Effect of Ectoparasitic Mites on Sexual Selection in a Sonoran Desert Fruit Fly

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EFFECT OF ECTOPARASITIC MITES ON SEXUAL SELECTION IN A SONORAN DESERT FRUIT FLY

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Abstract.—We conducted a field study and a laboratory experiment to test whether ectoparasitic mites, *Macrocheles subbadius*, generate parasite-mediated sexual selection in the Sonoran desert endemic fruit fly, *Drosophila nigrospiracula*. Male flies gather on the outer surfaces of necrotic saguaro cacti where they engage in male–male competitive interactions and vigorous female-directed courtship. At these sites, operational sex ratios were significantly skewed toward males. The degree to which mites were aggregated among flies varied across the 25 fly populations sampled. The degree of mite aggregation across fly populations was strongly positively related to the mean number of mites per fly (intensity of infestation). Both the intensity and prevalence of infestation (fraction of flies infested) increased with the age of the cactus necrosis. Infested flies of both sexes were significantly less likely to be found *in copula* than uninfested flies, and mean intensity of infestation was significantly more pronounced in noncopulating than in copulating flies. The effect of attached mites on copulatory success exhibited dose-dependency, and this effect was more stringent in males: males or females with more than two and four mites, respectively, were never found *in copula*. The magnitude of parasite-mediated sexual selection was estimated for 12 fly populations by calculating selection differentials for each sex separately. The relation between intensity of infestation and magnitude of parasite-mediated sexual selection was stronger in males but significant for both sexes. We also assayed copulatory success of field-caught males in the laboratory, both during infestation and after experimental removal of mites. Males infested with two mites copulated less frequently than uninfested individuals, and in mating trials after mites had been removed, previously infested males copulated as many times as flies with no history of infestation. These findings, and the lack of difference in the number of mite-induced scars on copulating and single individuals in nature, strongly suggest that the reduced copulatory success of infested flies is attributable to an effect of mites per se, rather than to a character correlated with parasitism or previous parasite infestation.

Key words.—Copulatory success, *Drosophila*, ectoparasitism, *Macrocheles*, sexual selection.

Virtually every organism will encounter parasites at sometime. This is not surprising because a large proportion of all known animals pursue parasitism as a way of life (Price 1980). Parasite-host associations result in some degree of harm to the host (Anderson and May 1979). For example, parasites reduce host body size, developmental stability, fecundity, growth rate, longevity, and mating success (Barnard and Benke 1990; Møller 1990, 1992; Read 1990; Hurd 1993; Polak 1993a). Some parasites achieve these effects via such dramatic devices as host castration and alteration of host behavior (Baudoin 1975; Moore 1984; Moore and Lasswell 1986; Combes 1991).

Significant reductions of host reproductive success together with evidence for heritability of resistance in a wide range of host taxa (Wakelin and Blackwell 1988) suggest that parasitism can exert a major effect on the evolution of host sexual behavior. In fact, it has been argued that sexual reproduction itself may be a consequence of selection imposed by parasitic organisms (Jaenike 1978; Hamilton 1980). Freeland (1976) was one of the first to implicate female preference for parasite-free mates as a determinant of male reproductive success. Freeland suggested that females incite "stressful competitive interaction among the males so as to determine which individuals have a high genetic resistance to disease" (1976, p. 19). A more recent "good-genes" hypothesis proposes a preference for males advertising innate resistance through the elaboration of secondary sexual characters such as colorful plumage, elaborate song, and behavioral courtship display (Hamilton and Zuk 1982; Folstad and Karter 1992). According to both of these models of parasite-mediated sexual selection (PMSS), indirect selection operates on female preferences because discriminating females enhance the viability of their offspring by securing genes that confer resistance against disease.

Empirical investigations of the good-genes models of PMSS, however, have yielded inconclusive results (Read 1990; Balmford and Read 1991; Kirkpatrick and Ryan 1991). An alternative competing female-choice hypothesis—that discriminating females accrue direct benefits, rather than genetic, or indirect ones—is also currently being debated (Reynolds and Gross 1990; Kirkpatrick and Ryan 1991). The hypothesis states that if important parasites (i.e., those imposing nontrivial fitness costs) are directly transmittable between individuals, then females that choose to mate with parasite-free males could avoid acquiring parasites themselves and avoid transmitting them to offspring (Borgia and Collis 1989). Moreover, if males take part in parental duties, and parasites diminish a male's ability to provide such care, then females selecting parasite-free partners could secure better care for their offspring (Hamilton 1990).

Yet another model of PMSS, distinct from those invoking female choice, predicts that effects of parasitism will generate variation in male reproductive success by affecting perfor-

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mance in intrasexual contests for mates (Howard and Minchella 1990). Various studies have attempted to distinguish among these models of PMSS (reviewed by Clayton 1991), all of which assume that parasites are causally related to reduced male reproductive success. However, the validity of this critical assumption is often not convincingly established, primarily because of practical impasses that limit experimental manipulation. Therefore, establishing this cause-and-effect relationship more convincingly is an essential challenge facing research into PMSS.

