

Layered sexual selection: a comparative analysis of sexual behaviour within an assemblage of piophilid flies

Russell Bonduriansky

Abstract: Although many theoretical and empirical studies have addressed the dynamics of sexual selection, little is known about the evolution of multiple sexual-selection mechanisms within the same system. I performed a qualitative comparative study of sexual behaviours in seven sympatric species of piophilid flies to identify and compare the apparent mechanisms of sexual selection operating in each system. In each of the seven species I observed several distinct types of male–male and male–female interactions, potentially representing multiple mechanisms of sexual selection. Male–male interactions included scramble competition and, in some species, bouts of intense combat. Male–female interactions exhibited two distinct patterns: (1) some form of premounting courtship followed by a simple copulatory sequence or (2) no premounting courtship but palpation behaviours during the copulatory sequence. Either pattern was combined with male–female struggle in some species. In two species, male mating success also appeared to depend on the ability to overcome a “revealing obstacle”, a female adaptation that exposed variation in male performance without direct assessment or struggle. In each species these mechanisms operated in a rough sequence and thus could be viewed as “layers” of sexual selection, with each layer potentially reducing the subset of individuals that have opportunities to compete in the next layer. A brief review of the literature suggests that layered sexual selection is the typical pattern in many animal groups and thus may have important evolutionary consequences.

Résumé : Bien que de nombreuses études théoriques et empiriques se soient intéressées à la dynamique de la sélection sexuelle, on connaît encore mal l'évolution des mécanismes multiples de la sélection sexuelle à l'intérieur d'un même système. Une étude qualitative et comparée des comportements sexuels de sept espèces sympatriques de piophilidés (Diptères) a permis d'identifier et de comparer les mécanismes apparents de sélection sexuelle dans chacun des systèmes. Chez chacune des sept espèces, on peut observer plusieurs types distincts d'interactions mâle–mâle et mâle–femelle, qui représentent potentiellement des mécanismes multiples de sélection sexuelle. Les interactions mâle–mâle comprennent de la compétition d'exploitation et, chez certaines espèces, des épisodes de combat intense. Les interactions mâle–femelle prennent deux formes distinctes : (1) un type de cour précédant l'accouplement suivi par une séquence de copulation simple ou (2) l'absence de cour avant l'accouplement, mais des comportements de palpation durant la séquence de copulation. Les deux formes s'accompagnent d'une lutte mâle–femelle chez certaines espèces. Chez deux espèces, le succès de l'accouplement chez le mâle semble aussi dépendre de sa capacité à surmonter un « obstacle révélateur », une adaptation de la femelle qui met en relief la variation dans la performance des mâles sans évaluation directe, ni combat. Chez toutes les espèces, ces mécanismes agissent selon une séquence grossière et ainsi peuvent être considérés comme des « niveaux » de sélection sexuelle; chaque niveau réduit potentiellement le sous-ensemble d'individus qui ont l'occasion de se faire compétition au niveau suivant. Une courte revue de la littérature indique que la sélection sexuelle par étapes est caractéristique de nombreux groupes animaux et qu'elle peut ainsi avoir d'importantes conséquences évolutives.

[Traduit par la Rédaction]

Introduction

The theory of sexual selection was originally based on two selective mechanisms: combat among males and mate choice by females (Darwin 1874). Although a great volume of work has now been devoted to sexual selection (see Andersson 1994), these two mechanisms have usually been treated separately, and other mechanisms have received little attention.

Although multiple sexual-selection mechanisms appear to operate in many species (see Andersson 1994; Berglund et al. 1996), it is not clear why multiple mechanisms should evolve, how they may interact, or what their evolutionary consequences might be (although see Berglund et al. 1996). The first step in answering these questions is an investigation of the patterns of co-occurrence of multiple mechanisms in nature.

The evolution of multiple courtship elements, such as ornaments and displays, has been investigated in several theoretical and empirical studies (e.g., Møller and Pomiankowski 1993; Pomiankowski and Iwasa 1993; Iwasa and Pomiankowski 1994; Omland 1996; Prum 1997; Johnson 2000). However, these studies addressed the dynamics of only one type of sexual selection mechanism — female mate choice —

Received 4 April 2002. Accepted 29 January 2003. Published on the NRC Research Press Web site at <http://cjz.nrc.ca> on 8 April 2003.

