly prefer pheromone-endowed males over those that are pheromone-free, and they also discriminate among males based on incremental differences in pheromone production (Conner et al. 1981; Iyengar et al. 2001). Previous laboratory studies have shown that a male's HD is also correlated with his body mass, spermatophore mass, PA transferred in the spermatophore, and systemic PA content (Conner et al. 1990; Dussourd et al. 1991). The male's spermatophore is a substantial nuptial gift amounting to up to 11% of his body mass (LaMunyon and Eisner 1994), and the female derives both direct phenotypic benefits and indirect genetic benefits by choosing males based on the pheromone. By selecting an HD-rich male, she ensures receipt of a large PA gift that she uses to better protect herself and her eggs from predators (direct benefit; Dussourd et al. 1988; González et al. 1999), a large nutrient gift that she uses to increase her fecundity by as much as 15% per mating (direct benefit; LaMunyon 1997), and genes that encode for large body size (indirect benefit; Iyengar and Eisner 1999a). Because body size is heritable, females mating with higher levels of HD have larger sons that are more successful in courtship and larger daughters that lay more eggs (Iyengar and Eisner 1999b).

There are many aspects of both male and female reproductive strategies in *U. ornatrix* that remain unresolved, as studies continue to reveal underlying complexities regarding both precopulatory choice and postcopulatory selection. Although previous work indicates that HD is the primary criterion of female choice (Iyengar et al. 2001), HD-free males achieve mating success at levels as high as 70% in sequential presentations to females (Conner et al. 1981). Additionally, Bezzerides (2004) found that, when the same pair of males was presented simultaneously to a series of different individual females, the same male often mated in numerous consecutive trials despite the permanent loss of PAs and body mass with each mating. These results suggest that factors other than HD may play a role in determining the mating success of males. There is also postcopulatory selection in *U. ornatrix*. Even though females are highly promiscuous, taking as many as 22 mates (11 on average) during their 3–4-week lifespan (Iyengar et al. 2001), they can use sperm selectively from the largest male while using nutrient and PAs from all mates (LaMunyon and Eisner 1993; Bezzerides and Eisner 2002). In experiments where large males were induced to produce subsized spermatophores, it was shown that relative spermatophore size, and not male body size, dictated the sperm used to fertilize the eggs (LaMunyon and Eisner 1994). In lepidopteran males, spermatophore size can be an accurate indicator of male size,

and the females may use stretch receptors in their bursae to indirectly gauge male size and quality (Sugawara 1979). Spermatophore size does not always directly reflect the size and quality of males in *U. ornatrix*, however, as males require at least 6 days to recover a full-size spermatophore with maximal nutrient and PA content (LaMunyon and Eisner 1994; Rossini et al. 2001) and males usually mate more frequently (Iyengar and Reeve, unpublished manuscript). Thus, although virgin males are capable of delivering a nuptial gift that accurately reflects their body size, the size of subsequent spermatophores is also a function of mating interval, which is likely to fluctuate based on female availability and the male's attractiveness.

Given the variation in male phenotypic quality based on mating history in *U. ornatrix*, I examined the role of male mating experience, courting experience, and mating interval on mating success to determine whether any of these factors were able to supersede male pheromone levels as the main criterion of female choice. One hypothesis was that virgin males or males with long mating intervals would be favored over recently mated males. Females choosing in this manner would receive direct benefits because, on average, they would receive larger spermatophores with more nutrient and PAs. Alternatively, experienced males could be favored over virgins if a male's prior mating experience reflects his high genetic quality and females seek to maximize indirect benefits. Courtship also involves a series of complex maneuvers in *U. ornatrix* (Conner et al. 1981), so it is possible that experienced males are better able to find and stimulate females and, thereby, profit from female choice based on those qualities. Finally, male experience may not be used at all as a choice criterion because females, by mating multiply, accrue direct benefits in the form of chemicals that *all* males are capable of providing to some degree regardless of their mating history. Further support for this hypothesis comes from the fact that there are postcopulatory mechanisms that allow females to profit directly from each partner while having the mate of highest quality fertilize the majority of her eggs. In fact, PAs from at least three different males can be found in a single egg, thus indicating the female's ability to pool resources from multiple matings to benefit offspring belonging to one sire (Bezzerrides and Eisner 2002). Here, I report the results from my experiments and provide potential explanations for the effects of male mating experience on female choice in *U. ornatrix*.

