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Original Article

Male courtship pheromones as indicators of genetic quality in an arctiid moth (*Utetheisa ornatrix*)

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One of the fundamental issues regarding sexual selection is whether females select males based on signals that represent direct phenotypic or indirect genetic benefits. In *Utetheisa ornatrix* (Lepidoptera: Arctiidae), females choose males based on a courtship pheromone, hydroxydanaidal (HD), derived from defensive pyrrolizidine alkaloids (PAs). At mating, virgin males transfer a spermatophore whose contents are proportional to the HD titer and body size; as a result, females receive both phenotypic benefits (more nutrients and PAs) and genotypic benefits (genes for larger body size inherited by the offspring). Previous data from field-collected individuals, however, indicated that the HD signal of nonvirgin males may not correlate with the spermatophore contents. Using chemical analyses, we determined that the HD signal does not change based on mating history, thereby supporting the importance of HD in advertising a male's genetic quality. Thus, male HD represents original body size and PA levels, and females, by choosing males based on this pheromone, are providing their offspring with genes to sequester chemicals that confer survival and reproductive advantages. We discuss the implications regarding the relative importance of direct and indirect selection in maintaining female preferences. *Key words:* Arctiidae, genetic benefits, mate choice, pheromone, pyrrolizidine alkaloid, sexual selection. *[Behav Ecol]*

INTRODUCTION

Sexual selection theory makes different predictions about the number of mates each sex should take to maximize lifetime reproductive success. Male fitness generally depends on access to multiple fertilizable females, due to their generally small investment of resources (Trivers 1972; Andersson 1994). Until recently, the opposite was widely believed to be true for females, who usually maximize their fitness by selectively taking fewer mates of high quality (Bateman 1948; Trivers 1972; Arnold and Duvall 1994). There are often significant costs associated with multiple mating, such as heightened predation risk (Magnhagen 1991), reduced feeding (Rowe 1994), increased injury (Chapman et al. 1995; Blanckenhorn et al. 2002; den Hollander and Gwynne 2009), and disease transmission (Thrall et al. 2002). Understanding the maintenance and high prevalence of this behavior has become an area of great interest for behavioral ecologists.

The fitness advantages that females derive from mating with multiple males are broadly classified as direct or indirect benefits. Direct, phenotypic, benefits are the result of nongenetic quantities that have a positive impact on the survivorship and offspring production of the female. Such benefits can include enhanced paternal care (Davies, 1992), the transmission of antipredator defensive compounds (Gonzalez et al. 1999), and the acquisition of nutrient rich nuptial gifts (Thornhill and Alcock 1983; Gwynne 1984). Lifetime egg production in insects tends to increase with an additional number of mates (reviewed in South and Lewis 2011); moreover, the usual costs of multiple mating may be greatly reduced or completely eliminated in species with nuptial gift-giving (i.e., male transfer of nongenetic material that increases female fitness). Indirect, genetic, benefits are those that are reaped in the next generation in the form of increased survivorship and mating success of sons and daughters (Andersson 1994). In many cases where females mate multiply, researchers have found evidence that sperm from the highest quality mate is used preferentially; this postcopulatory selection leaves open the possibility that females can accrue direct benefits from multiple males while having offspring with the highest genetic quality (Birkhead and Møller 1995; Keller and Reeve 1995; Eberhard 1996; Slatyer et al. 2011).

Utetheisa ornatrix is an arctiid moth that relies on plant-derived compounds for both defense and communication, and the complexity of the reproductive strategies of both sexes have made it a model system for studying sexual selection. *Utetheisa* larvae feed on plants of the genus *Crotalaria* (Fabaceae), which contain toxic pyrrolizidine alkaloids (PAs) that play a fundamental role in the life of both sexes (Eisner and Meinwald 2003). Larvae sequester PAs and retain them through metamorphosis into adulthood. Adult males invest PA along with other nutrients and sperm in spermatophores that can weigh up to 11% of their body mass (LaMunyon and Eisner 1994). Females allocate PA to the eggs, using not only some of the PA that she herself sequestered but also of the PA that she receives from the male via the spermatophore at mating (Dussourd et al. 1988). As a result, all life stages are rendered unpalatable to a variety of natural enemies (e.g., Eisner T and Eisner M 1991; Hare and Eisner 1993; Dussourd et al. 1988).

