Male antler flies (*Protopiophila litigata*; Diptera: Piophilidae) are more selective than females in mate choice

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Abstract: We investigated mate choice in the antler fly (*Protopiophila litigata* Bondurianksy), which forms mating aggregations and oviposits exclusively on discarded cervid antlers, by pairing males with nongravid females and by collecting copulating pairs on antlers. Because females probably receive larger ejaculates (which they partly ingest after mating) and more effective protection (mate guarding) from large males than from small ones, we expected females to prefer large males as mates. Because males experience high mating costs and often encounter females lacking mature eggs, we expected males to reject some females. Moreover, because relative abdomen width (fatness) predicts the number of mature eggs (egg load) of a female better than her body size does, we expected males to evaluate female quality by assessing female fatness. Of the 54 male–female pairings, 7 (13%) resulted in copulation, the female rejected the male in 6 (11%), and the male rejected the female in 41 (76%). We found no significant differences between individuals that mated and those that did not. However, females disproportionately rejected males smaller than themselves. Males exhibited a preference (quantified as duration of abdominal tapping bouts) for fat females as mates. Small males appeared to be less choosy than large males. Because males rejected potential mates more frequently than females (apparently) did, males may have been choosier than females. Coupled pairs collected on antlers exhibited positive assortment by body size and positive correlation of male body size with female egg load. These mate-choice and mating-assortment patterns may occur in many dipteran species, where copulation is costly for both sexes, females often lack mature eggs, and mating is initiated in dense aggregations of aggressive males.

Résumé : Nous avons étudié le choix d'un partenaire chez le piophile *Protopiophila litigata* Bondurianksy, qui forme des rassemblements au moment de la reproduction et pond exclusivement dans les bois rejetés des cervidés; nous avons accouplé des mâles à des femelles non gravides et observé des couples en copulation sur les bois de cervidés. Comme les femelles reçoivent probablement des éjaculats plus considérables (qu'elles ingèrent partiellement après l'accouplement) et une protection plus efficace (surveillance du partenaire) de mâles de grande taille que de mâles plus petits, nous avons posé en hypothèse que les femelles préféreraient s'accoupler à des mâles de grande taille. Or les mâles doivent faire face à des coûts reproducteurs élevés et rencontrent souvent des femelles dépourvues d'oeufs à maturité; nous nous attendions donc à ce que les mâles rejettent certaines femelles. De plus, comme la largeur relative de l'abdomen (« embonpoint ») laisse présager le nombre d'oeufs à maturité (« fardeau d'oeufs ») mieux que la taille corporelle, nous nous attendions à ce que les mâles évaluent la qualité d'une femelle à sont embonpoint. Des 54 appariements observés 7 (13%) ont abouti à un accouplement, la femelle a rejeté le mâle dans 6 (11%) cas et le mâle a rejeté la femelle dans 41 (76%) cas. Nous n'avons pas trouvé de différences significatives entre les individus qui se sont accouplés et ceux qui ne se sont pas accouplés. Cependant, les femelles ont rejeté surtout les mâles plus petits qu'elles. Les mâles ont montré une préférence (évaluée d'après la durée des tambourinements abdominaux) pour les femelles avec de l'embonpoint. Les petits mâles semblaient moins exigeants que les gros mâles dans leur choix. Comme les mâles rejettent des partenaires potentiels.

Introduction

Mate choice, the tendency to reject some potential mates, remains a controversial issue in sexual selection theory. According to the traditional view, the sex with the higher parental investment (usually the female) will be more discriminating in its acceptance of mating partners because it

has more to lose from mating with a suboptimal partner (e.g., Parker 1979; Maynard Smith 1991). However, Parker (1983) showed that choosiness, on the part of males or females, is favoured by a high degree of variance in mate quality among members of the other sex. Conversely, the ability to exercise mate choice is constrained by the costs (e.g., time) of finding and choosing a mate. When both sexes are choosy, low-quality individuals may be less choosy than high-quality ones, and this will result in assortative mating for the fitness trait. Gwynne (1991) pointed out that males may be choosy even when they outnumber and compete for access to females in mating aggregations, when costs of copulation (e.g., energy, time, risk of predation) are high enough to limit their potential lifetime mating success to a relatively small number of copulations. The usefulness of

Parker's (1983) and Gwynne's (1991) approach as an explanatory and predictive framework has yet to be adequately explored.

Hence, for female insects, mate choice is often adaptive because copulation is a costly parental investment of gametes and time, and constitutes a risk when males vary substantially in mate quality. For example, a male's body size may be correlated with the size of his nutritive "gift" (e.g., Steele 1986; Simmons and Parker 1989) or the effectiveness of the "mate guarding" (protection from harassment by other males) (Gwynne 1984; Alcock 1994) he provides during copulation, or with the fitness of the offspring he sires (e.g., Thornhill and Sauer 1992; Moore 1994). Conversely, high costs of mate choice, such as a high risk of injury in struggles associated with male-rejection attempts (Daly 1978; Reynolds and Gross 1990), may select for random mating.