R. Bonduriansky. Department of Zoology, University of Toronto, Toronto, ON M5S 3G5, Canada (e-mail: russell.bonduriansky@utoronto.ca).

asking why multiple male displays and multiple female preferences evolve. Likewise, in several studies two co-occurring mechanisms have been investigated: female mate choice and male–male combat (Moore 1990; Berglund et al. 1996; Moore and Moore 1999; Andersson et al. 2002; Sih et al. 2002; Bonduriansky and Rowe 2003). However, recent theory has extended the range of possible mechanisms of sexual selection. For example, “indirect” female mate choice occurs when female behaviour induces intrasexual competition among males (Wiley and Poston 1996), while “interaction-independent” sexual selection acts on metabolic efficiency or accuracy of timing exhibited by the competing sex (Murphy 1998). Additional mechanisms probably remain to be identified.

To investigate variation in the nature, co-occurrence, and sequence of sexual-selection mechanisms, I carried out a detailed qualitative study of sexual behaviour in seven related species of carrion flies (Diptera: Piophilidae, subtribe Piophilina) representing the complete piophilid assemblage in Algonquin Park, Ontario (see Bonduriansky and Brooks 1999a). The sexual behaviour of the antler fly, *Protopiophila litigata*, has been described previously (see Bonduriansky and Brooks 1998a, 1998b, 1999a, 1999b) and is summarized briefly in the Results for comparison with that of the other species. Very little is known of the sexual behaviour of any other piophilid species except the synanthropic “cheese skipper”, *Piophila casei* (Swammerdam 1758; Dufour 1844; Sacchi et al. 1978), and the “bone skipper”, *Centrophlebomyia furcata* (Freidberg 1981).

A sexual-selection mechanism is defined here as any factor that exposes variation among individuals in the performance of tasks associated with competition for mates. Thus, I identified as apparent sexual-selection mechanisms those interactions that appeared to expose variation among males in the performance of sexual behaviours. I also documented the apparent intensity of sexual competition, or the degree of variation in male performance, to examine the potential for each type of interaction to generate sexual selection. However, without knowledge of the variation in numbers of offspring sired that was due to variation in male performance, these results remain tentative. Based on the patterns observed, I discuss possible reasons for the evolution of multiple sexual-selection mechanisms and consider the potential evolutionary consequences of this phenomenon.

Methods

Sources and rearing of specimens

Larvae and adults of *Protopiophila latipes* (Meigen), *Liopiophila varipes* (Meigen), *Stearibia nigriceps* (Meigen), *Prochylyza xanthostoma* (Walker), and two undescribed species of *Parapiophila* McAlpine labeled sp. 1 and sp. 2 were obtained from carcasses of moose, deer, small mammals, and fish at the Wildlife Research Station, Algonquin Provincial Park, Ontario, Canada. Flies were identified using keys in McAlpine (1987) and Bonduriansky (1995). Based on an examination of collections of undescribed *Parapiophila* morphospecies (compiled by J.F. McAlpine) in the National Collection of Insects (Ottawa), *Parapiophila* sp. 1 appears to be close to *Parapiophila calceata* and *Parapiophila afrifrons*, and *Parapiophila* sp. 2 is close to *Parapiophila flavipes*

(from Sweden). All species were maintained in plastic population cages (15 × 23 × 35 cm) with mesh “windows” for ventilation, a 3 cm deep layer of soil, sugar cubes, sources of water, and petri dishes containing extra-lean ground beef for oviposition (provided on a layer of soil for the *Parapiophila* species). Final-instar larvae were transferred to individual pupation jars containing soil. Newly emerged females were transferred individually to 250-mL cages and males were transferred in groups of about 10 to 1.5-L cages. Adult flies were provided with sugar, ground beef, and water.

