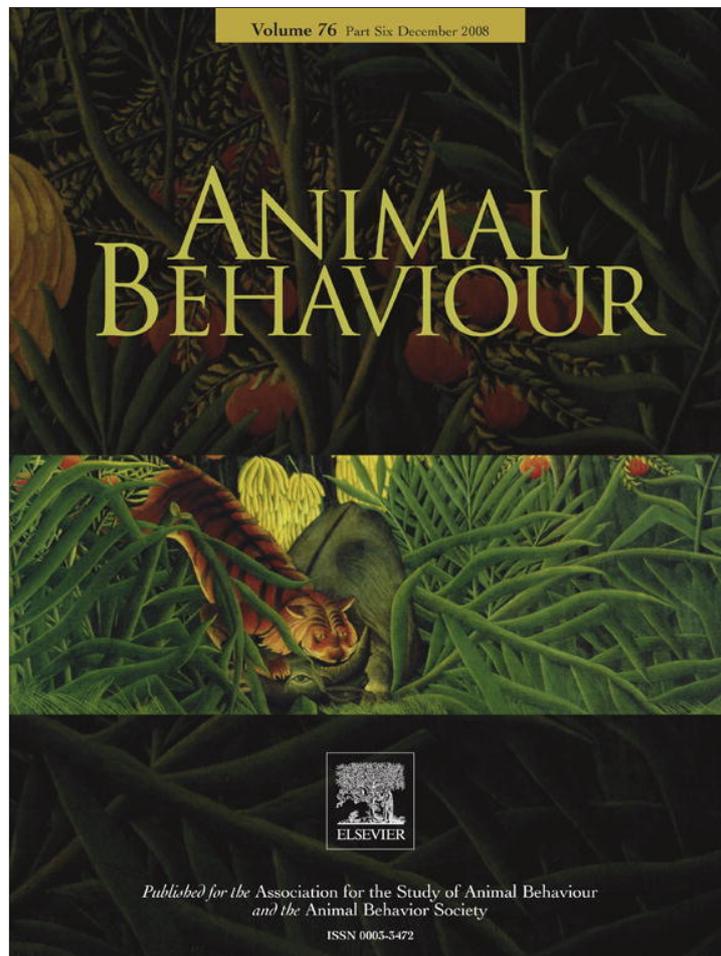


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Effects of male condition on fitness in two tropical tephritid flies with contrasting life histories

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We investigated the effects of male size and adult diet on male sexual competitiveness and lifetime reproductive success of female mates in two tephritid flies with contrasting natural histories, the Mexican fruit fly, *Anastrepha ludens*, and the guava fruit fly, *A. striata*. Small, medium and large males received either a low-quality diet (sucrose offered every third day) or a high-quality diet (sucrose and protein offered ad libitum). Regardless of size or species, males on the high-quality diet copulated significantly more often across the 4 observation days than males on the low-quality diet. For *A. ludens*, both size and diet influenced territory defence by resident males, but only size affected the likelihood of success by invading males. Females that copulated with a low-quality fed male had significantly shorter maximum longevity, although there was no effect of male diet on the proportion of eclosed eggs. For *A. striata*, diet, but not size, influenced territory defence by invading males. Females discriminated strongly against males fed a low-quality diet but not against smaller males. However, females that copulated with medium and small males had lower fecundities than those copulating with large males. We discuss the costs for females of mating with smaller males and their inability to distinguish between males of different conditions, as well as interspecific differences in mate choice and postcopulatory consequences for females in terms of the ecological differences that distinguish these two species.

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There have been many attempts to explain the factors that determine male sexual competitiveness in animals. Among these, size and adult diet have been shown to influence male mating success in a variety of vertebrates and invertebrates (Thornhill & Alcock 1983; Clutton-Brock 1988; Andersson 1994). In the case of insects, females of the stream water strider, *Aquarius remigis* (Sih & Krupa 1992), the dung fly *Sepsis cynipsea* (Ward 1983), *Drosophila melanogaster* (Partridge et al. 1987), the Mediterranean fruit fly *Ceratitis capitata* (Churchill-Stanland et al. 1986; Taylor & Yuval 1999; Kaspi et al. 2000) and the Caribbean fruit fly *Anastrepha suspensa* (Burk & Webb 1983) seem to prefer large males and tend to reject small males. However, in various other species of Diptera (e.g. swarming chironomids, McLachlan & Allen 1987), small size

and increased aerial agility may be advantageous in out-maneuvring sexual rivals. In species where large male size is an advantage, its benefits derive from female preference or greater prowess in male–male encounters that determine access to females (O'Neill 1983; Partridge et al. 1987).

Male diet may also be important and has often been indirectly implicated in the form of body mass as an indicator of nutrient and energy reserves. For example, in the barklouse *Lepinotus patruelis*, males fed on a poor diet tend to be rejected by females with a greater frequency than males fed on rich diets (Wearing-Wilde 1995). In fruit flies, the effect of diet on mating behaviour has been studied in *A. suspensa* (Landolt & Sivinski 1992), *C. capitata* (Blay & Yuval 1997; Kaspi et al. 2000) and in four species of *Anastrepha* (Aluja et al. 2001; Pérez-Staples et al. 2008). Diet also influences pheromone production in tephritids, which ultimately determines the male's ability to attract females (Landolt & Sivinski 1992; Epsky & Heath 1993).

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Male qualities, both genetic and phenotypic, can affect female fitness, and choosing an inferior quality mate could be extremely costly. For example, in *A. suspensa*, females kept with larger males have a shorter life span and similar lifetime fecundities than those kept with smaller males (Sivinski 1993). Male nutritional status may be especially relevant for female fitness in species where females receive nuptial gifts with nutritive value, including substances in the ejaculate (Cordero 1996; Sivinski et al. 2000 and references therein) or they may be adversely affected if seminal fluids are toxic (Chapman et al. 1995). A poor-quality diet can reduce mating investment by males (Gwynne 1993) either in the form of sperm numbers (e.g. Gage 1994) or accessory gland products (Craig 1967). For example, restricting the amount of protein in the adult diet can have negative effects on testes and accessory glands (Baker et al. 2003).

Not until recently have the effects of more than one variable on male competitiveness and female fitness been assessed simultaneously (e.g. Sih & Krupa 1992; Brown 1997; Taylor & Yuval 1999; Kaspi et al. 2000). In nature, independent variables probably interact synergistically or cumulatively to affect the reproductive success of a male and the fitness of females mating with such males, so our objective here was to test the simultaneous effects of adult diet and size on male mating success and the post-copulatory consequences for females of two fruit fly species with contrasting mating systems and natural histories. In most phytophagous insects, size is determined largely by the interaction of environmental factors such as larval diet or crowding conditions in the host and genetic components (Kaspi et al. 2002). Larval diet quality (i.e. host plant) can also influence adult condition (i.e. nutritional reserves, salivary gland development, rate of sexual maturation, immune system) and secondary sexual trait expression (Knell et al. 1999; Kaspi et al. 2002; Gwynne 2004; Engels & Sauer 2007 and references therein). Adult diet can in turn influence behaviour and performance in various ways but cannot affect body size, as most adult insects are unable to grow. Recently, working with the polyphagous fruit fly *A. obliqua*, we discovered that the interaction between larval and adult diet significantly influenced copulation duration. Protein-deprived males stemming from an exotic host (*Mangifera indica*) had the longest copulations, while those reared in a native host (*Spondias mombin*) had the shortest (Pérez-Staples et al. 2008). Although in most pterygote insects the effects of larval diet are fixed after the final moult, one could hypothesize that in the case of anautogenous insects, with no apparent secondary sexual traits (e.g. horns, stalked eyes), a genetically superior individual, affected by a poor larval diet or by extreme crowding conditions (i.e. of small size), could overcome the size handicap by efficiently foraging for high-quality adult food, which in turn would allow this individual to produce sexual pheromones of better quality (Epsky & Heath 1993), be more energetic while displaying to females (Kaspi et al. 2000) or signal a healthier immune condition (e.g. Contreras-Garduño et al. 2008).