Male mate choice may be at least as prevalent as female mate choice in the Diptera (e.g., Cook 1975; Hieber and Cohen 1983; Jarvis and Rutledge 1992). In species where females mate repeatedly (see Ridley 1988), male mate choice is likely to evolve if males often encounter females with no mature eggs. This is because, in most insects, eggs are fertilized just before they are laid (Wigglesworth 1972), enabling the female's last mating partner before oviposition to fertilize most of the clutch (Parker 1970a; Tsubaki et al. 1994). Thus, a male copulating with a nongravid female (i.e., a female lacking mature eggs) is likely to fertilize very few (if any) eggs because the female will probably mate at least once more before her next oviposition. If copulation is costly for males in this system, they will probably benefit by assessing female egg load and rejecting nongravid females.

The operation of male or female mate choice may result in patterns of mating assortment, i.e., covariation of male and female characteristics among coupled pairs (Parker 1983; Crespi 1989). For males, mating assortment may determine the mean number of eggs fertilized per copulation. For females, it may determine the mean value of direct benefits received from males (e.g., Sigurjónsdóttir and Parker 1981; Gwynne 1984, 1990). For either sex, it may determine mean offspring fitness (e.g., Moore 1994; Petrie 1994). Hence, mating assortment determines mean mate quality, an important component of sexual selection on males (Arnold and Wade 1984a, 1984b) and females (e.g., Grigolo et al. 1979; Kaitala and Wiklund 1994).

The antler fly, *Protopiophila litigata* Bonduriansky (1995), forms dense mating aggregations on discarded moose (*Alces alces* Linné) antlers, where males fight each other and sometimes defend territories (Bonduriansky 1996). After each copulation the female expels and ingests ejaculate materials and oviposits into cracks or pores in the antler. Throughout the copulation–oviposition cycle (mean duration ~2.3 h) the male remains on top of the female and guards her by warding off attacking single males with his wings (Bonduriansky and Brooks 1998).

Because large males probably provide larger ejaculates (partly ingested by the female) and more effective mate guarding, and may sire larger (higher quality) offspring than small males, we expected females to prefer large males as mates. Because copulation is costly for male *P. litigata* (Bonduriansky 1996), we expected males to be choosy if

they encounter large variation in female quality, such as mating aggregations in which many females lack mature eggs. Males may use female body size to assess female quality, and reject small females because these produce small clutches (Bonduriansky 1996) and, perhaps, small (low quality) offspring. However, the abdominal tapping bouts (ATBs) delivered to females prior to copulation (Bonduriansky and Brooks 1998) suggest that males assess female quality by female abdomen width, or abdomen width relative to body size (a condition index we will call fatness). If fatness predicts whether or not a female is gravid better than body size does, males are more likely to reject thin females than small ones. We tested these predictions using male–female pairings.

Although we compare directly those flies that mated with those that did not, the results represent an interaction of male and female mate-choice responses (see Discussion). Moreover, they do not take into account the relative mate quality of the paired individuals. Parker's (1983) model suggests that individuals evaluate the quality of potential mates relative to their own quality rather than an absolute threshold; in other words, they reject potential mates smaller than themselves. Hence, we quantified relative mate quality as the ratio of male to female head-capsule width, used the incidence of male-rejection behaviours to quantify female mate choice, and after showing that ATB duration is male-determined and reflects male interest, used ATB duration to quantify male mate choice.

Materials and methods

Female egg load and mating assortment

We collected 4 discarded moose (*Alces alces* Linné) antlers, ranging in age from <1 to 2 years during the study (May–August 1995), near the Wildlife Research Station (45°30′N, 78°40′W) in Algonquin Provincial Park, Ontario, Canada, and set each antler on a 0.8 m high wooden stand in the forest. To investigate mating assortment and variation in mate quality among females, we collected 99 copulating pairs and 93 single (i.e., noncoupled) females from the *P. litigata* population on the antlers by covering flies with small jars, and killed them by freezing. We also obtained 9 females newly emerged from puparia. Prior to dissection, we thawed each specimen and measured its body length (posterior tip of the abdomen to anterior tip of the antennal flagellum), head-capsule width, and abdomen width (females only) to 0.01 mm using an ocular micrometer in a dissecting microscope. The abdomen of each female was then severed with a microscalpel, placed in a drop of isotonic solution (pond water), and dissected with microprobes to determine the number of mature eggs (egg load). Because the data did not conform to parametric test assumptions (see Zar 1996), we compared mean egg loads of coupled and single females by means of the two-tailed Mann–Whitney U test. The p values in Table 2 were adjusted using the "classic" Bonferroni procedure (Wright 1992). Statistical tests were performed using Statistica (Release 5.0; © StatSoft, Inc., 1984–1995).

