

## Mate choice opportunity leads to shorter offspring development time in a desert insect

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### Abstract

We describe indirect genetic benefits of mate choice in two allopatric populations of cactophilic *Drosophila mojavensis*. By manipulating mate choice opportunity, we show that greater mate choice among sexually mature adults leads to shorter offspring egg-to-adult development times; the extent of this reduction was influenced by population origin and by host plant environment. We performed multiple-choice mating trials with individually marked flies to investigate whether differential male mating success was a consequence of female choice, male interaction, or both. We demonstrate that male copulation frequency was not random and instead, was determined by female choice. Virgin females in these trials were no less discriminating than females that had been previously exposed to males. These results suggest that there are indirect benefits of female mate choice that are population and environment specific, consistent with the hypothesis of ecologically influenced ‘good genes’ sexual selection.

### Introduction

Female discrimination among potential mates based on an ‘honest’ signal of superior quality can generate an indirect benefit to offspring (Andersson & Simmons, 2006). Although this ‘good genes’ mechanism for sexual selection is sometimes considered insufficient to overpower opposing direct selection on females (Bussiere *et al.*, 2008), phenotypic correlations between female choice and offspring fitness have been demonstrated (Simmons, 1987; Von Schantz *et al.*, 1989; Petrie, 1993; Andersson, 1994). Experimental manipulations of mate choice have been shown to influence offspring fitness when multiple males and females are allowed to interact freely, as opposed to being force-mated in single pairs. For example, female seaweed flies, *Coelopa frigida*, allowed a choice of mates in a population cage had higher fertility and produced offspring with higher viability than force-mated females (Crocker & Day, 1987). Similar influences have been observed in other

species in the field and in the laboratory (Martin & Hosken, 2003; Moore, 1994; Norris, 1993).

Environmental heterogeneity can influence behavioural traits, and ecological variation has been associated with differences in mate choice patterns among populations within a species. Variation in mate choice has been shown to be a consequence of adaptation to different environments in *Heliconius* butterflies, in which warning coloration patterns are genetically correlated with mate preference (Jiggins, 2008). In guppies, *Poecilia reticulata*, carotenoid colour is a geographically variable indicator value of a sexually selected male trait (Grether, 2000), and female preference for this trait is thought to have evolved via sensory bias (Rodd *et al.*, 2002). In sticklebacks, differences in water clarity influence the relative use of visual and olfactory cues in mate choice (Heuschele *et al.*, 2009). In cactophilic *Drosophila mojavensis*, a genetic correlation between egg-to-adult development time and premating isolation between allopatric populations is influenced by larval rearing environments (Etges, 1998). Thus, ecologically driven differences in mate success among populations may influence sexual isolation between populations.

Sexual selection is expected to deplete genetic variation for selected traits (Kirkpatrick & Ryan, 1991). One

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mechanism proposed to explain observed genetic variation for such traits is genotype  $\times$  environment interaction ( $G \times E$ ) (Greenfield & Rodriguez, 2004). For example, a superior male in one environment may be inferior in another, leading to lower offspring fitness if he encounters an environment different from the one where the female exercised choice. When environmental heterogeneity influences the fitness of a given genotype, disruptive selection across environments can maintain genetic variation (Ingleby *et al.*, 2010).  $G \times E$  and environmental influences on mate choice have been observed in predatory soil mites (Lesna & Sabelis, 1999), seaweed flies (Gilburn & Day, 1994), gray tree frogs (Welch, 2003), three-spine sticklebacks (Candolin *et al.*, 2007) and cactophilic *D. mojavensis* (Etges *et al.*, 2007, 2009).