## Methods

### Moth rearing and mating

All *U. ornatrix* used were from laboratory colonies established from wild stock collected in Highlands County,

FL. Larvae were reared on one of two types of diet: some moths were raised on a pinto bean-based diet free of PA [hereafter referred to as (–) moths], whereas others were raised on a pinto bean-based diet supplemented with PA-containing seeds *Crotalaria spectabilis*, a major foodplant of *U. ornatrix* [hereafter referred to as (+) moths]. As a result of these dietary treatments, (–) male moths did not have the courtship pheromone HD, which is derived from dietary alkaloid, whereas (+) moths have pheromone levels commensurate with other laboratory and field studies (0–37 µg; Conner et al. 1990). Adults were weighed 2 days after emergence, and were judged to be size-matched if they differed by less than 5% in body mass. The age in days given for the moths indicates time since emergence from the pupa (day 1 = day of emergence). Adults were isolated and given access to a 5% honey–water solution on a sponge that was moistened daily.

In some cases [experiments (Expts.) 1 and 3], the mating history of males was manipulated prior to use in experimental trials by allowing males to mate with females. The purpose of these matings was to alter either a male's mating frequency (i.e., number of previous mates), mating rate (i.e., interval between matings), or both. The males selected to be individually paired with females in these treatments were a random subset of the laboratory population. As a result, there were no inherent biases between virgin males and these experienced males in the experimental trials.

All experimental matings involved confining pairs of unrelated males with a virgin female in a screened cylindrical container (0.35 L) in the evening, when most mating occurs in nature (Conner et al. 1981). All pairs were monitored for at least 10 min to observe male courting behavior (as indicated by fluttering passes and corematal eversion). I only included trials where both males were courting to ensure that mating success was not biased by differences in sexual interest between the males. Since copulation typically lasts 10–12 h in *U. ornatrix* (LaMunyon and Eisner 1994), pairs were subsequently monitored under red light at 6-h intervals overnight to check on mating success (males were wing-marked for recognition purposes). There were 40–46 trials per experiment, and individuals were only used in one trial in one experiment.

### Effect of mating experience on male mating success

To determine whether male mating experience affected female choice (as measured by male mating success), I presented females simultaneously with two size-matched, 8-day-old males that differed in their mating history (mating frequency and/or mating rate). Naïve virgins were reared in isolation and had never been exposed to a female, and mated males had mated a different virgin female prior to use in the experimental trial. All experiments were

carried out in duplicate, using (+) and (–) pairs of males, to determine if male mating success differs based on the possession of the male pheromone.

#### *Experiment 1A*

Each female was presented with a naïve virgin and a once-mated male (mated on day 2). Given the 6-day mating interval for the mated male, both males were capable of delivering a full-sized spermatophore. As a result, I controlled for the size and content of the spermatophore, and the two males only differed in mating frequency.

#### *Experiment 1B*

Each female was presented with a naïve virgin and a twice-mated male (mated on days 2 and 5). Both mating frequency and mating rate varied between the males, which meant that the virgin male delivered a full-size spermatophore whereas the mated male was only capable of delivering a smaller subsized spermatophore.

#### *Experiment 1C*

Each female was presented with a once-mated (mated on day 5) and a twice-mated male (mated on days 2 and 5). Given the 3-day mating interval for both males, they each delivered equivalent subsized spermatophores and differed only in mating frequency.

#### Effect of courting experience on male mating success

To determine whether male courting experience affected female choice, I presented females simultaneously with two size-matched, 8-day-old virgin males that differed in their exposure to females. Naïve virgins were reared in isolation and had never been exposed to a female, and courting males had courted (but not mated) a different virgin female prior to use in the experimental trial. Courtship without mating was carried out by placing a male and a female in a cylindrical container where they were separated by mesh (1-mm<sup>2</sup> holes). All experiments were carried out in duplicate, using (+) and (–) pairs of males, to determine if mating success differs based on the possession of the male pheromone.

#### *Experiment 2A*

Each female was presented with a naïve virgin and a once-courting virgin male that courted a female on day 5. Given that neither male had mated prior to the experiment, both males were equally capable of delivering a full-size spermatophore.

#### *Experiment 2B*

Each female was presented with a naïve virgin and a twice-courting virgin male that courted females on days 2 and 5. Again, I controlled for both age and mating status of the males.