Male–female pairings

We investigated mate choice by observing male and female responses in 58 different male–female pairs, created using 21 males and 16 females, in a small container (described in Bonduriansky and Brooks 1998) on the stage of a dissecting microscope (July 11–12, 1995). We collected these flies on antlers by covering them with small jars, and measured them (as above) using the technique

Fig. 1. Mate-rejection responses by male and female *P. litigata* in male–female pairings; the vertical line separates domains of expected mate rejection by males and females (see the text); □, female rejected male; ●, male rejected female in <2.0 s; ○, no rejection.
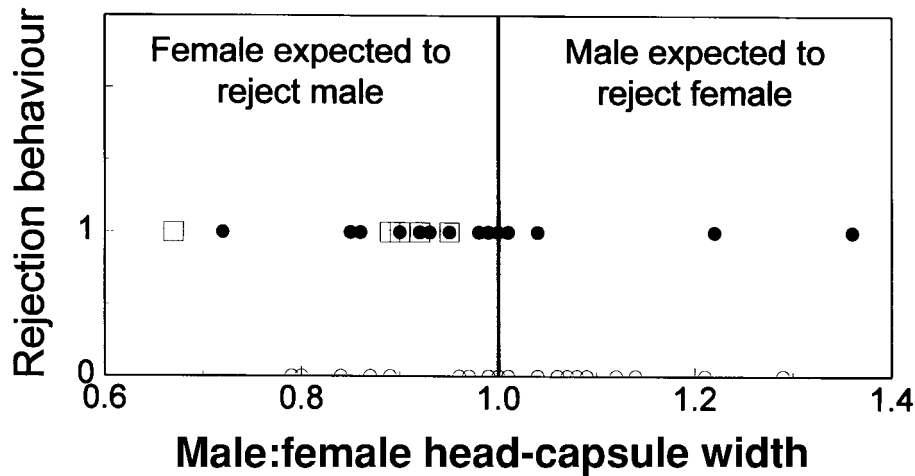
of Bonduriansky and Brooks (1997). Most individuals were paired with a total of 2–6 different partners. Each female was used in several consecutive pairings, killed by freezing, and dissected to assess her egg load. Males were marked with an individual code (see Bonduriansky and Brooks 1997), released at an antler, and recaptured for use in additional pairings.

For each male–female pairing we timed (mount to dismount) durations of the first 4 ATBs delivered by the male to the female, and recorded any male-rejection behaviours performed by the female. Of the 16 females used, only 1 was gravid; the 4 pairings involving this female were omitted from analyses of ATB duration to standardize egg load. In addition to direct comparisons of mated and nonmated individuals (see Results), the 47 pairings that did not result in copulation were grouped into sets of male partners for each female and sets of female partners for each male, excluding non-independent pairs (giving priority to earlier pairings). Because *P. litigata* is very tolerant of close observation, we also made "field observations" of mate-rejection behaviours on antlers throughout the study.

Results

Field observations

After alighting on an antler, a female usually walked through a dense aggregation of single males. All nearby males chased the female, mounted her (usually one by one), and tapped the sides of her abdomen with their mid and hind legs. After tapping for several seconds, a male either rejected the female and dismounted, or commenced genital stimulation. Some single females were rejected by every male they encountered. Less frequently, a female performed male-rejection behaviours such as fleeing (running, jumping, or flying) from a pursuing male (type 1), shaking vigorously to dislodge a mounted male (type 2), or curving her abdomen tip anteroventrally to prevent the male from delivering genital stimulation (type 3). Of these responses, type 1 appeared to be the most effective. Once mounted by a male, an unreceptive female using type 2 and (or) type 3 behaviours could delay genital locking by as much as 45 min, but was rarely able to prevent it.

Table 1. Proportions that were gravid and mean number of eggs per female for single and coupled *P. litigata* females collected on discarded moose antlers.

Female	N	No. gravid	% gravid	Mean no. of eggs/female
Single	93	17	18	3.70
In copula	99	72	73	20.60
Total	192	89	46	12.41

Male–female pairings: female mate choice

Do females prefer large males?

If females prefer large males as mates, males that mate may be larger, on average, than males that do not mate. However, males that mated ($N = 7$) did not have a greater body length (one-tailed Mann–Whitney U test, $U = 63.5$, $p > 0.1$) or head-capsule width ($U = 58.5$, $p > 0.2$) than those that did not mate ($N = 14$). If females prefer large males as mates, but evaluate male body size relative to their own body size, they will reject males smaller than themselves (male:female head-capsule width <1; Fig. 1). In each of the 6 pairings where male-rejection behaviours were observed, the female was the larger individual (one-tailed Mann–Whitney U test, $U = 53.5$, $n_1 = 6$, $n_2 = 46$, $p = 0.0154$), suggesting that females discriminated against males smaller than themselves.

Male–female pairings: male mate choice

Why and how might males choose females?

