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Paternal inheritance of a female moth's mating preference

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Females of the arctiid moth *Utetheisa ornatrix* mate preferentially with larger males, receiving both direct phenotypic and indirect genetic benefits¹. Here we demonstrate that the female's mating preference is inherited through the father rather than the mother, indicating that the preference gene or genes lie mostly or exclusively on the Z sex chromosome, which is strictly paternally inherited by daughters. Furthermore, we show that the preferred male trait and the female preference for that trait are correlated, as females with larger fathers have a stronger preference for larger males. These findings are predicted by the protected invasion theory^{2,3}, which asserts that male homogametic sex chromosome systems (ZZ/ZW) found in lepidopterans and

birds promote the evolution of exaggerated male traits through sexual selection. Specifically, the theory predicts that, because female preference alleles arising on the Z chromosome are transmitted to all sons that have the father's attractive trait rather than to only a fraction of the sons, such alleles will experience stronger positive selection and be less vulnerable to chance loss than would autosomal alleles.

The benefits accrued by female *Utetheisa* as a result of mating preferentially with larger males have been characterized. The phenotypic benefits take the form of nutrient and pyrrolizidine alkaloid transmitted seminally by the male to the female in quantities proportional to his size^{4–6}. The nutrient enables the female to increase her egg production⁷, and the alkaloid bestows chemical protection upon herself⁸ and her eggs^{1,5}. The genetic benefits, realized as a consequence of the heritability of body size⁹, ensure that females, by choosing larger males, have larger sons which are themselves more likely to be favoured in courtship, and larger daughters bound to be more fecund¹. The cumulative effect of these benefits is substantial: a female given a choice between males differing by 10% in body mass will have an estimated 25% more grandchildren by mating with the larger male. The strong selection for large males in *Utetheisa* may account for why in this species, contrary to the norm for Lepidoptera¹⁰, males are larger than females¹¹.

Sexual selection models generally assume heritable variation in both the trait and the mating preference, and a genetic correlation between trait and preference^{2,13}. In *Utetheisa*, the male trait (body size) is inherited from both parents⁹, but whether the female preference itself is genetically variable and a correlate of the male trait was unknown.

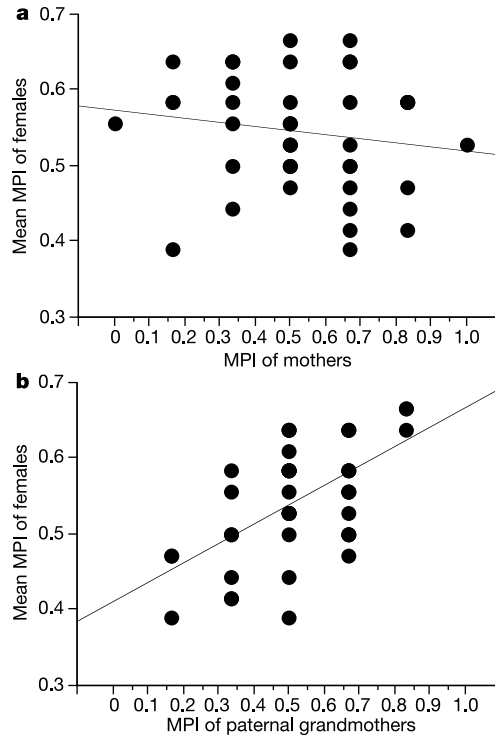


Figure 1 Mean mating preference index (MPI) of females (six full sisters) plotted as a function of the mating preference index of their mother and paternal grandmother ($n = 44$). **a**, The MPI values of females and their mothers are not correlated ($r^2 = 0.025$, $P = 0.305$, $y = 0.574 - 0.054x$), indicating that the mating preference is not inherited via the mother. **b**, The MPI values of females and their paternal grandmothers are positively correlated ($r^2 = 0.333$, $P < 0.0001$, $y = 0.41 + 0.257x$), indicating that the mating preference is paternally inherited by daughters [$h^2 = 0.513 \pm 0.112$ (mean \pm s.e.)].

First, we determined whether the genetically variable components of the female mating preference are inherited through the mother, the father, or both parents. To assess inheritance through the father, who does not express the female mating preference, we focused on the transmission of the mating preference from the female's paternal grandmother. We examined inheritance patterns by calculating mating preference indices (MPIs) for three generations of females ($n = 44$ families), as follows. We subjected each female to six sequential two-male choice tests, scoring the outcome of each test as either 0 (if the smaller male mated) or 1 (if the larger male mated). The resulting six values were then averaged to determine each female's MPI score. To estimate heritability of the mating preference—that is, the proportion of phenotypic variance attributable to additive genetic effects—we regressed female MPIs on paternal grandmother MPIs and mother MPIs to determine whether mating preferences are inherited from the paternal grandmother (an indication of inheritance through the father) and/or the mother.