#### Effect of mating interval on male mating success

To determine whether male mating interval affected female choice, I presented females simultaneously with two size-matched, 8-day-old males that differed in their time between matings. All experiments were carried out in duplicate, using (+) and (–) pairs of males, to determine if mating success differs based on the possession of the male pheromone.

#### *Experiment 3A*

Each female was presented with two once-mated males—one that mated on day 2 and another that mated on day 5. Thus, I controlled for both male age and mating frequency, and the two males differed only in the mating interval, which affects the size and contents of the spermatophore (the male that mated on day 2 could deliver a full-size spermatophore, whereas the other male would deliver a smaller spermatophore; LaMunyon and Eisner 1994; Rossini et al. 2001).

#### *Experiment 3B*

Each female was presented with two twice-mated males—one that mated on days 2 and 4 and another that mated on days 4 and 6. Although the two males transferred different-sized spermatophores (while I again controlled for both male age and mating frequency), this experiment differed from the previous one in that neither male was capable of delivering a full-sized spermatophore but instead delivered equivalent subsized spermatophores (LaMunyon and Eisner 1994; Rossini et al. 2001).

#### Male mating experience vs pheromone endowment

Based on the previous experiments that demonstrated an effect of mating experience on male mating success, I designed the following experiments to determine whether mating experience alone was enough to overcome the known advantages of pheromone possession securing a mate. I presented individual females simultaneously with two size-matched, 8-day-old males, where naïve (+) virgins were placed in competition with individual (–) males of varying mating status. We did not perform the reverse scenario [i.e., using experienced (+) males vs virgin (–)

males] because previous studies (Conner et al. 1981; Iyengar et al. 2001), along with the results of this study, have shown the (+) males are always favored over (–) males when males have similar mating experience. Given preliminary experiments indicating that experience increased the chances of mating success (Bezzarides 2004), experiments with experienced (+) males would not be illuminating since it was likely to further increase a highly significant advantage for (+) males over (–) males.

#### Experiment 4A

Each female was presented with a naïve (+) virgin and a naïve (–) virgin to confirm previous results demonstrating an advantage for (+) males endowed with the male pheromone HD. Although I used males of the same age, it was not necessary to control for mating interval based on the results of Expt. 3.

#### Experiment 4B

Each female was presented with a naïve (+) virgin and a once-mated (–) male (mated on day 6).

#### Experiment 4C

Each female was presented with a (+) naïve (+) virgin and a twice-mated (–) male (mated on days 4 and 6).

#### Experiment 4D

Each female was presented with a naïve (+) virgin and a thrice-mated (–) male (mated on days 2, 4, and 6).

## Data analyses

The data for each experiment (male mating success, expressed as males that mated vs those that did not) were analyzed using Chi-square goodness-of-fit tests (Zar 1999). Alpha levels were not adjusted because all insects were used only once (i.e., all experiments were independent). Differences in mate choice between trials were analyzed using Chi-square tests of independence, and for Expts. 4A–4D, which demonstrated significant differences across all four experiments, I conducted pairwise comparisons using a multiple comparisons test for proportions (Zar 1999). All analyses were performed using the statistical program JMP®5.1 (SAS Institute 2004).

## Results

### Effect of mating experience on male mating success

There were no differences in the mating success of naïve virgins and once-mated males for either diet (Expt. 1A; Table 1). In fact, there were no differences in the relative mating success of naïve vs once-mated males based on the male's diet and possession of the pheromone [Expt. 1A, (+) vs (–):  $\chi^2=0.998$ ,  $df=1$ ,  $p=0.318$ ]. Twice-mated males, on the other hand, did have an advantage over their virgin counterparts, but only in the absence of the pheromone (Expt. 1B; Table 1). Furthermore, there was a significant difference in relative mating success of naïve vs twice-mated males based on diet [Expt. 1B, (+) vs (–):  $\chi^2=4.260$ ,  $df=1$ ,  $p=0.039$ ]. However, the mating success between once-mated and twice-mated males did not differ for either

**Table 1** Mating incidence (number of males that mated per total number of trials) of (+) and (–) males, presented as pairs to single females, in courtship trials