Male–male interactions

Male–male interactions were observed in the wild, on the ovipositional substrate and the surrounding vegetation, in each species except *Parapiophila* sp. 2 (see Results). In addition, I observed male scramble and combat behaviours in the laboratory in clear-plastic 1.5-L population cages containing ~10 males each. I report maximum estimates of the frequency and duration of male–male interactions in the wild (i.e., for areas of greatest mating activity at the height of the season). Because of extreme local and seasonal variation, maxima are much easier to estimate than means or medians, and permit better resolution of interspecific differences. Maxima may also represent the most intense intrasexual selection occurring in each species. For *P. xanthostoma* I also conducted 180 male–male pairings inside a 200-mL transparent plastic arena containing a dry spruce twig, sugar cube, and source of water, and illuminated from one side by incandescent and broad-spectrum lights. “Boxing” involves head-to-head butting and hitting or jabbing with the forelegs.

Male–female interactions

Male–female interactions were observed in the wild, on the ovipositional substrate and the surrounding vegetation, in each species except *Parapiophila* sp. 2 (see Results). To investigate these interactions in greater detail, I paired laboratory-reared males and females of each species (for sample sizes see Table 1) inside a clear-plastic arena (3 cm diameter, 1 cm depth) under a dissecting microscope. An opaque cylinder was placed over the arena to minimize visual disturbance for the flies. Pairings were terminated after 20–30 min if no copulation occurred or 20–30 min after genital separation. Between pairings, the inside of the arena was wiped with a lint-free tissue moistened with alcohol to remove any chemical residues.

In each species, six females were paired repeatedly (on days 2, 3, 4, and 5 after adult emergence), but subsequent pairings were conducted using new individuals for each pairing. Means and coefficients of variation (CV) for behaviours were calculated using each pair once, and are based on laboratory observations (unless noted otherwise). In *P. xanthostoma*, the precopulatory side-to-side (zigzag) courtship steps performed by the male were counted using a manual counter. Male–female interactions in this species were also recorded and examined using digital video. To examine the droplets of ejaculate deposited by females on the lid of the pairing arena, I removed the female before she could ingest the droplet and transferred the lid to the stage of a compound microscope.

Results

Relevant morphological, copulatory, and ecological characteristics of each species are summarized in Table 1. Male–female interactions are subdivided into three sections: *initiation* describes the male’s approach or courtship towards the female; *nonmating outcome* describes interactions that do not result in copulation; and *mating outcome* describes interactions that lead to copulation.

Protophihila litigata

Male–male interactions

Males defended territories or searched for females on discarded cervid antlers, usually attacking any male within ~15 cm. Typically, males spread one wing, circled about each other, then charged and boxed. When a female alighted on the antler, every male within ~15 cm charged and attempted to leap on her. Following copulation, the male remained on the female and guarded her by pushing away single males with his wings and body.

Male–female interactions

Initiation: The male charged the female and leaped on her from any direction. The male then tapped the sides of the female’s abdomen, apparently to assess her egg load. *Nonmating outcome:* If the female’s abdomen was thin (i.e., she had no mature eggs), the male usually dismounted after a few seconds of abdominal tapping. The female occasionally resisted by shaking the male and (or) lowering her abdomen tip. *Mating outcome:* If the male accepted the female, he rubbed her abdomen tip with his hind legs and gonopods until she extended her ovipositor slightly. To establish genital lock, the male pushed several times against the female’s abdomen with his hind legs, then rubbed the female’s genitalia briefly with his hind tarsae (Fig. 1A). The mean combined duration of the precopulatory phases was 836 s, with considerable variation among pairs. During copulation, the male pushed against the female’s abdomen with his abdomen at intervals of ~34 s. Finally, the male rubbed the female’s genitalia again and withdrew his aedeagus. Several minutes later, the female extended her ovipositor and deposited and ingested one or two droplets of fluid containing sperm. The female then oviposited into the antler while the male guarded her (summarized from Bonduriansky and Brooks 1998a, 1998b, 1999a, 1999b).

Protophihila latipes

Male–male interactions

Males searching for females or defending territories on a carcass charged any fly within a radius of ~15 cm, leaping on females and attacking males. In combat, males oriented to each other, often spread one wing and swayed from side to side or waved both wings asynchronously, then charged and boxed. The winner then chased the loser for up to 20 cm. Single males also attempted to dislodge males guarding ovipositing females, while the latter used their bodies and wings to push the single males away.