Females are highly promiscuous over their 3- to 4-week lifespan, taking an average of 11 and as many as 22 mates, each of

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whom delivers a substantial spermatophore containing both genetic (sperm; LaMunyon and Eisner 1993) and nongenetic material (nutrients and PAs; Dussourd et al. 1988; LaMunyon 1997). Females choose males based on a courtship pheromone, hydroxydanaidal (HD), that is derived from defensive PAs acquired during the larval stage and is contained in a pair of brush-like glandular structures (coremata) that evert during courtship (Conner et al. 1981; Dussourd et al. 1991). Previous experiments independently manipulating PA, HD, and body size demonstrated that HD is the only criterion of mate choice used by females (Iyengar et al. 2001). Earlier work with virgins showed that the male's HD titer correlates positively with both the male's systemic PA content and the amount of PA that the male transmits to the female as a gift, a result leading to the postulation that the female might use the male's HD titer as a parameter for gauging genetic or phenotypic quality (Dussourd et al. 1991). Furthermore, because the male's PA content also correlated with male body mass (as well as mass of spermatophore transferred), it seemed that, through assessment of the male's HD, female could gauge male size (Conner et al. 1990; Iyengar and Eisner 1999a) This is important because both male body size and female mating preferences are heritable in *U. ornatrix* (Iyengar and Eisner 1999a; Iyengar et al. 2002). The pheromonal scent, therefore, could provide the female with a means for assessing, on the one hand, the direct phenotypic benefits that she might accrue from a male (i.e., the amount of nongenetic material including PA and nutrient contained in his spermatophore) and on the other, the indirect genetic benefits (by favoring large males the female could assure that she produced larger sons more successful in courtship and larger daughters able to produce more eggs; Iyengar and Eisner 1999b; Eisner and Meinwald 2003). In other words, female choice based on the male pheromone allows her to profit on multiple levels because HD has the potential to represent both phenotypic and genetic quality.

The data described above, pertaining to female choice in *U. ornatrix*, were obtained using virgin males. The correlation between male body mass, spermatophore mass, body PA content, and HD titer was descriptive of newly emerged males yet to invest in reproduction—that is, adults still in full possession of the entire alkaloidal and nutritive reserves acquired as larvae. However, because alkaloid and nutrients cannot be replenished as adults, it is important to understand the relationship among these parameters in mated males, which are likely to comprise the majority of the population. Is there still a correlation between these parameters after the male has mated and lost some of his body mass and PA through mating (LaMunyon and Eisner 1994)? Specifically, we sought to determine whether HD titer in mated males remained a correlate of virginal PA and therefore a predictor of genetic quality or whether HD changes based on mating history.

A recent field study conducted at the Archbold Biological Station in Florida revealed that the male parameters of body mass, spermatophore mass, PA transferred in the spermatophore, and systemic PA content were all positively correlated; however, none were correlated to corematal HD content (Bezzerides et al. 2005). Assuming that most of these field males were not virgins, which seem reasonable given their apparent age (based on wing wear), there are some aspects of male signaling that remain unclear. As Bezzerides et al. (2005) were not able to control for the number of times males had mated, it is uncertain how the male's signal is affected by the number of matings. Is it possible that male's HD signal changes over time?

We here attempt to reconcile the lab data on virgin males and the field data on nonvirgins by determining whether the male's pheromonal (HD) signal changes over an individual's lifetime. Specifically, we examined the HD and PA levels of same-sized brothers of differing, yet controlled, mating history to determine the degree to which both quantities change over time. Determining whether HD levels remain constant over a male's life should reveal the primary benefits that the female obtains via mating with a previously mated male. For example, if pheromone titer decreases over time in proportion to the male's systemic PA (and PA gift-giving capacity), then it would suggest that the male is advertising his current phenotype and that the female, by selecting males based on HD, receives direct phenotypic benefits. On the other hand, if the pheromone remains constant over the male's lifespan and never decreases, then HD may represent the male's original body size, a heritable trait that is also correlated with the larval acquisition of PA (Iyengar and Eisner 1999a; Del Campo et al. 2005). Another possibility is that males low in systemic PA misrepresent themselves in courtship by producing exaggerated levels of HD that would, in turn, increase their chances of securing a mating. If we found an increase in HD only among males low in PA, then there would be evidence for such a conditional strategy similar to the "last ditch effort" strategy seen in sticklebacks (Candolin 1999) or the terminal investment in male sex pheromones by mealworm beetles (Sadd et al. 2006). In general, one expects signals to be an honest representation of quality—either phenotypic or genetic—if they are costly (Zahavi 1975; Grafen 1990). Given the overwhelming positive effects on mating success of producing as much HD as possible (HD is the sole criterion of choice; Iyengar et al. 2001), the fact that virgin males on average convert less than 4% of their PA to HD suggests a cost associated with HD production that makes cheating unlikely. We investigated the relationship between HD and PA among mated males to gain insight into the selective forces driving the evolution and maintenance of female choice in *U. ornatrix*.