Populations of *D. mojavensis* are ideal subjects for the investigation of genetic and ecological influences on patterns of mate choice. Baja California populations of this species are ancestral to those in mainland Mexico and Arizona and are thought to have colonized the mainland via a host plant shift from pitaya agria cactus, *Stenocereus gummosus*, to organ pipe cactus, *Stenocereus thurberi* (Heed, 1978, 1981; Etges, 1990). Mainland populations express longer and more variable egg-to-adult development times (DEVT), particularly when cultured on organ pipe cactus; DEVT is known to be heritable (Etges, 1993, 1998). Mainland flies reared on organ pipe showed the greatest response to artificial selection on DEVT, and selected flies exhibited decreased premating isolation with a Baja population (Etges, 1998). Thus, there is a genetic correlation between premating isolation and DEVT that differs in magnitude among populations in an environment-specific manner.

We investigated the effects of rearing substrates on mate success and assessed two components of offspring fitness: egg-to-adult viability and development time. We used multiple-choice mating trials, because they closely resemble mating congregations around cactus rots in nature (Krebs & Bean, 1991), and host–cactus rearing environment is known to influence mating behaviour (Brazner, 1983; Brazner & Etges, 1993). To investigate the mechanism of sexual selection, we conducted multiple-choice mating trials with individually marked cactus-reared males and females and monitored all inter- and intra-sexual interactions to examine the cause of any deviations from random male-mating success. To assess the influence of previous female social experience on male mating success, we included both virgin females and females that had been previously exposed to other sexually mature adults in these multiple-choice trials.

## Materials and methods

### Stocks and husbandry

Populations of *D. mojavensis* were collected over baits and from cactus rots in the field, returned to the laboratory

and mass reared on banana food (Brazner & Etges, 1993) in 8-dr shell vials at room temperature. A population collected in November 2007 from Punta Onah, Sonora, originated from 472 baited adults plus *ca.* 80 adults aspirated from cactus rots, and a population from Punta Prieta, Baja California, collected in January 2008 was established from 465 baited adults. For five generations, stocks were maintained in large numbers on banana food in 8-dr vials (Brazner & Etges, 1993) in an incubator at 27 °C during the day and 17 °C at night on a 14 : 10 LD cycle. Stocks were reared on laboratory food at medium larval density in half-pint bottles for one generation in an effort to remove possible maternal effects resulting from vial rearing.

### Cactus rearing

Half-pint bottles containing 75 g of aquarium gravel covered with a 5.5-cm-diameter piece of filter paper (Etges, 1998) were autoclaved. To this, 60 g of either agria or organ pipe tissue was added and then the bottle was autoclaved again for 10 min. After cooling to room temperature, each culture was inoculated with 0.5 mL of a pectolytic bacterium, *Erwinia cacticida* and 1.0 mL of a mixture of seven yeast species common in natural agria and organ pipe rots: *Dipodascus starmeri*, *Candida sonorensis*, *Starmera amethionina*, *Candida valida*, *Pichia cactophila*, *Pichia mexicana* and *Sporopachydermia cereana*. Eggs were collected for 10 h from each group of aged adults and washed in deionized water, in 70% ethanol and again in sterile deionized water. Eggs were counted out in groups of 200, transferred to a 1-cm<sup>2</sup> piece of sterilized filter paper and placed on fermenting cactus in the incubator (Etges, 1992). We measured egg-to-adult development time (DEVT) in days and viability (calculated as the number of flies eclosed/number of hatched eggs) for each culture. All emerged adults were collected daily from each culture, separated by sex and housed in small groups in vials on banana food in the same incubator until sexually mature (10–12 days).

### Mating-choice manipulation experiment

Four bottles containing agria tissue and four bottles containing organ pipe tissue were established for each population. Flies emerging from two bottles of each cactus/population combination were randomly assigned to mating treatments: one allowing free interaction among courting adults ('multiple-choice') and the other in which males and females were paired by us and allowed to individually mate ('no-choice'). In the multiple-choice treatment, 50 flies of each sex were allowed to interact for 30 min in a 250-mL Erlenmeyer flask during the morning active period (8 AM), during which approximately half of the flies copulated. Each copulating pair was removed, placed into an empty 8-dram vial and allowed to mate for 48 h in an incubator set on a

27 : 17 °C, 14 : 10 LD cycle. In the no-choice treatment, 25 randomly formed pairs were housed individually in 8-dram vials for 48 h and allowed to mate. Mated females from each treatment were allowed to oviposit and eggs from each treatment group were placed onto cactus tissue as described earlier. Offspring from these cultures were collected and subjected to the multiple-choice and no-choice experiments. DEVT and egg-to-adult viability were scored for both generations. Data were log-transformed, and analysis of variance (SAS-Institute 2004) was used to assess the main effects of population, cactus, mate-choice treatment, and sex, and their interactions.

