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Ejaculate feeding and female fitness in the sexually dimorphic fly *Prochyliza xanthostoma* (Diptera: Piophilidae)

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Recent theory and empirical evidence suggest a role for sexual conflict in the evolution of male ejaculates, including ‘nuptial gifts’. According to the sexual conflict hypothesis, the greater the probability of female remating, the stronger the selection on males to induce an elevated reproductive rate in their mates, even if this reduces some components of female fitness, such as survivorship. Piophilid flies show an unusual form of nuptial gift: following copulation, females expel and ingest much of the sperm and accessory fluids received from the male. We investigated the effects of ejaculate ingestion on female fitness in the piophilid carrion fly *Prochyliza xanthostoma* by manipulating females’ opportunity to ingest the ejaculate under two background food levels (fed ad libitum versus starved). We also estimated females’ probability of remating. Few *P. xanthostoma* females mated more than once in repeated pairings with males. Females permitted to ingest the ejaculate oviposited sooner and laid more eggs than females prevented from ingesting the ejaculate, but ejaculate ingestion did not affect female survival. Hence, ejaculate feeding appeared to increase females’ net fitness. However, these effects were evident only in fed females, suggesting that ejaculate meals provide mainly stimulation rather than nutrition. The benign effects of ejaculate feeding on female fitness, and the lack of evidence of sexual conflict, are consistent with the low female remating rate in this species. None the less, our results suggest that sexual conflict has played a role in the evolution of these traits.

Sexual conflict theory proposes that the fundamental divergence in the reproductive strategies of males and females results in sex differences in phenotypic optima for a wide variety of traits (Parker 1979; Rice 1984). Thus, whereas male–female interactions were previously regarded as evolutionary manifestations of cooperation for mutual benefit (see Darwin 1874), they are increasingly regarded as expressions of sexual antagonism and conflict of interest (Chapman et al. 2003). In particular, some species show male traits that appear to harm females, either mechanically (Crudgington & Siva-Jothy 2000; Stutt & Siva-Jothy 2001) or chemically (Chapman et al. 1995). Although it remains unclear what factors drive the coevolution of such male traits and female responses to these traits, the rate of female polyandry and consequent intensity of male sperm competition are generally regarded as key parameters in this process (see Arnqvist & Nilsson 2000; Johnstone & Keller 2000; Hosken et al. 2003). At the centre of this debate is the evolution of male ejaculates, including those thought to function as ‘nuptial gifts’.

Although ‘nuptial gifts’ have long been regarded as female-benefiting products of male–female coevolution, recent reappraisals in light of sexual conflict theory suggest that nuptial gifts may increase males’ fitness at the expense of their mates (Arnqvist & Nilsson 2000). This hypothesis suggests that males may benefit by inducing an elevated reproductive output in their mates following copulation, even if this reduces females’ net fitness through negative effects on survival or late-life reproduction (see Wolfner 1997; Arnqvist & Nilsson 2000; Hosken et al. 2003). Selection on males to elevate the reproductive rate of their mates is expected to increase with females’ mean probability of remating (Fig. 1). The sexual conflict hypothesis is consistent with evidence that male accessory gland substances may elevate female oviposition rate but reduce female survivorship in *Drosophila melanogaster* (Chapman et al. 1995; Chapman 2001). However, the role of sexual conflict in the evolution of nuptial gifts has not been explored adequately in theoretical or empirical work. In particular, there have been few attempts to relate females’ probability of remating to the effects of ejaculate components transferred by males. Thus, it is not clear

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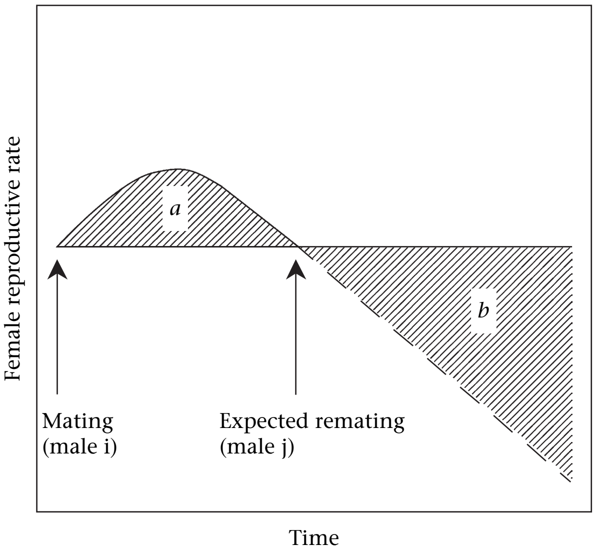