None of the 9 newly emerged females, and a minority of single females collected on antlers, had mature eggs (Table 1). Single females had, on average, fewer mature eggs than coupled females did (two-tailed Mann–Whitney U test, $U = 1905$, $z = 7.01$, $p < 0.0001$). Hence, males often encounter females lacking mature eggs, and would probably benefit by rejecting some potential mates. Of five potential phenotypic "predictors" of the number of mature eggs carried by a

Table 2. Regressions of egg load (number of mature eggs) on indices of body size and breadth for female *P. litigata* collected from discarded moose antlers (*p* values are Bonferroni-adjusted).

Phenotypic index	*N*	*r*	*F*	*p*
Body length (BL)	211	0.17	6.224	0.067
Head-capsule width (HCW)	211	0.09	1.553	>0.5
Abdomen width (AW)	171	0.28	14.173	0.001
AW/BL	171	0.28	14.395	0.001
AW/HCW	171	0.32	19.672	0.0002

Table 3. Mean durations of abdominal tapping bouts (ATBs) delivered by male *P. litigata* to the females with which they were paired, for three categories of pairings defined by the ratio of male to female head-capsule width (HCW): A, male much larger than female; B, male and female of nearly equal body sizes; C, male much smaller than female.

Category	Male:female HCW	*N* (pairings)	ATB duration (s) Mean	ATB duration (s) SD
A	1.1–1.36	6	4.55	2.88
B	0.9–1.09	28	6.53	11.84
C	0.67–0.89	13	7.38	14.41

Table 4. Mean durations of abdominal tapping bouts (ATBs) delivered to the thinnest and fattest female *P. litigata* with which each of 6 males had been paired.

Male No.	Mean duration (s) of ATBs delivered by male to: thinnest female	Mean duration (s) of ATBs delivered by male to: fattest female
146	1.94	5.25
287	2.77	6.13
420	1.51	1.68
254	3.68	4.73
126	1.06	10.8
278	2.13	3.44

female (Table 2), abdomen width/head-capsule width (fatness) yielded the highest *r* and lowest *p* values (Fig. 2). Fatness was not correlated significantly with head-capsule width ($N = 171$, $r = –0.076$, $F = 0.985$, $p > 0.3$) or body length ($N = 171$, $r = 0.15$, $F = 3.718$, $p > 0.05$). Hence, fatness is a better indicator than body size of a female's egg load, and males may use fatness as a proximate criterion of female quality.

Does ATB duration reflect the male's interest in the female?

Is ATB duration determined primarily by the male or the female? If ATB duration is determined by the female (i.e., all rejections are initiated by the female), we expect more rapid rejection when the male is smaller than the female because females discriminate against males smaller than themselves (see above) and because smaller males are probably more easily dislodged. However, rejection was not more rapid in pairings where male:female head-capsule width <1 (one-tailed Mann–Whitney *U* test, $U = 20$, $n_1 = 6$, $n_2 = 5$,

Fig. 2. Fatness (abdomen width/head-capsule width) as a predictor of egg load (number of mature eggs) for female *P. litigata* collected from discarded moose antlers (see Table 2).
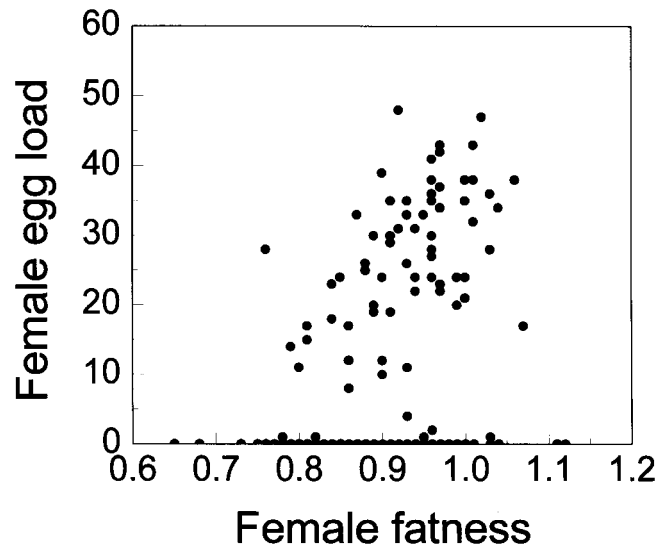
$p > 0.2$). Among all pairings, the opposite appeared to be the case: ATB duration appeared to increase with decreasing male:female head-capsule width (Table 3). These patterns suggest that ATB duration is male-determined. Moreover, if a longer ATB indicates greater male interest in the female, ATBs leading to copulation will be longer than ATBs leading to rejection. The mean duration of ATBs leading to copulation ($N = 5$, mean = 135.3 s, SD = 150.2 s) was greater (one-tailed Mann-Whitney *U* test, $U = 193$, $p < 0.015$) than the mean duration of ATBs leading to rejection ($N = 47$, mean = 6.5 s, SD = 11.8 s). Hence, ATB duration is suitable as an index of a male's interest in a female.

Do males prefer fat females?