### Male-mating success trials

Eight cultures of each type of fermenting cactus were established for each population. To assess the influence of previous female social experience on male mating success, half (16) of these trials used females that had been combined with mature males for 24 h (and then separated for 24 h because mated females typically do not remate for approximately 24 h at 25 °C, Markow (1982)) and the other half of the trials used virgin females. We conducted four replicate trials of each of the eight population/cactus/female 'experience' treatment combinations, for a total of  $n = 32$  trials. Treatments were assigned randomly to day of trial, so that four trials were conducted each day over an 8-day period. Trials were conducted during the morning period of activity (Krebs & Bean, 1991). Ten adults of each sex were individually marked with different coloured fluorescent dusts (Radiant Color, Richmond, CA, USA), combined in a small (60 × 15 mm) petri dish after light CO<sub>2</sub> anaesthesia, and allowed to court and to mate. We recorded the number of matings per individual, female discrimination behaviours, male–male interactions and the time

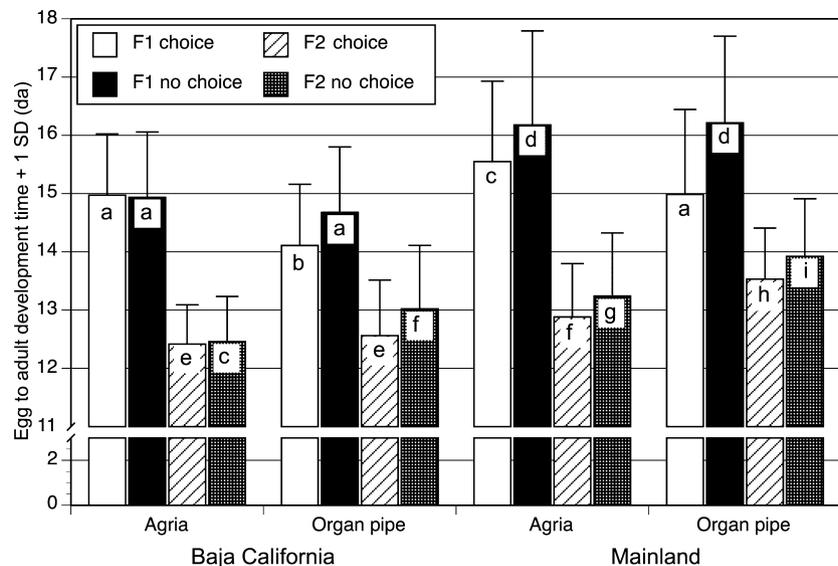
it took for each male to successfully copulate. The female discrimination behaviour 'rejection' was defined as an instance in which a female was courted by a male but did not allow him to mount, either by remaining stationary or walking, running or flying away. A male–male interaction was defined as an instance in which a male approached a courting pair, either by lining up directly posterior to the courting male or by walking up to the side of the courting male and attempting to interfere by licking the female genitalia. In all instances, such interactions resulted in the female walking away from both males; this was also scored as a female rejection event.

The observed distribution of matings per male for each trial was compared to the expected distribution, as derived from a Poisson distribution (Jones *et al.*, 2006). The null hypothesis was that a male could mate up to 10 times in each trial (the maximum number of matings by a single male observed in the laboratory is 7, H. Alonson-Pimentel *et al.*, unpublished data). Expected values were calculated using the Poisson formula with an average equal to the observed number of successes. Significance of the goodness-of-fit test statistic was determined using the Monte Carlo procedure of Hope (1968); we used the *chisq.test* procedure in R (2010).