Figure 1. Sexual conflict over reproductive allocation: the solid horizontal line represents the reproductive rate resulting from the optimal schedule of reproductive allocation for a hypothetical female. According to the sexual conflict hypothesis, male i will benefit by inducing an elevated reproductive rate in the female (indicated by the solid curve) because this will maximize his fertilization gain before the female remates with a different partner (male j), who will gain some or all of the subsequent fertilizations. Because the correlation between the fitness of male i and the female's rate of fitness gain declines over time as the female's probability of remating increases, male i will benefit from this strategy even if it indirectly causes a subsequent decline in the female's reproductive rate (indicated by the dashed line) and reduces her lifetime fitness. Male i is considered to 'harm' his mate if his manipulation ultimately causes her to suffer a fitness loss (hatched area *b*) that exceeds her initial fitness gain (hatched area *a*). Conversely, if the female's probability of remating is zero, male i will never benefit by harming her because his fitness depends on her lifetime fitness. Thus, in this case, male i would maximize his own fitness by maximizing the lifetime fitness of his mates.

what rate of female remating is sufficient to yield evidence of sexual conflict in the effects of ejaculates on female fitness.

Nuptial gifts of accessory gland substances can be transmitted to females via the reproductive tract, or via the gastrointestinal tract. The former appears to be widespread in Lepidoptera (e.g. Wiklund et al. 2001), Megaloptera (Hayashi 1998) and Diptera (e.g. Markow & Ankney 1984; Bownes & Partridge 1987; Markow et al. 1990, 2001). The latter ('ejaculate feeding') is widespread in crickets and katydids (Orthoptera), where sperm is transferred in specialized edible capsules called spermatophylaxes (Vahed 1998). Ejaculate feeding also occurs in some molluscs, where females ingest a portion of the sperm received from the male (Srilakshmi 1991). In other animals, ejaculate expulsion by females is common (e.g. Eberhard 1994; Polak et al. 1998; Cordoba-Aguilar 1999; Pizzari & Birkhead 2000; Reinhardt & Meister 2000), but the ingestion of expelled ejaculates appears to be extremely rare (but see Hamm 1933; Preston-Mafham 1999).

None the less, an unusual form of ejaculate feeding occurs in several species of piophilid flies (Bonduriansky & Brooks 1998; Bonduriansky 2003). Several minutes after the end of copulation, once their two small spermathecae have been filled with sperm, piophilid females usually expel the remaining contents of their bursa copulatrix from the ovipositor in the form of one or more droplets, and immediately ingest them (Fig. 2). In the sexually dimorphic 'waltzing fly' *Prochyliza xanthostoma*, these ejaculate meals contain accessory gland fluids and large, dense bundles of sperm (R. Bonduriansky, unpublished data). The capacity of the muscular bursa copulatrix greatly exceeds that of the two sclerotized spermathecae (Fig. 3), and dissections of females before and after ejaculate expulsion suggest that a large proportion of the ejaculate fluid and sperm are ingested (R. Bonduriansky, unpublished data). Although ejaculate feeding in *P. xanthostoma* has only been observed in the laboratory, this behaviour has been observed in the wild in several related species (e.g. Bonduriansky & Brooks 1998; Bonduriansky 2003). The effects of ejaculate feeding on fitness of piophilid females have never been investigated before.

We asked how ejaculate feeding affects three components of female fitness in *P. xanthostoma*: time until first oviposition ('oviposition delay'), probability of oviposition following mating and number of eggs laid ('fecundity') and survival. We also estimated female remating rate. If female remating rate is high, the sexual conflict hypothesis suggests that ejaculate feeding may reduce some components of female fitness, such as survivorship, while increasing other components, such as fecundity. Males may also benefit by inducing their mates to oviposit sooner (thus reducing the probability of female remating prior to oviposition). Indeed, if reproductive traits are far from evolutionary equilibrium as a result of rapid, conflict-driven coevolutionary cycles (see Iwasa & Pomiankowski 1995; Gavrilets et al. 2001; Houle & Kondrashov 2001), it is possible that females would ingest ejaculates even if this reduced their lifetime fitness. A greater or more rapid egg output may reduce females' net fitness if it is associated with reduced survivorship. Conversely, if female remating rate is low, then ejaculate feeding is expected to have