Expt.	Male diet	Treatment: male 1/male 2	N	Mating incidence: male 1/male 2	$\chi^2$ (df=1)	P
1A	(+)	Naïve virgin/once-mated	44	23/21	0.091	0.763
	(–)	Naïve virgin/once-mated	41	17/24	1.201	0.273
1B	(+)	Naïve virgin/twice-mated	43	22/21	0.023	0.879
	(–)	Naïve virgin/twice-mated	44	13/31	7.584	0.006
1C	(+)	Once-mated/twice-mated	42	22/20	0.095	0.758
	(–)	Once-mated/twice-mated	40	17/23	0.903	0.342
2A	(+)	Naïve virgin/once-courting	43	21/22	0.023	0.879
	(–)	Naïve virgin/once-courting	46	25/21	0.348	0.555
2B	(+)	Naïve virgin/twice-courting	41	22/19	0.220	0.639
	(–)	Naïve virgin/twice-courting	41	18/23	0.611	0.434
3A	(+)	Once-mated, long interval/once-mated, short interval	44	23/21	0.091	0.763
	(–)	Once-mated, long interval/once-mated, short interval	43	19/24	0.582	0.445
3B	(+)	Twice-mated, long interval/twice-mated, short interval	41	22/19	0.220	0.639
	(–)	Twice-mated, long interval/twice-mated, short interval	42	21/21	0.000	1.000

diet (Expt. 1C; Table 1), and there were no differences between the two diets [Expt. 1C, (+) vs (-):  $\chi^2=0.803$ ,  $df=1$ ,  $p=0.370$ ].

#### Effect of Courting Experience on Male Mating Success

There were no differences in the mating success of virgin males based on their courting experience, regardless of their diet or whether they courted once or twice (Expts. 2A and B; Table 1). I also found no difference in relative mating success between the two diets [Expt. 2A, (+) vs (-):  $\chi^2=0.270$ ,  $df=1$ ,  $p=0.603$ ; Expt. 2B, (+) vs (-):  $\chi^2=0.782$ ,  $df=1$ ,  $p=0.377$ ].

#### Effect of mating interval on male mating success

There were no differences in the mating success of males based on mating interval, regardless of their diet or mating status (Expts. 3A and B; Table 1). Comparisons of relative mating success between the two diets revealed no differences [Expt. 3A, (+) vs (-):  $\chi^2=0.110$ ,  $df=1$ ,  $p=0.740$ ; Expt. 3B, (+) vs (-):  $\chi^2=0.111$ ,  $df=1$ ,  $p=0.739$ ].

#### Male mating experience vs pheromone endowment

As expected based on previous studies, naïve (+) virgins that possessed the male pheromone were strongly favored over naïve (-) virgins that did not (Expt. 4A: 34 vs 11,  $\chi^2=12.330$ ,  $df=1$ ,  $p=0.0004$ ; Fig. 1). Naïve (+) virgins were also favored over once-mated (-) males (Expt. 4B: 29 vs 14,  $\chi^2=5.343$ ,  $df=1$ ,  $p=0.021$ ; Fig. 1). However, there were no differences in mating success between naïve (+) virgins and

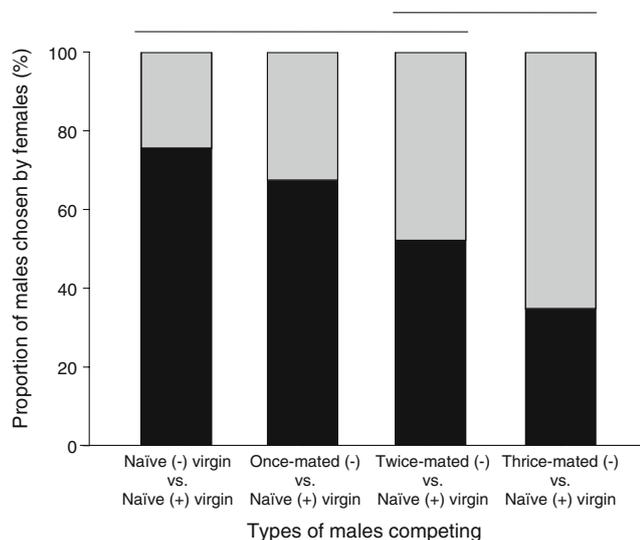
twice-mated (-) males (Expt. 4C: 24 vs 22,  $\chi^2=0.087$ ,  $df=1$ ,  $p=0.768$ ; Fig. 1). In a reversal of the commonly observed mating pattern, females bypassed naïve (+) virgins to preferentially mate with thrice-mated (-) males (Expt. 4D: 16 vs 30,  $\chi^2=4.329$ ,  $df=1$ ,  $p=0.038$ ; Fig. 1).