Here, we examine two *Anastrepha* species that show contrasting mating systems and natural histories.

Anastrepha ludens belongs to the *fraterculus* and *A. striata* to the *serpentina* species group (Norrbon et al. 2000; Norrbom 2002). *Anastrepha ludens* males attract females by wing fanning and emitting a pheromone from 1700 to 1900 hours (Aluja et al. 2000). Males may call alone or from leks formed on adjacent leaves (Aluja et al. 1983). Copulation lasts an average \pm SE of 73.4 ± 6.6 min (Aluja et al. 2000) and females lay clutches of eggs (up to 40 eggs/clutch) shortly after mating (Díaz-Fleischer & Aluja 2003b). In contrast, *A. striata* males only occasionally aggregate to call and are commonly seen wing fanning alone from 1000 to 1700 hours (Aluja et al. 2000). Once female *A. striata* approach the male, an elaborate courtship takes place including the transfer of fluids through trophallaxis (courtship feeding). This fluid may have a nutritive value, as *A. striata* females that mate with virgin males live longer, possibly because of the transfer of less depleted nutritive materials via trophallaxis or accessory gland products (Pérez-Staples & Aluja 2004). There are an average \pm SE of 13 short (7.3 ± 0.3 s) mating attempts before the female finally accepts a male (copulation lasts 29.2 ± 2.3 min) (Aluja et al. 1993). Females usually lay one egg per oviposition bout, but clutches of up to three eggs are sometimes recorded. Forced copulations are unlikely in either species because of the necessity of the female presenting her genitalia prior to penetration (Aluja et al. 2000). Thus, in both species, male attractiveness plays a critical role in female choice (Aluja et al. 2001).

Since *A. striata* shows trophallaxis and *A. ludens* does not, we predicted that male nutritional status, and presumably the quality of transferred nutrients (as opposed to the volume), would have a particularly important influence on female mate choice in *A. striata*. On the other hand, male mating performance in *A. ludens* is not affected by adult diet (Aluja et al. 2001), possibly leading females to base mating choices on male body size. These qualities could be displayed during territory defence while calling in pairs and, thus, we predicted that male size would be more important to females of *A. ludens* than to those of *A. striata*. For similar reasons, we predicted that female fitness in *A. striata* would be affected by male diet more than size, while female fitness in *A. ludens* would be principally affected by male size. Finally, we predicted that in both species, the costs of being small could be circumvented by a high-quality adult diet.

METHODS

Study Insects

Infested wild oranges (*Citrus aurantium* L.) and guavas (*Psidium guajava* L.) were collected from the vicinity of Xalapa, Veracruz, Mexico and transported to the Instituto de Ecología, A.C. to obtain *A. ludens* and *A. striata*, respectively. Oranges and guavas were placed in separate containers with vermiculite until the larvae pupated. Pupae were weighed individually and divided into three categories: 15–20 mg (small), 21–25 mg (medium) and 26–30 mg (large).

Handling of Adult Flies

Once adults emerged from either of the three size categories, they were separated by sex and size and transferred to 30 × 30 × 30 cm Plexiglas cages. As we were interested in determining how adult diet may influence behaviour and how body size (effect of larval rearing environment) interacted with adult diet, male adults from each size category (details above) were offered water and one of two diets, sucrose and protein (3:1) or sucrose alone. Sucrose and protein (a high-quality diet) was provided continuously (i.e. ad libitum), while sucrose (a low-quality diet) was provided every third day until adults were 10 days of age. The rationale of additionally stressing males offered the low-quality diet (i.e. they only had access to food every third day) was based on the fact that Aluja et al. (2001) found that offering *A. ludens* males ad libitum access to only sucrose did not negatively affect their mating performance. Males were separated into six categories: protein-fed large, medium or small, and protein-deprived large, medium or small (see Table 1). All females were medium sized (21–25 mg) and fed protein and sucrose and water ad libitum (i.e. same high-quality diet offered to half of the males used in experiment).

Field Cage Experiments

Observations of *A. ludens* were carried out in a 3 m (diam) × 3 m (height) cylindrical field cage similar to the one described by Calkins & Webb (1983). The cage was situated at the Instituto de Ecología, A.C. headquarters in Xalapa, Veracruz, Mexico (19°30'N, 96°55'W, 1300 m above sea level). In the case of *A. striata*, a field cage of the same type was situated in Apazapan, Veracruz (19°19'N and 2°21'E, 347 m above sea level). This provided each species with optimum climatic conditions since *A. ludens* is commonly found in areas above 1000 m, while *A. striata* is more common at lower altitudes (Aluja et al. 1996; Sivinski et al. 2004). Each field cage was lined with potted mango (*Mangifera indica*), sapodilla (*Manilkara zapota*), guava (*Psidium guajava*) and citrus (*C. aurantium*) trees, 1–2 m tall, so that the whole cage was filled with foliage. Both *C. aurantium* and *M. indica* are hosts of *A. ludens* (not infested by *A. striata* in nature), while *P. guajava* is a host of *A. striata* (not infested by *A. ludens* in nature) (Aluja et al. 2000). *Anastrepha ludens* was observed from April through May while *A. striata* was observed from November to December, during the time of the year when these species are abundant in the field.

All potted trees had been thoroughly rinsed with water by using a high-pressure hose so as to remove any honeydew, bird faeces or bacteria growing on these substrates, as they could have provided food sources for flies (Aluja 1994). Trees were also inspected for presence of scale insects or aphids, in which case, they were replaced by other trees free of such insects that are known to produce honeydew. Note, however, that adult flies graze for leachates (i.e. leaf exudates) on leaf surfaces that primarily contain minerals (no protein or carbohydrates; Hendrichs et al. 1993), and that despite the thorough rinse, such leachates could have been produced by leaves.

When male adults reached 8 days of age, all individuals were marked with a unique colour on their pronotum using water-based paint (Vinci, Vinci de Mexico S.A. de C.V. Mexico). When reaching 10 days of age, 24 males of each size and diet treatment (three sizes, two diets) were released into the field cage. Only water was provided to these males. Two days later, some individuals were randomly captured so as to leave only six males per treatment. We released more males than needed because typically some die shortly after being released as a result of handling or other unknown causes. In total, 36 males were observed per replicate (five replicates for *A. ludens* and six for *A. striata*). Immediately after removing some of the excess males, 18 virgin females were released into the field cage (as noted before, females were all the same size and fed on the high-quality diet). As before, only water was provided to males and females once they were released into the field cage. We are fully aware that our design could potentially complicate the interpretation of some of our results, given that the capacity of a female to choose may decline with her nutritional state (i.e. once females fed on a high-quality diet were released into the experimental arena they were not provided with food over the next 4 days). The alternative we had was to remove all females at the end of the day and replace them the next day with freshly fed females. We chose not to do so, since we were interested in the starvation effect over time.