If males prefer fat females as mates, females that mate may be fatter, on average, than females that do not mate. However, females that mated ($N = 6$) were not significantly fatter (one-tailed Mann-Whitney *U* test, $U = 42.5$, $p = 0.1$) than females that did not mate ($N = 10$). When we compared the mean durations of ATBs delivered by each male to the thinnest and fattest female with which he had been paired (Table 4), we found that longer ATBs were delivered to fatter females (Wilcoxon's test, $N = 6$, $T_- = 0$, $p = 0.0277$), suggesting that males preferred fat females as mates.

Do males prefer large females?

If males prefer large females as mates, females that mate may be larger, on average, than females that do not mate. However, females that mated ($N = 6$) did not have greater body length (one-tailed Mann-Whitney *U* test, $U = 40.0$, $p > 0.1$) or head-capsule width ($U = 35.5$, $p > 0.2$) than females that did not mate ($N = 10$). When we compared the mean durations of ATBs delivered by each male to the longest and shortest female with which he had been paired (Table 5), we found that males did not deliver longer ATBs to longer females (Wilcoxon's test, $N = 6$, $T_- = 10$, $p > 0.5$). If males prefer large females as mates, but evaluate female body size relative to their own body size, they will reject females smaller than themselves (male:female head-capsule width

Table 5. Mean durations of abdominal tapping bouts (ATBs) delivered to the shortest and longest female *P. litigata* with which each of 6 males had been paired.

Male No.	Mean duration (s) of ATBs delivered by male to:	
	shortest female	longest female
146	4.74	5.25
287	6.13	1.42
420	1.68	3.84
81	50.71	13.11
126	4.20	10.80
278	1.05	3.44

Table 6. Mean durations of abdominal tapping bouts (ATBs) delivered to each of 7 female *P. litigata* by the smallest and largest male with which they were paired.

Female No.	Mean duration (s) of ATBs delivered to female by:	
	smallest male	largest male
142	5.95	3.50
143	1.66	1.42
147	1.36	1.51
149	54.6	1.68
150	3.88	3.54
152	4.20	3.13
154	1.42	0.94

Fig. 3. Body lengths of male and female *P. litigata* in copulating pairs collected on discarded moose antlers, July 1–11, 1995.

>1; Fig. 1). However, rapid rejection of the female (defined here as mean ATB duration <2.0 s) was not more likely to occur in pairings with large male:female head-capsule width ratios than in other pairings (one-tailed Mann–Whitney U test, $U = 259.0$, $n_1 = 16$, $n_2 = 36$, $p > 0.5$). The results of all the above tests suggest that males did not prefer large females as mates.

However, the only gravid female used in male–female pairings (body size ~ 15th percentile) was rejected in <2.0 s by each of the 4 males with which she was paired, even though she had 12 mature eggs (~2/3 expected clutch size) and was relatively fat (~50th percentile). Hence, males may discriminate against very small females.
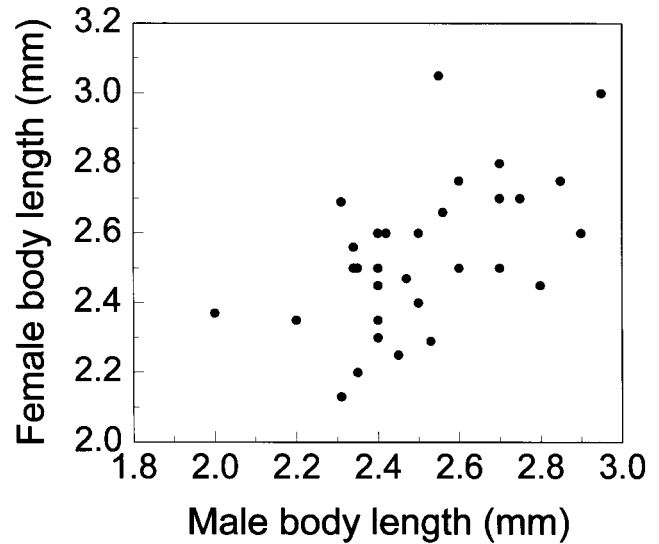
Are large males choosier than small males?

We compared the mean durations of ATBs delivered to each female by the smallest and largest male with which she had been paired (Table 6). The smallest male delivered longer ATBs than the largest male (Wilcoxon's test, $N = 7$, $T_- = 1$, $p = 0.0280$), suggesting that the larger male was more choosy.

Are males choosier than females?

Of the 47 pairings where no copulation occurred, the female performed visible male-rejection behaviours in 6 pairings, dislodging the male by vigorous shaking (4 pairings) or attempting to flee from him (2 pairings). Conversely, in 41

of the 47 pairings, the female performed no visible male-rejection behaviours, whereas the male dismounted the female (apparently "voluntarily") after tapping her abdomen. Hence, males rejected females (41/47 pairings) much more frequently (sign test, $z = 4.96$, $p < 0.0001$) than females rejected males (6/47 pairings), suggesting that males were more choosy than females.