## Results

### Manipulation of mate choice

In both generations, multiple-choice flies produced progeny with significantly shorter DEVT than no-choice flies ( $\alpha = 0.05$ , Fig. 1). The magnitude of the decrease was both population- and cactus-specific, as revealed by the significant three-way interaction in the ANOVA (Table 1). Variation in DEVT due to mate choice was greatest in the mainland population reared on organ pipe



**Fig. 1** Offspring development time in days for two allopatric populations of *Drosophila mojavensis* reared on two different cactus substrates. Different letters (a–i) denote significant differences ( $\alpha = 0.05$ ) among least square means for each generation analysed separately.

Source of variation	$F^*$ (F <sub>1</sub> gen)	$P$ (F <sub>1</sub> gen)	$F^*$ (F <sub>2</sub> gen)	$P$ (F <sub>2</sub> gen)
Population	425.8	< 0.0001	385.31	< 0.0001
Cactus	34.86	< 0.0001	136.33	< 0.0001
Population × cactus	8.49	0.004	17.72	< 0.0001
Sex	0.06	0.809	2.77	0.096
Population × sex	4.66	0.031	0.27	0.606
Cactus × sex	2.98	0.084	1.05	0.306
Population × cactus × sex	1.69	0.194	0.40	0.528
Mate	74.88	< 0.0001	56.55	< 0.0001
Population × mate	31.34	< 0.0001	0.64	0.423
Cactus × mate	85.17	< 0.0001	11.01	0.001
Population × cactus × mate	36.47	< 0.0001	7.14	0.008
Sex × mate	4.63	0.031	0.04	0.834
Population × sex × mate	0.03	0.858	2.88	0.090
Cactus × sex × mate	0.07	0.797	2.03	0.154
Population × cactus × sex × mate	4.83	0.028	0.05	0.816
Replicate	16.29	< 0.0001	3.81	0.051
Replicate × population	21.96	< 0.0001	35.06	< 0.0001
Replicate × cactus	41.92	< 0.0001	4.58	0.032
Replicate × population × cactus	38.08	< 0.0001	2.44	0.118
Replicate × sex	0.29	0.590	0.25	0.620
Replicate × population × sex	1.96	0.162	0.14	0.710
Replicate × cactus × sex	1.08	0.298	0.4	0.526
Replicate × population × cactus × sex	1.86	0.173	0.86	0.354
Replicate × mate	19.52	< 0.0001	17.20	< 0.0001
Replicate × population × mate	87.32	< 0.0001	3.37	0.067
Replicate × cactus × mate	29.83	< 0.0001	2.56	0.110
Replicate × sex × mate	0.04	0.836	0.00	1.00
Replicate × population × sex × mate	0.21	0.650	0.42	0.518
Replicate × cactus × sex × mate	3.03	0.082	0.11	0.738

\* $F$ -test ratio d.f. for all effects and interactions: F<sub>1</sub> gen. (1,2240); F<sub>2</sub> gen. (1,2313).

cactus, whereas there was no significant difference in the Baja population reared on agria. Baja flies reared on organ pipe and mainland flies reared on agria cactus showed statistically significant, intermediate differences in DEVT associated with mate choice opportunity (Fig. 1). The F<sub>2</sub> flies showed significantly shorter overall DEVT ( $P < 0.0001$ ), so each generation was analysed separately. ANOVA revealed no significant viability differences among treatments. Thus, indirect fitness effects of mating choice expressed as DEVT differences were population and rearing-environment specific.

### Male mating success

Across all 16 trials with virgin adults, males mated 0, 1 or 2 times. The probability of any given triplet combination of number of matings was calculated as:

$$\frac{10!}{n_0!n_1!n_2!} p_0^{n_0} p_1^{n_1} p_2^{n_2}$$

where  $n_i$  is the number of males mating  $i$  times and  $p_i$  is the Poisson probability that a male obtained  $i$  mates (assuming an expected number of matings equal to 1). The observed distribution of triplets of mating success differed significantly from the expected distribution (goodness-of-fit test statistic 204.69,  $P = 0.0005$ ).