According to the calling activities of each species, observations of *A. ludens* began at 1700 hours and those for *A. striata* began at 1000 hours (Aluja et al. 2000), and observations of both species ended the same day at sunset or when the last copulating pair separated. Each cohort of males and females (i.e. replicate) was continuously observed over 4 consecutive days. Such an approach, allowed us not only to measure total reproductive male success (i.e. total number of copulations per male), but to analyse success variance over time, presumably as nutritional condition deteriorated. Furthermore, we were able to monitor the effect of male condition (e.g. large and fed on a high-quality diet versus small and fed on a poor-quality diet) on other measures of success such as the ability to defend a territory (see below).

In each field cage, we recorded the number of males calling, defined as wing fanning and emitting a pheromone by abdominal pleural distension (Aluja & Norrbom 2000). Males could be calling individually, in pairs (two males on adjacent leaves of the same branch) or in leks (i.e. three or more males calling within a 30 cm³ space). We also recorded the number and duration of copulations by each male, and the number and outcome of male–male disputes over the ownership of leaf-territory signalling sites. Encounters were scored as being won by either a resident or an invading male. Resident males were those who called from an established territory (underside of leaf), while invaders were males that arrived at an established territory where a male was already calling and engaged in aggressive interactions (wing displays and/or head butting accompanied by wing buzzing with the resident male; Aluja et al. 2000). The male that remained on the leaf after such an encounter was scored as having won. Once a female had copulated, she was promptly removed

from the cage and replaced the following day with another virgin female of the same age. Note that these females had the same nutritional history as the females originally introduced into the field cages. Inside these cages, we had also placed a rinsed *C. aurantium* branch with leaves to mimic the condition of females inside field cages.

Consequences on Female Fitness

As described above, each female was allowed to freely choose and mate with males of six types (three sizes and two diets). At the end of an observation period, all the females that had copulated were transported to our laboratories at the Instituto de Ecología, A.C. in Xalapa, Veracruz (no more than a 1 h drive). Females from each replicate that had mated with the same type of male were placed together in Plexiglas cages (30 × 30 × 30 cm). That is, we added the females from days 2, 3 and 4 to the cage from day 1. We housed females in groups because grouped (eight females per cage) *A. ludens* females lay significantly more eggs than do solitary females (Díaz-Fleischer & Aluja 2003a) and our main goal was to obtain as many eggs as possible to compare among treatments while determining fertility.

Females were provided with the high-quality diet offered ad libitum until death and artificial fruit (i.e. 2.5 cm diameter agar spheres) as oviposition substrates (Díaz-Fleischer & Aluja 2003a). One sphere was provided for every five females in each cage. Spheres were made with agar (Bacteriological Agar, BD Bioxon, Becton Dickinson de Mexico, Cuatitlán Izcalli, Edo. de Mex., Mexico), coloured with green food dye (McCormick-Herdez S.A. de C.V., San Luis Potosí, San Luis Potosí, Mexico) and wrapped in Parafilm 'M' (American National Can, Chicago, IL, U.S.A.). In the case of *A. striata*, an artificial guava drink flavour (Be-Light de Fresqui Bon, Dulco S.A. de C.V., Cuatitlán Izcalli, Edo. de México, Mexico) was added to the agar to make spheres more attractive to ovipositing females (Pérez-Staples & Aluja 2004). Spheres were dissected daily to count all eggs in them. Once counted, 100 eggs were placed in humidity chambers to quantify eclosion (Jácome et al. 1999). Six days later, the proportion of hatched eggs was assessed. Females were monitored until they died. Following Carey (1993), we calculated female life expectancy, maximum longevity, gross fecundity and proportion of eclosed eggs.

Data Analysis

All data related to male competitiveness were transformed to ranks (Conover & Iman 1981). Each species was analysed separately. Data on male calling activity and copulations were summed across the 4 observation days for each replicate and according to male condition (size and diet). The total number of males calling in leks, in pairs or alone and the total number of copulations obtained by males were each analysed by a two-way ANOVA with male diet and size as independent variables. In addition, data on the number of males calling each day and the number of copulations obtained per male over the 4-day observation period (repeated observations on same

individual) were each analysed using a repeated measures ANOVA. Copula duration was analysed by means of a two-way ANOVA. For males with multiple copulations, the durations of the first and second copulation were subjected to a Wilcoxon signed-ranks test. The proportion of agonistic interactions won by resident or invader males was averaged across males from each treatment and then analysed using a two-way ANOVA. Data on mean gross female fecundity and mean proportion of eclosed eggs were square-root ($\sqrt{+1}$) and arcsine-transformed, respectively, and analysed using a two-way ANOVA with diet and size as independent variables. Mean clutch size for *A. ludens* was rank-transformed and subjected to a two-way ANOVA. Given that life span distributions of both species passed both normality and equal variance tests, untransformed data was analysed by means of a two-way ANOVA. Tukey tests were used for post hoc comparisons in all cases. All data were analysed using Statistica (1999).

RESULTS

Male–Male Aggressive Encounters

In *A. ludens*, the probability of obtaining and defending a calling territory as a resident was related to both diet and size (two-way ANOVA: diet: $F_{1,24} = 4.70$, $P = 0.040$; size: $F_{2,24} = 4.29$, $P = 0.025$; diet × size: $F_{2,24} = 2.23$, $P = 0.130$). Males fed on a high-quality diet had a higher probability of obtaining a territory than those fed on a low-quality diet, and medium-sized males tended to win more agonistic interactions than small-sized males ($P = 0.025$). In contrast, when these same males were invaders, there was no effect of diet on the number of victories obtained but there was a significant effect of size (two-way ANOVA: diet: $F_{1,24} = 2.16$, $P = 0.154$; size: $F_{2,24} = 11.24$, $P = 0.0004$; diet × size: $F_{2,24} = 1.60$, $P = 0.223$).

For *A. striata*, neither diet nor size had an effect on the number of victories obtained by resident males (two-way ANOVA: diet: $F_{1,30} = 0.0014$, $P = 0.91$; size: $F_{2,30} = 2.69$, $P = 0.083$; diet × size: $F_{2,30} = 1.29$, $P = 0.29$). However, when males were invaders, only those that fed on a high-quality diet managed to displace the resident male from its territory (two-way ANOVA: diet: $F_{1,30} = 4.89$, $P = 0.0348$; size: $F_{2,30} = 1.45$, $P = 0.25$; diet × size: $F_{2,30} = 0.67$, $P = 0.52$).

Calling Activity and Copulation Success

Overall patterns of calling activity, separated by modality (i.e. alone, in pairs or in leks) are illustrated in Fig. 1. Few *A. ludens* males joined leks during the course of the experiment, and neither diet nor size affected their participation (two-way ANOVA: diet: $F_{1,24} = 0.81$, $P = 0.38$; size: $F_{2,24} = 0.92$, $P = 0.41$; diet × size: $F_{2,24} = 0.98$, $P = 0.39$). Individually calling males were significantly more likely to be those fed a high-quality diet, but size and the interaction of size and diet had no effect (two-way ANOVA: diet: $F_{1,24} = 8.42$, $P = 0.008$; size: $F_{2,24} = 0.74$, $P = 0.49$; diet × size: $F_{2,24} = 0.40$, $P = 0.67$). Large males were more

likely to call in pairs, but diet and the interaction of diet and size had no significant effect (two-way ANOVA: diet: $F_{1,24} = 2.14$, $P = 0.16$; size: $F_{2,24} = 6.66$, $P = 0.005$; diet \times size: $F_{2,24} = 0.31$, $P = 0.74$). In *A. striata*, no leks or calling pairs were observed (i.e. all males called singly).