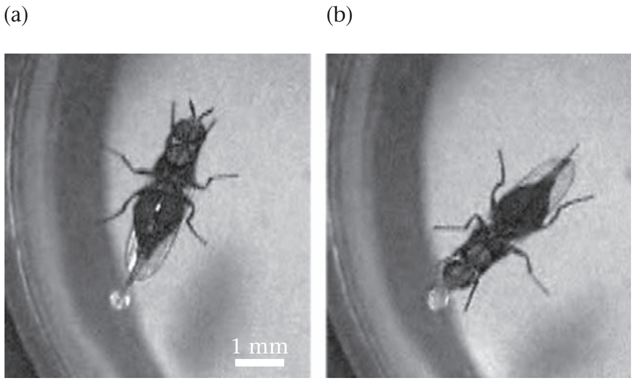

Figure 2. Ventral view of a *Prochyliza xanthostoma* female under a dissecting microscope, expelling (a) and ingesting (b) ejaculate fluids and sperm following copulation. At 20 °C, ejaculate expulsion typically occurs 5–10 min after the end of copulation, and ejaculate ingestion takes 20–40 s.

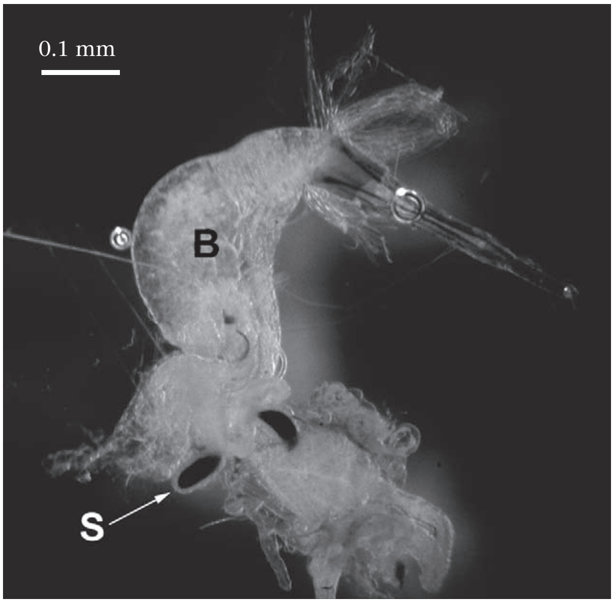

Figure 3. Dissected reproductive system of a *Prochyliza xanthostoma* female following copulation but before ejaculate expulsion, showing the bursa copulatrix (B) filled with ejaculate fluid and sperm, and the two small, darkly sclerotized spermathecae (S). The ovipositor can be seen extending to the right of the distal tip of the bursa copulatrix.

benign or beneficial effects on all components of female fitness. To determine whether ejaculate meals provide mainly nutrition or stimulation, we also manipulated background food availability. Any nutritional benefits should be more evident in food-deprived females.

METHODS

Sources and Rearing of Flies

Gravid *P. xanthostoma* females were collected on carcasses of moose (*Alces alces*) at the Wildlife Research Station in Algonquin Park, Ontario. They were transferred to population cages with mesh windows, containing sucrose, sources of distilled water, and petri dishes filled with 'organic' (i.e. nonhormone treated) ground beef for oviposition. Cages were illuminated with both incandescent and broad-spectrum fluorescent lights, and maintained under a 16:8 h light:dark cycle at 18 °C. Progeny were transferred as pupae to larger population cages, and their F1 offspring were transferred as pupae to individual vials. F1 adults were maintained in same-sex groups of ~10 in population cages containing beef, sugar and water prior to use in the experiment.

Female Remating Rate

To estimate female remating rate, we paired 55 virgin females (5–6 days old) with 55 virgin males (5–7 days old) in scintillation vials. All pairs mated within 30 min. We maintained these females with ad libitum access to sugar, water and ground beef, and re-paired them one or more times (each time with a different virgin male) at different ages to determine whether they would remate, and whether the length of time elapsed since mating affects the probability of remating: four females were re-paired at ages 7, 8, 9 and 16 days, 20 females were re-paired at ages 7, 14, 21 and 26 days, and 31 females were re-paired at the age of 20 days. We also investigated female remating rate in the wild by observing male–female interactions on moose carcass and the surrounding vegetation during the period of *P. xanthostoma* adult activity in Algonquin Park (late April to early June).

Ejaculate Feeding and Food Level Manipulation

At age 7–11 days, we randomly paired males and females in glass scintillation vials, and observed them until the end of copulation. A few pairs failed to mate within 30 min, and were discarded. After copulation, we removed the male and observed the female for about 30 min more, a sufficient amount of time to complete the expulsion of the ejaculate (Bonduriansky 2003). Of the 121 females that mated, five (4%) did not expel any ejaculate within 30 min, and were discarded. For the remaining females, we prevented some from ingesting the ejaculate droplet(s) that they deposited by tapping the scintillation vial, which caused females to become disoriented; then we transferred them to individual cages (see below). We permitted others to ingest the ejaculate, then tapped the vial as above and transferred them to individual cages. These treatments were applied in alternating order. One female subsequently escaped.