Previous attempts to do so have involved experimental manipulation of parasite loads on some vertebrates (e.g., Milinski and Bakker 1990; Møller 1990; Spurrier et al. 1991). For example, one experimental study of guppies and their monogenean parasites evaluated relative female preference for uninfected versus experimentally infected brothers (Houde and Torio 1992). Females actively avoided infected males, and when courted, females responded more often to displays of uninfected individuals.

Nonexperimental studies of mating systems of vertebrates (e.g. Ressel and Schall 1989; Gibson 1990) and of insects (Zuk 1987, 1988; Jaenike 1988; Simmons 1990; Forbes 1991; Simmons and Zuk 1992) have invariably observed covariance between degree of parasitism and various measures of male reproductive success and have drawn inferences regarding the operation of PMSS. Correlational analyses, however, can be difficult to interpret, because it is often not possible to exclude other underlying causes, correlated with parasitism, that may independently result in reduced host reproductive success (Grafen 1988; Read 1990). For example, inbreeding leading to reduced genomic heterozygosity may increase susceptibility to various disease organisms (Hughes and Nei 1988; O'Brien and Evermann 1988) and reduce male mating ability (Sharp 1984; Partridge et al. 1985; Miller and Hedrick 1993). In addition, host age is also known to influence male reproductive success (Forsyth and Montgomery 1987; Polak 1993b) and to covary positively with parasite load (Kirkpatrick et al. 1991). Therefore, parasitism and mating success may covary only as an incidental result of behavioral and immunological manifestations of inbreeding depression or senescence or with any other host "condition" that affects the rate of parasitism.

These potentially confounding variables compromise the effectiveness of correlational studies in PMSS. Experimental studies are therefore needed to determine whether reductions in male reproductive success are caused by parasitic organisms per se. Despite previous suggestions that insect-parasite associations can represent model systems with unique experimental opportunities (Levin et al. 1982; Jaenike 1992), only rarely have workers integrated efforts in the field and the laboratory to address questions in PMSS. A noteworthy exception is a series of laboratory experiments showing that male *Drosophila testacea* parasitized by allantonematid nematodes, *Howardula aoronymphium*, were 33% less likely to mate than were uninfected males (Jaenike 1988). However, a subsequent field study, designed to evaluate the impact of these worms on host sexual selection under natural conditions, failed to generate significant results (James and Jaenike 1992).

In the present study, we first report results from field work to test whether ectoparasitic mites, *Macrocheles subbadius* (Berlese), influence sexual selection in their host, the Sonoran-desert endemic fruit fly *D. nigrospiracula* (Patterson and Wheeler). We tested whether mite infestation affects the frequency at which individual flies copulate and we calculated the opportunity for PMSS in different host populations. But we also performed a laboratory experiment to evaluate the importance of possible correlated factors that could confound our interpretation that mites per se cause observed reductions in male copulatory success.

Macrocheles subbadius (Acarina: Macrochelidae) is a cosmopolitan species of mite occurring on a variety of substrates including animal dung and rotting plant tissue (G. W. Krantz pers. comm. 1992). To exploit such ephemeral substrates, mites attach to adult members of numerous dipteran species which provide them an essential means of transport to fresh substrates where mites feed on rhabditid nematodes, fly eggs and small arthropods (Filipponi and Pegazzano 1963; Krantz 1983; Polak unpubl. data). The present study focuses on the effect of attached mites on sexual selection in *Drosophila nigrospiracula* (Diptera: Drosophilidae), a fruit fly that is endemic to the Sonoran desert of North America and that breeds in necroses of saguaro (*Carnegia gigantea*) and cardon cacti (*Pachycereus pringlei*) (Heed 1978). Adult female mites, and occasionally deuteronymphs, attach to the ventral surface of the fly abdomen of both male and female flies (Polak 1993a). Mites attach near the junction with the thorax by embedding their chelicerae into abdominal tissue, but when numerous mites (>2) are present on a single fly, some are commonly found attached more distally along their host's abdomen. Approximately 5% of attached mites are found on the neck and face of the fly. *Macrocheles subbadius* mites are orange-brown in color and large enough that they can be distinguished with the unaided human eye when attached to their host. A laboratory experiment to track radioactive carbon isotope from labeled flies to mites has revealed that mites ingest fly hemolymph (and perhaps other host tissue), and establishes the parasitic nature of this association (Polak MS). Flies previously parasitized by mites can be distinguished by abdominal scars, darkened puncture wounds inflicted by mites with their mouthparts.

MATERIALS AND METHODS

Patterns of Mite Dispersion within Fly Populations

All samples of flies collected to assess dispersion patterns of mites and the impact of mites on host sexual selection were sampled from the dry, outer surfaces of necrotic cacti. Courtship and mating occur primarily on these sites (Markow 1988). Feeding and resting flies are found along rivulets of ooze and inside moist cavities that tunnel through necrotic tissue. Therefore, in this study we assume that sex ratios of sampled flies closely approximate operational sex ratios (OSRs, Emlen and Oring 1977).

