

Exploring the consequences of postmating–prezygotic interactions between the sexes

L. Lacey Knowles^{1*}, B. Brodie Hernandez² and Therese A. Markow²

¹Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA

²Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721-0088, USA

* Author for correspondence (knowlesl@umich.edu).

Recd 30.01.04; Accptd 23.02.04; Published online 07.04.04

The role of male–female interactions in the divergence of postmating–prezygotic reproductive traits has recently focused on sexual conflict as the selective force. While an association between mating costs and benefits suggests that antagonistic interactions may be important, a mosaic of processes may actually mediate the evolutionary dynamics of postmating–prezygotic interactions. Our study examines the ecological relevance of mating effects on females. We test this critical but often overlooked assumption in desert *Drosophila*, where the species' ecology provides a framework for evaluating the evolutionary implications of such mating consequences. We show that mating has a profound impact on an ecologically critical character—desiccation resistance. To explore what selective factors might underlie the observed population differences in mating effects, we test whether trends in the data match predictions based on the species' ecology. While these preliminary data are consistent with the expectation that the mating benefits are positively correlated with environmental conditions, further examination reveals an additional unanticipated association with the reproductive environment. This unexpected association challenges existing assumptions regarding the forces driving divergence of traits involved in mating.

Keywords: antagonistic coevolution; *Drosophila*; sexual conflict

1. INTRODUCTION

The diversity of physiological and behavioural consequences of postmating–prezygotic reproductive interactions (Wolfner 2002) coupled with an association between mating costs and benefits (Fowler & Partridge 1989; Chapman *et al.* 1995; Holland & Rice 1999) suggests that sexual conflicts may drive the evolution of reproductive characters. For example, in *D. melanogaster*, postmating–female mortality is correlated with males' sperm-competitive abilities (Civetta & Clark 2000). If the male-induced harm to females is a pleiotropic effect of the character conferring a male-mating advantage, as females

evolve resistance to the deleterious effects of mating and males then counteradapt to maintain a mating advantage, a dynamic cycle of perpetual coevolution between the sexes can result. Although it may appear that a process of antagonistic coevolution could be inferred if male-mating advantage is associated with male-induced harm to females, such a correlation, by itself, does not identify the actual evolutionary implications of these mating consequences (Partridge & Hurst 1998) or exclude other explanations (Gavrilets & Waxman 2002). Moreover, although there is an association between male-mating advantage and male-induced harm in *D. melanogaster* (Wolfner 2002), we lack empirical evidence that this pleiotropic link generates selective pressures that are strong enough to drive the rapid divergence observed in postmating–prezygotic characters, such as male seminal proteins (Howard 1999).

Desert *Drosophila* can offer remarkable insights into the evolutionary implications of these postmating–prezygotic reproductive interactions because both the opportunity for, and relative effectiveness of selection have been established. Not only do males transfer enormous ejaculates, but the females of some desert *Drosophila* species also actively incorporate the seminal products (Pitnick *et al.* 1997). Thus, postmating–prezygotic interactions abound and most probably account for the extraordinarily large male ejaculates (Eberhard 1996). The role of postmating–prezygotic interactions has also been identified as the mechanism driving the evolution of a variety of characters, including coevolutionary morphological divergence of the reproductive tract (Pitnick *et al.* 1999; Knowles & Markow 2001). Finally, there is a clear ecological framework for examining the consequences of mating in the desert species, and thus, for evaluating the evolutionary implications of postmating–prezygotic interactions (Partridge & Hurst 1998; Gavrilets & Waxman 2002).

This study represents a preliminary exploration into this critical but often overlooked question of whether it is possible that the consequences of mating for females are of sufficient magnitude to promote divergence in the male traits involved in mating. This presumption, especially as applied to purported examples of sexual conflict, is yet to be tested. We address this question by examining the effects of mating in two desert *Drosophila* species, *D. mojavensis* and *D. arizonae*. We also explore whether there is evidence of other factors, and specifically environmental ones, which mediate the evolution of qualities of male ejaculates that affect female fitness. These species both occur in the Sonoran desert, yet populations across their ranges experience varying degrees of desiccation stress owing to precipitation and temperature differences (see electronic Appendix A). We use this natural variation to (i) evaluate the ecological relevance of postmating–prezygotic interactions in these flies; and (ii) explore what processes underlie the observed variation in mating effects among populations.

