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Parasites physically block host copulation: a potent mechanism of parasite-mediated sexual selection

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Research on the role of parasites in sexual selection has focused mainly on host mate choice favoring relatively unparasitized males. But parasites can also generate variance in host reproductive success by influencing the ability of individual hosts to directly compete among themselves for mates or fertilizations, a subject area that has received far less attention. We demonstrate experimentally that parasitism by mites can drive sexual selection by way of a novel mechanism involving male competition: physical inhibition of host copulation. Mite resistance in natural populations is heritable, emphasizing the evolutionary potential of parasite-mediated sexual selection in this system and indicating that females should be receiving indirect fitness benefits as a result of this process. We show that parasitism by mites, *Macrocheles subbadius*, reduces mating success of male *Drosophila nigrospiracula*. Smaller males were more strongly compromised, identifying host body size as a tolerance trait. As parasite load increased, the rate at which males attempted to copulate but failed because of obstruction by mites increased. When mites were removed from infested males, host mating success was restored. Thus, the physical presence of the mites per se generates differential mating success, in this case by interrupting the normal flow of mating behaviors. This study elucidates a potent mechanism of parasite-mediated sexual selection in a system wherein parasite resistance is demonstrably heritable, and as such expands our understanding of the evolutionary potential of sexual selection. *Key words: Drosophila, Macrocheles*, mate competition, parasite-mediated sexual selection, parasitism, sexual selection. *[Behav Ecol 18:952–957 (2007)]*

Sexual selection arises from variation in fitness due to competition for mates (Andersson 1994). Because differential fitness of genotypes is a primary agent of evolution, understanding the competitive mechanisms generating differential mating success in either sex is an active area of sexual selection research (Andersson 1994; Arnqvist and Rowe 2005; Andersson and Simmons 2006). Parasites, and host immunological responses to them, have received considerable attention in this context because of the relationship they can have to host fitness in general and to mating success in particular (e.g., Price 1980; Hamilton and Zuk 1982; Read 1988, 1990; Møller 1990; Clayton 1991; Loehle 1997; Schmid-Hempel 1998; Koskimäki et al. 2004; Rantala and Kortet 2004; Simmons and Roberts 2005; Ahtiainen et al. 2006). Indeed, parasites can influence host sexual selection by way of 2 nonmutually exclusive mechanisms: in general, they may reduce individual attractiveness to members of the opposite sex or alter the outcome of competition among members of the same sex for access to mates and fertilizations (Howard and Minchella 1990).

In interactions among males, parasitism may generate nonrandom mating by way of various specific mechanisms. In mice and in some primates, for example, parasites can disrupt dominance hierarchies among sexually competitive males (Freeland 1976; Rau 1984). Parasites may also reduce male weapon and body size, physiological vigor, energy reserves, sperm and physical competitive ability, and indicators of both strength and stamina signaled to rivals (e.g., Jaenike 1988; Howard and Minchella 1990; Forbes 1991; Simmons 1993, 2001; Rolff et al. 2000; Bollache et al. 2001; Marden and Cobb 2004). Whereas intrasexual competition is generally recognized to be a potent mechanism generating nonrandom mating (Andersson 1994), interest in this subject has nevertheless figured less prominently than mate choice in the parasite-mediated sexual selection literature (Howard and Minchella 1990; Clayton 1991; Bollache et al. 2001), thus narrowing our understanding of the scope of parasitic effects on host evolution. Compounding this shortage of studies has been the view that intrasexual selection does not require parasite resistance to be heritable (Howard and Minchella 1990; Clayton 1991). Whereas this point is of heuristic value because it draws a distinction between competing models describing the role of parasites in host sexual selection, it has limited the number of heritability estimates for resistance in systems that do not involve host mate choice (Clayton 1991; Sorci et al. 1997). This dearth of genetic information further compromises our ability to predict the evolutionary consequences of parasite-mediated sexual selection.

Here we demonstrate a novel mechanism of parasite-mediated sexual selection: physical inhibition of host copulation, whereby parasites block male attempts to engage their genitalia with that of the female, thus reducing male mating success. We focus on the *Drosophila–Macrocheles* mite association (Polak 1996), which occurs naturally within the North American Sonoran Desert. Courtship and mating occur on necrotic cacti, where large numbers of individuals of both sexes typically aggregate, and males vigorously compete for access to mates (Markow 1988; Polak and Markow 1995). Infested males that are unable to copulate with a receptive female because of physical inhibition by mites would quickly lose their would-be mate to a rival male. In this way, parasitism would interact with elements of the existing mating system (i.e., high male density and male–male competition) to accentuate differential mating success in natural populations.