Mean intensity of infestation was calculated as the total number of mites in a sample of flies divided by the total number of flies within that sample. This parameter was calculated for 25 host populations sampled on different days at 12 different cacti from August 1992 to March 1993. On each

day of collection, 15 to 535 flies were netted or aspirated directly from the substrate and placed into 8-dram vials containing banana-agar food medium (vials throughout this study contained food medium). On the day of collection, flies were returned to the laboratory and scored individually for number of attached mites. The ratio between the variance in mite burden and mean intensity of infestation (s^2/x) was calculated for each of these 25 samples for which sexes were pooled. Degree of dispersion of parasites within a given host population is manifested by this ratio (Anderson and Gordon 1982): for underdispersed (regular) distributions of parasites, s^2/x values are less than unity, whereas for overdispersed (aggregated or contagious) distributions, s^2/x values are greater than unity. Random patterns of dispersion, in turn, are described by s^2/x values approximately equal to unity (for random, Poisson, distributions, the variance = the mean).

The fly population at one cactus, which was known to have fallen on August 28, 1992, was sampled at 2–5-d intervals for a total of 33 d to monitor patterns of mite dispersion among flies as the cactus tissue deteriorated until only its desiccated skeleton remained. At this advanced stage, it supported few flies. Mean intensity (see above) and prevalence (fraction of the fly population infested) were calculated for each sample.

We tested whether infestation by mites affects sexual selection in natural populations of flies by contrasting copulating versus single flies with respect to the (1) frequency at which members of the two groups carried one or more mites, and (2) mean intensity of infestation. Samples of copulating and single flies were collected from the outer surface of rotting saguaro cacti on 12 different days from May 3 to September 7, 1992, at locations within 45 mi of Phoenix, Arizona. Five different cacti were visited on one to four occasions. On each visit to a cactus supporting flies, beginning shortly after sunrise, an approximately 1.5-m portion of either a fallen or a standing necrotic cactus was continuously monitored for copulating pairs. When a pair was discovered, single individuals within 5 to 8 cm of the pair were aspirated into 8-dram banana-agar food vials. The copulating pair was then collected and aspirated into a separate food vial. Vials were individually labeled.

Scoring and Analysis of Mite Load

On the day of collection, copulating and single individuals were returned to the laboratory. Each fly was sexed and the number of attached mites it carried was counted under a dissecting microscope. Flies carrying no mites were scored for mite-induced scars. Body size was estimated by measuring thorax length using an ocular micrometer (Robertson and Reeve 1952). Flies collected from May 3 to May 26 were significantly larger than those from June 11 to June 15. Therefore, contrasts of mean number of mites and scars between copulating and single individuals are reported separately for these two periods. Thorax length of flies collected between August 28 and September 7 was not measured in order to increase the rate at which flies could be scored for mites and scars. Data from this third sampling period were also analyzed separately. To determine whether frequency of infestation data from these three periods could be combined, dif-

ferences among the sampling period were assessed using heterogeneity χ^2 analysis. This analysis tests the null hypothesis that different samples could have come from the same population (i.e., H_0: samples are homogeneous) (Zar 1984).

For each of the 12 samples of flies, we calculated the selection differential (S), as the difference between mean number of mites on copulating individuals and the mean number for the total population (copulating and noncopulating individuals) (Arnold and Wade 1984; Falconer 1989). This parameter, which can be regarded as the deviation between mean phenotypic value before and after selection, was used as an estimate of the magnitude of sexual selection operating through differences in mite load among individual flies. We then determined the relation between S and the intensity of parasitism across fly populations, and contrasted this relation between the sexes. Because no single females were found in two samples, S for females was calculated for 10 samples as opposed to 12 for males.

Interaction effects between intensity of infestation by mites and host sex were assessed by constructing a regression model with intensity as one independent variable (quantitative variable) and sex as a qualitative variable. We tested whether $\beta_3 = 0$ (β_3, the regression coefficient of the cross-product term between intensity and sex) in order to determine whether the slope for males differed from that for females (Neter et al. 1990).

Laboratory Assay of Male Copulatory Ability

A laboratory experiment was conducted to determine the effect of a previous episode of infestation on male copulatory success. Males used in the experiment were collected at two different saguaro cacti on 3 days during March 1993. Flies were collected by sweeping an insect net over necrotic sections of cacti and aspirating flies into 8-dram vials. In the laboratory, males infested with either one or two mites were gently aspirated into separate vials. An equal number of males without mites and mite-induced scars were also separated. All vials contained ad libitum food for males, including live yeast and autoclaved, necrotic cactus tissue. Vials containing males were stored in an incubator for 5 d with a 12L : 12D photoperiod and a 26°C day and 21°C night temperature cycle. We chose a 5-d period to ensure that males reached sexual maturity before the mating experiment (Pitnick and Markow unpubl. data). All flies were transferred to new food vials once during the 5-d maturation period.