Male–female interactions

Initiation: The male charged the female and leaped on her from any direction. *Nonmating outcome:* The female nearly always arched her body and shook the male violently from side to side, while the male lowered his wings as a brace against the substrate (Fig. 1B). The male was dislodged after an average of 3.6 s (CV = 2.10, $N = 38$), although wrestling matches lasting up to 2 min were sometimes observed in the wild. The female sometimes also extended her ovipositor and lowered or raised it out of the male’s reach. Nonmating outcomes appeared to result from female resistance rather than male mate choice. *Mating outcome:* Approximately 77% of receptive females shook the male prior to copulation, with a mean struggle duration of 4.0 s (CV = 1.29, $N = 17$). Once the female ceased shaking the male, he spread her wings, rubbed his mid and hind legs over the sides of her abdomen, and tried to grasp her abdomen tip with his gonopods and parameres. After an average of 98 s (CV = 0.46, $N = 16$), the female slightly extended her abdomen tip and the male grasped it with his genitalia. The male then rubbed the sides of the female’s abdomen with his hind legs in rapid bursts (~1–2 bursts/s), which gradually decreased in duration and amplitude and ceased after an average of 8.7 min (CV = 0.41, $N = 16$). Finally, the male stepped over the female’s body a few times, rubbed her genitalia with his hind legs for several seconds, and withdrew his aedeagus. The male then either dismounted immediately or was shaken off by the female or he stayed mounted and guarded the female while she oviposited.

Stearibia nigriceps

Male–male interactions

Males sat motionless, or searched for females, on carcasses or nearby vegetation. When two males met, they sometimes oriented to each other, spread and waved their wings, and reached forwards with spread forelegs, swaying slowly from side to side. Often the males then boxed each other, after which the winner chased the loser briefly.

Male–female interactions

Initiation: The male either “stalked” the female briefly from behind, or simply charged her and leaped on her from any direction. *Nonmating outcome:* Often the male dismounted after 1–2 s without struggle, apparently rejecting the female. However, in ~49% of pairings the male stayed mounted and the flies engaged in a peculiar struggle: the male spread the female’s wings with his hind legs, vigorously rubbed the sides of her abdomen with his hind legs, the top of her abdomen with his midlegs, and the sides of her thorax with his forelegs, and moved his abdomen side to side over her abdomen. At the same time, the female extended and arched her abdomen, raised her forelegs over her head, and pummeled the male’s head and thorax (Figs. 1C, 1D) until the male dismounted. *Mating outcome:* Struggle occurred in ~85% of pairings that resulted in copulation (mean duration = 73 s, CV = 0.79, range = 4–166 s, $N = 7$). If the female stopped pummeling the male and began to relax her abdomen, the male continued rubbing her with his legs and abdomen while slowly reaching for her abdomen tip with his genitalia. Mean total time to genital lock was 163 s (CV = 0.59,

Table 1. Ecological, morphological, and sexual characteristics, and numbers of male–female pairings performed, for seven piophilid species (see the text).

	Larval substrate ^d	Operational sex ratio bias ^d	Morphology	Sexual dimorphism in body shape	No. of male–female pairings	Mating duration (min)		Ejaculate ingestion by female?	Mate guarding?
						Mean ± SD	N		
<i>Protophihila litigata</i> ^b <i>Protophihila latipes</i>	Antlers	Male	Sleek, greyish pruinose	None	87	137 ± 52	22	Yes	Yes
	Carcasses	Male	Sleek, shiny black; forecoxa white, rest of foreleg black	None	46	21.5 ± 3.6	17	Yes	Yes
<i>Stearibia nigriceps</i>	Carcasses	None	Rotund, shiny black; bristles reduced	None	77	26.90 ± 5.65	14	Yes	No
<i>Liopiophila varipes</i>	Carcasses	Male	Sleek, shiny black; face yellow	None	40	3.56 ± 1.02	15	Yes	No
<i>Prochyliza xanthostoma</i>	Carcasses	None	Narrow, shiny black; face yellow, with silvery strip below compound eye	Male head, antennae, and fore-legs elongated	100	4.95 ± 1.96	40	Yes	No
<i>Parapiophila</i> sp. 1	Antlers, carcasses	Female	Rotund, shiny black; foretarsal segment 1 white, rest of tarsus black	None	32	1.77 ± 1.00	22	Yes	Yes?
<i>Parapiophila</i> sp. 2	Carcasses	Female	Rotund, shiny black; foretarsus black distally, rest of foreleg yellow; gonopods enlarged	None	47	4.42 ± 1.90	15	No ^c	Yes?