Mating assortment

Body lengths of males and females in copulating pairs collected over the season (June–August 1995) were positively correlated ($N = 86$, $r = 0.30$, $F = 8.295$, $p = 0.0050$), but this may be an artifact of the decrease in mean adult body size over the season (Bonduriansky 1996). However, body lengths of males and females were also correlated among copulating pairs collected between July 1 and 11 ($Y = 0.30X + 1.81$; $N = 36$, $r = 0.56$, $F = 15.443$, $p = 0.0004$; Fig. 3). This pattern was not an artifact of changing mean adult body size because the order in which these pairs were collected over the 11-d period was not correlated with body length of males ($N = 36$, $r = -0.25$, $F = 2.178$, $p > 0.1$) or females ($N = 36$, $r = 0.03$, $F = 0.022$, $p > 0.5$). Although, among pairs collected over the season (June 9 – August 16, 1995), male body length was not significantly correlated with female egg load ($Y = 10.11X - 4.78$; $N = 87$, $r = 0.14$, $F = 1.607$, $p > 0.2$), the relationship was significant among pairs in which the female had mature eggs ($N = 63$, $r = 0.26$, $F = 4.563$, $p = 0.0367$), and among pairs collected between July 1 and 11 ($Y = 23.73X - 35.98$; $N = 36$, $r = 0.39$, $F = 5.928$, $p = 0.0203$; Fig. 4).

Discussion

Female mate choice

In male–female pairings, males that mated were not significantly larger than males that did not mate. Although this suggests that females did not prefer large males as mates, interpretation of this result is equivocal because whether or not a copulation occurred depended on the receptiveness of both individuals and, hence, integrated the female's and

Fig. 4. Male body length and female egg load (number of mature eggs) in copulating pairs of *P. litigata* collected on discarded moose antlers, July 1–11, 1995.
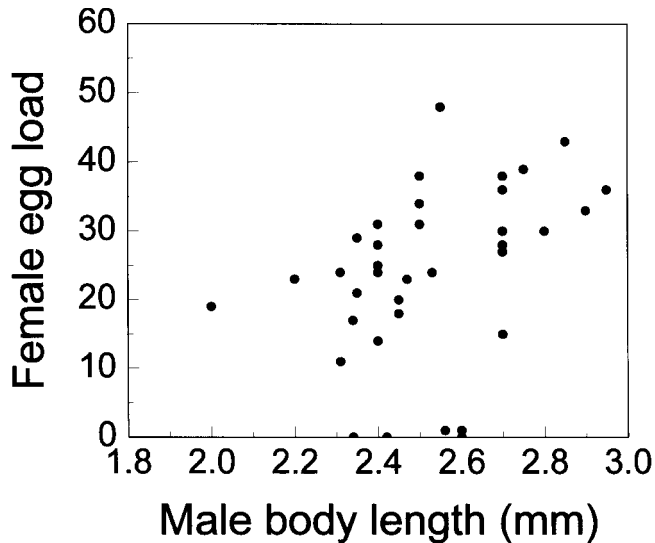
male's mate-choice responses. If copulation occurred, the male accepted the female, but the female may or may not have accepted the male (i.e., mated "willingly"). If copulation did not occur, the male may have rejected the female, the female may have rejected the male, or both. Thus, the sets "males that mated" and "males that did not mate" both include individuals acceptable and unacceptable to their female partners. The above result is to be expected if, for example, large (high quality) males are more choosy and, hence, more likely to reject nongravid (low quality) females.

Females disproportionately rejected males smaller than themselves, suggesting that they preferred males larger than themselves as mates. Nevertheless, female mate choice appears to exert weaker selective pressure for large male body size than male–male competition (Bonduriansky 1996), because females rejected males relatively infrequently and not always successfully. However, even when their ability to prevent coupling is limited, females may discriminate among males via cryptic female choice (Thornhill 1983; Eberhard 1991). For example, female *P. litigata* may vary the proportions of different ejaculates expelled and ingested after each copulation (Bonduriansky and Brooks 1998). If this occurs, cryptic female mate choice may play an important role in sexual selection in *P. litigata*.

Female mate choice is adaptive when females receive greater "direct benefits" from certain males (Reynolds and Gross 1990). For female *P. litigata*, a high-quality mate is one that provides effective mate guarding, resulting in a reduced risk of injury in male–male struggles for access to the female, and a large ejaculate, resulting in more food and, perhaps, a higher fertilization rate (Bonduriansky 1996). In addition, if body size is heritable, mate choice is adaptive for a female because a clutch fertilized by a low-quality male represents a large reduction in her fitness. Because large males usually defeat small males in agonistic contests (Bonduriansky 1996), they are likely to guard females more effectively than small males. Large males also probably pro-

vide larger ejaculates than small males. Thus, because females receive greater direct benefits from large males, they are selected to prefer large males as mates.