On the day of the mating experiment, one infested and one uninfested male were placed together into a clean 8-dram vial. A total of 32 and 38 males carrying one and two mites, respectively, were tested. Before an individual female was introduced to each vial, males were allowed to acclimate to vial conditions for 2 min. Females were derived from stock cultures and mated the previous evening. Each vial was laid horizontally and was observed until a male copulated, typically within less than 1 min. After copulation, males were anesthetized with CO_2 only long enough to allow removal of mites using fine forceps and a dissecting microscope. All males were then separately placed into vials containing necrotic cactus and held for 2 d. Since males of this species will re-mate within a 15-min period in the laboratory, we
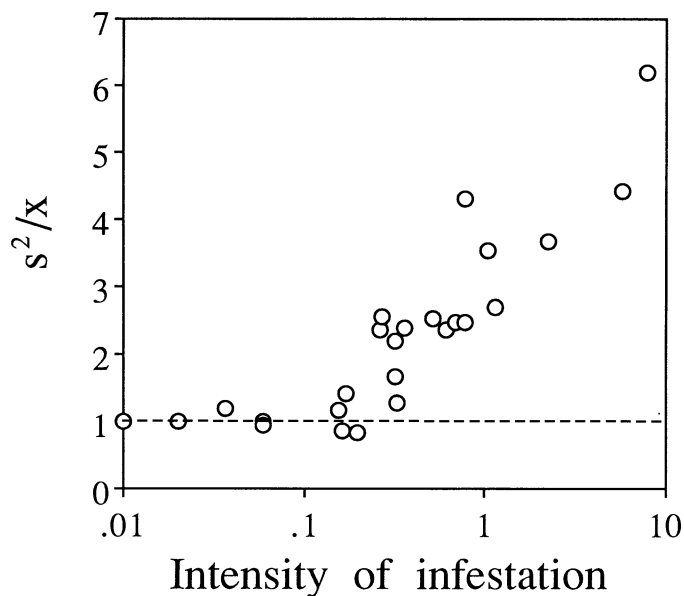
FIG. 1. The relation between ratio of variance to mean intensity of infestation and mean intensity of infestation for 25 fly populations. Mean intensity is plotted on $\log_{10}$ scale to better visualize the data. Data points consist of 15–535 flies of both sexes ($\bar{x}$ = 127, SE = 35.5).

assume that a 2-d recovery period is ample time to remove any handicap that may have been induced by a previous copulation. Incubator conditions were as described above. Following this period of recovery, previously parasitized males were again placed into agar vials with the same unparasitized males with which they were pitted in the first trial. A non-virgin female was then introduced separately into each vial, as described above. Once a copulation occurred, the pair was aspirated from the vial, and the copulating male was identified under a dissecting microscope. Previously parasitized males were distinguished by mite scars.

RESULTS

Sex Ratios at Mating Sites

Courtship and mating in *Drosophila nigrospiracula* occur predominantly on the outer surface of rotting saguaro cacti, although mating pairs of flies were also observed within cavities. All populations (n = 12) of adult flies sampled from outer surfaces of cacti were characterized by a sex ratio biased toward males (sign test, P < 0.0001). Pooling data from samples collected during each of the three periods of the study, female:male ratios (0.30 : 1, 0.17 : 1, 0.31 : 1) differed greatly from unity (χ^2 = 39.5, 79.4, 101.1, P < 0.0001). Pooling was justified as samples in each period were found to be homogeneous by heterogeneity χ^2 testing.

Distribution of Mites among Flies

Degree of mite aggregation was assessed using s^2/x ratios calculated for each of 25 fly populations sampled across a 7-mo period. Ratios varied from 0.95 to 6.2, indicating that mite distributions ranged from being nearly random to strongly aggregated within host populations. The degree of mite
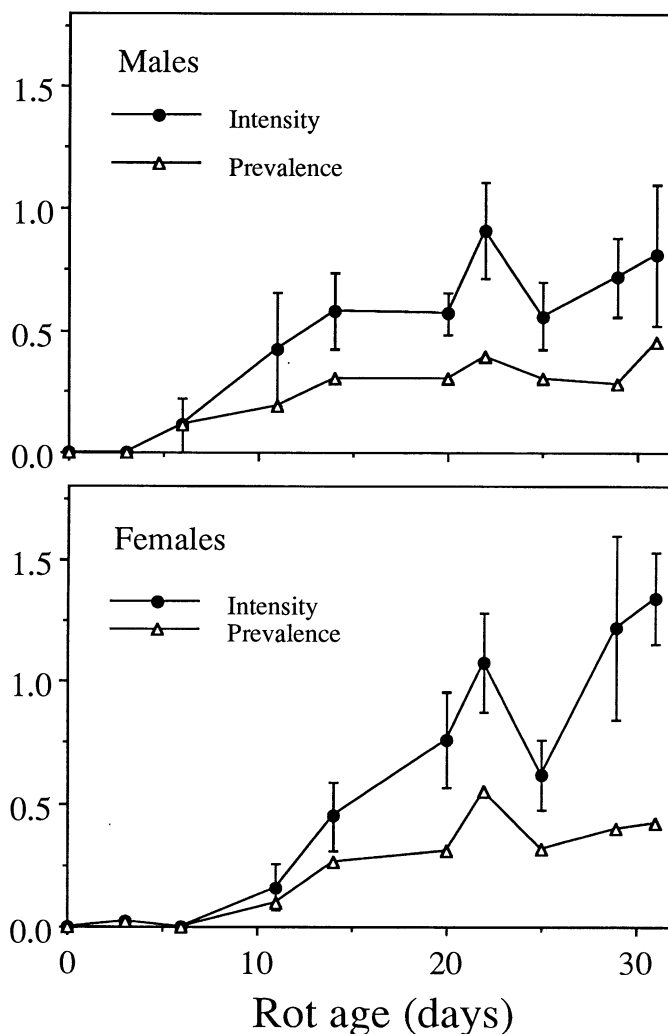