^aFrom Bonduriansky and Brooks (1999a).^bFrom Bonduriansky (1995); Bonduriansky and Brooks (1998a).^cNo ejaculate expulsion or ingestion within 30 min of the end of copulation.

range = 50–425 s, $N = 13$). In genital lock, the male rubbed the female's abdomen for an average of 198 s more (CV = 0.46, range = 60–309 s, $N = 13$). Prior to genital separation, the male often resumed rubbing for several minutes. Finally, the male dismounted and the flies walked in opposite directions until they were separated. Many females (~62%) tried to dislodge the male before he dismounted, using their hind legs to push at the male's legs and pummeling his head with their forelegs (mean struggle duration = 247 s, CV = 1.66, range = 29–1320 s, $N = 9$). Several minutes later, the female extended her ovipositor and deposited and ingested a large droplet of fluid containing pieces of thick, gelatinous matter and sperm.

Liopiophila varipes

Male–male interactions

Males searched for females or defended territories on carcasses, charging any fly within a radius of ~10 cm. When males met, they oriented to each other, raised and waved their wings and swayed slowly from side to side for several seconds, then charged and boxed. The winner then chased the loser for a distance of up to 20 cm.

Male–female interactions

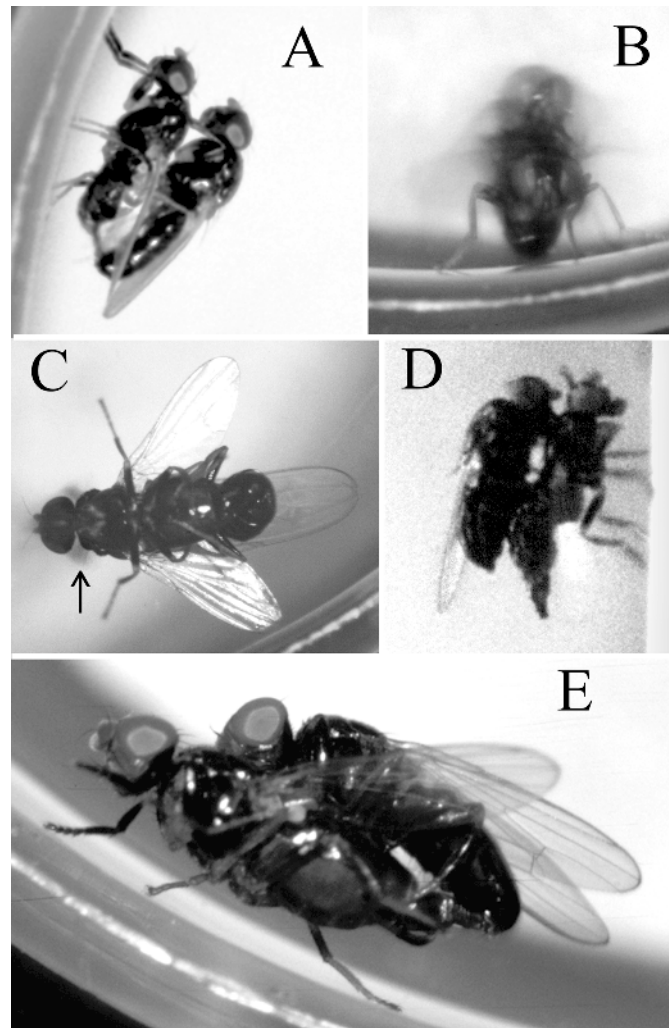
Initiation: The male “stalked” the female from behind with rapid, jerky steps for ~5–60 s (field observations) as the female walked over the carcass. Finally, the male leaped on the female from behind. **Nonmating outcome:** If the female was not receptive, she bent her abdomen downwards, crossed her hind legs over her abdomen tip, and kicked backwards, nearly always dislodging the male within 1 s of contact. Some females (~15%) also raised their wings and boxed the male. Male–female struggle (i.e., the female shaking or wrestling with the male) occurred in only 2 of 40 pairings. Nonmating outcomes appeared to result from female resistance rather than male mate choice. **Mating outcome:** If the female permitted the male to remain on her back, the male established genital lock after an average of 5 s (CV = 2.46, range = 2–10 s, $N = 14$). During the brief copulation, the pair usually remained motionless (Fig. 1E), although 2 of 14 males rubbed the female's abdomen with their hind legs for 2–3 s. Finally, the male performed several hard, convulsive abdominal pumps of increasing intensity, withdrew his aedeagus, and dismounted. However, in 2 of the 14 copulations, the female attempted to dislodge the male by shaking or pushing at him with her hind legs before he extracted his aedeagus. Several minutes after separation, the female extended her ovipositor and deposited and ingested one to several small droplets of fluid (produced at 5- to 10-min intervals) containing particulate matter and sperm.

