Nonantagonistic interactions between the sexes revealed by the ecological consequences of reproductive traits

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Abstract

In addition to the obvious role reproductive traits play in mating-system evolution, reproductive characters can also have critical ecological or life history consequences. In this study we examine the ecological consequences of mating for female cactophilic *Drosophila* to test different hypotheses about the processes driving divergence in reproductive characters. Comparisons between intra- and interpopulation matings suggest that population differences in mating benefits, namely increased desiccation resistance in mated females, is not solely attributable to either a male or female-specific reproductive trait. Instead, the results indicate that increased desiccation resistance is a product of a male–female postmating–prezygotic interactions. The results underscore that postmating–prezygotic interactions can serve as an arena for the evolution of male characters that confer substantial benefits to females, not just costs arising from sexual conflict. Variation in the relative benefits conferred by mating between intra- and interpopulation matings also suggests that the relationship between speciation and divergence in reproductive characters via male–female interaction will be difficult to predict.

Introduction

The forces that drive the evolution of characters involved in postmating–prezygotic interactions, like other reproductive traits (e.g. Emlen, 2001; Kurdziel & Knowles, 2002), are not limited to any single functional context (Eberhard, 1996; Cordero & Eberhard, 2003; Knowles et al., 2004). An association between mating costs and benefits suggests antagonistic interactions may be important; however, a mosaic of processes may actually mediate the evolutionary dynamics of postmating–prezygotic interactions. A correlation between male-mating advantage and male-induced harm to females (reviewed in Wolfner, 2002), by itself, does not exclude other explanations (Gavrilets & Waxman, 2002; Eberhard &

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Cordero, 2003) nor does it identify the actual evolutionary implications of such mating consequences (Partridge & Hurst, 1998).

Evidence of male-induced harm to females (Fowler & Partridge, 1989; Holland & Rice, 1999; Civetta & Clark, 2000) and the now frequently invoked process of antagonistic coevolution may be only one of several different mechanisms contributing to the evolution of reproductive characters involved in postmating–prezygotic interactions, such as male seminal proteins (Civetta & Singh, 1995; Swanson et al., 2001). For example, male desert *Drosophila* transfer enormous ejaculates, and the females of some desert *Drosophila* species actively incorporate the seminal products (Pitnick et al., 1999). A reproductive cost to females in *Drosophila mojavensis* and *Drosophila arizonae* (Knowles & Markow, 2001) suggests sexual conflict may play a role in the evolution of post-mating–prezygotic characters in these cactophilic species. However, reproductive interactions can also have important ecological and life history consequences

(e.g. Gross & Charnov, 1980; Civetta & Clark, 2000; Fu et al., 2001; Kurdziel & Knowles, 2002), and indeed this broader context revealed a beneficial effect of mating in these desert flies – increased desiccation resistance. The transfer of male seminal products to females in both *D. mojavensis* and *D. arizonae* increases female survival to desiccation stress by as much as 20 h, an increase of 62% (see Fig. 1, Knowles et al., 2004). Both species breed in necrotic tissue of specific columnar cacti endemic to the Sonoran Desert (Heed, 1978). Because the host cacti are patchily distributed and the breeding sites (i.e. necrotic patches) are both spatially and temporally infrequent (Breitmeyer & Markow, 1988), the flies are exposed to the harsh environmental conditions for extended periods during dispersal (Markow & Castrezana, 2000). The limited availability of resources coupled with the physiological stresses imposed by the desert environment suggests that such differences in desiccation resistance could indeed significantly impact female fitness in the field (Knowles et al., 2004).

In this study we take advantage of previously described population differences in the benefits mating confers to females to examine the processes driving divergence in the reproductive characters affecting the ecologically critical trait, desiccation resistance. We test three different hypotheses about how differences in the reproductive characters conferring desiccation resistance might have evolved in these desert *Drosophila*, specifically that this variation reflects differences in: (i) a female-specific character, such as the ability to take-up or utilize male seminal products, (ii) a male-specific character, such as the quality or quantity of ejaculates, or (iii) male–female, reproductive interactions. If either female or male-specific traits alone are responsible for observed population differences in the benefits conferred by mating (Knowles et al., 2004), then female desiccation resistance from intrapopulation matings should not differ from interpopulation matings. That is, the degree of desiccation resistance in mated females will not be sensitive to whether or not the males are from the same population (i.e. that males and females have coevolved). In contrast, patterns of desiccation resistance are predicted to differ between intra- and interpopulation matings if population differences in mating benefits (Knowles et al., 2004) reflects divergence in male–female reproductive interactions.