MATERIALS AND METHODS

Moth colony

All *Utetheisa* moths were reared in the laboratory at Villanova University. The colony was derived from the stock collected at the Archbold Biological Station in Lake Placid, Florida, in accordance with methods described by Conner et al. (1981). All larvae were fed a pinto bean-based diet that was supplemented with seeds of *Crotalaria spectabilis* (10% "spectabilis" diet), the major food plant of *Utetheisa*, which provides the larvae with the alkaloid, monocrotaline. To standardize the larval environment, larvae were maintained at the same density in each container and provided the same quantity of artificial diet. Adults were given access to water only. Mating pairs were used to establish distinct families that gave rise to groups of full siblings.

Experiment 1: relationship of HD and PA among unrelated virgins and size-matched brothers

To compare our results for quantification of HD and PA with those from the literature (Dussourd et al. 1991), 36 unrelated virgin males were analyzed for HD and PA on day 2 of their adult life cycle. Males were weighed on day 7 of the pupal stage and again just before extraction.

To determine that the relationships among PA, HD, and body size previously demonstrated for unrelated males (Dussourd et al. 1991) also held true for size-matched brothers, 24 pairs of size-matched virgin brothers were extracted and analyzed for PA and HD on day 2 after eclosion. Size matching (±5 mg) was based on 7-day pupal mass, and males were weighed again on day 2 prior to extraction. The average weight of adult brother pairs ranged from 59 to 112 mg. Each pair

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represented one family, and no families were represented more than once.

Experiment 2: effect of mating on HD and PA

To determine how mating affects HD and PA content of males, size-matched (7-day pupal mass within 5 mg) pairs of brothers in which each individual was randomly into the "virgin" or "mated" group (pairs ranged from 58 to 119 mg). Twenty-six pairs of brothers were used, and no families were represented more than once. Virgin males were weighed again on day 2 prior to extraction and mated males were weighed on day 2 and then again on the day after all matings were complete. Virgin brothers were naive and never interacted with a female. Virgins were sampled and analyzed on day 2. Mated brothers were isolated with 2 different virgin females everyday starting on day 2. We used PA-laden females to promote normal mating behavior. Although this meant we could not measure PAs in the spermatophore, previous work has demonstrated that PAs in the spermatophore are a direct reflection of the overall PAs in the body for both virgin and mated males (Iyengar 2001). Males were observed courting (via visual inspection for corematal eversion) in all pairings, and mating was confirmed by observation (the extra unmated female was removed once a pair was in copula). Males were given new females and an opportunity for mating everyday, allowing males to complete 1–4 successful matings. We could not assign males to treatment groups based on the number of matings beforehand due to the extreme variability in male promiscuity (Iyengar and Reeve 2010), which would have subsequently compromised our sample size. However, because females are in control of mating (males cannot force copulation; Conner et al. 1981) and female promiscuity also has a heritable component (Iyengar and Reeve 2010), the randomization of potential female partners to each male, in conjunction with ensuring that each male courted, effectively means that males were randomized with respect to their mating category (i.e., the number of matings). Furthermore, because all males were confirmed to have courted each day, there were no observable differences in vigor among the males. Pairs were placed together for about 18 h, and copulation typically took place for 8–12 h. Mated males were sampled and analyzed on completion of all copulations.