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The ability to avoid infestation by mites is significantly heritable (Polak 2003), underscoring the evolutionary potential of parasite-mediated sexual selection in this system. Polak and Markow (1995) have previously demonstrated that these mites reduce copulatory success in field caught males but where potentially confounding variables such as male age and condition were not controlled. Here we present an extension to this work by testing for a cause-and-effect relationship between parasitism and mating success under controlled experimental conditions; in so doing, we also quantify synergistic effects between parasitism and male body size on mating success and thus for the possibility that parasite-mediated sexual selection favors the joint evolution of genes influencing these different traits. We test the following predictions of the physical inhibition hypothesis: 1) experimentally parasitized males should exhibit reductions in mating success relative to parasite-free males; 2) parasitized males that fail to copulate should attempt copulation but exhibit a higher proportion of failed attempts because of obstruction by parasites; and 3) experimental removal of the parasites should ameliorate, if not restore, mating success. By measuring mating success immediately following parasite removal, we were also able to ascertain the extent to which any loss of mating success among parasitized males results from the physiological drain caused by ectoparasitism. Such costs may accrue from both nutrient extraction by the feeding mites (Polak 1996) and from the host's immunological response (Rolff and Siva-Jothy 2003), occurring here in the form of melanin deposition (scar formation) at the wound site (Polak and Markow 1995).

MATERIALS AND METHODS

Rearing of flies and mites

A stock culture of *Drosophila nigrospiracula* was initiated with flies netted at saguaro cacti (*Carnegia gigantea*) approximately 72 km East of Phoenix, Arizona. Flies were maintained in the laboratory for no more than 5 generations before commencement of experiments. Flies were cultured as described previously (Polak 1996) and maintained in an incubator with a 12:12 light:dark photoperiod at 25 °C. A mite culture was initiated with individual female mites removed from the abdomens of infested flies captured at the same time and site as described above and cultured (Polak 1996).

Experiment 1: effect of mites on mating frequency

Male flies were harvested from culture bottles on the day of emergence, and held in an incubator for 4 days in vials containing a standard banana agar substrate in groups of 20 males without females. Fifty flies were aspirated into an experimental infestation chamber (Polak 2003) containing culture medium with mites and recovered after 6 h. These recovered flies were either parasitized or not. The fraction without mites served as uninfested controls. On the first 2 days of the experiment (there were a total of 6 days), unexposed control flies were similarly held in infestation chambers for 6 h containing identical culture medium but without mites; thus, these unexposed controls were naive flies having never encountered mites in their lifetimes. The 2 categories of uninfested flies did not differ in their mating success (analysis of covariance with thorax length as covariate: $F_{1,15} = 1.7$, $P = 0.21$; least squares mean [standard error {SE}, n] for exposed males = 4.0 [0.53, 11] and for naive males = 5.0 [0.68, 7]); exposed, unparasitized males were used as controls on the remaining 4 days of the experiment. Thus, whereas these exposed males were in essence "self-selected," this lack of a significant difference indicates that exposed flies can serve as a control group under the present conditions (for a similar result pertaining to fly longevity, where self-selected and naive groups did not differ in this trait either, see Polak 1996, p. 1383).

Treatment groups (i.e., parasitized and control males) were held for 36 h in banana agar vials without females prior to the mating assay. On the morning of the mating assay, males were individually aspirated into clean, sterile vials and allowed to acclimate for 10 min. Vials with the different males were interdigitated in a row along a desktop. Two mature virgin females 8–10 days old were then introduced to each vial with an aspirator. Because all females in this study were virgins well past the age at which they reach sexual maturity (ca., 4 days), they were eager to mate. The total number of copulations achieved by each male in exactly 2 h from the time females were introduced was recorded. Copulation duration lasts several minutes in this species, so no copulations were missed. Immediately following copulation, both females were replaced with 2 fresh virgins and put aside for later dissection. A total of 6 such daily sessions were conducted for a total of 148 males (97 infested and 51 uninfested). During 2 of these sessions, in which 23 and 18 males were assayed, behavioral data on the frequency of failed copulation attempts by males and on female responses to courting males were recorded. Vials were scanned successively throughout the assay, and each was observed for 5 s per scan. During each 5-s observation period, the occurrence of any focal behaviors was noted. A failed attempt consisted of a male climbing onto a female from behind her and either dismounting again by moving off the female backwards or sliding off her abdomen to either side of her body. If a male was engaged in close pursuit courtship (i.e., the male was either touching the female or up to 0.5 body lengths away from her, and for a detailed description of courtship in this species, see Markow 1988), the observer noted whether or not the female displayed receptivity by spreading her wings slightly forward.