**Table 1** ANOVA results for development time owing to the main effects of population, cactus, mate choice (mate), sex, replicate and all interactions for both generations.

Across the 16 trials with experienced females, males mated 0 or 1 time. The number of males that were successful in obtaining a single mating was significantly greater in the trials with virgin females than with experienced females (mean  $\pm$  1 SE;  $14.5 \pm 1.04$  vs.  $1.5 \pm 0.43$ , two-tailed Student's  $t = 69.07$ ,  $P < 0.0001$ ), indicating that previous female experience significantly influenced male mating success.

### Female choice, male competition, or both?

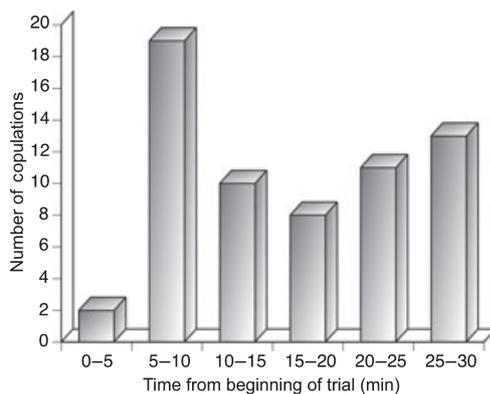
Across all 32 trials, the number of female rejection events was significantly greater than the number of male competition events (Table 2; two-tailed Student's  $t = 30.91$ ,  $P < 0.0001$ ). The rarity of male–male interactions as compared to female rejection events suggests that male mating success was primarily determined by female choice. Time to copulation for each male was recorded from the start of each trial. Rates of copulation were not influenced by population, cactus, or mating status (ANOVA, all  $P > 0.05$ ) so these data were pooled, and time to copulation was fairly constant over all trials (mean  $\pm$  1 SD =  $17.0 \pm 7.9$  min), with 33% of all matings occurring in the first 10 min (Fig. 2). Rarely, two males were observed to simultaneously attempt courtship by following closely and licking the female's genitalia. In these

**Table 2** Number and percentage of successful matings, numbers of female rejections and numbers of male–male interactions for each population/cactus/‘experience’ combination in the male mating success trials.

Female mating status	Population and substrate	Number (%) of successful matings	Number of female rejections	Number of male aggressions
Virgin females	SQ08 AG	17 (43)	20	6
	SQ08 OP	16 (40)	27	3
	PO07 AG	15 (38)	23	1
	PO07 OP	10 (25)	33	1
Mated females	SQ08 AG	0 (0)	19	0
	SQ08 OP	1 (2.5)	32	2
	PO07 AG	2 (5)	31	5
	PO07 OP	3 (7.5)	37	1
			27.75 ± 3.96*	2.38 ± 0.34*

SQ08, San Quintin 2008; PO07, Punta Onah 2007; AG, agria; OP, organ pipe cactus.

\*Mean ± 1 SE.



**Fig. 2** Time to copulation over five-min intervals recorded for each male. Timing began when the flies were introduced into the mating chamber and was monitored continuously until the trial was terminated after 30 min.

cases, females never indicated receptivity by wing spreading or allowing either male to mount, and such encounters never led to copulation. This was the only type of intra-sexual interaction observed, in contrast to aggressive or competitive male interactions observed in other *Drosophila* species (Chen *et al.*, 2002). There was no significant difference between the number of female rejection events in the trials with experienced females as compared to those with virgin flies (Student's  $t = 0.22$ ;  $P = 0.42$ ), which indicates that females that had been previously exposed to males were no more discriminating than virgin females.