FIG. 2. Increase in mean intensity (number of mites per fly) and prevalence (fraction of flies infested) of infestation with rot age. Error bars on intensity represent standard errors.

aggregation is strongly positively related to the intensity of infestation (r_s = 0.88, P < 0.0001) (fig. 1). Greatest levels of aggregation occurred within fly populations experiencing the greatest intensities. For example, in the population with the most extreme mean intensity of 7.8 mites per fly (range 0–25, n = 33 flies), the most heavily infested quartile of flies harbored 55% of the population of attached mites. This fly population was sampled at a cactus in a very late stage of necrosis (very little moist tissue remained). Figure 2 illustrates the increase of prevalence and intensity of mite infestation with rot age monitored across a 33-d period.

Mites and Copulatory Success of Flies

In the first period of the study, the difference in mite load on copulating versus single individuals was statistically nonsignificant, although in the last period, characterized by the highest intensity of infestation, this difference was significant in both males and females (table 1).

The presence of one or more attached mites had a marked

TABLE 1. Mean mite load and mean number of scars on copulating and single flies sampled on saguaro cacti. Values in parentheses are range and n, respectively. Mann-Whitney Z is corrected for ties. One-tailed P values are presented for mite load, and two-tailed P values for scars.

	Males			Females		
	Copulating	Single	Z, P	Copulating	Single	Z, P
May 3–26						
Mite load	0.053	0.079	0.28, 0.39	0.11	0.13	0.35, 0.36
	(0–1, 76)	(0–2, 114)		(0–2, 54)	(0–2, 31)	
Scars	1.15	0.70	0.026, 0.98	0.81	0.95	0.05, 0.96
	(0–14, 39)	(0–11, 87)		(0–6, 42)	(0–10, 21)	
June 11–15						
Mite load	0.18	0.39	1.49, 0.067	0.11	0.92	0.89, 0.18
	(0–1, 56)	(0–6, 136)		(0–2, 44)	(0–6, 12)	
Scars	0.56	0.76	0.39, 0.69	1.02	0.60	0.21, 0.83
	(0–6, 43)	(0–14, 66)		(0–9, 41)	(0–4, 15)	
Aug. 28–Sept. 7						
Mite load	0.17	0.99	4.73, 0.0001	0.60	1.28	1.97, 0.024
	(0–2, 95)	(0–10, 374)		(0–4, 84)	(0–11, 101)	

effect on copulatory success of both sexes, but this effect was stronger in males than in females (table 2). Of all copulating females scored for mites, 21.3% of these were infested, whereas among copulating males, this value was 12.7%. Data in table 2 are pooled across the three periods of the study; heterogeneity χ^2 was not significant for females ($\chi^2 = 2.53$, df $= 2$, $P > 0.25$), and only marginally so for males ($\chi^2 = 5.99$, df $= 2, 0.05 < P < 0.025$). When frequency data for males in the first period (that with the lowest intensity of infestation, table 1) were excluded from this analysis, homogeneity between periods was restored (heterogeneity$\chi^2 = 0.98$, $P > 0.25$) and results of the χ^2 analysis reported in table 2 remained virtually unaffected (χ^2 for males $= 22.34$, df $= 1$, $P < 0.0001$).

Since one previous field study of *D. nigrospiracula* has suggested that copulating males can be larger than unpaired individuals (Markow 1988), we tested for a relation between body size and mite load. There was no difference in body size between infested and uninfested males or females (table 3), and in a previous study the correlation between male body size and number of attached mites per male was also nonsignificant (Polak 1993a).

Among infested males, the distribution of mites on single individuals was skewed significantly more toward high mite loads than that on copulating males (fig. 3, Kolmogorov-Smirnov $D = 0.373$, $P < 0.005$). Among females, however, the difference between distributions was not significant (fig. 3, Kolmogorov-Smirnov $D = 0.128$, $P > 0.75$). Of the 27 infested males found *in copula,* none carried more than two attached mites, whereas up to four mites were found on copulating females (fig. 3).

Mean number of abdominally located mite scars counted on both sexes (range 0–14) were typically more than tenfold the mean number of attached mites per fly (table 1). Yet, among currently uninfested flies, there was no difference in the number of scars on copulating versus single individuals (table 1), suggesting that flies surviving an episode of infestation are as likely to copulate as previously unparasitized flies.

For both males and females, the intensity of infestation was positively and significantly related to the selection differential calculated for each fly population sampled (fig. 4). The significant difference between the regression slopes ($\beta_3 = 0.34$, $t = 3.14$, $P = 0.0057$) indicates that this relationship was significantly more pronounced in males than in females. Therefore, for both sexes, but more markedly for males, the opportunity for sexual selection increased significantly as a function of intensity of infestation. These findings indicate

TABLE 2. Frequency at which copulating and single males and females were found with and without mites on saguaro cacti. Numerals in parentheses are expected values. Entries in table are pooled frequencies across three sampling periods.