The 115 experimental females were maintained individually in glass cages (3 × 9 cm) containing a source of water, a punctured eppendorf tube containing 'organic' ground beef for oviposition (which females could reach with the ovipositor, but not with the proboscis), and either ad libitum sucrose and ground beef ('fed' treatment) or no food ('starved' treatment). Food treatments were also applied in alternating order. Females were not starved before copulation because food is required for ovule maturation and receptivity (R. Bonduriansky, unpublished data). All pairings were conducted over 5 days, transfers were performed without anaesthesia, and cages were illuminated as described above. During the first 3 weeks after pairings, we inspected each cage daily: we estimated the number of eggs laid to the nearest 10 and recorded female mortality. After 3 weeks (by which time very little oviposition was taking place), we inspected cages twice per week. Following death, we estimated female body size by measuring thorax length using a dissecting microscope with an ocular micrometer.

Analysis

Because female thorax length and survival data were normally distributed, survival data were analysed by analysis of covariance (ANCOVA) with ejaculate ingestion (permitted or denied) and food level (fed or starved) as factors and female thorax length as a covariate. Survival data were also analysed using survival analysis.

Oviposition delay and fecundity data were non-normally distributed, and were analysed using nonparametric tests. Female age, which may affect fitness measures, did not differ between treatment groups. Although copulation duration is somewhat variable in *P. xanthostoma* (Bonduriansky 2003), ejaculate volume appears to be unrelated to copulation duration in this species (R. Bonduriansky, unpublished data). All analyses were performed using Statistica (StatSoft 1998).

RESULTS

Female Remating Rate

Of 55 females paired repeatedly in the laboratory, only two (4%) mated more than once. One female remated 1 day after her first mating (when 7 days old), and one female remated 15 days after her first mating (when 20 days old). Of 460 courtships observed in the field, none resulted in copulation. These field data are consistent with a low female remating rate: if most females in the population are unreceptive, whereas males are eager to mate, the vast majority of courtships will fail to result in copulation. None the less, despite the brief copulation duration in this species (~5 min at 20°C; Bonduriansky 2003), we observed two copulating pairs near oviposition substrates, confirming that our field observations were conducted in appropriate locations to observe mating.

Oviposition Delay

Among all females, oviposition delay was significantly shorter in females that ingested the ejaculate (median delay = 0 days, $N = 57$) than in females that did not (median delay = 1 days, $N = 52$) (Gehan's Wilcoxon test: $Z = 2.205$, $P = 0.0275$; Fig. 4). We did not test for this effect within food levels because sample sizes were too small. There was no evidence of an effect of food level on oviposition delay (Gehan's Wilcoxon test: $Z = 0.32$, $N = 22$ fed, $N = 6$ starved, $P = 0.75$), although the power of this test was low because so few of the starved females oviposited.

Fecundity

Fed females laid more eggs than starved females (fed: upper quartile = 13, $N = 54$; starved: upper quartile = 0, $N = 55$; Mann–Whitney U test: $Z = 3.210$, $P = 0.0013$), and were nearly five times more likely to oviposit than starved females ($Z = 3.672$, $P = 0.0002$; Fig. 5). Females that were permitted to ingest ejaculate laid more eggs than females that were prevented from doing so, but this result was driven by the response of fed females only, since ejaculate ingestion had no effect on starved females (Table 1; Fig. 5). Females that were permitted to ingest ejaculate were 1.7 times as likely to oviposit as females that were prevented from doing so ($Z = -1.379$, $P = 0.17$).

Survival

ANCOVA indicated that fed females lived longer than starved females, but ejaculate ingestion had no effect on female survival, and there was no evidence of an interaction between factors (Table 2; Fig. 6). Qualitatively identical results were obtained with female fecundity