Prochyliza xanthostoma

Male–male interactions

Males defended territories and searched for females on branches near carcasses or occasionally on carcass surfaces. When two males met, or encountered a female simultaneously, they usually attacked each other. Most male–male interactions were brief (<2 s): the males approached each other while rapidly waving their forelegs, after which one male fled or the males engaged in a brief boxing match.

Fig. 1. Sexual behaviours of piophilid flies. (A) *Protopiophila litigata*, copulating pair (male on top) viewed from the side, showing the male rubbing the female's genitalia. (B) *Protopiophila latipes*, pair (male on top) viewed from the front, showing male–female struggle. (C) *Stearibia nigriceps*, pair (male on top) viewed from below, showing the female pummeling the male's head with her forelegs (indicated on one side by an arrow). (D) The same behaviour viewed from the side. (E) *Liopiophila varipes*, copulating pair (male on top) viewed from the side (see the text for details).



However, escalated interactions were not infrequent: each male raised his body, spread his forelegs with the flat medial surface rotated down, locked foretarsae (sometimes also antennae) with his opponent (Fig. 2A), and attempted to strike down at the other male with his forelegs and antennae. Eventually, one male fell off the branch or bone or ran away, chased briefly by his opponent.

Male–female interactions

Initiation: Males typically encountered and courted females basking on branches in sunspots near a carcass. The male oriented to the female with his body at ~45° to the substrate, his antennae erect and apart, and performed repeated side-to-side (zigzag) steps, striking down with his abdomen

at each step. The rate and lateral displacement of zigzag steps varied at least 10-fold among males. After several zigzag steps, the male sometimes raised one or both forelegs and vibrated the foreleg(s) very rapidly for ~0.5 s ("vibrating display") (Fig. 2B). Vibrating-display frequency varied from 2 to 72/min (mean = 12.5/min, CV = 1.4, $N = 31$). Most males (~70%) also occasionally charged the female, touched her with both forelegs, and retreated. *Nonmating outcome*: If the female was not (immediately) receptive, she typically turned and walked away a few steps. Often, the male then froze, lowered his body slowly towards the substrate, and began to "creep" slowly towards the female. When the male's approach elicited a movement from the female, he resumed the zigzag dance. Unreceptive females usually flew away after one or more bouts of zigzag dance and creeping approach. There was no evidence of male mate choice. *Mating outcome*: If the female was receptive, she oriented to the male and appeared to observe his courtship. The male always responded with several consecutive vibrating displays directly in front of her (Fig. 2B). The female then reached out to the male with her forelegs, simultaneously extending her abdomen tip slightly. The male responded by touching the female's foretarsae with his own, then braced one foreleg against the substrate and leaped (vaulted) forwards, attempting to turn 180°, land on the female's back, and immediately establish genital lock. About 87% of post-acceptance leaps resulted in the male missing or falling off the female, so ~72% of males required >1 leap (mean = 2.2 leaps, CV = 1.06, $N = 33$) to achieve copulation. Males sometimes leaped without acceptance by the female or (rarely) from behind, but this never resulted in copulation. However, successful post-acceptance leaps led to genital lock within ~1 s. The mean duration of courtship prior to copulation was 484 s (CV = 1.05, range = 5–1980 s, $N = 32$), with an average of 699 zigzag steps performed (CV = 1.16, range = 14–3200 steps, $N = 32$). During copulation, the male grasped the female's thorax and abdomen with his legs, making occasional pumping motions with his abdomen (Fig. 2C). Finally, the male withdrew his aedeagus and dismounted. Several minutes later, the female usually extended her ovipositor and deposited and ingested one or more droplets containing dense bundles of sperm.