When males called in leks, neither diet (ANOVA: $F_{1,24} = 0.52$, $P = 0.41$) nor size ($F_{2,24} = 0.37$, $P = 0.35$) significantly influenced the number of copulations obtained. Four large, eight medium and one small male mated. Among individually calling *A. ludens* males, those fed a high-quality diet obtained a significantly greater number of copulations than those fed a low-quality diet (74 versus 36, respectively) (ANOVA: $F_{1,24} = 11.56$, $P = 0.002$) but size had no effect (ANOVA: $F_{2,24} = 0.68$, $P = 0.515$). In contrast, when males called in pairs, diet had no significant effect on the number of copulations obtained (ANOVA: $F_{1,24} = 2.31$, $P = 0.141$), but size did (ANOVA: $F_{2,24} = 6.66$, $P = 0.005$). Twelve of the large, two of the medium and one of the small paired males mated.

Diet in *A. ludens* had a significant effect on the total number of males that called across the 4 observation days, while size and the interaction of size and diet had no effect (repeated measures ANOVA: diet: $F_{1,24} = 11.40$, $P = 0.025$; size: $F_{2,24} = 0.82$, $P = 0.45$; diet \times size: $F_{2,24} = 0.10$, $P = 0.90$). Males that had access to the low-quality diet every third day tended to call less than males that had ad libitum access to the high-quality diet (mixture of sucrose and protein) (Fig. 2a). For *A. striata*, there was no effect of diet or size on the total number of males that called across the 4 days (repeated measures ANOVA: diet: $F_{1,30} = 0.88$, $P = 0.35$; size: $F_{2,30} = 0.03$, $P = 0.97$; diet \times size: $F_{2,30} = 0.03$, $P = 0.96$; Fig. 2b).

Effect of Male Diet and Size on Number of Copulations Obtained

Anastrepha ludens males fed a high-quality diet obtained significantly more copulations than those fed a low-quality diet, but male size or the interaction between diet and size had no effect (two-way ANOVA: diet: $F_{1,24} = 11.88$,

$P = 0.002$; size: $F_{2,24} = 2.59$, $P = 0.096$; diet \times size: $F_{2,24} = 0.15$, $P = 0.86$; Fig. 3a). For *A. striata*, diet also greatly influenced the number of copulations obtained by males, but male size and the interaction of size and diet had no effect (two-way ANOVA: diet: $F_{1,30} = 18.80$, $P = 0.0001$; size: $F_{2,30} = 2.28$, $P = 0.12$; diet \times size: $F_{2,30} = 0.05$, $P = 0.948$; Fig. 3b). Males fed a high-quality diet obtained significantly more copulations than those fed a low-quality diet.

For *A. ludens*, the total number of males that copulated during the 4 days was affected by diet but not by male size or the interaction of size and diet (repeated measures ANOVA: diet: $F_{1,24} = 11.87$, $P = 0.002$; size: $F_{2,24} = 2.97$, $P = 0.071$; diet \times size: $F_{2,24} = 0.17$, $P = 0.84$; Fig. 4a). Similarly, for *A. striata*, the total number of males that copulated during the 4 days was affected by male diet but not by male size or the interaction of size and diet (repeated measures ANOVA: diet: $F_{1,30} = 15.30$, $P = 0.0005$; size: $F_{2,30} = 1.07$, $P = 0.35$; diet \times size: $F_{2,30} = 1.08$, $P = 0.35$; Fig. 4b). Significant differences occurred between medium and large males ($P = 0.006$). Large males fed a high-quality diet obtained 35.7% of all copulations during the first 3 days, but their success declined sharply during the fourth day. By the fourth day of observation, success of medium males fed both diets was greater than that of males fed a high-quality diet. Interestingly, small males fed a high-quality diet obtained similar numbers of copulations as large males fed a high-quality diet during the first day, but their success decreased over subsequent days (Fig. 4b).

With respect to multiple matings by single males, in *A. ludens* this was only significantly influenced by diet (two-way ANOVA: diet: $F_{1,174} = 16.2$, $P \leq 0.001$; size: $F_{2,174} = 2.35$, $P = 0.1$; diet \times size: $F_{2,174} = 0.37$, $P = 0.7$). Only those males fed on the high-quality diet mated up to four times. Furthermore, most males fed on the low-quality diet never mated (Fig. 5a). In the case of *A. striata*, the number of times that a male mated repeatedly was significantly influenced by both diet and size, but not by the interaction of these factors (two-way ANOVA: diet: $F_{1,210} = 24.55$, $P \leq 0.001$; size: $F_{2,210} = 4.24$, $P = 0.016$;

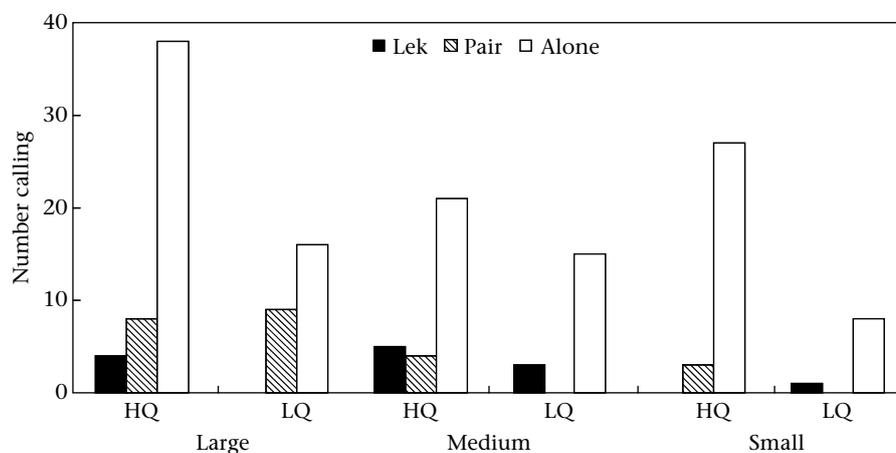


Figure 1. Total number of large, medium and small *A. ludens* males on high and low-quality diets that called in leks, in pairs or alone. HQ: high-quality diet of protein and sucrose ad libitum; LQ: low-quality diet of sucrose every third day. Data for *A. striata* not shown as no leks or calling pairs were observed (i.e. all males called singly).