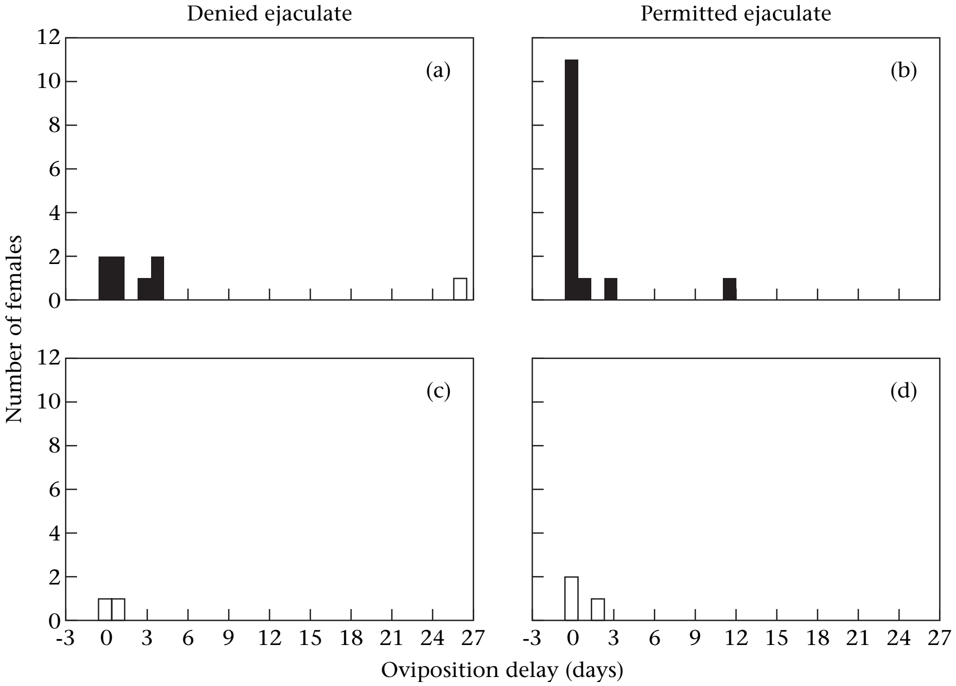

Figure 4. Frequency distributions of female oviposition delay (number of days from mating until first eggs laid) for females assigned to fed (■) and starved (□) background food treatments that were either denied ejaculate (a, c) or permitted ejaculate (b, d).

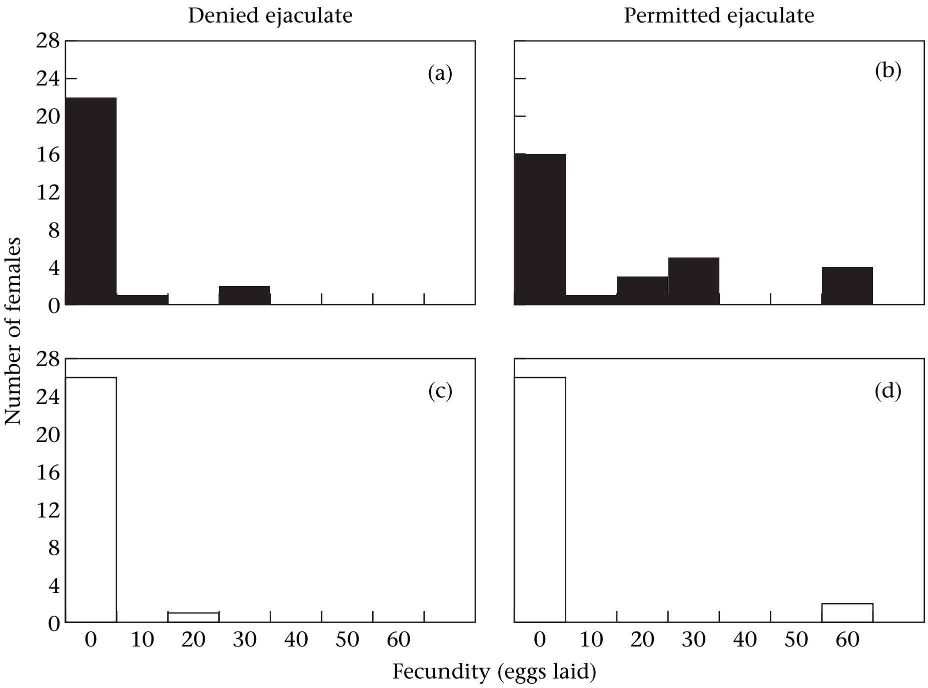

Figure 5. Frequency distributions of female fecundity (total number of eggs laid) within each treatment combination. See Fig. 4 for treatment designations.

included as a second covariate, indicating that there was no detectable trade-off between oviposition rate and survival. Survival analysis yielded similar results (Fig. 7): fed females survived longer than starved females (Gehan's Wilcoxon test: $Z = 9.29$, $N = 54$ fed, $N = 55$ starved, $P < 0.0001$), but there was no effect of ejaculate ingestion on female survival (Gehan's Wilcoxon test: $Z = -0.80$, $N = 57$ permitted ejaculate, $N = 52$ denied ejaculate, $P = 0.42$).