Following the mating assay on any given day, male thorax length (estimate of male body size) and number of attached mites were determined for each male under a stereomicroscope, and all females that had been exposed to males were dissected in a drop of physiological saline to ascertain whether they had been inseminated by checking for sperm within the reproductive tract. "Copulation success" refers to the total number of copulations achieved by a male in the 2-h observation period, whereas "mating success" is the number of copulations that resulted in sperm transfer.

The effect of mite load on mating performance was evaluated using multiple regression, in which male thorax length and both the linear and quadratic terms for mite burden were included. For graphical presentation, residual mating success was plotted against mite burden, where the residuals were from linear regression of male mating success on thorax length. To test for synergistic effects between host body size and mite burden on mating success, males were divided into 2 groups on the basis of whether they fell above or below mean thorax length of the sample ($\bar{x} = 1.23$ mm). A regression model with thorax length treated as a categorical (binary) variable (i.e., high vs. low) tested H_o: $b_3 = 0$, where b_3 is the coefficient of the cross-product term between mite burden and thorax length (Neter et al. 1990). Male mounting attempts were not distributed normally, so we utilized nonparametric statistics in this case.

Experiment 2: mite removal

A second experiment was conducted to determine the effect of mite removal on male mating success. Males were harvested from culture bottles, aged, and experimentally parasitized as described above. On the morning of the assay, a random

subset of the parasitized group was chosen for the removal treatment. These males were lightly anesthetized with CO_2 and all their mites removed with fine forceps. The males whose mites were not removed, and which constituted the parasitized group, were not anesthetized (otherwise some mites invariably would disengage). Control males (of the unexposed variety, see above) were anesthetized and prodded with forceps. Males were given 1 h to recover from anesthesia, and their mating success assayed, as above, simultaneously with the parasitized group. In this experiment, observations were made "blind" with respect to the treatment to which males belonged (which was not possible in Experiment 1, as attached mites are readily visible). The total sample size was 51 males, broken down as follows: 33 with mites experimentally removed, 8 with mites, and 10 controls. Multiple regression analyses evaluated the effect of mite burden (previous or current in separate analyses) and thorax length on mating success. A statistical comparison of slopes of functions relating previous or current mite burden on mating success was done using the binary variable technique described above. For graphical representation, residuals in mating success were generated as above. All statistical analyses were conducted in SAS (SAS Institute 2002).

RESULTS

In Experiment 1, we examined the effect of experimentally manipulated ectoparasite burden on the mating success of 6- and 7-day-old males under laboratory conditions. The number of copulations and matings achieved by males during the 2-h observation period ranged from 0 to 11 (mean, SE = 4.45, 0.18) and 0 to 10 (3.81, 0.16), respectively. Because the correlation between these 2 measures of sexual performance was highly significant, both in the present experiment ($r = 0.92$, $n = 148$, $P < 0.0001$) and in Experiment 2 ($r = 0.96$, $n = 51$, $P < 0.0001$), henceforth we report analyses on mating success only. Among infested males in the present experiment, mite burden ranged from 1 to 6 (median = 2, $n = 97$), and the regression of mite load on thorax length was not significant ($\hat{b} = -1.22$, SE = 2.55, $F_{1,95} = 0.23$, $P = 0.63$).

Figure 1A shows that as mite burden increased, the percentage of males that failed to copulate during the 2-h observation period increased steadily to 100% at 6 mites per male. We next examined the relationship between the number of matings achieved by these males and mite burden using multiple regression. This analysis revealed that male mating success was significantly positively related to thorax length and that both the linear and quadratic mite burden terms were strongly significant (Table 1, full data set). However, the effect of the quadratic term was more pronounced, explaining 2.8 times more mating success variation than the linear term (consult coefficient of partial determination values, Table 1, full data set). The shape of the quadratic function (Figure 1B) indicates that the effect on mating success was mildly positive at low mite loads (≤2 mites), becoming strongly negative with increasing parasite burden. When data for the 3 flies with 6 mites were excluded, the analysis remained virtually unchanged (Table 1, data set without males with 6 mites), indicating that the results are robust to the loss of the relatively few males with the most extreme mite burden.