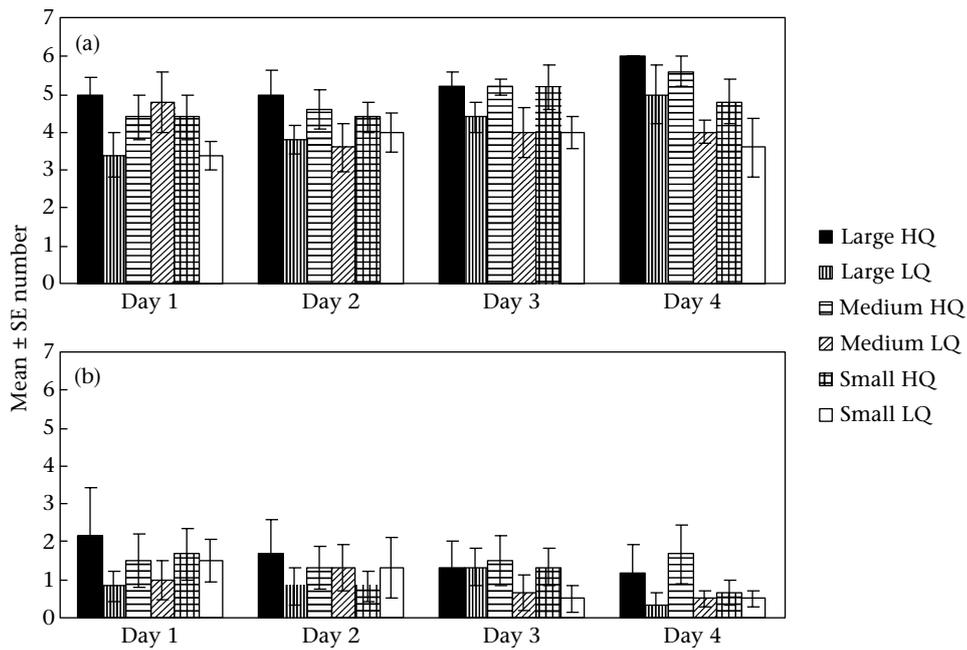


Figure 2. Mean \pm SE number of (a) *A. ludens* and (b) *A. striata* males in each size class (small, medium, large) and diet treatment (HQ: high-quality, protein and sucrose ad libitum; LQ: low-quality, sucrose every third day) that called throughout the 4-day observation period.

diet \times size: $F_{2,210} = 0.84$, $P = 0.4$). Most males fed on the poor-quality diet never mated, and among those that did, the maximum number of copulations achieved by a single male was two. In sharp contrast, some males fed on the high-quality diet achieved up to six copulations (Fig. 5b), and the maximum number of times that a single male copulated was significantly greater for large males than for medium and small ones ($P = 0.04$).

Duration of Copulations

For *A. ludens*, male diet had a significant effect on the mean duration of copulations while size and the interaction of size and diet had no effect (two-way ANOVA: diet: $F_{1,24} = 11.92$, $P = 0.002$; size: $F_{2,24} = 0.92$, $P = 0.41$; diet \times size: $F_{2,24} = 0.22$, $P = 0.80$). The same was true for *A. striata*, (two-way ANOVA: diet: $F_{1,30} = 14.31$,

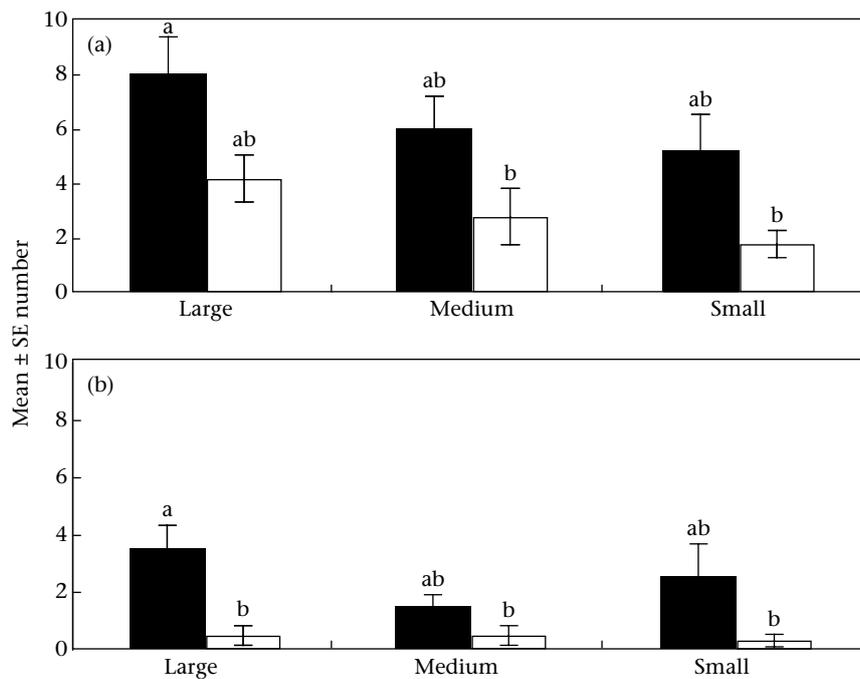


Figure 3. Mean \pm SE number of copulations obtained by male (a) *A. ludens* ($N = 142$) and (b) *A. striata* ($N = 54$) in each size class (small, medium, large) and diet treatment (■: high-quality, protein and sucrose ad libitum; □: low-quality, sucrose every third day). Bars with the same letter were not statistically different.

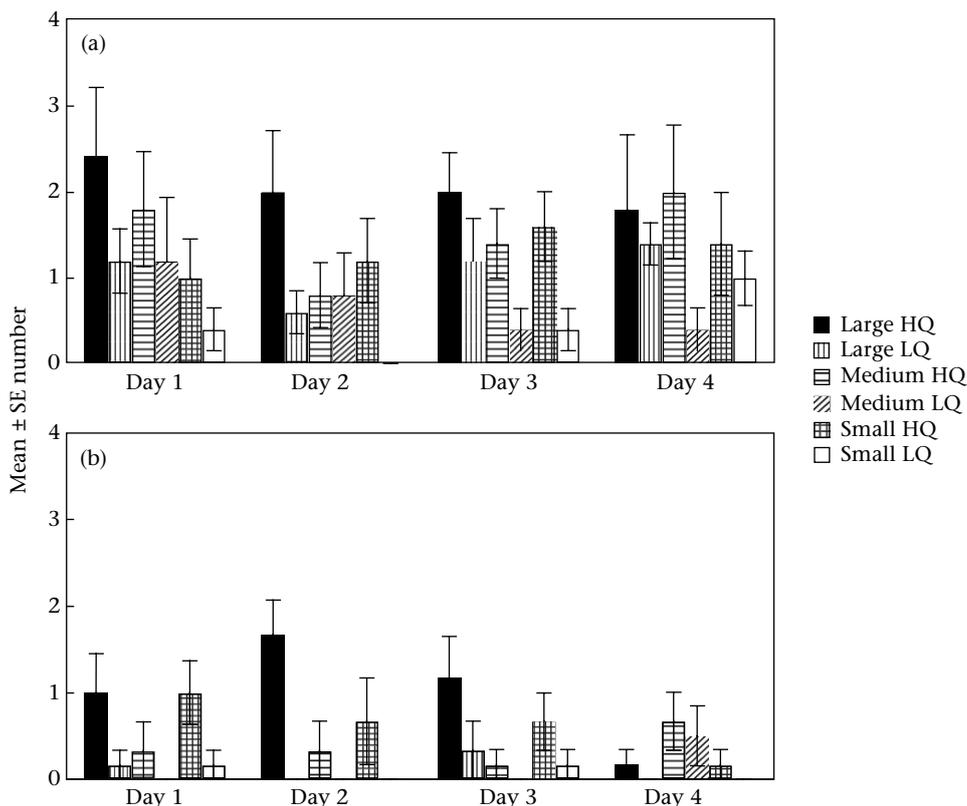


Figure 4. Mean \pm SE number of copulations obtained by males of each size class (small, medium, large) throughout the 4-day observation period for (a) *A. ludens* ($N = 142$) and (b) *A. striata* ($N = 54$). HQ: males fed on sucrose and protein ad libitum (high-quality diet). LQ: males fed on sucrose every third day (low-quality diet).

$P = 0.0007$; size: $F_{2,30} = 0.89$, $P = 0.42$; diet \times size: $F_{2,30} = 0.24$, $P = 0.78$). For both species, mean copulation duration was significantly longer for males fed a high-quality diet than for those fed a low-quality diet.