DISCUSSION

Female *P. xanthostoma* that were allowed to ingest ejaculate oviposited sooner and laid more eggs than females that were not allowed to ingest the ejaculate, suggesting that ejaculate feeding provides direct benefits for females. Moreover, because ejaculate feeding did not affect female survival, there was no evidence that these benefits were offset by viability costs. These benefits were detectable in fed females, but not in starved ones. Both field observations and laboratory pairings suggested that few females mate more than once in their lives.

Ejaculates and Female Fitness

Because females that were permitted to ingest ejaculate oviposited significantly more and sooner, but did not die sooner, than females that were denied the ejaculate meal, we conclude that ejaculate ingestion increases females' net fitness. Increased egg output clearly represents a fitness advantage for females, since it is likely to translate into more offspring. Reduced oviposition delay is also likely to be advantageous: given the very high daily mortality risk faced by wild flies (see Bonduriansky & Brassil 2002), ovipositing 1 day sooner may yield an average fitness gain of 10% or more for females. These advantages did not appear to be balanced by reductions in survivorship in our captive population. Note, however, that the effects of ejaculate ingestion on female viability may be altered by ambient conditions, and it is possible that viability costs would become apparent if our experiment were repeated under more stressful conditions, such as those probably experienced by wild flies. The effects of ejaculate ingestion on females also represent a fitness gain for their mates. Males are likely to benefit from females' increased egg output, as well as the lower probability of female remating

Table 1. Mann–Whitney *U* tests for effects of ejaculate ingestion on fecundity of *Prochyliza xanthostoma* females

	Permitted		Denied		Tests		
	Upper quartile	*N*	Upper quartile	*N*	*U*	*Z*	*P*
All females	7	57	0	52	1194.5	−2.639	0.0083
Fed females	30	29	0	25	238.5	−2.668	0.0076
Starved females	0	28	0	27	364.0	−0.599	>0.5

Table 2. Analysis of covariance for the effects of ejaculate ingestion and food level on survival of *Prochyliza xanthostoma* females

Factor*	df effect	MS effect	df error	MS error	*F*	*P*
Ejaculate	1	17.950	110	44.313	0.405	0.5258
Food level	1	31 868.84	110	44.313	719.18	<0.0001
Interaction	1	0.320	110	44.313	0.007	0.9320

*Female thorax length was included as a covariate. Significant factors are highlighted in bold.

resulting from reduced oviposition delay. None the less, the effects of ejaculate ingestion do not provide clear evidence of male coercion of females in this species.

Although many females failed to oviposit in all treatment combinations, this proportion was much smaller among females that were fed and permitted to ingest ejaculate (Fig. 5). Thus, there is no evidence that our conclusions are confounded by the failure of some females to oviposit. The fecundity distribution observed in this experiment is typical for this species in the laboratory (R. Bonduriansky, unpublished data). Although the fecundity distribution for wild females is not known, it is likely that many wild females also fail to oviposit, or produce very few eggs, as a result of food limitation or other factors. Thus, our results appear to be representative of the biology of this species, and probably reveal the directions of the fitness effects of ejaculate ingestion. None the less, reliable estimates of the magnitudes of these effects can only be obtained in the field (see Bonduriansky & Rowe 2003).

The effects of ejaculates on female fitness represent an important puzzle in the study of male–female coevolution. Sexual conflict theory suggests that males may benefit by inducing an elevated reproductive rate in their mates when some females mate multiply, even if this reduces females' net fitness (see Fig. 1). This theory is supported by evidence of negative effects of ejaculate fluids on female fitness components (Chapman et al. 1995; Wolfner 1997; Andrés & Arnqvist 2001; Chapman 2001; but see Wagner et al. 2001). On the other hand, females usually appear to benefit from specialized 'nuptial gifts', such as the spermatophylaxes of many orthopterans (Gwynne 1984; Vahed 1998). Moreover, even in species lacking specialized nuptial gifts, females often benefit from multiple mating (e.g. Ridley 1988; Arnqvist & Nilsson 2000; Wagner et al. 2001; Worden & Parker 2001), although it is generally unclear whether this is an effect of ejaculate fluids or other factors, such as increased sperm supply, genetic diversity, or some unmeasured correlate of multiple mating. Thus, the role of sexual conflict in the evolution of ejaculates and nuptial gifts remains poorly understood.