Regression analysis, in which body size was entered as a categorical variable, revealed that the slope of the relationship between mating success and ectoparasite burden was significantly steeper in smaller males ($\hat{b} = -0.606$, SE = 0.11, $t = 5.31$, $P < 0.0001$, $n = 71$) than in larger males ($\hat{b} = -0.276$, SE = 0.14, $t = 2.0$, $P = 0.049$, $n = 77$; $\hat{b}_3 = 0.64$, SE = 0.22, $t = 2.9$, $P = 0.0042$). Thus, the dose-dependent negative effect of mite burden on male mating success is stronger among smaller males in the population, identifying body side as a tolerance trait in this host species.

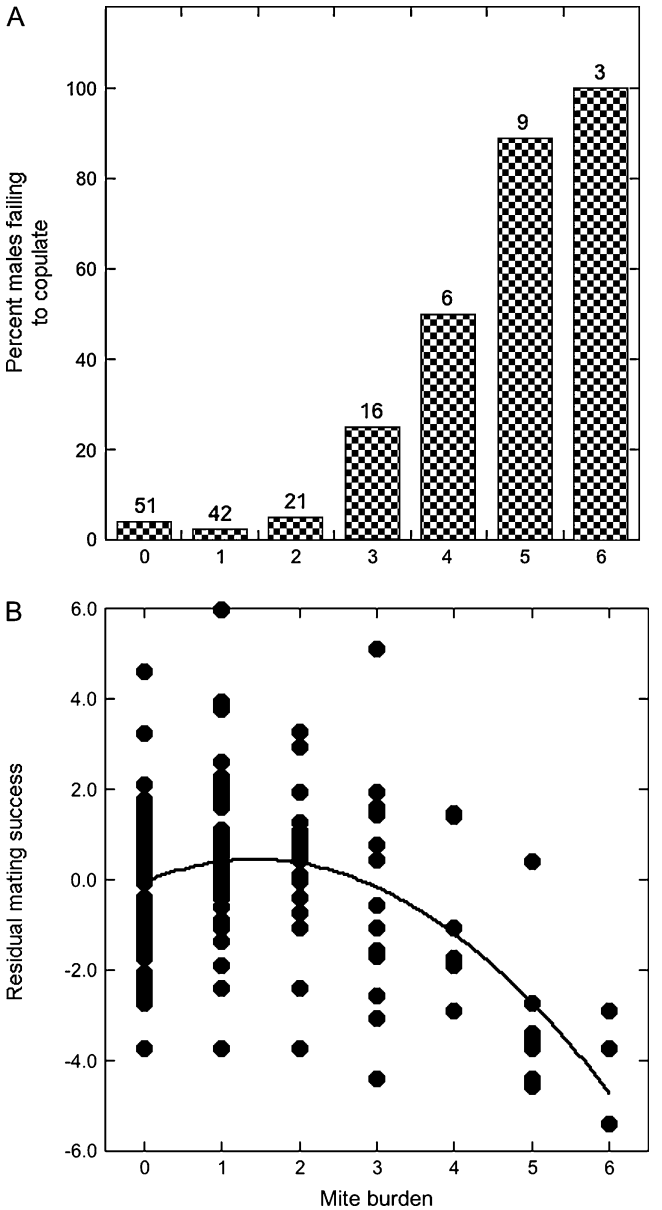


Figure 1
(A) The increase in the percentage of (%) males failing to copulate during 2 h of observation across mite burned categories, in Experiment 1. Numeral above each bar is the number of males. (B) Effect of mite burden on residual mating success ($n = 148$ males). Residuals are from regression of male mating success on thorax length. Consult Table 1A for the coefficients of the regression function.

Interactions between 41 males and females were closely monitored: 14 males were unparasitized and 27 were parasitized (range = 1–6 mites). The female always responded to the courtship effort of males by stopping her forward path of locomotion and spreading both her wings slightly forward, indicating her receptivity (Polak and Markow 1995). For uninfested and infested males, the median number of failed copulation attempts was 0 (range = 0–5, $n = 14$) and 2 (range = 0–76, $n = 27$), respectively (Wilcoxon $Z = 2.38$, $P = 0.017$). A successful attempt occurred when a male mounted from behind and engaged his genitalia with that of the female.