2. METHODS

Virgin adults were collected following eclosion and stored in sex-specific, yeasted culture vials. Only sexually mature flies were used (i.e. flies aged 9 days), 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 mated flies, as well as unmated flies, were placed in individual vials and housed in a desiccator where the relative humidity was maintained

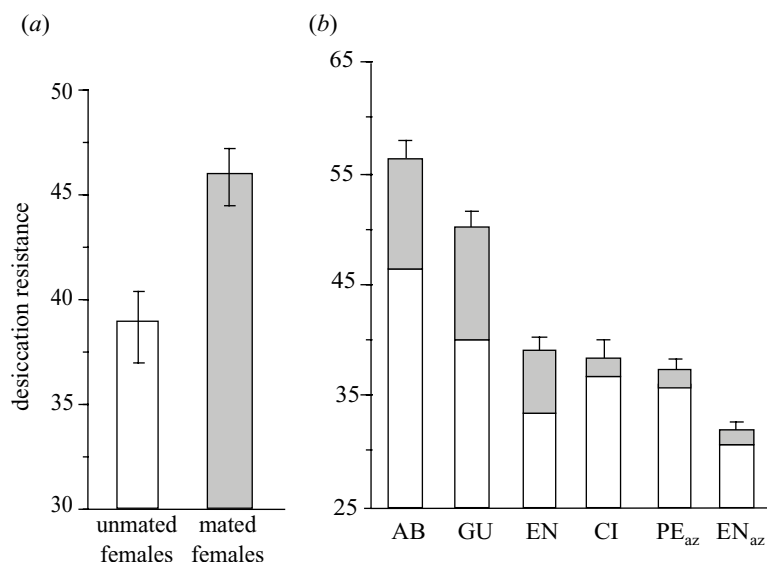


Figure 1. Comparison of desiccation resistance (measured in hours) (a) between mated (filled bars) and unmated (open bars) females, and (b) among populations. The relative contribution of mating in each population is identified by comparison with unmated females, indicated by the open bars.

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 and two *D. arizonae* populations (see electronic Appendix A). All experiments were repeated four times for each population (i.e. a total of 80 mated and 80 unmated females were examined in each of the six populations). An analysis of variance was used to compare the tolerance to desiccation stress of females among populations; initial analyses determined that there was no significant relationship between body size and desiccation resistance for mated or unmated females ($r^2 = 0.0002$ and $p = 0.94$, $r^2 = 0.0002$ and $p = 0.92$, respectively). A significant population effect indicates that the effect of mating (measured by female desiccation resistance) differed significantly among populations (i.e. AB, CI, EN, GU, EN_{az} and PE_{az}; see electronic Appendix A). To correct for heteroscedasticity and non-normality, all data were transformed prior to analyses using a square-root transformation. Spearman's ρ was used as a non-parametric measure of the association between effects of mating on female desiccation resistance: (i) the stress imposed by the desert environment; and (ii) costs associated with the reproductive environment.

3. RESULTS AND DISCUSSION

Mating significantly increased female resistance to desiccation stress ($F_{1,106} = 11.05$, $p < 0.001$; figure 1a). Mating increased female survival by as much as 20 h, an increase of 62%. Mated females were also consistently more resistant to desiccation stress than unmated females across all populations of *D. mojavensis* and *D. arizonae* (figure 1b). The males of these species transfer exceptionally large ejaculates that are actively incorporated by the females (Pitnick *et al.* 1997), suggesting that the benefit conferred by mating may be a derivative of a male reproductive character; namely, male seminal products. If such large ejaculates are costly for males to produce, we would expect that the benefits conferred by mating will differ among populations and correspond to the varied selective pressures on female desiccation resistance.