The apparently benign ejaculates of *P. xanthostoma* males may have evolved as a result of low female remating rate. Both field and laboratory results suggest that a large majority of *P. xanthostoma* females mate only once in their lives. This conclusion is also supported by comparison with other piophilid species (e.g. *Protopiophila litigata*), in which females readily remate in the laboratory and matings are often observed in the field (Bonduriansky & Brooks 1998). Male adaptations to sperm competition

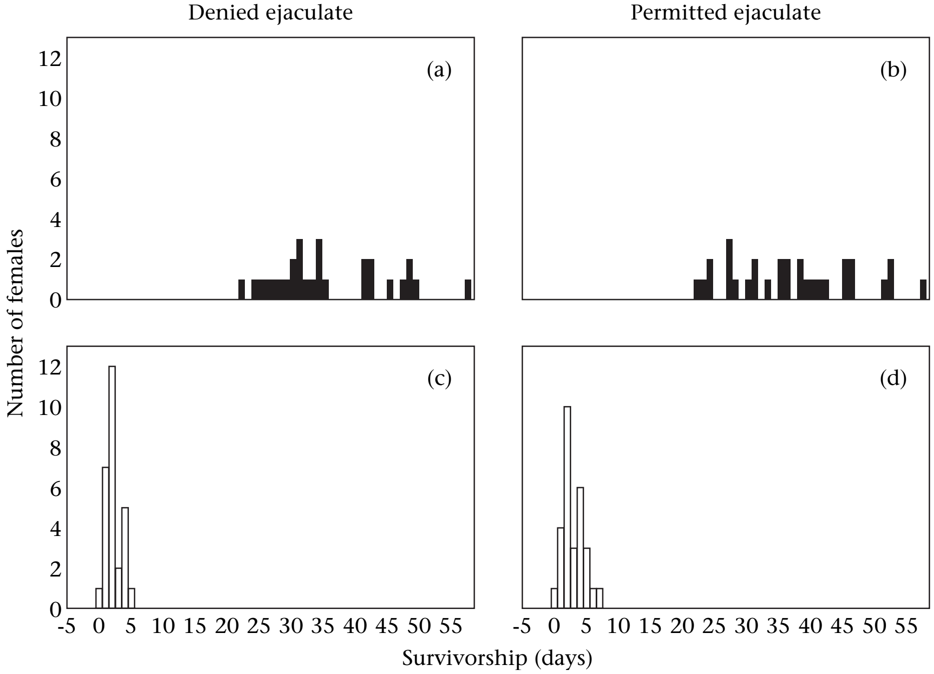

Figure 6. Frequency distributions of female survival (days from adult emergence until death) within each treatment combination. See Fig. 4 for treatment designations.

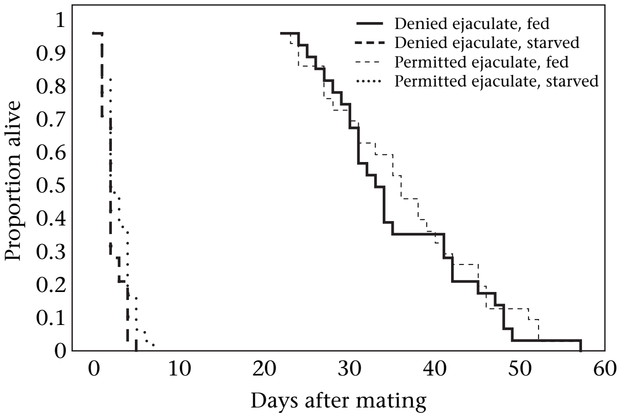

Figure 7. Female survival curves for each treatment combination, showing the proportion of females alive on each day following copulation.

may select indirectly for ejaculate toxicity (Rice 1996; Wolfner 1997; Holland & Rice 1999; Johnstone & Keller 2000; Chapman 2001), or for ejaculate volumes that greatly exceed females' requirements for fertilization (Parker 1970; Eady 1994; Martin & Hosken 2002; but see Simmons & Kvarnemo 1997), and females may retaliate by expelling portions of ejaculates. Conversely, since few *P. xanthostoma* females mate multiply, sperm competition is likely to have much less importance. In such a system, a male's fitness will depend on the lifetime fitness of his mates, and this will favour benign or beneficial ejaculates.

However, sexual conflict may play a more cryptic role. First, females' reluctance to remate may itself be a result of male coercion mediated by ejaculate components (Arnqvist & Nilsson 2000; Andrés & Arnqvist 2001). Indeed, if ejaculates are simply benign gifts conferring cumulative benefits, it is not clear why females should tend to avoid second matings instead of foraging for additional ejaculates (see Bonduriansky 2001). It is possible that substances present in the ejaculate reduce female receptivity and, hence, female probability of remating. This would represent a form of male manipulation of female reproductive behaviour that benefits the male by reducing sperm competition, but potentially harms the female by denying her opportunities to obtain additional ejaculate nutrients and sperm. Second, ejaculate feeding also occurs in piophilid species with high female remating rates, such as the antler fly (Bonduriansky & Brooks 1998). This suggests the possibility of complex coevolutionary chases involving male ejaculate traits and female mating rate and ejaculate feeding behaviour, with different piophilid species showing different combinations of these traits. Thus, while our results provide no direct evidence of sexual conflict in the effects of ejaculate feeding on *P. xanthostoma* females, sexual conflict is likely to have played a role in the coevolution of male ejaculates and female behaviours in the piophilid flies. Given that ejaculate feeding occurs in combination with large interspecific variation in female remating rate in this group, the piophilid flies are a valuable model system for research on the role of sexual conflict in the evolution of ejaculates and nuptial gifts.