The MPIs of females did not correlate with those of their mothers, indicating that mating preferences are not inherited from the mother (Fig. 1a). However, the MPIs of females and their paternal grandmothers were significantly positively correlated, and the regression slope was significantly greater than zero (Fig. 1b). Moreover, the regression slopes of female–mother and female–paternal-grandmother MPI values were significantly different (analysis of covariance (ANCOVA), $F_{1,84} = 15.832$, $P < 0.0001$). These results indicate that variable female mating preference genes are inherited by daughters primarily from the father. Because the preference is sex-linked and females inherit one-half of their sex chromosomes from their paternal grandparents (see Fig. 2), heritability from the paternal grandmother was taken to be two rather than four times the regression slope¹⁴.

Second, we examined female MPI values relative to their father's body size to determine whether there was a genetic correlation between the female mating preference and the male trait. We found a significant positive correlation between female MPIs and their father's body size (Fig. 3), that is, daughters of larger fathers have a stronger preference for larger males. In contrast, there was no correlation between a female's MPI and her mother's or paternal grandmother's body size (female–mother: $r^2 = 0.008$, $n = 44$, $P = 0.56$; $y = 0.507 + 0.0004x$; female–paternal grandmother: $r^2 = 0.023$, $n = 44$, $P = 0.33$; $y = 0.593 - 0.001x$). Furthermore, a female's mating preference was not related to her own body size ($r^2 = 0.043$, $n = 44$, $P = 0.18$; $y = 0.422 + 0.001x$), as larger females were not choosier than smaller females. The positive genetic correlation between the female preference and the male trait in *Utetheisa* is required by the two major alternative models of indirect

sexual selection (fisherian and 'good genes')¹³.

Recent studies of mating preferences focused on the inheritance and sex-linkage of male traits and left unanswered the question of the chromosomal location of the gene(s) that encode the mating preference^{15–19}. *Utetheisa*, like all lepidopterans, has a 'reversed' genetic architecture (ZZ/ZW) where males are homogametic (ZZ) and females heterogametic (ZW). This sex determination system, coupled with the observed inheritance patterns for the female preference genes, that is, the daughter's receipt of such genes from the father only, permits assignment of the preference genes of *Utetheisa* to the Z chromosome. There is no evidence for the autosomal location of preference genes, as indicated by the lack of heritability between mother and daughters. In contrast, the male trait (body size) is inherited from both parents equally⁹, indicating primarily autosomal genetic determination. Given that arctiids have, on average, 31 pairs of chromosomes²⁰ and that sex chromosomes in Lepidoptera are usually comparable in size to autosomes²¹, the probability of all mating preference genes being on the Z chromosome by chance is small (about $(1/62)^n$, where n is the number of genes). Thus, we conclude that the occurrence of the female mating preference gene(s) on the Z chromosome, instead of autosomes, is the consequence of a nonrandom, underlying evolutionary process.

The strong evidence for Z-linked, and not autosomal, female preference gene(s) supports the protected invasion theory, which predicts that mutant female preference genes arising on the Z chromosome will be especially protected from random loss when rare (H.K.R. and D.W. Pfennig, unpublished work). This protection arises from the fact that females transmit a Z-linked female preference gene to all their sons, at least half of which also receive the male trait that is the target of the preference. Sons with the male trait have enhanced mating success, and the enhanced mating success of the sons who also possess the female preference gene will cause that gene to rise in frequency in the next generation, reducing the probability of its random loss from the population. In contrast, no sons are guaranteed possession of both the female preference gene and the male trait gene when genes are X-linked, and only a quarter of sons have such assurance when genes are autosomal (H.K.R. and D.W. Pfennig, unpublished work). Thus, the protected invasion theory predicts that species with Z-linked genetic systems, such as lepidopterans and birds, will be especially prone to evolve exaggerated male ornaments through sexual selection (H.K.R. and D.W. Pfennig, unpublished work). Z-linked mating preferences may in fact play a role in speciation itself, as female progeny of interspecific crosses between two species of sexually dimorphic *Colias* butterflies have been shown to prefer males of the paternal species²². Thus, the Z-linkage of female mating

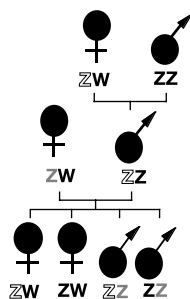


Figure 2 Inheritance of a Z-linked mating preference. The white Z represents the sex chromosome bearing the preference gene(s), shown here to be introduced into the lineage by the paternal grandmother. The grey Z is that possessed by the mother but not transmitted to daughters. Therefore, granddaughters can only acquire Z-linked gene(s) by way of their father.