Female and male body sizes were positively correlated among coupled pairs. Because the relationship (Fig. 3) is homoscedastic and symmetrical about the line of best fit, it represents "true" mating assortment (Arnqvist et al. 1996; also see Crespi 1989). According to Crespi (1989), assortative mating by body size may be explained by three hypotheses: (1) mate choice; (2) mate availability; (3) mating constraints. Hypothesis 2 is probably not important in *P. litigata* because mating aggregations normally included active males and females spanning nearly the full range of body sizes in the population (Bonduriansky 1996). Similarly, hypothesis 3 does not appear to be important because individuals of very different body sizes were clearly capable of copulating, both in experimental male–female pairings and on antlers. Assortative mating by body size in *P. litigata* is best explained by hypothesis 1, particularly females' preference for males larger than themselves, relatively indiscriminate mating by small males, and rejection by males of very small females. For reasons described above, this pattern of mating assortment represents a substantial advantage for large females relative to small ones.

Because male *P. litigata* are aggressive and densely aggregated on antlers, the ability of females to reject males may be constrained by the high costs of male–female struggles, in which females risk injury (e.g., damage to wings or legs; Bonduriansky and Brooks 1998) and infection by parasites (Bonduriansky 1996) and may be at greater risk of predation (e.g., Rowe 1994). Similar factors may constrain female mate choice in other Diptera. Female mate choice has been observed in a variety of insects (e.g., Thornhill 1983, 1984; Hedrick 1988; Gwynne 1993; Tuckerman et al. 1993; Moore 1994; Rowe 1994; Wiernasz 1995), including Diptera (e.g., *Scatophaga stercoraria*, Borgia 1981; *Drosophila* spp., Steele 1986; Kaneshiro and Boake 1987; Hoikkala and Aspi 1993). However, the ability of females to avoid coupling with certain males appears to be limited or absent in many dipteran species (e.g., the stratiomyid *Hermetia comstocki*, Alcock 1990, 1993; a Hawaiian *Drosophila* species; Droney 1992, 1994; the bibionid *Plecia nearctica*, Hieber and Cohen 1983; the tabanid *Hybomitra illota*, Taylor and Smith 1990; the bombyliid *Lordotus pulchrissimus*, Toft 1989), perhaps because males are often highly aggressive and mating tends to occur in dense swarms or leks (e.g., Hieber and Cohen 1983; Droney 1992, 1994; Bonduriansky 1996). This suggests that female mate choice plays a limited role in many Diptera, although further investigation of cryptic female mate choice in Diptera may yet alter this picture.

Male mate choice

Females that mated were neither significantly fatter nor larger than females that did not mate. Although this may suggest that males did not prefer either fat or large females as mates, this result is difficult to interpret because the set "females that did not mate" includes females both acceptable (i.e., female rejected males) and unacceptable (i.e., males rejected female) to potential mates. Because the ATB delivered by a male *P. litigata* to a female after mounting

(Bonduriansky and Brooks 1998) appeared to be similar to the abdominal "probing" involved in male mate choice in *Dryomyza anilis* (Otronen 1984), ATB duration appeared to be male-determined (see below), and ATBs leading to copulation were longer than those leading to female-rejection, we concluded that ATB duration reflected male interest. Using ATB duration as an index of male preference, we found that males rejected thin females more quickly than fat ones. Hence, males appeared to prefer fat females as mates. Males may use female fatness (abdomen width relative to body size) as a proximate index of female quality because it predicts a female's egg load better than her body size does. In contrast, males showed no preference for large females, or females larger than themselves, as mates. However, one very small gravid female was rapidly rejected by all 4 of the males with which she was paired, suggesting that males may discriminate against the smallest females in the population. In addition, large males rejected females more rapidly than small males did, suggesting that large (high quality) males are more discriminating in mate choice, as predicted by Parker's (1983) mate-choice model.

A potential alternative interpretation of the ATBs delivered by male *P. litigata* to females is as "copulatory courtship" (Eberhard 1991, 1994) rather than as male mate-choice behaviour. Eberhard (1994) presented evidence of "courtship during copulation" in 131 species of insects and spiders, representing "attempts by males to influence cryptic female choice." If ATBs represent copulatory courtship, then our ATB durations must be reinterpreted. For example, fat females may have been tapped longer than thin ones because fat (high quality) females required more copulatory courtship, whereas small males tapped longer than large males did to compensate for their small body size (low quality). Interpretation of ATBs as copulatory courtship suggests that ATB duration was female-controlled, and in all male–female pairings where copulation did not occur, the female rejected the male. However, this interpretation is not supported, because (*i*) females discriminate against males smaller than themselves (see above) and could probably dislodge a small male more easily than a large male, but there is no evidence that rejection occurred more rapidly in pairings where the male was smaller than the female; (*ii*) it is unlikely that females can dislodge males much larger than themselves (category A in Table 3) in <5 s without any visible rejection behaviours or struggle. Because ATB duration was probably male-determined, the most likely function of ATBs in *P. litigata* is assessment of female egg load rather than copulatory courtship.