For males with multiple matings, we compared the duration of the first copulation with the duration of the second copulation on the following day (males that copulated twice during the same day were excluded). For *A. ludens*, only males fed a high-quality diet could be considered since there were not enough cases of multiple matings for males fed a low-quality diet. When males were large, there were no significant differences between the first and second copulations (Wilcoxon signed-ranks test: $T = 7.0$, $N = 6$, $P = 0.463$). However, for medium-sized males, duration of the second copulation was significantly shorter than the first (Wilcoxon signed-ranks test: $T = 0.0$, $N = 5$, $P = 0.043$). For small males, there were no statistically significant differences between the duration of the first and second copulations (Wilcoxon signed-ranks test: $T = 3.00$, $N = 4$, $P = 0.460$). In the case of *A. striata*, there were not enough cases to be analysed.

Female Fitness

Female gross fecundity in *A. ludens* was not affected by male diet or size or their interaction (two-way ANOVA: diet: $F_{1,16} = 0.35$, $P = 0.56$; size: $F_{2,16} = 0.08$, $P = 0.93$; diet \times size: $F_{2,16} = 2.06$, $P = 0.16$; Fig. 6a). In contrast, gross

fecundity for female *A. striata* was affected by male size, although there was a marginally nonsignificant effect of male diet (two-way ANOVA: diet: $F_{1,14} = 4.04$, $P = 0.06$; size: $F_{1,14} = 5.81$, $P = 0.01$; diet \times size: $F_{1,14} = 2.09$, $P = 0.16$). Post hoc tests indicated that females laid significantly more eggs when they copulated with large males than when they copulated with small ($P = 0.03$) or medium males ($P = 0.04$) (Fig. 6b).

In *A. ludens*, mean clutch size was significantly influenced by the interaction of diet and size (two-way ANOVA: diet: $F_{1,17} = 0.94$, $P = 0.35$; size: $F_{2,17} = 1.21$, $P = 0.32$; diet \times size: $F_{2,17} = 4.06$, $P = 0.036$; Fig. 7). The average clutch size in females that had copulated with large males fed a high-quality diet was significantly larger than the clutch size oviposited by females that copulated with large males fed a low-quality diet ($P = 0.04$). There was also a difference between the average clutch size oviposited by females that copulated with large or medium males fed a high-quality diet ($P = 0.01$). This analysis could not be performed with *A. striata* as females usually lay only one egg per oviposition bout.

For *A. ludens*, male size had a significant effect on the proportion of eclosed eggs (two-way ANOVA: diet: $F_{1,17} = 0.001$, $P = 0.97$; size: $F_{2,17} = 4.07$, $P = 0.036$; diet \times size: $F_{2,17} = 1.72$, $P = 0.21$; Fig. 8a). A post hoc Tukey test revealed that the proportion of eclosed eggs was significantly greater in females that copulated with medium-sized males when compared to those that mated with large males

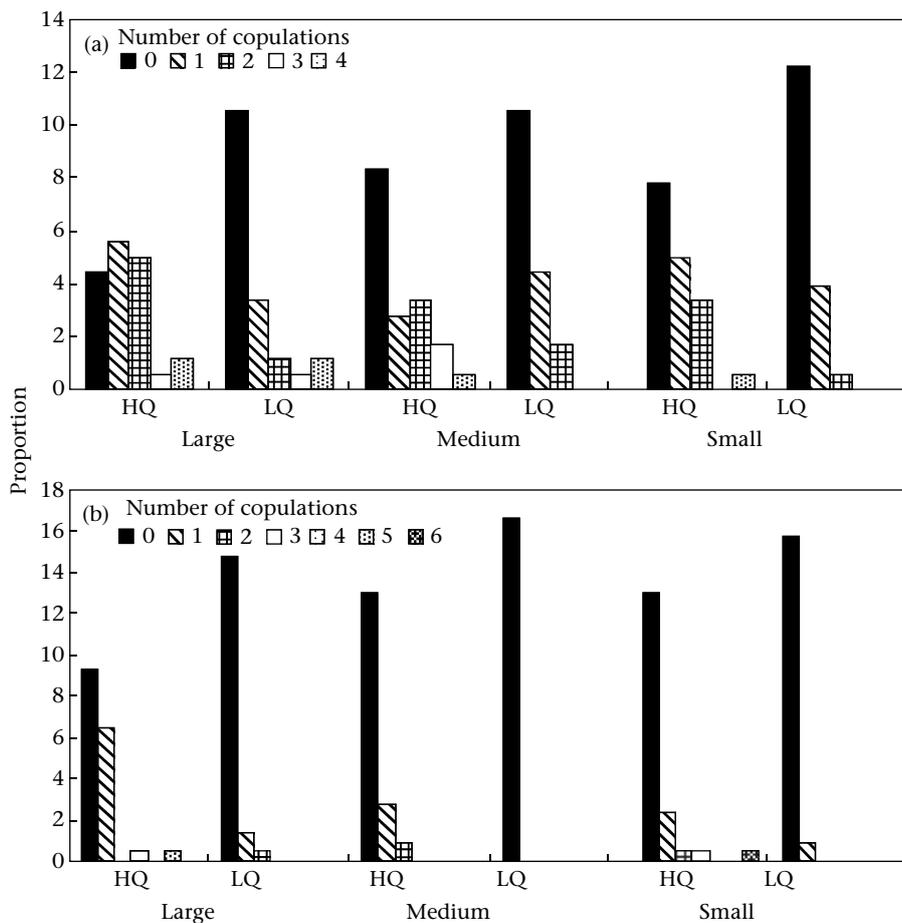


Figure 5. Number of copulations (proportion of total observed) obtained by individually marked *A. ludens* (a) ($N = 180$) and *A. striata* (b) ($N = 216$) males of each size class (small, medium, large) that were offered either a high-quality protein and sucrose diet ad libitum (HQ) or a low-quality sucrose diet every third day (LQ).

($P = 0.03$). For *A. striata*, male condition had no significant effect on the proportion of eclosed eggs even though the effect of size was only marginally nonsignificant (two-way ANOVA: diet: $F_{1,14} = 0.04$, $P = 0.85$; size: $F_{2,14} = 3.52$, $P = 0.06$; diet \times size: $F_{2,14} = 0.98$, $P = 0.40$; Fig. 8b). In this species, the highest proportion of eclosed eggs was observed in large males, independent of diet.

Male condition affected the maximum longevity (longest lived individual per replicate) of *A. ludens* females.

Females that copulated with males fed a high-quality diet lived longer than females that copulated with males fed a lower-quality diet (two-way ANOVA: diet: $F_{1,17} = 4.96$, $P = 0.040$; size: $F_{2,17} = 0.245$, $P = 0.785$; diet \times size: $F_{2,17} = 0.354$, $P = 0.707$; Table 1). For *A. striata*, there was no statistically significant difference between female longevity and male diet or size (two-way ANOVA: diet: $F_{1,14} = 3.22$, $P = 0.094$; size: $F_{2,14} = 0.226$, $P = 0.801$; diet \times size: $F_{2,14} = 0.490$, $P = 0.623$; Table 1).