HD analysis

The HD titer retained by males was determined from whole coremata that were destructively sampled. Both male coremata were excised from each moth with forceps and frozen (−20°C) until analysis. Pairs of coremata were added to a 400-µl vial insert with 150 µl of methylene chloride. Additionally, 50 µl of an internal standard (220 ppm benzophenone in methylene chloride) and a micro-stir bar were added to each vial insert. Samples were extracted overnight with stirring and then analyzed by gas chromatography with flame ionization detection (GC/FID) on a Hewlett Packard 5890 gas chromatograph (Bezzerides 2004). Two microliters of the HD extract in chloroform were injected in the splitless mode (injector temperature 250°C) onto a 5% phenyl polydimethylsiloxane fused silica capillary column (0.25 mm inner diameter, 30 m length, and 0.25 µm phase thickness), using helium as the carrier gas at 1.0 ml/min. The oven was programmed at 50°C for 2 min and ramped to 250°C at 20°C/min. The retention times for HD and benzophenone were 6.04 and 7.2 min, respectively. HD was synthesized using a previously reported method from monocrotaline starting material (Bell and Meinwald, 1986). HD purity was found to be >99% by Gas chromatography–mass spectrometry. An internal standard calibration curve using benzophenone was prepared that spanned the range of HD peak areas observed in the extracts analyzed.

PA analysis

PAs were quantified in whole adults minus coremata. Adults were macerated in an Eppendorf tube with an Eppendorf fitted pestle in 0.5 ml of 0.1% formic acid and added to 3 ml of aqueous 0.1% formic acid. The pestles were rinsed with an additional 0.5 ml of 0.1% formic acid, and the Eppendorf tubes were rinsed with 1.0 ml 0.1% formic acid into the vial. Five milliliters of chloroform was added to each vial to remove nonpolar compounds. Vials were shaken and then stirred for approximately 24 h. The vials were then centrifuged for 10 min at 4500 rpm. One milliliter of the upper aqueous layer was added to 9 ml of 0.1% formic acid. A zinc reduction was performed by the addition of granular zinc (JT Baker) with stirring for 3 h to convert the *N*-oxide forms of PAs to the free base form. Approximately 0.5 ml of this solution was filtered with a 0.22 µm syringe filter into a 400 µl glass insert for analysis. PA was analyzed using flow injection-tandem mass spectrometry (FI-MS/MS). PA extracts (10 µl) were injected using a Shimadzu Prominence LC system into a 50/50 (methanol/water 0.1% formic acid) flowing at 0.5 ml/min. PA was ionized using electrospray ionization (ESI) in the source of an Applied BioSystems API 2000 triple quadruple mass spectrometer. ESI source voltages, temperatures, and gas flows were optimized using pure monocrotaline. Monocrotaline (PA) was purchased from Sigma-Aldrich and was used as received. Monocrotaline was identified and quantified by multiple reaction monitoring (MRM). MRM isolates the 326 *m/z* ion precursor (protonated monocrotaline) in the first quadrupole (Q1), fragments the precursor in Q2, and isolates the120 *m/z* ion in Q3 for quantification. The 326/120 *m/z* MRM analysis provides both selective and sensitive detection of monocrotaline, and therefore, chromatographic separation was not necessary for monocrotaline identification and quantification. An external standard calibration curve was prepared that spanned the range of monocrotaline observed in the extracts analyzed.

Statistical analyses

Model I regressions were used to determine the relationship between total content of PA and levels of HD for unrelated virgin males. Intraclass correlations were used to determine the relationship for both PA and HD among size-matched brothers as this does not require the males be placed in arbitrary categories. In addition, model II regressions created a common slope for both relationships, and these slopes were compared with a slope equal to 1 with chi-square tests. Paired *t*-tests compared size-matched virgin and mated brothers for PA and HD levels. Because data from the 3 experiments were normally distributed (Shapiro–Wilk, $P > 0.10$ for all parameters measured), parametric statistics were used for all analyses. All analyses were performed in JMP IN 4.0 (SAS Institute 2001).