Table 1

Multiple regression results on mating success achieved by male *Drosophila nigrospiracula* in 2 h of laboratory observation using 2 data sets

Covariate	Coefficient (SE)	T	P	COPD[a]
Full data set[b]				
Thorax length	16.33 (2.50)	6.53	<0.0001	0.23
Mites	0.70 (0.26)	2.66	0.0087	0.047
Mites^2	−0.25 (0.053)	4.70	<0.0001	0.13
Data set without males with 6 mites[c]				
Thorax length	16.69 (2.54)	6.57	<0.0001	0.23
Mites	0.83 (0.29)	2.82	0.0055	0.053
Mites^2	−0.28 (0.064)	4.39	<0.0001	0.12

[a] Coefficient of partial determination (COPD) calculated using Type II sums of squares.
[b] Overall model: $F_{3,144} = 31.13$, $P < 0.0001$, $r^2 = 0.39$.
[c] Overall model: $F_{3,141} = 26.84$, $P < 0.0001$, $r^2 = 0.36$.

Of the 14 uninfested males, 2 males failed to copulate once each, whereas one male failed 5 times. In all these cases, the male mounted and probed with his genitalia but for no apparent reason dismounted backwards without copulating. All 3 of these males nevertheless successfully copulated multiple times during the 2-h observation period.

In contrast, unsuccessful infested males dismounted by moving backward off the female or sliding off to either side of her body. Males carrying 3 or more mites made repeated attempts to copulate by bending their abdomen forward and probing for access to the female's genitalia but could not engage in copulation because mites were seen to physically block these attempts. For example, 2 heavily infested males (6 mites per male) made 49 and 76 attempts to copulate, with the mites visibly obstructing genital coupling; neither male secured a copulation during the observation period. Figure 2 demonstrates the decrease in mating success as a function of mite burden ($r = -0.58$, $P < 0.0001$, $n = 41$), concomitant with an increase in the failure rate ($r_s = 0.61$, $P < 0.0001$, $n = 41$). Thus, ectoparasitism increases a male's failure rate in dose-dependent fashion through the directly observable effect of physical inhibition of copulation.