Methods

Virgin adults were collected following eclosion and stored in sex-specific, yeasted culture vials. Only sexually mature flies were used (i.e. flies of 9 days of age), and matings were performed in the morning, the typical mating time in natural populations. One female was aspirated into each culture vial with a male, and after copulation the male and female were transferred to separate vials. Twenty females from the intrapopulation matings, as well as twenty females from the corresponding
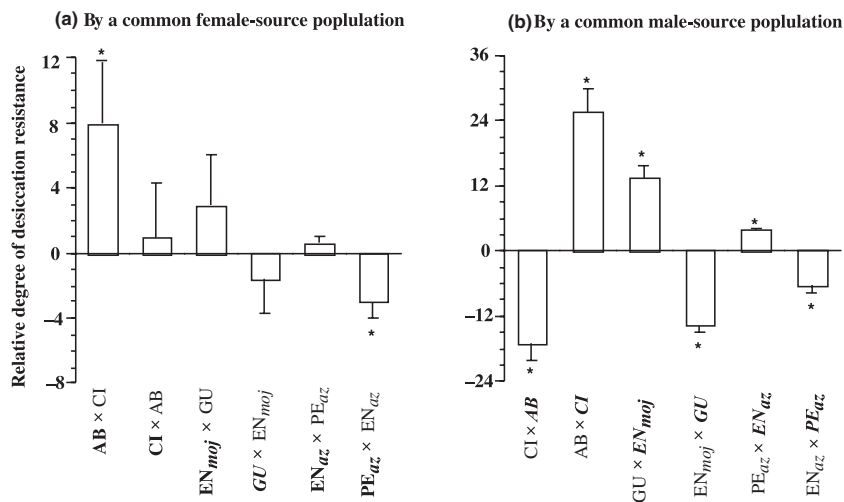
Fig. 1 Degree of desiccation resistance in females from reciprocal crosses relative to those from intrapopulation matings for the four different populations of *Drosophila mojavensis* (i.e. AB, CI, EN_{moj}, and GU) and two *Drosophila arizonae* populations (i.e. EN_{az}, PE_{az}). Contrasts between the intrapopulation mating (identified by the population name in bold and italicized) and reciprocal crosses according to a common source population for females are shown on the left (a), and for males on the right (b) (see Methods for details). The females' population is listed first and intrapopulation matings are scaled to 0 such that negative and positive values indicate greater desiccation resistance in intra- and interpopulation matings, respectively. If mating effects depend exclusively upon male or female-specific traits, there should be no difference between intra- and interpopulation matings (i.e. expected value is 0); significant differences between intra- and interpopulation matings are marked with asterisks.

interpopulation crosses, were placed in individual vials and housed in a desiccator where the relative humidity was maintained at 0%. Flies were checked hourly and the time of death was recorded for each fly.

Desiccation resistance was characterized for four *D. mojavensis* populations (AB = Anza Borrego, southern California; CI = Santa Catalina Island; GU = Guaymas, Sonora Mexico; EN_{moj} = Ensenada de los Muertos, Cape region of Baja California), two *D. arizonae* populations (EN_{az}; PE_{az} = Peralta Canyon, Arizona) (Knowles *et al.*, 2004), and reciprocal crosses between the AB and CI, and GU and EN_{moj} *D. mojavensis* populations, and between the two *D. arizonae* populations, PE_{az} and EN_{az} . All experiments were repeated four times for each intra- and interpopulation mating (i.e. a total of 80 mated females were examined in each of the different intra- and interpopulation matings).

A nested analysis of variance was used to test whether female desiccation resistance from intra- and interpopulation matings differed; this design was used to avoid confounding two separate sources of variation (i.e. the variation among inter- and intrapopulation mating type that is attributable to population differences). Separate analyses were conducted to test for a female and male-specific effect on desiccation resistance in mated females. To test for a female-effect, desiccation resistance in mated females from an intrapopulation mating (e.g. AB × AB, where the females' population is listed first) was compared only with interpopulation crosses involving females from that same population (e.g. AB × CI). Likewise, to test for a male-effect, contrasts of resistance in mated females included only those crosses where the males were from the same population as the intrapopulation mating (e.g. comparing AB × AB with CI × AB). Initial analyses determined there was no significant relationship between body size and desiccation resistance for mated or unmated females ($r^2 = 0.0002$, $n = 41$, and $P = 0.94$, $r^2 = 0.0002$, $n = 41$, and $P = 0.92$, respectively) (Knowles *et al.*, 2004). To correct for heteroscadasticity and non-normality, all data were transformed prior to analyses using a square-root transformation.