There was a significant difference in female preferences among all four experiments ( $\chi^2=18.322$ ,  $df=3$ ,  $p=0.0004$ ; Fig. 1). I then performed pairwise comparisons to determine if there was a threshold at which the number of matings by a (-) male (i.e., mating experience) could overcome the mating advantage usually achieved by (+) males based on their possession of the courtship pheromone. Pairwise comparisons revealed that the only significant differences among the experiments were between Expts. 4A vs 4D and Expts. 4B vs 4D, thus demonstrating the importance of mating three times in achieving mating success when lacking the courtship pheromone.

## Discussion

Females often exercise mate choice based on traits that reflect male phenotypic or genetic quality (Andersson 1994). Given that a male's mating history may affect his phenotype and, thus, the direct benefits he can provide to females, I investigated the role of male mating experience, courtship experience, and mating interval on mating success in the moth *U. ornatrix*. When females were given a choice between pairs of males reared on the same diet but differing in mating frequency, females only favored experienced males over virgins when both males lacked the courtship pheromone, HD, and when males were much more experienced. The absence of choice among the size-matched, pheromone-possessing males differing only in mating frequency indicates that the courtship pheromone remains the primary criterion for male mating success, as its presence, at least in high quantities, masks any effects of mating experience on female choice. However, when the pheromone was absent from both males, twice-mated males were favored over virgins. Apparently, males must mate at least twice to gain a competitive advantage over virgins, but there was no difference in the mating success of twice-mated vs once-mated males. Although we did not test more experienced males, these results suggest that one successful copulation provides enough experience for males to compete favorably against more experienced rivals. It is important to note that a successful mating is critical, as mere courting experience is not sufficient to help males gain a future mating advantage.

Many lepidopteran females prefer virgins over mated males because virgins are usually capable of providing a more substantive spermatophore that can enhance female fecundity and fertility (reviewed in Torres-Vila and



**Fig. 1** Proportion of (-) males of varying mating history (gray) and naïve (+) virgins (black) chosen by females (Expts. 4A–4D;  $N \geq 43$  for each). There were significant differences among the four experiments, and the lines above the columns indicate experiments that did not differ based on pairwise comparisons

Jennions 2005). My results indicate that *U. ornatrix* females did not mate preferentially based on a male's ability to provide more phenotypic benefits despite the substantial advantages to be gained by doing so. In the absence of the pheromone, females preferred twice-mated males over virgins despite the fact that these experienced males transferred smaller spermatophores than their virgin rivals due to a shortened mating interval. When controlling for the mating frequency and altering mating interval only, females did not distinguish among the males despite substantial differences in spermatophore size and content (LaMunyon and Eisner 1994; Rossini et al. 2001).

The female's disregard for direct phenotypic gain is particularly evident in the results from the experiments demonstrating that experienced, pheromone-free males can be favored over males possessing the pheromone and, therefore, the defensive chemicals (PAs) from which it is derived. Despite the normally large advantage observed for males possessing the pheromone, twice-mated, pheromone-free males were able to level the playing field by virtue of their mating experience, and thrice-mated pheromone-free males were actually favored over pheromone-possessing virgins. This complete reversal of the pattern normally observed (Conner et al. 1981; Iyengar et al. 2001) means that females are choosing males that are not only completely devoid of defensive PAs, but also likely to transfer subsized spermatophores with less fecundity-boosting nutrient (LaMunyon 1997) and potentially lower quantities of sperm (e.g., Vahed and Gilbert 1996; Kendall et al. 2002). Why would a female ignore these potential direct benefits when choosing males? In *U. ornatrix*, females are highly promiscuous, perhaps because, despite significant variability in spermatophore contents among field-caught males (Conner et al. 1990; Bezzerides et al. 2005), all males are likely to transfer some PAs and nutrient that can be used to confer protection and enhance fecundity, respectively. Also, since a single male usually transfers more than enough sperm to fertilize all of a female's eggs (Curtil and LaMunyon 2006; Bezzerides et al. 2008), it is unlikely that females mating with males transferring subsized spermatophores would suffer reduced fertility. Thus, as observed in the almond moth *Cadra cautella* (McNamara 2007), perhaps there is no bias against experienced males in *U. ornatrix* because there is no cost in fecundity or fertility. Furthermore, postcopulatory selection allows females to use PAs and nutrient from multiple mates (Bezzerides and Eisner 2002) while using sperm selectively (LaMunyon and Eisner 1993, 1994). As a result, there may be no selection on females to distinguish among males based on phenotypic quality because female *U. ornatrix*, like other polyandrous insects (reviewed in Arnqvist and Nilsson 2000), accrue some direct benefits regardless of their partner's mating history.