Parapiophila sp. 1

Male-male interactions

Males were rarely seen interacting in the wild. When encountering each other, males often oriented to each other and waved their forelegs and occasionally hit each other with their forelegs. Pre-combat charges and post-combat chases rarely exceeded distances of 1 cm.

Male-female interactions

Initiation: The male oriented to the female and "beckoned" to her with his spread forelegs for ~3 s (Fig. 2D). The female often responded with the same behaviour. After this, the male leaped or climbed onto the female's abdomen. *Nonmating outcome*: The female sometimes responded by kicking and pushing at the male with her hind legs or by lowering her ovipositor, usually causing him to fall off or dismount after an average of 2.4 s (CV = 0.61, $N = 7$).

Occasionally, males appeared to dismount voluntarily (i.e., reject the female). *Mating outcome*: If the male was able to hold on to the female's abdomen, he established genital lock after an average of 2.6 s (CV = 0.58, $N = 5$), holding his body posterior and ventral to the female's. Once in genital lock, the male gradually released his hold of the female's abdomen until he was attached to her only through the genitalia. After an average of 91 s (CV = 0.25, $N = 12$), the female usually (in 80% of pairings) began to kick, push, and drag the male (Fig. 2E). This struggle continued until he withdrew his aedeagus and fell off (mean struggle duration = 59 s, range = 7–124 s, CV = 0.59, $N = 12$). One to two minutes after separation, the female extended her ovipositor and deposited and ingested a single large droplet of fluid containing particles of gelatinous matter and sperm.

Parapiophila sp. 2

Male-male interactions

Because the operational sex ratio is extremely female-biased in this species (Bonduriansky and Brooks 1999a), sexual behaviours were observed only in the laboratory. It is not known why males of this species are so rarely observed in the wild, or how they locate females. In the laboratory, male-male interactions closely resembled those of *Parapiophila* sp. 1.