Spearman's rank correlation was also used to test for an association between the effects of intra- and interpopulation matings. To examine the effects of mating on female desiccation resistance specifically and to control for innate differences in desiccation resistance among populations (Knowles *et al.*, 2004) the residuals from a regression analysis of mated and unmated flies ($R^2 = 0.47$, $n = 46$, $P < 0.0001$) were used to test for a correspondence between the rank order of resistance among intra- and interpopulation matings. As females may incur benefits differently in intra- vs. interpopulation matings (especially if particular proteins evolved by a tight coevolution between males and females within populations), intra- and interpopulation matings were also analysed separately. Results from these separate regressions did not differ from the joint analysis of intra- and interpopulation matings, and are therefore not presented. The analyses were conducted on the combined data from *D. mojavensis* and *D. arizonae* because the average benefit of mating did not differ between the species ($F_{21} = 0.86$, $P = 0.36$). All analyses were carried out using the statistical program JMP (ver. 3.4.1, SAS Institute, 1996, Cary, NC, USA).
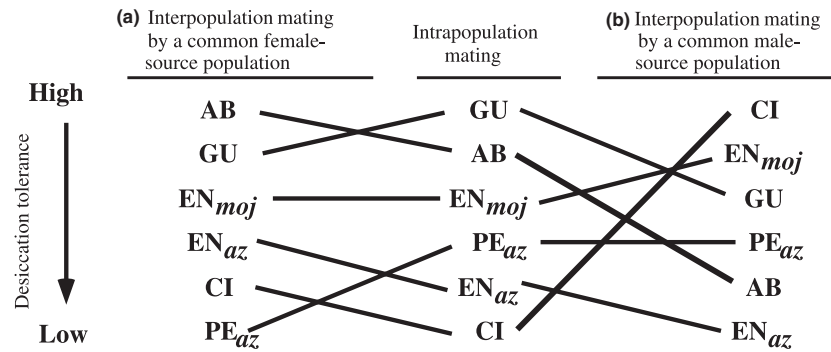
Results

Comparison between the desiccation resistance of females from intra and interpopulation matings showed that the mating effects differed significantly between intra- and interpopulation matings (Table 1). These significant differences were observed in comparisons based on a common female-source population (e.g. AB × AB vs. AB × CI, where the female's population is listed first) and a common male-source population (e.g. AB × AB vs. CI × AB).

There was also a significant difference among populations in the effect of mating in female desiccation resistance between intra- and interpopulation matings (Table 1). The increase in desiccation resistance conferred by intraspecific matings was greater than interpopulation matings in some populations (e.g. in females from AB), whereas females from other populations exhibited higher tolerance to desiccation stress in interpopulation matings (Fig. 1). Likewise, there was no correspondence between the rank order of populations by degree of desiccation resistance between the intra- and interpopulation matings (Fig. 2; $\rho = 0.65$, $n = 6$, $P < 0.17$ and $\rho = -0.14$, $n = 6$, $P < 0.78$ for comparisons

Table 1 Comparisons of desiccation resistance in females from intra- and interpopulation matings. A nested analysis of variance was carried out separately to test for female and male-specific effects (see Fig. 2).

Source	d.f.	F
By a common females' source population		
Mating type (population)	6	2.65*
Population	5	71.71**
Error	555	
By a common males' source population		
Mating type (population)	6	24.89**
Population	5	17.09**
Error	555	

Significant effects are marked with asterisks (*$P < 0.01$ and **$P < 0.001$). A significant mating-type effect indicates that desiccation resistance differed significantly between intra- and interpopulation matings; a significant population effect reflects differences in the resistance of mated females among the populations (i.e. AB, CI, EN_{moj}, GU, EN_{az}, and PE_{az}).

Fig. 2 The rank order of desiccation resistance of females among populations in intra- and interpopulation matings, where reciprocal crosses according to a common source population for females (a) are on the left, and those for a common source population for males (b) on the left. The crossing lines between populations from intra- and interpopulation matings indicate that desiccation resistance in females from intrapopulation matings does not correspond to either reciprocal cross.

according to a common females' and males' source population, respectively).