Although advantages for experienced males have been frequently observed in insects (e.g., Van Dongen et al. 1999; Schlaepfer and McNeil 2000; Dukas 2005; Krupke et al. 2008), the mechanism by which previously mated males are achieving mating success over virgins is often unknown. In speculating on possible explanations for my results, it is important to note that, in *U. ornatrix*, there are no forced copulations because males require the cooperation of females to be successful in courtship (Conner et al. 1981). Also, males generally do not compete directly with other males in the field because they are more likely to arrive at signaling females sequentially, with the female choosing to accept or reject each mate following a brief courtship (Conner et al. 1981). Experienced males in insects such as the blowfly *Lucilia cuprina* achieve mating success by directing aggression at less-experienced rivals (Cook 1995); however, despite the fact that my confinement of two males created more opportunities for intrasexual competition, I did not observe any aggressive interactions among the pairs of *U. ornatrix* males in my experimental trials. It is likely that experienced males achieve greater mating success by improving on their ability to stimulate females. For example, lepidopteran males, by virtue of losing mass via spermatophore transfer at mating, often increase their speed and acrobatic capabilities due to lower wing-loading, thereby increasing their mating success (Marshall 1988; Liggett et al. 1993). Males can affect the outcome of female choice by increasing the intensity of their courtship (Kaitala and Wiklund 1995; Hosken et al. 2003), and many studies have shown that males can learn through prior mating experience by altering many aspects of their courtship behavior (Sakata et al. 2002; Dukas 2005). In *U. ornatrix*, experienced males may be favored by a similar mechanism because courtship has numerous stages that require both acrobatics (fluttering near a stationary female in darkness) and precise timing (to evert pheromonal glands over the female's antennae). Although most courtship variables were not quantified during the experimental trials, I am currently investigating potentially important behaviors that may account for the success of experienced males including latency to courtship, timing of glandular eversion, wing-beats per minute, and body positioning.

My results demonstrate that sexual selection can be more complex than it initially appears, and that there may be subtleties that will only be revealed through careful manipulation of multiple variables. Although female choice may be based on a single trait or a suite of traits (Andersson 1994), previous work in *U. ornatrix* focused primarily on female mating preferences based on the male's courtship pheromone. The current study does not dispute previous findings, as it indeed supports the notion that a male's pheromonal endowment is the primary criterion of female choice. Females did not discriminate among males based on

mating histories under any circumstances when they both possessed the pheromone, and it is exceptionally rare to find a male in nature that lacks the pheromone (Conner et al. 1990; Bezzerides et al. 2005). Furthermore, pairwise comparisons revealed that the threshold for the normal mating preferences (based on the pheromone) to be reversed was only observed for very disparate levels of mating experience (thrice-mated vs virgin males). Given the fact that there is an even sex ratio in nature (Bezzerides 2004), female virgins are rare (Bezzerides and Eisner 2002), and males may court and mate on eight consecutive nights (Iyengar, unpublished manuscript), it is not clear how important mating experience may be in a population with very few virgins where one successful copulation provides sufficient experience.

Although many studies show an effect of mating experience among males of similar phenotypic quality (e.g., Van Dongen et al. 1999; Schlaepfer and McNeil 2000; Dukas 2005; Krupke et al. 2008), this study demonstrates that mating experience is capable of superseding a trait (the male pheromone) that serves as the primary criterion of mate choice. My results further illustrate that courtship is often composed of a complex series of actions, and the ability to improve upon any aspect has the potential to impact mating success. Given the increased evidence on the ability of organisms to learn through experience (including mate copying; reviewed in Westneat et al. 2000), it is likely that further investigations regarding mating experience will show that less obvious factors may play an important role in sexual selection.

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