	Pairing status			
Attached mite(s)	Copulating (expected)	Single (expected)	Total	χ^2 (df, P)
Males				
Yes	27 (56.5)	185 (155.5)	212	28.04 (1, <0.0001)
No	200 (170.5)	439 (468.5)	639	
Total	227	624	851	
Females				
Yes	42 (52.8)	51 (40.2)	93	6.98 (1, 0.008)
No	155 (144.2)	99 (109.8)	254	
Total	197	150	347	

TABLE 3. Mean thorax length (mm) of males and females sampled with and without mites. With each mean sample size and standard error are presented in parentheses.

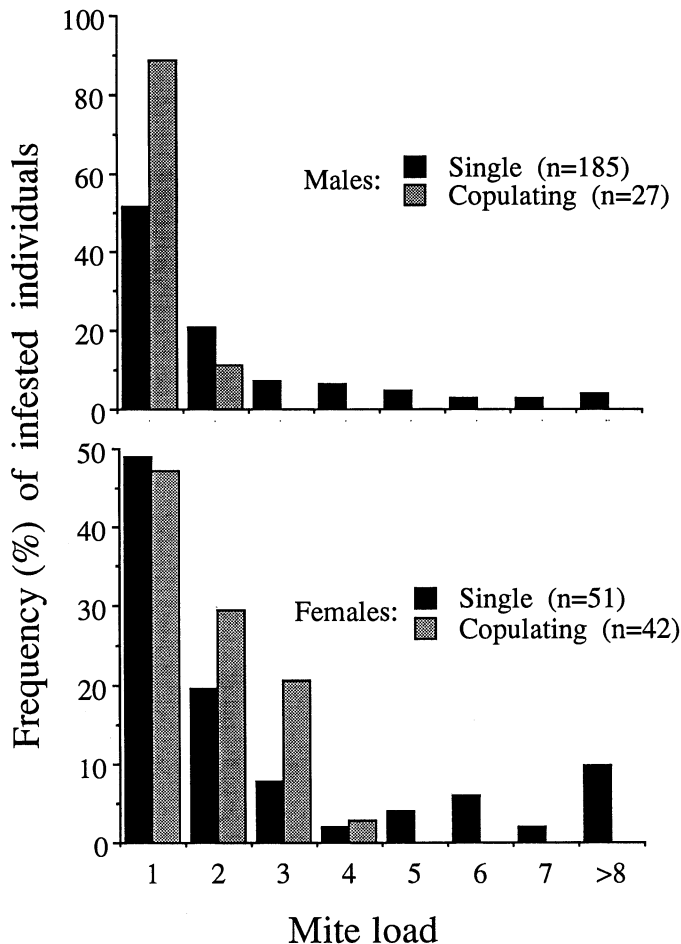|	Infested	Uninfested	t (df, P)
May 3–26			
Males	1.24 (11, 0.032)	1.30 (11, 0.028)	1.45 (20, 0.16)
Females	1.45 (8, 0.033)	1.44 (8, 0.029)	0.52 (14, 0.61)
June 11–15			
Males	1.15 (38, 0.012)	1.16 (38, 0.012)	0.68 (74, 0.50)
Females	1.35 (18, 0.020)	1.32 (18, 0.011)	1.26 (34, 0.26)

FIG. 3. Frequency at which males and females sampled *in copula* or singly where infested with one to eight (or more) mites.
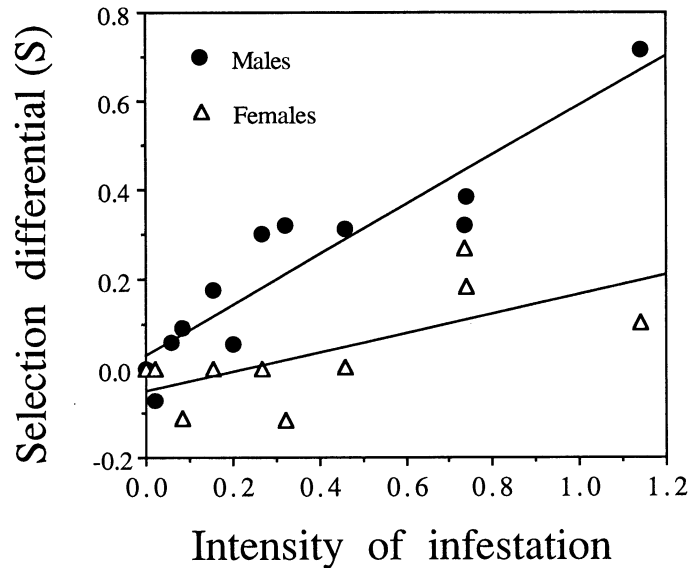

FIG. 4. The relation between the selection differential and intensity of infestation for males (filled circles) and females (open triangles). Males: slope = 0.56, SE = 0.072, R^2=0.86, P = 0.0001; females: slope = 0.22, SE = 0.084, R^2=0.49, P = 0.032.

that the magnitude of sexual selection is correlated with rot phenology and is strongest at older, more desiccated rots.