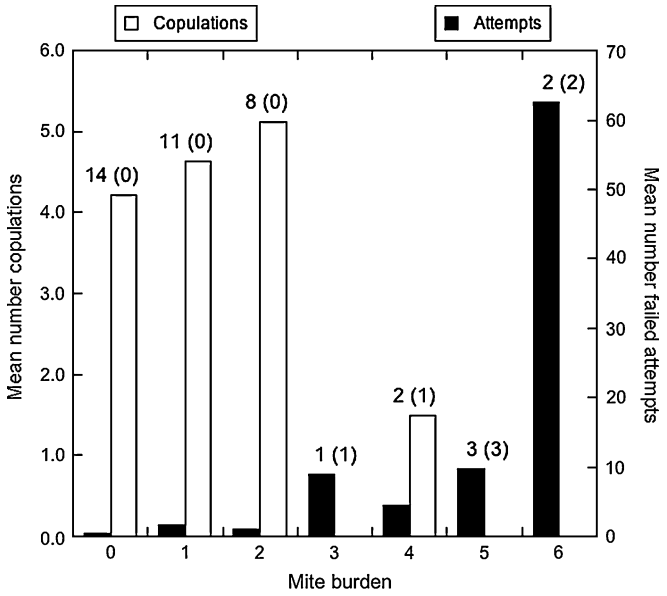


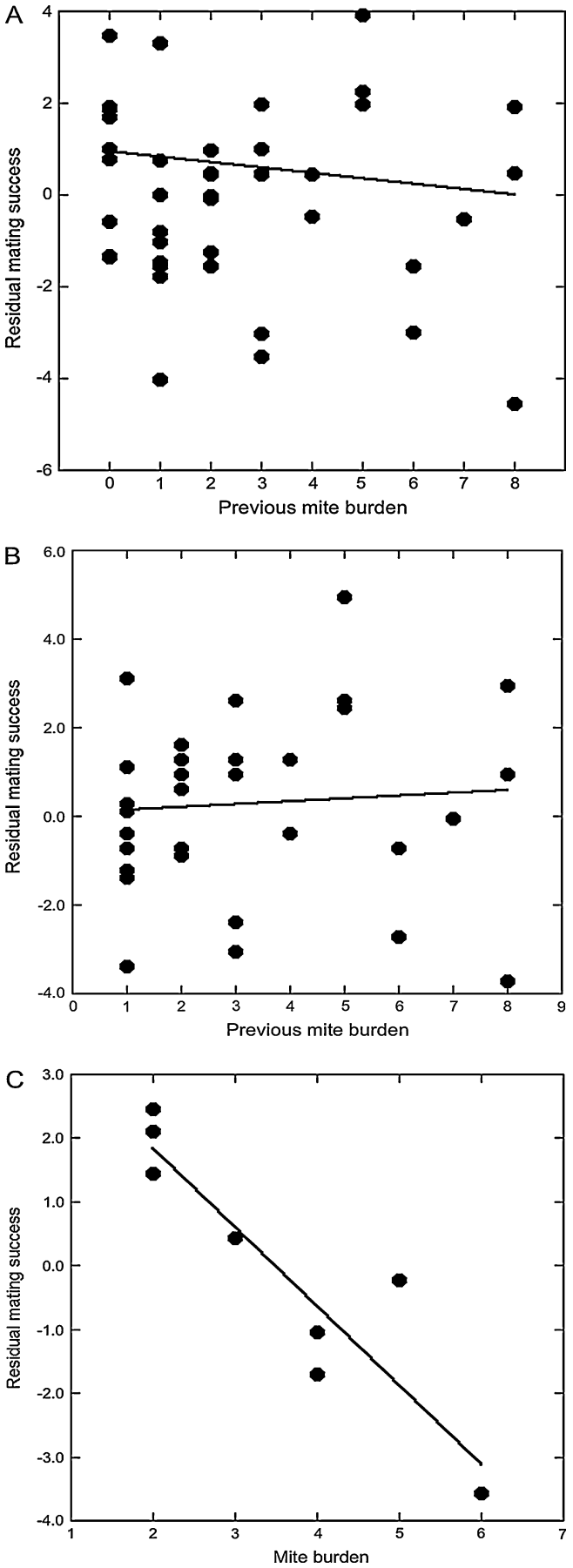
Figure 2
Mean number of copulations achieved by males in Experiment 1 contrasted against mean number of attempts that failed to result in copulation across mite burden. Numerals above bars are number of males, and numerals in parentheses are the number of males that failed to copulate.

In Experiment 2, where mites were experimentally removed, the relationship between previous mite load and mating success was not significant ($\hat{b} = -0.10$, SE = 0.13, $F_{1,41} = 0.60$, $P = 0.44$, Figure 3A), despite males in this experiment having a greater range of parasite loads (0–8 mites) than infested males in the present experiment and in the larger assay of Experiment 1 (both ranges: 0–6 mites). Figure 3B presents the results of a similar analysis, but with unparasitized males excluded, revealing a likewise nonsignificant relationship ($\hat{b} = 0.061$, SE = 0.16, $F_{1,31} = 0.15$, $P = 0.70$). Figure 3C, in contrast, reveals as in Experiment 1 the strong negative effect of mite burden on mating success among males harboring mites ($\hat{b} = -1.24$, SE = 0.22, $F_{1,6} = 130.5$, $P = 0.0015$); slopes of the functions depicted in Figures 3B and 3C are significantly different ($\hat{b}_3 = -1.30$, SE = 0.49, $t = 2.6$, $P = 0.012$). Thus, the experimental removal of mites restored male mating success, satisfying the third and final prediction of the physical inhibition hypothesis presented in the Introduction.

DISCUSSION

The literature dealing with parasitic effects on host sexual selection is focused mainly on mate choice (Read 1988; Endler and Lyles 1989; Hamilton 1990; Zuk et al. 1990; Clayton 1991; Andersson 1994; Andersson and Simmons 2006). Moreover, because the presence of heritable variation for resistance is a key assumption specific to good genes models of sexual selection (Clayton 1991), the few heritability estimates relevant to understanding parasite-mediated sexual selection in natural populations also mainly come from systems involving mate choice (Sorci et al. 1997). The present study is therefore unique in demonstrating the operation of a potent mechanism of parasite-mediated sexual selection accentuated by mate competition in a system wherein parasite resistance is heritable (Polak 2003; Luong and Polak 2007). Thus, females are likely to be gaining indirect benefits in the form of resistance transmitted to offspring as an incidental outcome of this mechanism (see Howard and Minchella 1990). These indirect (genetic) benefits may at times be considerable, as parasitism by mites has pronounced negative effects on major fitness components not just restricted to mating success but also on fecundity and adult survivorship (Polak 1996, 1998); mites in this system are not sexually transmitted (Polak and Starmer 1998; cf., Abbot and Dill 2001; Webberley et al. 2002).