Stimulation or Nutrition?

The benefits of ejaculate feeding could only be detected in females provided with food (see Fig. 5). The strong effect of ejaculate ingestion on the fecundity of fed females suggests that ejaculate meals are more important as sources of oviposition stimulants than as sources of nutrients. This result is not an artefact of short life spans in starved females because most females that oviposited did so within 24 h after copulation (Fig. 4), whereas most starved females survived for several days (Fig. 6). However, since food availability appeared to affect fecundity, we may have failed to detect the nutritional contribution of ejaculate meals if this was insufficient to provide starved females with the minimum food requirements for oviposition. Although the starvation treatment appears to be very severe, many adult females may experience food limitation (e.g. lack of sugars) in the wild, so the starvation treatment may represent more 'natural' conditions than the ad libitum food treatment.

The Evolution of Ejaculate Feeding

Piophilid ejaculate feeding behaviour is unusual: it is the only known example of such behaviour within the Diptera (but see Hamm 1933; Preston-Mafham 1999), and one of the only known examples of females ingesting apparently unspecialized ejaculate fluids and sperm. Ejaculate feeding is widespread in the subtribe Piophilina, having been observed in species of *Liopiophila*, *Prochyliza*, *Parapiophila*, *Protopiophila* and *Stearibia* (Bonduriansky & Brooks 1998; Bonduriansky 2003). Interestingly, the predominant visible, solid component of ejaculate meals is sperm in *Prochyliza xanthostoma* and *Protopiophila litigata*, but a gelatinous material in *Stearibia nigriceps* (Bonduriansky & Brooks 1998; Bonduriansky 2003). Ejaculate feeding also occurs in the empidid fly *Empis trigramma* (Hamm 1933; Preston-Mafham 1999), in orthopterans (Vahed 1998), and in some gastropod molluscs (Srilakshmi 1991). Many other insects show other types of nuptial gifts, or other modes of gift transfer. For example, females absorb ejaculate components through the reproductive tract in some species of *Drosophila* (Markow & Ankney 1984; Bownes & Partridge 1987; Markow et al. 1990, 2001), Lepidoptera (Wiklund et al. 2001) and Megaloptera (Hayashi 1998). Some *Drosophila* females receive fluid droplets from the male's proboscis during courtship (Steele 1986). Females may also feed on males' haemolymph, specialized external appendages, or prey gifts (see Vahed 1998 for review).

Given that ejaculates represent costly energetic and material expenditures for males (Dewsbury 1982; Bonduriansky 2001; Preston et al. 2001), opportunities for females to exploit these male 'donations' for direct benefits exist in many species. Indeed, males take advantage of opportunities to ingest rivals' ejaculates in some species (Ono et al. 1989; Bonduriansky & Brooks 1998), presumably to obtain nutrients and fluids. Thus, the apparent rarity of ejaculate ingestion by females calls for an explanation. Because ejaculate feeding is likely to select on ejaculate volume and composition, the ingestion of apparently unspecialized ejaculate fluids and sperm by piophilid females may represent an early stage in the evolution of specialized nuptial gifts.

If the fitness effects of ejaculate meals are dose dependent, females may benefit by discriminating among males on the basis of phenotypic correlates of ejaculate size, potentially leading to the evolution of complex signal–receiver systems. An association between ejaculate traits and phenotypic 'signals' may result from condition dependence of both types of traits (Rowe & Houle 1996; Kotiaho 2002; Simmons & Kotiaho 2002). This may be the case in *P. xanthostoma*, where the quantity of sperm males transfer is signalled by a condition-dependent aspect of their head shape, and females prefer the long-headed male phenotype associated with large sperm volume (Bonduriansky & Rowe 2003). The condition dependence of ejaculate traits and the effects of ejaculate variation on female fitness (including both offspring number and quality) have yet to be assessed directly in this species. It is also not known how ejaculate composition varies between *P. xanthostoma* males, or whether it is correlated with male morphological or courtship traits.

Acknowledgments

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