Interestingly, the relative benefit conferred by mating differs significantly among the desert fly populations ($F_{3,35} = 3.16$, $p < 0.01$; figure 1b). However, divergence of a reproductive character cannot occur because of the trait's effect on the ecology (or life history) of the opposite sex unless the mating consequences are of sufficient magnitude to constitute an effective selective mechanism

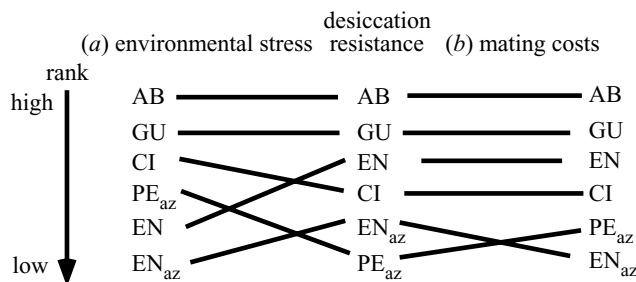


Figure 2. Comparisons between the rank orders of populations according to the degrees of desiccation resistance and (a) stress imposed by the environment ($\rho = 0.77$, $p = 0.07$), and (b) costs arising from sexual conflict ($\rho = 0.94$, $p < 0.01$). The population ranks according to degree of environmental stress were inferred from the desiccation resistance of an unmated female from each population, which also generally matches average annual temperature and precipitation profiles for the different areas (see electronic Appendix A). Postmating–prezygotic interactions obviously cannot contribute to the significant differences in desiccation resistance of unmated females among populations ($F_{3,35} = 8.86$, $p < 0.001$). Instead, these differences presumably reflect the varied selective pressures experienced by the different populations because of differing environmental pressures. The population ranks according to mating costs were based on the size and duration of the insemination reaction mass, which are indicative of the negative effects of mating on females (Knowles & Markow 2001).

(Partridge & Hurst 1998). The question is whether the ecological consequences of mating for females have contributed to differences in the qualities of male ejaculates among populations. Comparing the rank order of populations according to the stress imposed by the physical environment with the relative degree of desiccation resistance of mated females shows that the mating benefits are positively correlated with environmental conditions (figure 2a). This pattern suggests that indirect selective pressures associated with the physical environment do indeed drive the evolution of male ejaculate qualities that confer

benefits to females under specific environmental conditions, namely extreme desiccation stress.

While the preliminary results provide compelling evidence for the importance of considering the ecological environment, this is but one of what might be a mosaic of processes contributing to the evolution of characters involved in postmating–prezygotic interactions (Eberhard 1996). For example, in addition to the benefits of mating, our previous work suggests that sexual conflicts in these desert flies impose a measurable load (Knowles & Markow 2001). Mating in *D. mojavensis* and *D. arizonae* is associated with the formation of an insemination reaction mass—a swelling of the female's uterus. The size and duration of the reaction mass are positively correlated with reduced remating and oviposition rates. Both effects have potentially deleterious consequences for females in these sperm-limited species, whereas males benefit from reduced competition over fertilization success (Knowles & Markow 2001). Thus, qualities of the male ejaculates that reduce the deleterious effects of mating on female fitness (i.e. that offset the costs of mating) would be favoured. In fact, comparison between the relative degree of desiccation resistance of mated females and the relative mating costs among populations demonstrates that these two factors are also positively correlated (figure 2*b*). This pattern supports the hypothesis that the negative fitness consequences that arise from antagonistic coevolution may contribute to the evolution of reproductive traits that are advantageous under certain ecological conditions.

Thus, the reproductive and physical environments both appear potentially to contribute to the evolution of sexual characters that upon mating confer desiccation resistance. At this point, it is not possible to determine which of these two factors (or even possibly a correlated, but as yet, unconsidered third factor) is responsible for the evolution and maintenance of the male ejaculate qualities conferring desiccation resistance. The results nonetheless highlight the caution that needs to be taken when inferring which forces might actually contribute to divergence of the characters involved in mating.