We demonstrated experimentally that parasitism by mites has an adverse effect on host mating success by way of physical inhibition of host copulation. The relatively high rate of failure to copulate among infested males, coupled with direct observations of the mites blocking male attempts to achieve intromission, demonstrates unambiguously that the presence of the mites obstructs copulation. Indeed, the failure rate increased with mite burden, concomitant with a pronounced decrease in mating success. In nature, large numbers of males and females aggregate at necrotic desert cacti. Prevalence of parasitism varies widely, but predictably, with the age of the cactus necrosis (Polak and Markow 1995): both the prevalence and intensity of parasitism, as well as the degree to which parasites are aggregated in the host population, increase with rot age. At one old necrosis, for example, intensity rose to 7.8 mites per fly, and the most heavily infested quartile of flies harbored 55% of the attached mites (Polak and Markow 1995). It is on the outer skin of cacti that males can readily be seen to engage in vigorous agonistic interactions



and where they chase, court, and copulate with females (Markow 1988; Polak and Markow 1995). Thus, the critical inference linking the present work to natural populations is that infested males that fail to copulate with a receptive female because of physical interference by mites will quickly lose their would-be mate to a nearby rival. Fly populations occupying the older rots in the environment are likely to be experiencing the greatest parasite-mediated sexual selection pressure resulting from this effect.

This inhibitory effect of mites is dose dependent, revealed by the pronounced curvilinear relationship between mating success and mite load. At low mite burdens, there was a modest rise in mating success, reflecting the upregulation of male mating effort in response to parasitism (Forbes 1993) revealed previously in a study tracking male courtship rates in this species (Polak and Starmer 1998). But with increasing mite loads above 2 mites, the parasites come to occupy an ever-greater proportion of the fly's abdominal area, increasingly interfering with the male's ability to bend his abdomen down and forward and to engage the female's genitalia. There was thus a significant interaction between parasitism and male body size, such that the decline in mating success with increasing mite load was significantly stronger among smaller males than larger males; for any given mite load, small males have a larger surface area of their abdomens occupied by the bodies of mites. Male body size is therefore acting as a "tolerance trait," in the sense that large size mitigates the negative effects of parasitism on mating success. Parasite-mediated sexual selection in this system simultaneously favors genes conferring parasite resistance and large host body size, which as a result may be coevolving.

Experimental removal of the mites restored male mating success. This result verifies that the parasite-mediated reduction in mating success was due to the presence of the mites per se, thus satisfying the final prediction of the proposed physical interference hypothesis (in the Introduction). Moreover, this finding also eliminates 1) the possibility that the observed loss of mating success could have been due to nutrient drain brought about by the feeding parasites and 2) the possible effects of costs accruing from the host's immunological response that occurs as melanin deposition at the sites where mites have breeched the host's integument (Polak and Markow 1995), a host response that can be physiologically costly (Rolff and Siva-Jothy 2003). We note, however, that in the field, the presence of additional stress factors (e.g., food deprivation, desiccation, and prolonged physical activity) are likely to interact with the effects of parasitism, such that physiological debilitation resulting from parasitism (due both to nutrient extraction and the additional weight burden of the parasites) may exert stronger effects than manifested in this laboratory setting.

In summary, the present work demonstrates that parasites physically block host copulation, thus supporting the physical inhibition mechanism of parasite-mediated sexual selection proposed by Polak and Markow (1995). The present study helps expand the scope of the parasite-mediated sexual selection

Figure 3
(A) Effect of previous mite burden on residual mating success in Experiment 2: the mite removal experiment ($n = 43$). Residuals are from regression of male mating success on thorax length. The line is drawn for ease of contrast to functions depicted in other figures; the quadratic term was nonsignificant ($P = 0.80$). (B) A linear function fitted to the same data as in (A) but without the 10 parasite-free males ($n = 33$). (C) Effect of mite burden on residual mating success in the mite removal experiment ($n = 8$). These 8 males are not represented in Figure 1. A linear function is fitted; the quadratic term in the regression model was not significant ($P = 0.76$).

literature by documenting a potent negative effect of parasitism on host mating success operating synergistically with intrasexual competition in a system wherein parasite resistance is demonstrably heritable.

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