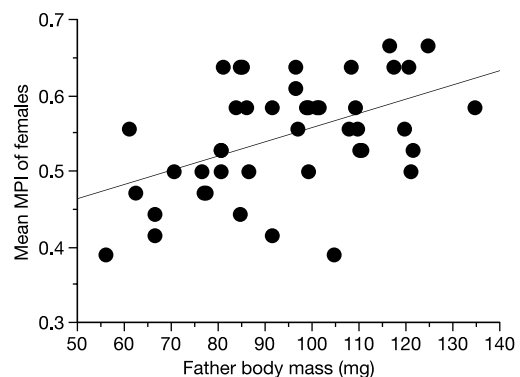


Figure 3 Correlation between mean mating preference index (MPI) of females (six full sisters) and the body mass of their father ($n = 44$). Female MPI values and their father's body mass were positively correlated ($r^2 = 0.232$, $P < 0.001$).

preferences is potentially a general phenomenon in the Lepidoptera. What we show here for *Utetheisa*, and attribute to genetic architecture, might in fact apply beyond lepidopterans and birds to any group that exhibits male homogamy. The protected invasion theory, therefore, might be invoked broadly to account for taxonomic variation in the degree to which sexual dimorphism is manifested in nature. □

Methods

Utetheisa rearing and mating

Larvae were reared as previously described²³ on a pinto-bean-based diet supplemented with seeds of *Crotalaria spectabilis*, a natural food plant of *Utetheisa*. For any set of progeny, two groups of 8–10 larvae each were raised under identical conditions to adulthood. As in earlier studies²⁴, all matings involved presenting females with a choice of two males for 24 h in humidified cylindrical containers (0.35 l). For the present purposes, the males were related neither to the female nor to each other. Males and females were of known body mass at the time of mating, and the males were all 3-day-old virgins. Events in the mating enclosures were monitored on an ongoing basis for the first hour (to ensure that both males actively courted) and then at intervals of 6 h (to check on the occurrence of mating). A record was kept of the male that mated (the males were wing-marked for recognition purposes).

The experimental protocol required that individual females be presented daily with an unfamiliar pair of males until they mated a total of six times. The two males in the pair initially offered were chosen such that, in a randomly selected half of cases, they differed by 5% in body mass, and by 10% in the remaining cases. Subsequent presentations to any one female were dependent on whether the female mated. If she did not mate, she was next presented males showing the same mass difference; if she did, her next choice was between males of the alternative mass difference.

Mating Preference Index

For experimental purposes, we raised three generations of 44 families (derived from wild *Utetheisa* females caught in Highlands County, Florida, USA). Within each family, mating preferences were assessed for six full sisters, their mother and their paternal grandmother. The mating preference index (MPI) was calculated for each female as the average of her six mating choices. Individual choices were scored as 0 if the female favoured the smaller male, and as 1 if she chose the larger male. MPI values therefore fell within the range of 0 to 1.

Statistical analyses

To estimate heritability (in the narrow sense)¹⁴, we regressed the average MPI value of six full sisters on the MPIs of their mother and paternal grandmother. A significant positive correlation was evident in the female–paternal-grandmother regression only, indicating that the mating preference is sex-linked rather than autosomal. For calculation of heritability, therefore, the slope of the regression was multiplied by two rather than four because females share half of their sex chromosomes, and only a quarter of their autosomes, with their paternal grandparents¹⁴. The data were not transformed because they met the assumptions of normality (normal probability plot) and linearity (Lewontin test). Furthermore, there was no need to adjust regression coefficients or standard errors for unequal variances because the variances were the same for mother and paternal-grandmother MPI values (two-tailed variance ratio test, $F = 1.728$, $P = 0.08$). Heritabilities (regression slopes) were compared using ANCOVAs¹⁴.

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Detecting recent positive selection in the human genome from haplotype structure

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The ability to detect recent natural selection in the human population would have profound implications for the study of human history and for medicine. Here, we introduce a framework for detecting the genetic imprint of recent positive selection by analysing long-range haplotypes in human populations. We first identify haplotypes at a locus of interest (core haplotypes). We then assess the age of each core haplotype by the decay of its association to alleles at various distances from the locus, as measured by extended haplotype homozygosity (EHH). Core haplotypes that have unusually high EHH and a high population frequency indicate the presence of a mutation that rose to prominence in the human gene pool faster than expected under