If large variation in female quality exists and males are able to assess it, they will benefit by responding differently to different females (Parker 1983; Otronen 1990). Hence, mate choice is advantageous for male *P. litigata* because females are vary greatly in quality as mates: only 18% of single females on antlers, and none of the 9 newly emerged females, had mature eggs. Because last-male sperm precedence is probably high (see Bonduriansky 1996), a nongravid female, which will be re-inseminated before depositing her next clutch, constitutes a low-quality mate (Parker 1984). However, a male is likely to reject some females only when costs of mating are high enough to limit

his potential lifetime mating success to relatively few copulations (Gwynne 1991). For male *P. litigata*, mating costs include energy and nutrients invested in the ejaculate, energy and risk of injury associated with copulation and mate guarding (Bonduriansky and Brooks 1998), and loss of opportunities (time) to search for better females (Parker 1978). Because an average male has a total of 38 h available for mating (6.44 d adult life-span × 5.9 h/d on antlers) and a copulation–oviposition cycle takes ~2.3 h (Bonduriansky 1996), time costs alone limit his potential fecundity to <16 clutches. Energy costs probably impose a substantially lower limit. Additional selective pressure for male choosiness may result from a "sexual conflict" (Parker 1979). Because ejaculates are a source of food and water for females, they may actually "forage" for ejaculates by seeking to copulate even when they are not gravid. This possibility is supported by the results of an experimental manipulation: when all males were removed from an antler, females appeared to search for and solicit mates (Bonduriansky 1996). These factors represent selective pressures for male mate choice.

In contrast, the costs of finding or selecting a mate represent selective pressures for indiscriminate mating (Parker 1983). For male *P. litigata*, search costs may be moderate because the female arrival rate is fairly high (1 every 2 min on a large moose antler) and the overall sex ratio on antlers is only 3 males to 1female (Bonduriansky 1996). The most important cost of mate choice may be risk of error (Parker 1983): in this case, rejection of a gravid female. This cost can be reduced by improving egg load assessment mechanisms or, more likely, adopting a "safe-bet" strategy (i.e., if in doubt, mate). A safe-bet strategy would be advantageous because rejection of a gravid female ("false negative") is far more costly than acceptance of a nongravid female ("false positive"). Because an average male has 38 h available for mating (see above) and achieves 2.37 copulations (Bonduriansky 1996), a false negative costs >40% of lifetime mating opportunities but a false positive (assuming full mating) costs only 6% of available time. The use of a safe-bet strategy, which reduces the frequency of false negatives by increasing the frequency of false positives, is supported by the fairly high proportion of copulations with nongravid females (27%), and may explain how male mate choice is maintained in this system.

Large male *P. litigata* tended to mate with large females, as is expected when high-quality individuals are choosier than low-quality ones (Parker 1983), and is observed in many insect species (e.g., Partridge 1983; Crespi 1989; Toft 1989; Wiernasz 1995; Arnqvist et al. 1996). As a result, large males may benefit through production of large (high quality) offspring. Moreover, male body size was positively correlated with female egg load among coupled pairs, probably as a result of male preference for fat females, relatively indiscriminate mating by small males, and a positive correlation between female fatness and egg load. Because male fecundity is a product of mating success (number of copulations) and mean mate quality (Arnold and Wade 1984*a*, 1984*b*), of which female egg load (and possibly body size) is an important component, this pattern of mating assortment represents a great advantage for large males relative to small ones.

Male mate choice occurs in many Diptera. Like male *P. litigata*, male *D. anilis* "probe" a female's abdomen to assess her egg load (Otronen 1984). The widespread occurrence of male mate choice in this order (e.g., the drosophilid *Drosophila melanogaster*, Cook 1975; the scatophagid *Scatophaga stercoraria*, Parker 1970*b*; the bibionid *Plecia nearctica*, Hieber and Cohen 1983; the psychodid *Lutzomyia longipalpis*, Jarvis and Rutledge 1992; and the empidid *Empis borealis*, Svensson and Petersson 1992) suggests that males of many dipteran species experience high mating costs relative to search costs and a high probability of encountering nongravid females.

Conclusions

Protopiophila litigata may be an unusual insect in that both males and females reject some potential mates. Moreover, males appear to be choosier than females (although the existence and importance of cryptic female choice in this species await investigation). Large individuals of both sexes benefit through mate choice and resulting patterns of mating assortment. Male mate choice and the limited ability of females to prevent coupling may be common features of dipteran mating systems where coupling begins in dense aggregations of aggressive males, copulation involves a large investment of time or nutrients, and males often encounter females lacking mature eggs. Our findings support Parker (1983) and Gwynne (1991), who argued that both males and females may be choosy, even when males compete for access to females, when mating is costly, variance in quality is large, and search costs are moderate for both sexes.

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