RESULTS

Experiment 1: relationship of HD and PA among unrelated virgins and size-matched brothers

The male's systematic PA content had a positive linear association with the male's HD titer for unrelated virgins ($F_{1,34} = 77.08$, $r^2 = 0.694$, $P < 0.001$; Figure 1a). In addition, for same-sized virgin brothers, total PA content was positively correlated (ICC = 0.90, $N = 24$, $P < 0.001$; Figure 1b) and levels of HD were positively correlated (ICC = 0.97, $N = 24$, $P < 0.001$; Figure 1c) between Brother 1 and Brother 2. Model II

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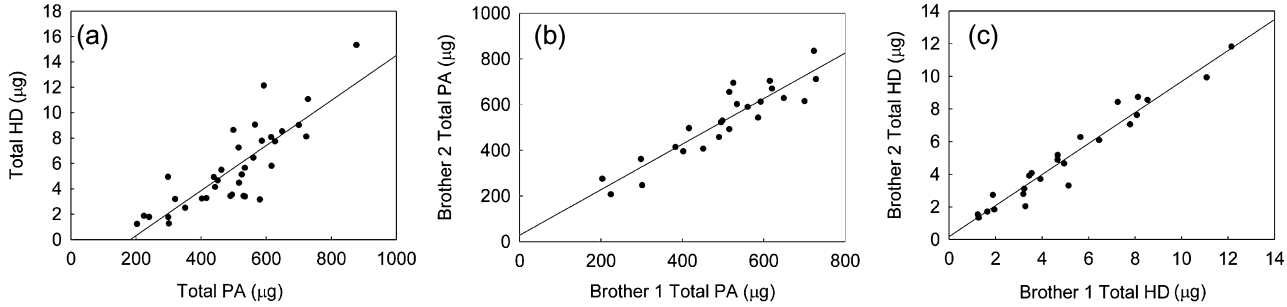

Figure 1
(a) A Model I regression of nonbrother virgin total PA (μg) and total HD (μg); (b) the relationship of total PA (μg) for 24 pairs of same-sized virgin brothers; (c) the relationship of total HD (μg) for 24 pairs of same-sized virgin brothers.

regression analyses provided a common slope for the 2 possible lines (Brother 1 on Brother 2 and Brother 2 on Brother 1) for both PA and HD analyses (Warton and Weber 2002). The slopes of the common line for both PA and HD were not significantly different than one, indicating that same-sized brothers have the same amounts of PA and HD on eclosion (PA: $\chi^2_1 = 1.9$, $P = 0.170$; HD: $\chi^2_1 = = 0.5$, $P = 0.49$). Therefore, we can assume that initial PA and HD will be matched in sibling pairs used in Experiment 2.

Experiment 2: effect of mating on HD and PA

Nine males mated twice, 13 males mated 3 times, and 4 males mated 4 times. Mated brothers weighed significantly less than their virgin brothers (paired $t_{25} = 11.494$, $P < 0.001$), despite having similar weights prior to mating (paired $t_{25} = 0.838$, $P = 0.775$), confirming that spermatophores were successfully transferred to females during copulation. Mated males had significantly less PA than their virgin brothers (paired $t_{25} = 6.533$, $P < 0.001$; Figure 2a), which is the expected result of spermatophore transfer. However, there was no significant difference in HD titer between mated and virgin brothers (paired $t_{25} = 0.682$, $P = 0.501$; Figure 2b). Both pupal mass and virgin mass show significant positive linear associations with postmating HD content (pupal mass: $F_{1,24} = 29.85$, $r^2 = 0.554$ $P < 0.001$; virgin mass: $F_{1,24} = 19.85$, $r^2 = 0.453$ $P < 0.001$). However, there was a positive linear association between PA and HD for mated males ($F_{1,24} = 5.94$, $r^2 = 0.198$, $P = 0.023$), just as there was for their virgin brothers ($F_{1,24} = 45.16$, $r^2 = 0.653$, $P < 0.001$). An analysis of covariance comparison of the slope of HD against PA for mated brothers to the slope for the virgin brothers indicated that the 2 slopes were not different ($F_{1,48} = 10.62$, $P = 0.354$).