Laboratory Assay of Male Copulatory Ability

The presence of a single attached mite did not lower the probability of copulation for males in the laboratory experiment in which infested males competed against those without mites. Of the 32 males infested with one mite, infested males did not copulate successfully more than uninfested males (table 4). Of the 32 males that failed to copulate in the first competitive mating trial, 17 mated, whereas 15 did not mate in the mating trial 2 d after mites were removed (χ^2 = 0.12, df = 1, P > 0.5).

In the second experiment, in which males carrying two mites competed against uninfested males, significantly fewer infested males (31%) copulated compared to uninfested males (68%) (table 4). Removal of mites followed by a 2-d recovery period with ad libitum food restored the competitive ability of previously infested males: 47% of infested and 53% of uninfested males copulated. This experimental finding demonstrates that a burden of two mites significantly reduced a male's chance at copulating, but that mite removal and a 2-d recovery period eliminated the mating handicap. This result

suggests that mites per se reduce male copulatory success and that, in nature, infested flies suffer reductions in copulatory success as a result of being infested, rather than as a consequence of correlated effects.

DISCUSSION

The ectoparasitic mite, *Macrocheles subbadius*, reduces the copulatory success of its host, *Drosophila nigrospiracula*. In nature, flies of both sexes burdened with mites were significantly less likely to be found in copula than uninfested individuals. Moreover, this effect was more pronounced in males than in females: of all males and females found in copula, 12.7% and 21.3% were infested, respectively, whereas the proportion of single males and females harboring mites was 31.3% and 34.0%, respectively. The number of attached mites above which an individual was never found *in copula* also differs between the sexes, being more stringent in males (two mites) than in females (four mites).

Our data indicate that reduced host copulatory success is caused by mites per se. Two lines of evidence show that flies that had been infested, but whose mites had since detached, copulated as frequently as flies with no history of infestation. First, copulating and single individuals in nature did not differ with respect to number of scars (table 3). Second, males from which mites had been experimentally removed copulated as frequently (after a 2-d recovery) as uninfested, unscarred individuals (table 4).

The differential effect of mites between the sexes is further demonstrated by contrasting regression functions that relate the selection differential (S), which expresses the magnitude of sexual selection (Arnold and Wade 1984), with the mean number of mites per male (intensity) (fig. 4). Among males, intensity explains a greater portion of the variation in S (86%) than in females (49%), and more importantly, the slope of

TABLE 4. Frequency at which infested and uninfested males mated with laboratory females. Numerals in parentheses are expected values.

Removal of mite(s)	One mite	Uninfested	χ^2 (df = 1), P	Two mites	Uninfested	χ^2 (df = 1), P
Before	18 (16)	14 (16)	0.50, >0.5	12 (19)	26 (19)	5.16, 0.01 < P <0.025
After	16 (16)	16 (16)	0.0, 0.99	17 (18)	19 (18)	0.11, >0.75

this function is significantly steeper in males than in females. Because the strength of selection is a function of the degree of phenotypic variation in a given trait (Falconer 1989), the relation between selection and intensity can be explained by the strong positive relation between intensity and variance in mite load (fig. 3). Our data complement those of one interspecific study of New Guinean passerine birds, which has reported a strong positive correlation between the opportunity for selection and intensity of infection by blood protozoa (Clayton et al. 1992).

Whereas the present study has focussed on the effect of mites on host sexual selection, attached mites also increase host mortality and reduce host longevity and fecundity (Polak MS). Mites therefore also represent agents of natural selection within fly populations. If fly populations harbor genetic variation for susceptibility to infestation by mites, we might expect the evolution of flies that are better able to evade infestation. An important next step toward characterizing the Drosophila–Macrocheles association could therefore be to estimate the magnitude of genetic variation for the ability to evade infestation among flies, and consequently, the potential for evolutionary change in mite burden within fly populations.

Our results indicate that the strength of sexual selection is highly variable and strongly correlated to intensity of infestation, and therefore, to rot age. This finding underscores another novel feature of our study, the link between the magnitude of sexual selection and a highly variable ecological parameter, substrate suitability. The positive relation between substrate suitability and intensity of infestation is probably a consequence of numerous factors, including the number of mites that are unattached to a host relative to the number of flies present at a rot, as well as the "motivation" of mites to infest their host. Nevertheless, an important implication of such fluctuating selective pressures is their potential for maintaining heritability for parasite resistance by preventing (or slowing) the fixation of resistance-conferring alleles, especially if multiple loci are involved (Eshel and Hamilton 1984; Atkinson 1991). This outcome would be more likely to arise if even a minor trade-off exists between resistance and life-history variables such as host fecundity and survivorship (Minchella 1985; Stearns 1992).

Our finding that all fly populations were characterized by ratios of variance to mean intensity greater than unity, except for those harboring a minimal number of mites, is consistent with the occurrence of heterogeneity in genetic factors underlying resistance (Anderson 1988). Nonrandom parasite distributions are typical of most host species studied (Anderson 1988), including other Drosophila (Jaenike 1992; Jaenike and Anderson 1992). A study examining the distribution of tapeworms in deer mice, Peromyscus maniculatus, provides

an illustration of such genetic effects (Wassom et al. 1986). In laboratory populations of vulnerable mice, parasites were approximately randomly distributed, whereas parasites became aggregated in heterogeneous samples consisting of both resistant and vulnerable hosts (resistance in this case is controlled by a single, autosomal, dominant gene).