Discussion

If the varied mating effects among populations reflect differences in female-specific characters, such as the females' abilities to take-up or utilize male seminal products, the benefits conferred by mating will be similar irrespective of whether the female is mated with a male from a different population. Similarly, if divergence in male-specific traits (e.g. quantity or quality of the male ejaculate) is responsible, the benefits conferred by mating will be similar even if the female is from a different population. Contrary to these predictions, comparison between the desiccation resistance of females from intra and interpopulation matings showed that the mating effects differed significantly between intra- and interpopulation matings (Fig. 1). These significant differences were observed in comparisons based on a common female-source population (e.g. AB × AB vs. AB × CI, where the female's population is listed first) and a common male-source population (e.g. AB × AB vs. CI × AB) (Table 1), indicating that population differences in the benefits conferred by mating are not simply attributable to divergence in male or female-specific traits alone. Likewise, there was no correspondence between the rank order of populations by degree of desiccation resistance between the intra- and interpopulation matings (Fig. 2). These results support the hypothesis that male–female reproductive interactions contribute significantly to differences in desiccation resistance among populations (Knowles *et al.*, 2004), rather than divergence in one of the two sexes alone.

The exact mechanisms by which females derive benefits from mating are not known in *D. mojavensis* or *D. arizonae* and could be multifarious, possibly involving aspects of courtship as well as male seminal products.

Seminal fluid proteins are numerous and diverse, as are their effects on female physiology and behaviour (Swanson *et al.*, 2001). Moreover, postmating–prezygotic interactions are not limited to the female reproductive tract (Bertram *et al.*, 1996). Some male-derived substances act in the female hemolymph and at sites other than the reproductive tract (Richmond *et al.*, 1990; Kubli, 1996; Wolfner, 2002). Nevertheless, the importance of male–female interactions in the divergence of reproductive characters have been identified in these as well as other *Drosophila*. Experiments on male-reproductive proteins have also detected significant interactions between male and female genotypes (Clark & Begun, 1998; Clark *et al.*, 2000; Andrés & Arnqvist, 2001). In *D. mojavensis*, *D. arizonae* and closely related species, divergence in male–female postmating–prezygotic interactions has been linked to differentiation in other mating system traits (Knowles & Markow, 2001), including correlated morphological changes in reproductive characters between the sexes (Pitnick *et al.*, 1999).

When coevolutionary divergence of males and females drive differences in reproductive characters, one commonly invoked prediction is that such divergence could eventually lead to speciation (e.g. Rice, 1996; Gavrilets & Waxman, 2002). That is, the process underlying the divergence in reproductive characters could itself contribute to or act as a barrier to gene flow, thereby generating specific evolutionary implications. In the context of mating benefits related to increased desiccation resistance, a process of divergence involving male–female interactions would imply that differences in the tolerance of desiccation stress in mated females among populations could potentially contribute to or act as barriers among populations to the extent that desiccation resistance in these desert flies has significant fitness consequences, as it most certainly does (Hadley, 1994; Knowles & Markow, 2001). However, the relative difference in mating

benefits between intra- and interpopulation matings varied among populations (Fig. 1). Consequently, coevolutionary divergence of males and females will not necessarily lead to reduced gene flow and will depend on both the female-response functions and male traits that evolved in each population. These results appear to support recent theoretical treatments that conclude that the relationship between speciation and divergence in reproductive characters via male–female interaction (including antagonistic coevolution) will be difficult to predict (Rowe *et al.*, 2003).

Significant differences in female desiccation resistance between intra- and interpopulation matings demonstrate that variation in the beneficial effects of mating among populations are not attributed solely to either (i) divergence in male characters, such as among male ejaculates, or (ii) variation in the females, such as the ability to take-up or convert male seminal products into desiccation tolerance. Instead, contrasts between the intra- and interpopulation matings show that differences in female desiccation resistance among populations are consistent with divergence in reproductive interactions between males and females. However, variation in the relative mating benefits between intra- and interpopulation matings indicate that differentiation of the reproductive characters conferring desiccation resistance will not necessarily eventually lead to decreased gene flow among populations, as often assumed when divergence involves male-female coevolution. It remains to be determined whether the ecological consequences of these reproductive interactions are reflected in any patterns of genetic differentiation and/or gene flow among populations.

Acknowledgments

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