## Discussion

Mate choice opportunity in cactus-reared *D. mojavensis* influenced progeny development time, which is a component of fitness (Etges, 2002). Increased opportunity for choosing mates was associated with decreased development time, suggesting that sexual selection involving environment-specific ‘good genes’ may counter natural selection for longer development time in populations using organ pipe cacti. The shorter development time of

Baja California populations has been ascribed to the shorter duration of agria rots in nature because of more rapid tissue degradation and smaller stem diameters than organ pipe rots (Etges, 1989; Heed & Mangan, 1986). However, decreases in development time due to female-biased mate choice were not significant in Baja California progeny reared on agria, but all other pairwise comparisons were significant (Fig. 1).

The population and host–cactus specificity of these indirect genetic consequences raises some interesting questions concerning the role that  $G \times E$  interactions for mating signals may play in maintaining genetic variation for these traits and highlights the influence of ecology on mating systems in this species. Theoretical analysis suggests that the potential for  $G \times E$  to maintain variation in sexual traits is greatest when there is ecological crossover of genotypic fitnesses (Kokko & Heubel, 2008). However, if there is limited mixing of reproductive individuals that have developed in different environments, then weaker forms of  $G \times E$  can help maintain additive genetic variability and hence support persistence of female preference (Higginson & Reader, 2009). Further,  $G \times E$  for sexual traits can influence the ‘honesty’ of the signal for genetic quality, which is a prerequisite for ‘good genes’ sexual selection. Thus, the prevalence of indirect benefits of sexual selection in *D. mojavensis* requires an understanding of the role of preadult rearing environments on adult mate choice behaviours and expression of life history variation.

The significant population  $\times$  cactus  $\times$  mate interaction revealed by ANOVA (Table 1) indicated that the indirect benefits of mate choice varied among populations and rearing environments, a pattern that was repeated over two generations. Uncontrolled variation in cactus tissues can influence growth and development of larvae (Etges, 1989) and could have caused the shorter development time (Etges, 1993) in the offspring of the second generation. Alternatively, this result could have been caused by the mating scheme in this experiment. Further experimentation will be required to determine the source of this effect. Here, mainland flies reared on organ pipe exhibited the largest reduction in offspring

development time due to mate choice. Natural selection may have increased development time in mainland populations that use organ pipe cactus because tissue fermentation is slower (Etges, 1989). Thus, adaptation of derived mainland populations to a secondary host plant, organ pipe cactus, has caused longer development time, whereas mate choice caused shorter development time (Fig. 1). Therefore, the potential for further life history evolution may be constrained by sexual selection, suggesting natural and sexual selection act antagonistically in this species and as a result, genetic variation for development time may be maintained (Etges, 1993, 1998).

Some investigations of sexual selection in *D. mojavensis* have used flies reared on artificial media (Knowles *et al.*, 2004, 2005; Oneal *et al.*, 2007). These studies suggest that sexual selection may influence the two sexes differently. However, interpopulation mating trials using flies raised on cacti (Etges & Tripodi, 2008) revealed different levels of pre-mating isolation than those found in studies using flies raised on artificial media (Brazner, 1983; Brazner & Etges, 1993). Cactus-reared flies showed lower levels of pre-mating isolation than flies reared on artificial media and organ-pipe-reared flies showed increased pre-mating isolation compared to agria-reared flies (Etges, 1992; Brazner & Etges, 1993). Thus, patterns of mating success were influenced by preadult rearing environments. This suggests that studies using flies reared on artificial media may be misleading.

Careful experimental design has helped to disentangle the various mechanisms that contribute to variance in mating success (Kotiaho & Puurtinen, 2007; Fitze *et al.*, 2008). Laboratory investigations of sexual selection have been criticized for drawing ambiguous conclusions about the mechanism of selection (Partridge, 1980; Promislow *et al.*, 1998). Partridge (1980) showed that free interaction between multiple adults was associated with greater offspring viability in *D. melanogaster*, but male–male competition may have masked female choice, confounding the effects of these two variables (Kingett *et al.*, 1981). All flies should prefer the same mates, as either fitter males have a competitive advantage, indicating male–male competition, or females can detect heritable fitness in males, indicating female choice (Partridge, 1981). Indeed, competition among males, whether direct (interference competition) or indirect (via differences in male activity), can have the same consequences as female choice, consistent with ‘good genes’ sexual selection (Pekkala *et al.*, 2009).