It is also possible that in D. nigrospiracula, certain ecological (e.g., choice of feeding site), demographic (e.g., age) and other genetic (e.g., inbreeding) factors, acting alone or in concert, predispose some individuals to a greater probability of infestation. These factors could contribute to the observed aggregated mite distributions (Anderson and Gordon 1982). For example, variability in the probability of infestation across rots of different ages could lead to an aggregated distribution of mites within fly populations. Moreover, the weight of attached mites probably depresses a fly's ability to run and to decamp rapidly from the substrate when faced with further threat of infestation. Running speed, for example, is negatively correlated with weight burden carried by leafcutter ants, Atta colombica (Lighton et al. 1987). If the probability of further infestation rises with mite burden, it would contribute to aggregated mite distributions in fly populations (see, e.g., Poulin et al. 1991).

Mechanisms of Mite-Induced Reductions in Host Copulatory Success

Several lines of evidence have shown that the strength of sexual selection imposed by mites is greater in males than in females, probably because males must work closer to their physiological limits relative to females in mate acquisition. Sexual selection theory predicts relatively greater degrees of competition among males when more males are present relative to females within the available pool of receptive individuals (Emlen and Oring 1977; Thornhill 1986). In the present study, operational sex ratios of all populations sampled were significantly skewed toward males, and extreme ratios neared six males to one female.

Reduced copulatory success of males infested with mites is probably attributable to impaired competitive ability, courtship vigor, and physical inhibition of genitalic coupling. Because competitive interactions and female-directed courtship are energetically expensive, they are likely to become compromised when these large, feeding mites are attached to their host. Competitive interactions between males at natural aggregation sites include reciprocal chases and what appear to be short abortive bouts of courtship. When a receptive female alights in an active mating area on a necrotic saguaro, nearby males immediately run toward her, and often pursue her until she either decamps from the substrate or accepts one of her suitors. Competitive interactions among rival males involve

mutual pushing as one male runs past the other in order to remain close to the distal end of the female's abdomen. Females signal receptivity by spreading and moving their wings slightly forward, at which point the male directly behind her is most likely to mount and copulate. Male copulatory success is therefore mediated by the ability to maintain close contact with a receptive female and to exclude competing males from this position of advantage.

Parasite-induced mechanical constraints on male copulatory ability are a previously unidentified mechanism of PMSS. To achieve copulation, male flies must bend their abdomen and position its terminal end against that of the female. On eight occasions during our laboratory study, abdominally attached mites were observed to physically obstruct this process, and after several aborted attempts, males abandoned their would-be mates. Smaller males may be more strongly affected, since a greater fraction of their abdominal surface area is occupied by mites relative to larger individuals.

Active avoidance by females could also contribute to the reduced copulatory success of infested males. Direct (material) and indirect (good genes) benefits accrued by discriminating females could explain the evolution of such avoidance behavior (reviewed in Kirkpatrick and Ryan 1991). A potential benefit to a discriminating female is avoidance of direct transmission of mites to herself. However, of the 30 copulations with infested males witnessed during this study (and of many more subsequent to this work), no female ever acquired a mite from her partner.

Alternatively, females that mate with an uninfested male could benefit through passing genes conferring resistance to offspring (Hamilton and Zuk 1982). Whether heritable variation exists within fly populations that influences individual susceptibility to mites in not known at present, although strong fitness consequences from parasitism, together with variable intensities of parasitism, could serve to maintain the needed genetic variation within the host population for this form of adaptive mate choice (Cade 1984; Atkinson 1991). No female, however, was ever observed actively avoiding males bearing mite(s), nor was any female witnessed attempting to dislodge an infested male during any phase of mounting or copulating. To test thoroughly the possibility of active female choice of this sort, analyses of video recordings of courtship and mating will be required.

Our results indicate that mites also reduce female copulatory success. One explanation for this observation is that infestation renders females unreceptive. In fact, there is a strong negative correlation between female fecundity and number of attached mites. Moreover, all of 14 females dissected that were burdened with four or more mites were sterile (Polak MS).

Male mate choice may also contribute to reduced female copulatory success. Read (1990) suggested that male choice for parasite-free mates could occur in species in which males provide parental care or in which sperm production is limited. In fact, whenever the costs associated with courtship and copulation are nontrivial, males become predisposed to discriminate among females, especially if considerable variation exists in female quality. Although male *D. nigrospiracula* contribute little but gametes toward offspring, their sperm

are long (6.30 ± 0.06 mm; Pitnick unpubl. data), and a laboratory study of this species showed that sperm transfer decreases across three subsequent copulations when males are given ad libitum access to receptive females (Polak unpubl. data). In a comparative study that examined other *Drosophila* species exhibiting impressive variation in sperm length, (Pitnick and Markow 1994) species with long sperm have been shown to produce and transfer fewer gametes per copulation relative to species in which males produce shorter sperm. Therefore, male *D. nigrospiracula* may have been selected against inseminating infested, infertile females, in order to prevent sperm wastage and depletion of sperm stores. Especially since females of this species mate multiply on any given day (Markow 1982), sperm competition could favor males that pass the greatest number of sperm per ejaculate to fecund, high-quality females.

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