DISCUSSION

These experiments successfully reconciled laboratory data on the PA and HD content of virgins with field data on nonvirgin males using controlled matings and chemical methods. Previous studies with laboratory virgins demonstrated that HD was positively correlated with many aspects of male quality, including body size, spermatophore size, and PA load (Dussourd et al. 1991), whereas the HD of field-collected males was not correlated to any of these parameters (Bezzerides et al. 2005). We expected that HD would not change with mating history because the coremata are everted for milliseconds at a time, suggesting that there is not much HD loss during courtship (Conner et al. 1981). Furthermore, unlike many lepidopteran pheromones, including that from female *Utetheisa*, male HD is not particularly volatile, as evidenced by the fact that males court females in close proximity to the female's antennae (without making contact; Schultz 2009). Our results show that HD does not change with respect to mating history, thereby providing evidence that HD may represent the male's genetic quality. Because the HD signal does not appear to change over their lifetime, males are always advertising at a level correlated with original size and PA content, and thus, there is no evidence that HD represents the male's direct

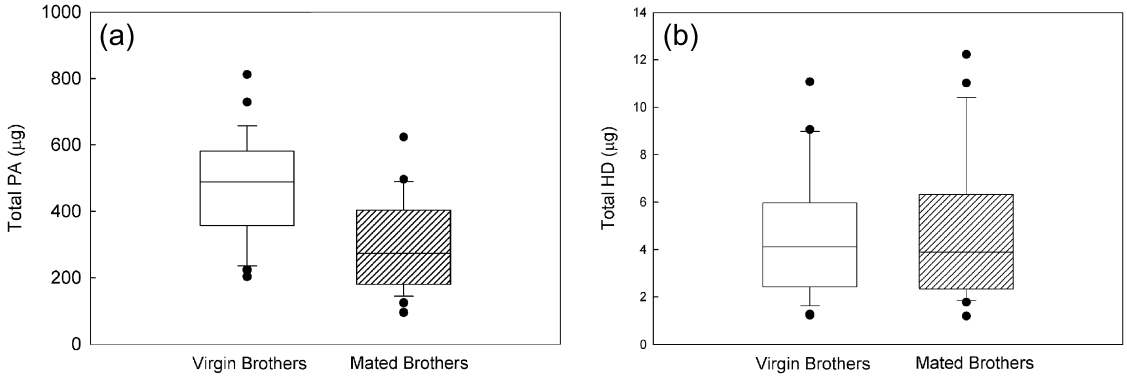

Figure 2
(a) Comparison of the amount of PA (μg) in size-matched virgin and mated brothers. Median + interquartile range (IQR) = 488.5 + 205.6 μg for virgin brother and 273.5 + 209.9 μg for mated brother; (b) comparison of the total HD (μg) titer in size-matched virgin and mated brothers. Median + IQR = 4.1 + 3.29 μg for virgin brother and 3.9 + 3.8 μg for mated brother. The horizontal line is the median, the upper and lower edges of the boxes are the 25th and 75th percentiles, and the lines indicate the 10th and 90th percentiles. Points beyond the lines are outliers.

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benefits (e.g., PA nuptial gift). Also, there is no support for the hypothesis that the HD signal is subject to artificial inflation (i.e., cheating) because HD levels did not increase disproportionally to PA. The lack of an increase in HD during adulthood also provides evidence that the pheromone is not synthesized after eclosion, although this supposition has yet to be empirically tested. Nevertheless, our results show that females, by choosing males based on HD only (Iyengar et al. 2001), receive indirect benefits by selecting mates of higher genetic quality.

Given the unchanging HD signal and its correlation with the male's body size and PA content at eclosion, female choice is ultimately based on male parameters that reflect larval feeding. Larvae obtain the majority of their PA when feeding on *Crotalaria* pods, where PA is most concentrated. Individual larvae often take up residence in an entire pod and larvae must thus compete with one another for this valuable resource. Those larvae that were unable to claim a pod must instead obtain PA from leaves and will not sequester the same high quantity of PAs as those feeding on the seeds (Conner et al. 1990). A male's original body size, as advertised by HD, is therefore an indicator of larval success that is likely to have a genetic component because offspring may inherit genes that enable them to outcompete other larvae. Although this is critical for both sexes, it is especially important for the male larvae to have greater access to PA so that they can grow larger (PA is a phagostimulant that increases feeding rate; Del Campo et al. 2005) and produce a larger HD signal as adults (thereby increasing mating success; Iyengar et al. 2001). Our results support previous studies that females could be using HD as an indirect assessment of the male's original body size, a heritable trait with known fitness consequences for her offspring (Iyengar and Eisner 1999a, 1999b).