In systems where males invest less in gamete production than females and there is no after-birth parental investment from either sex, parental investment theory predicts that males are less discriminating when choosing mates than females (Williams, 1966; Trivers, 1972). It is likely that in any given species, both male–male competition and female choice may be operating (Andersson, 1994). A common view of *Drosophila* mating behaviour is

that males take an active and females take a passive role in courtship (Brown, 1964, 1965). Experiments have confirmed female choice to be common in some species (Hoikkala & Aspi, 1993; Scott, 1994; Droney & Hock, 1998; Hoikkala *et al.*, 1998), whereas male competition and aggression in lekking species is common in some Hawaiian species (Boake *et al.*, 1997) that also exhibit strong female choice (e.g. *D. silvestris*, Spiess & Carson, 1981). Thus, the roles of female choice and male–male interaction in determining indirect benefits of sexual selection are species specific.

Experiments designed to control for either inter- or intra-sexual interactions have been used to address this problem (Anderson *et al.*, 2007). One of these is the ‘choice’ approach, in which a female is allowed to choose among males in arenas where intra-sexual combat and inter-sexual coercion have been eliminated (Anderson *et al.*, 2007). Another is the ‘no-choice’ approach, in which the time until males achieve copulation is used to infer female choice (while controlling for male–male competition, see Gowaty *et al.*, 2002; Oneal *et al.*, 2007). Criticisms of such approaches are that they do not allow for the separation of active mate choice from passive attraction, each of which can lead to identical indirect benefits (Kotiaho & Puurtinen, 2007). Here, we tested the null hypothesis of random male-mating success using a multiple-choice design because *D. mojavensis* feed and breed in groups in and around cactus rots in nature (Krebs & Bean, 1991). Also, previous trials with cactus-reared flies revealed little evidence of differences in male mating propensity, suggesting that males do not directly outcompete each other for mates (Brazner & Etges, 1993; Etges, 1998; Etges & Tripodi, 2008).

Consistent with our observation of indirect benefits of sexual selection, the null hypothesis of random male mating success was rejected. We observed male–male interaction to be very rare, and we observed few aggressive or competitive male behaviours independent of female mating status. Female discrimination was apparent, as female rejection events were significantly more frequent than male–male interactions (Table 2). The lack of difference in the number of female rejections together with the large difference in the number of successful matings between virgin and mated females (Table 2) suggests that male *D. mojavensis* avoid previously mated females. These results indicate that female choice during courtship leads to the indirect fitness benefits of shorter development times in most cases when cactus-reared males and females interact freely in courtship trials.

The results of this study clearly demonstrate that intra-population sexual selection operates in *D. mojavensis*, with consequences that vary among population and environment. Sexual selection can lead to diversification in mating preferences within (Turner & Burrows, 1995) and between populations (Lande, 1981, 1988; Schluter & Price, 1993). The effect of within-population sexual selection on development time and the genetic associa-

tion between development time and cuticular hydrocarbons that mediate male mating success (Etges *et al.*, 2010) suggest that female choice-based sexual selection may also influence the evolution of life history traits in *D. mojavensis*.

We have demonstrated that preadult rearing conditions influence mate choice (Etges, 1992; Etges *et al.*, 2009) and that this influences offspring fitness (Fig. 1). Therefore, the consequences of 'good genes' sexual selection may be overlooked if geographical and environmental variability are ignored. Condition dependence of mate choice has been observed in other organisms (Gilburn & Day, 1994; Lesna & Sabelis, 1999; Welch, 2003; Greenfield & Rodriguez, 2004) and may be more commonly observed once multiple environments are accounted for in sexual selection studies. Identification of the specific traits involved in sexual selection will allow for further tests of hypotheses concerning the potential contribution of sexual selection to speciation and the role of G × E in maintaining genetic variation for these traits in nature.

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