4. CONCLUSIONS

The significant effect of mating on female desiccation tolerance reveals the potential role of natural selection in shaping the evolution of sexual characters involved in postmating–prezygotic interactions. Moreover, these preliminary results suggest that the ecological consequences of mating for females may actually drive divergence in the male reproductive characters that confer varying levels of resistance to desiccation among populations. While there is correspondence between the degree of desiccation resistance conferred by mating and the stress imposed by the physical environment, an unexpected correlation between the increase in desiccation resistance after mating and the deleterious effects of mating associated with the insemination reaction mass in females was also observed. These results reinforce the mosaic processes potentially underlying the evolution of reproductive characters involved in postmating–prezygotic interactions.

An important implication of the study pertains to how inferences are made about the potential mechanisms underlying the evolution of postmating–prezygotic characters (Partridge & Hurst 1998; Gavrillets & Waxman 2002).

It was only in a broader biological context that we were able to detect (i) that there was more than one potential selective basis for the qualities of male ejaculates that confer a mating benefit; and (ii) that there may be beneficial effects of mating in addition to previously documented negative ones (Knowles & Markow 2001). This disparity is predicted by models that demonstrate that the costs and benefits to females will change under different environmental conditions (Partridge & Hurst 1998; Holland & Rice 1999). Evidence of male-induced harm to females in *D. melanogaster* (Fowler & Partridge 1989; Holland & Rice 1999; Civetta & Clark 2000) may well be indicative of only one of several possible mechanisms that might contribute to the evolution of reproductive characters involved in postmating–prezygotic interactions, such as of male seminal proteins (Civetta & Singh 1995; Swanson *et al.* 2001). As with other reproductive traits (Emlen 2001; Kurdziel & Knowles 2002), the forces that drive the evolution of characters involved in postmating–prezygotic interactions are clearly not necessarily limited to any single functional context.

Acknowledgements

This research was supported by a grant to B.B.H. from the Howard Hughes Medical Institute, and an NSF grant to T.A.M.

- Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995 Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**, 241–244.
- Civetta, A. & Clark, A. G. 2000 Correlated effects of sperm competition and postmating female mortality. *Proc. Natl Acad. Sci. USA* **97**, 13 162–13 165.
- Civetta, A. & Singh, R. S. 1995 High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* group species. *J. Mol. Evol.* **41**, 1085–1095.
- Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.
- Emlen, D. J. 2001 Costs and the diversification of exaggerated animal structures. *Science* **291**, 1534–1536.
- Fowler, K. & Partridge, L. 1989 A cost of mating in female fruitflies. *Nature* **338**, 760–761.
- Gavrillets, S. & Waxman, D. 2002 Sympatric speciation by sexual conflict. *Proc. Natl Acad. Sci. USA* **99**, 10 533–10 538.
- Holland, B. & Rice, W. R. 1999 Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl Acad. Sci. USA* **96**, 5083–5088.
- Howard, D. J. 1999 Conspecific sperm and pollen precedence and speciation. *A. Rev. Ecol. Syst.* **30**, 109–132.
- Knowles, L. L. & Markow, T. A. 2001 Sexually antagonistic coevolution of a postmating–prezygotic reproductive character in desert *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 8692–8696.
- Kurdziel, J. P. & Knowles, L. L. 2002 Mechanisms of morph determination in the marine amphipod *Jassa*: implications for the evolution of alternative male phenotypes. *Proc. R. Soc. Lond. B* **269**, 1749–1754. (DOI 10.1098/rspb.2002.2089.)
- Partridge, L. & Hurst, L. D. 1998 Sex and conflict. *Science* **281**, 2003–2008.
- Pitnick, S., Markow, T. & Spicer, G. S. 1997 Phylogenetic examination of female incorporation of ejaculate in *Drosophila*. *Evolution* **51**, 833–845.
- Pitnick, S., Markow, T. & Spicer, G. S. 1999 Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. *Evolution* **53**, 1804–1822.
- Swanson, W. J., Clark, A. G., Waldrip-Dali, H. M., Wolfner, M. F. & Aquadro, C. F. 2001 Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 7375–7379.
- Wolfner, M. F. 2002 The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88**, 85–93.

Visit www.journals.royalsoc.ac.uk and navigate to this article through *Biology Letters* to see the accompanying electronic appendix.