We understand that using body size to represent quality may have limitations given that such correlations between a single trait and total fitness may be undermined life history tradeoffs and genotype-by-environment interactions (Hunt et al. 2004). Although all these aspects have not been explicitly tested in *U. ornatrix* to address those concerns, we know that females mating with larger males have an accelerated rate of oviposition (Del Campo et al. 2005) and lay larger eggs that develop faster (Iyengar and Eisner 2002). Indeed, previous research on this moth suggests a multitude of benefits to large size as this single fitness component appears to be correlated with total lifetime reproductive success in *U. ornatrix*. By selecting for larger males, females receive genes for large body size that confer reproductive benefits for all her offspring as larger daughters are more fecund and larger sons sire more offspring (Iyengar and Eisner 1999b). Overall, it was demonstrated that *U. ornatrix* females, by mating with a male based on his pheromone levels, will have 25% more grandoffspring by mating with a male that is 10% larger due to both direct and indirect benefits (Iyengar and Eisner 1999b). This strong directional selection raises the question of what is maintaining heritable genetic variation, which has been demonstrated to be a relatively common phenomenon with many potential explanations (reviewed in Andersson and Simmons 2006). Although such questions remain unanswered in *U. ornatrix*, we suspect that smaller males can and do achieve paternity, by flying faster, being more maneuverable in courtship, or living longer. Regardless of such unresolved issues, previous research suggests that pheromone levels, insofar as they represent male body size, constitute a useful proxy for male quality in *U. ornatrix*.

Females mate multiply in *Utetheisa*, which is not surprising given their ability to gain PAs and nutrients with each mating while having a single sire of high quality (LaMunyon and Eisner 1993). Given female polyandry and known mating frequency in both laboratory and field settings (LaMunyon and Eisner 1993; Iyengar and Reeve 2010), we can infer that males also mate multiply. Evidence from field caught males suggests that most males have transferred spermatophores, as indicated by variation in mass beyond the expected spontaneous mass loss (Bezzerides et al. 2005). Likewise, laboratory studies show that males will mate for 6 consecutive nights and do not appear limited by size or sperm availability (Iyengar and Reeve 2010). This suggests that females in the field will more frequently encounter males who have mated several times relative to virgin males. When mating with virgin males, females are able to maximize direct benefits in addition to indirect benefits as those males are able to provide relatively higher quantities of both nutrient and alkaloid in the nuptial gift. Although it is common for lepidopteran females to prefer males that can provide greater direct benefits in their nuptial gifts (reviewed in Torres-Vila and Jennions 2005), such opportunities are likely to be rare for *U. ornatrix* in nature. Furthermore, whereas virgin males can produce a spermatophore over one-tenth of their total mass, mated males require 6–7 days between matings to produce a comparable spermatophore (LaMunyon and Eisner 1994). Given that males are willing to mate daily (based on corematal eversion; Iyengar and Reeve 2010), at which time they can only deliver a sub-sized spermatophore with fewer nutrients and alkaloid, our results indicate that the pheromonal signal of mated males is an honest indicator of original body size and PA, not current body size and PA. *U. ornatrix* are by no means the only lepidopteran in which females appear to be selecting males based on indirect benefits; for example, in the lekking arctiid moths *Creatonotos transiens* and *C. gangis*, males also require larval access to PAs to produce HD (Schneider et al. 1982) and females use HD as an indicator of larval diet and thus genetic quality.

Overall, our finding that the pheromone signal did not change with male mating history suggests that HD represents original PA levels, which are likely to reflect a genetically based ability to acquire and sequester chemicals important in defense and reproduction. By selecting males based on HD titer, females are able to produce offspring that have higher mating success (Iyengar and Eisner 199b). These data support the hypothesis that the HD signal serves, at least in part, as an honest indicator of genetic quality and maintains female preference for a larger HD signal. For many organisms, it is still unclear whether phenotypic or genetic benefits drive female choice. Previous work suggests that direct selection is more important than indirect selection in the evolution of choice when both occur simultaneously (Kirkpatrick and Barton 1997) and that the evolutionary maintenance of polyandry is driven by direct benefits (Arnqvist and Nilsson 2000). Our research indicates that, by teasing apart genetic and nongenetic benefits, indirect selection may also play an important role in the maintenance and evolution of female choice.

Ethical Standards

The authors declare that these experiments comply with the current laws of the country in which they were performed, the United States of America.

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