Table 1. Maximum (max.) (days survived by longest living individual) and mean \pm SE life span for *A. ludens* and *A. striata* females that copulated with males of different sizes and adult nutritional histories

Male diet	Male size	<i>A. ludens</i>		<i>A. striata</i>	
		Max.	Mean \pm SE	Max.	Mean \pm SE
High quality	Large	137	95.0 \pm 14.2 ^a	111	79.4 \pm 12.8 ^a
Low quality	Large	70	52.3 \pm 8.5 ^b	51	50.5 \pm 0.5 ^a
High quality	Medium	133	91.0 \pm 14.6 ^a	116	83.3 \pm 16.4 ^a
Low quality	Medium	100	73.5 \pm 12.7 ^b	64	61.0 \pm 2.5 ^a
High quality	Small	155	96.5 \pm 25.0 ^a	83	67.0 \pm 6.7 ^a
Low quality	Small	86	71.0 \pm 10.4 ^b	81	62.5 \pm 18.5 ^a

High quality: continuous access to sucrose and protein (3:1); low quality: access to sucrose every third day. Numbers within a column followed by the same letter were not significantly different (two-way ANOVA: $P > 0.05$). Means separated with Tukey test.

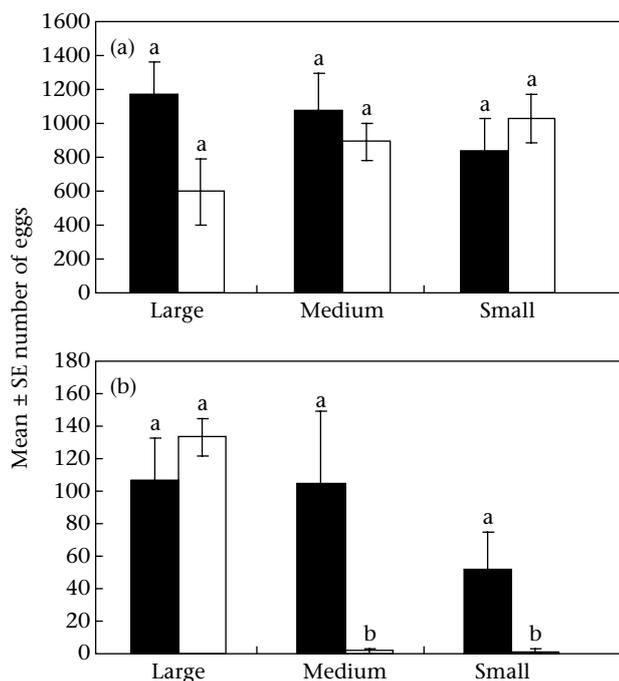


Figure 6. Mean ± SE gross fecundity (number of eggs laid per female) for (a) *A. ludens* and (b) *A. striata* females that copulated with small, medium or large males that were offered a high-quality protein and sucrose diet ad libitum (■) or a low-quality sucrose diet every third day (□). Bars with the same letter were not statistically different.

There was also no significant difference in the mean life expectancy of females that copulated with any type of male (two-way ANOVA: $P > 0.05$ for all cases; Table 1).

DISCUSSION

In partial contrast to our predictions, diet was the factor that had the greatest influence on overall male competitiveness for both the lekking *A. ludens* and the trophalactic *A. striata*. However, under certain contexts (e.g. calling in pairs or winning male–male aggressive encounters as residents in the case of *A. ludens*), size also played a role in determining male competitiveness. Importantly, we also found that in both species some small

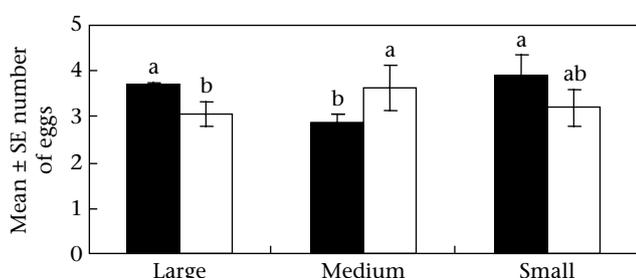


Figure 7. Mean ± SE clutch size (number of eggs laid per clutch) for *A. ludens* females that copulated with small, medium or large males offered a high-quality protein and sucrose diet ad libitum (■) or a low-quality sucrose diet every third day (□). Bars with the same letter were not statistically different. Data for *A. striata* are not shown because females of this species tend to lay only one egg per oviposition bout.

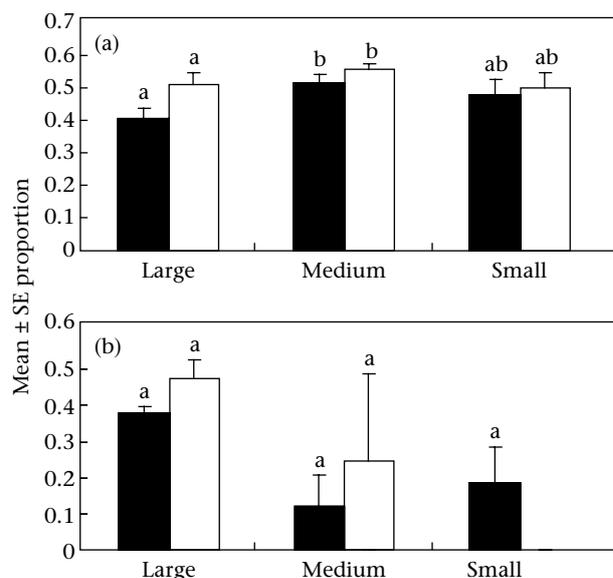


Figure 8. Mean ± SE proportion of enclosed eggs for (a) *A. ludens* and (b) *A. striata* females that copulated with small, medium or large males offered a high-quality protein and sucrose diet ad libitum (■) or a low-quality sucrose diet every third day (□). Bars with the same letter were not statistically different.

males were, as predicted, able to circumvent their size handicap by ingesting a higher-quality diet. Furthermore, male condition also affected female fitness. For example, in *A. ludens*, male diet significantly affected female longevity, and clutch size increased significantly when females mated with large males fed a high-quality diet. In addition, we found that male size had a significant effect on fecundity in *A. striata* and on the proportion of enclosed eggs in *A. ludens*. However, despite costs to female fitness, females did not effectively discriminate between males of different sizes; thus, sexual conflict may be taking place over mating decisions. We discuss our results in terms of the ecological differences that distinguish these two species.

Anastrepha ludens

Calling activity was significantly influenced by diet and size, and this in turn depended on the calling context (e.g. alone or in pairs). Males fed a high-quality diet called more often and over more prolonged periods (i.e. days) than those fed a low-quality diet. When males called in pairs, size determined the number of copulations that males obtained. Yet when males called alone, diet was the most important factor determining a male's success securing copulations. This relationship may be especially relevant for female choice at lek sites as it suggests that two males calling side by side may facilitate female comparison of males, biasing mating success towards larger males. When males are calling alone, females may not be able to accurately compare male sizes and, thus, small males could exploit this situation to obtain copulations.

On the other hand, the quality of adult diet might be more easily expressed by single males. Pheromones and acoustic signals in related species are influenced by carbohydrate and protein consumption. In *A. suspensa*, diet influences male calling-song quality and pheromone production (Landolt & Sivinski 1992; Epsky & Heath 1993). Energy assays of protein-deprived *C. capitata* males suggest that they expend less energy in courtship than protein-fed males (Warburg & Yuval 1996) and therefore have fewer opportunities to copulate (Kaspi et al. 2000; Yuval et al. 2002). Perhaps, diet-influenced pheromones and acoustic signals that are produced by adjacent males present a confused set of clues, so single males might be easier to assess on the basis of odour and sound.

Males fed on a high-quality diet had longer copulations and consistently obtained more copulations across the 4 observation days. This is in contrast to what was previously found by Aluja et al. (2001), where *A. ludens* mating success and copulation duration were unaffected by diet. However, male adult diet in both studies was not comparable. Here, lower-quality diets were composed of sucrose offered only every 3 days as opposed to sucrose offered ad libitum in earlier studies. Furthermore, our results concur with those on other tephritids where males fed on a protein-supplemented diet had higher mating success than males fed on other diets (Blay & Yuval 1997; Aluja et al. 2001; Pérez-Staples et al. 2007). Only males fed a high-quality diet were able to mate up to four times. In addition, we found that some small males were able to circumvent their size handicap by ingesting a higher-quality diet. Small males fed on a high-quality diet were as successful as large males fed on a low-quality diet (Fig. 3a). Energy reserves are more strongly correlated with winning contests in damselflies than in size (Marden & Waage 1990), whereas we found that male condition, in terms of both diet and size, was important in determining a male's ability to win territorial contests, and thus presumably influenced male mating success.

Consequences on female fitness

In *A. ludens*, male size and diet had no effect on female reproductive success in terms of gross fecundity. However, mean clutch size was significantly influenced by the interaction of male diet and size. Likewise, in the spruce budworm, *Choristoneura fumiferana*, females mated with males fed a low-quality diet had lower fertility and lower total fecundity than females mated to males fed a high-quality diet (Delisle & Hardy 1997). Thus, females may bias paternity preferentially towards large males fed a high-quality diet and/or male-adaptive sperm mechanisms may be at play. For example, in *C. capitata*, females mated to large, protein-fed males stored more sperm than females mated to small, protein-deprived males (Taylor & Yuval 1999). Nutritional effects on the quantity or quality of sperm have been reported in katydids, mormon crickets, moths and water striders among others (Gwynne 1990, 1993; Gage & Cook 1994; Arnqvist & Danielsson 1999).

In our study, male diet also had an important effect on female longevity. Females that copulated with males fed

a low-quality diet had a subsequent decrease in their longevity (approximately a 30-day difference). Although *A. ludens* males do not offer prenuptial gifts or engage in trophallaxis, males may transmit substances during copulation that eventually affect female longevity (Eberhard & Cordero 1995). Indeed, ejaculate-derived nutrients increase female longevity in butterflies, bruchid beetles and crickets (Wiklund et al. 1993; Fox 1993; Brown 1997; Wagner et al. 2001; Wagner & Harper 2003). In light of the negative consequences for females of copulating with males fed a low-quality diet and our finding that females mated preferentially with males fed a high-quality diet, there seems to be strong selective pressure for females to discriminate among males of different nutritional conditions. A male in good condition, irrespective of its size, may reflect a higher capacity to forage for foods containing protein in nature (e.g. bird faeces).

Anastrepha striata

Male mating success in *A. striata* was also determined by diet. Previously, Aluja et al. (2001) found that diet significantly influenced calling frequency. Here, we also found that males fed a high-quality diet (i.e. containing protein) were able to call over longer periods (i.e. more days) than males fed a low-quality, protein-deprived diet. Consequently, males fed a high-quality diet were also more likely to obtain copulations because females were more attracted to them. Only males fed a high-quality diet were able to mate more than four times, while most males fed a low-quality diet never mated (Fig. 5). This result is similar to reports for *C. capitata* (Blay & Yuval 1997) and is consistent with lower energy reserves in malnourished males (Jácome et al. 1995). Importantly, and as was the case with *A. ludens*, some small males were able to circumvent their size handicap when they were fed a high-quality diet (Figs 2b, 3b). For example, medium-sized males fed a high-quality diet obtained more copulations after 4 days of exposure to virgin females than did poorly fed large males (Fig. 3b).

In contrast to *A. ludens*, size or diet in *A. striata* did not determine the success of resident males in aggressive encounters. However, invading males benefited from a high-quality diet. Differences between these two species can be explained in terms of their contrasting mating systems. *Anastrepha ludens* males are more prone to join leks compared to *A. striata*. In other lekking species such as *C. capitata*, protein-deprived males are not able to participate in leks (Yuval et al. 1998). Thus, in *A. ludens*, diet may be especially relevant for resident males in maintaining a territory within a lek. In contrast, *A. striata* rarely call in leks and, therefore, territories may not be as contested. Hence, male size was of no measurable importance in determining male–male aggressive encounters in this species.

Consequences on female fitness

In species that provide nuptial gifts, male condition is particularly important for female demographic parameters and offspring size or quality (Boggs 1995; Savalli & Fox

1999; Bonduriansky et al. 2005). In *A. striata*, males transfer an oral substance to females through trophallaxis before mating. The nutritional value of the courtship gift is unknown, yet in the fruit fly *Drosophila subobscura* and the tree cricket *Oecanthus nigricornis*, courtship feeding positively influences female fitness (Steele 1986; Brown 1997). Here, contrary to our predictions, male diet had no effect on female reproductive parameters or longevity. Likewise, Pérez-Staples & Aluja (2004) found no effect of male diet on female fecundity or fertility, but they found that female life span was longer when females were mated to virgin males compared to when females subsequently mated with the same male (nonvirgin). In other words, when looking at the effect of male diet on female reproductive parameters, it is important to consider male mating history.

Females benefit from mating with larger males, as shown by the positive effect of male size on fecundity in this study. This result suggests that larger males may provide females with a larger quantity or better quality courtship gift or ejaculate that females can use for egg production. Likewise, in the seed beetle, *Stator limbatus*, irrespective of female size, females mated to large males lay more eggs than females mated to small males (Fox et al. 1995), and larger males produce larger ejaculates than small males (Savalli & Fox 1998).

Given that egg production of females that mated with medium and small males was on average less than half that of females that mated with large males, and that *A. striata* females are very choosy (females receive on average 13 mating attempts before they accept a male for copulation), one wonders why some costly mistakes were made. Females that mate with small males may oviposit for a short period and then remate with larger males. However, similarly to *A. ludens*, female remating inhibition lasts at least 2 weeks after the initial mating (M. Aluja, personal observation). Thus, the costs of an imprudent first choice in a mate are quite high. It seems likely that in both of these species, there may be sexual conflict over mating decisions (Arnqvist & Rowe 2005) as small and medium-sized males may be effective in manipulating females to mate with them despite the cost to females in terms of lower fecundity. Likewise, in *C. capitata*, females are unable to distinguish between virgin and nonvirgin males even though they have lower fecundities when mating with nonvirgin males (Shelly & Whittier 1993). Here, despite fitness costs to females, *A. striata* females, like *A. ludens*, also seemed unable to distinguish between males of different sizes, as there was no significant difference in the number of copulations obtained by males according to size. Unlike males fed a low-quality diet, perhaps medium and small *A. striata* males are able to produce adequate substances to transfer through trophallaxis and court females satisfactorily, manipulating females into mating with them.

In conclusion, this study highlights the complex interaction of male size (largely determined by the interaction of environmental factors such as larval diet or crowding conditions in the host and genetic components) and adult diet in determining sexual competitiveness and

the fitness consequences for females mating with males of different conditions. It also addresses the importance of studying these issues in closely related species that have distinct natural histories and reproductive behaviours. Our results suggest that nutritional status is especially important in influencing female choice. Females confronted with males at lek sites may be unable to distinguish between males of different sizes (or are indifferent), and seem to be more adept at discriminating between males of different nutritional conditions. Also, we found that *A. striata* females that chose to mate with smaller males suffered a cost in terms of gross fecundity. This result suggests that there may be sexual conflict at play over mating decisions. Nevertheless, we cannot discard the possibility that there may be indirect fitness benefits for females mating with these males that may outweigh the costs (e.g. Cordero 1996).

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