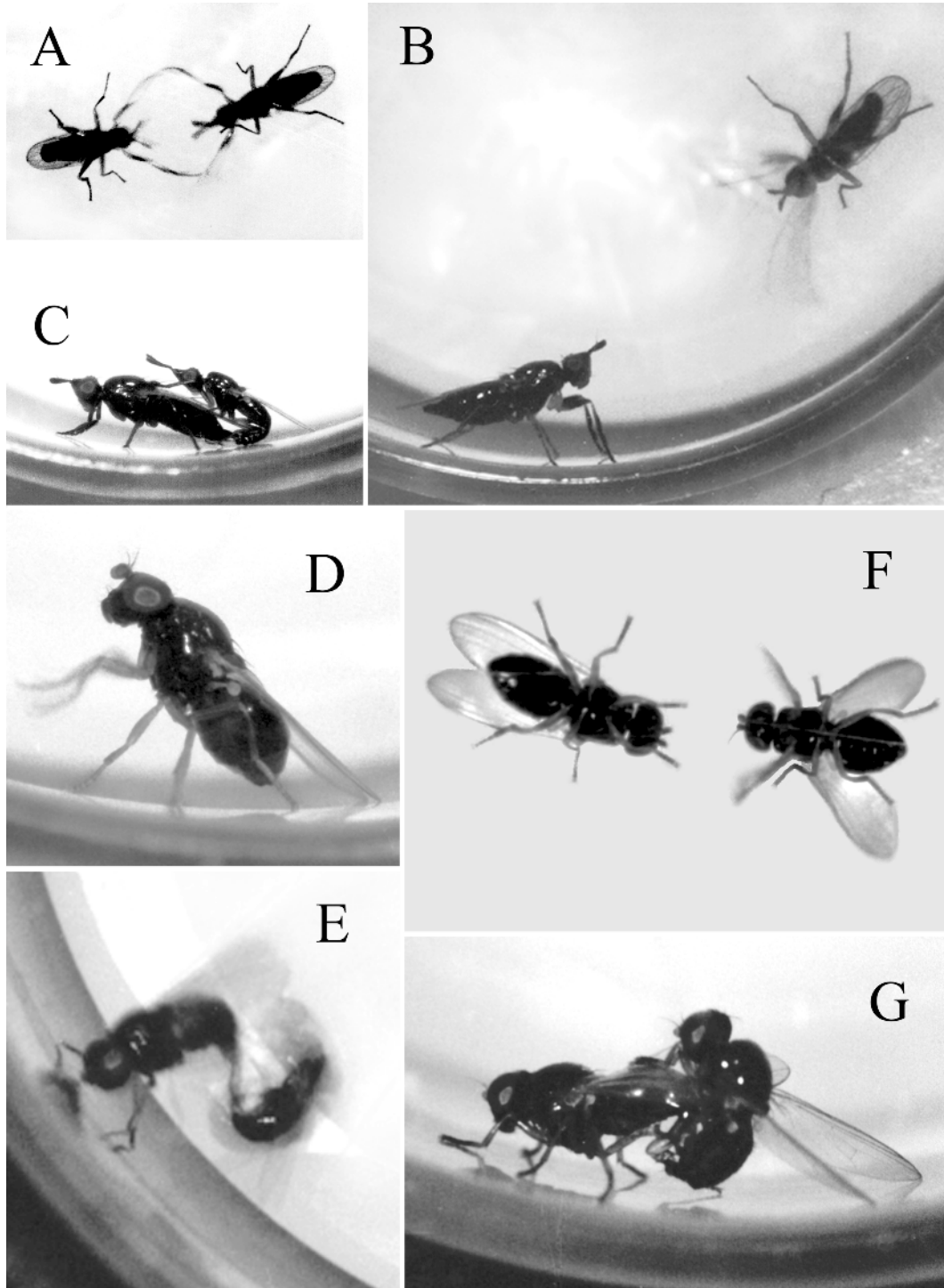
Male-female interactions

Initiation: The male oriented to the female and stopped, then spread his wings and vibrated them at high frequency for ~1–30 s while performing several abrupt swaying motions of his body with simultaneous up-and-down motions of the forelegs (Fig. 2F). Mean total courtship duration was 10.3 s (CV = 0.73, range = 3–30 s, $N = 18$). The female often responded by orienting to the male, sometimes waving her forelegs slowly. The male then climbed or leaped onto the female and attempted to cling to her abdomen. If the female did not orient to, or ran from, the male, he sometimes gave chase and leaped from behind. *Nonmating outcome*: If the female was unreceptive, she usually began to kick and push the male with her hind legs, dislodging him after an average of 5.5 s (CV = 0.46, $N = 9$). Occasionally, males appeared to dismount voluntarily (i.e., reject the female). *Mating outcome*: If the female extended her abdomen tip, the male tried to grasp it with his gonopods and establish genital lock (Fig. 2G). After an average of 97 s (CV = 0.52, $N = 16$) of copulation, the female began to kick and push the male with her hind legs, often while dragging him over the substrate. The male struggled to cling to her abdomen with his legs but eventually withdrew his aedeagus and dismounted or fell off the female (mean struggle duration = 168 s, range = 48–500 s, CV = 0.78, $N = 16$). No ejaculate expulsion or ingestion occurred within 30 min of genital separation.

Discussion

The seven piophilid species examined in this study exhibit remarkable variation in sexual behaviour. Male-male interactions involve scramble competition in all species, in combination with fierce combat in several species. Male-female

Fig. 2. Sexual behaviours of piophilid flies. (A) Male *Prochyliza xanthostoma* locking foretarsae prior to combat, viewed from below. (B) A male *P. xanthostoma* (seen from below) performing the zigzag dance and vibrating display in front of an observing female (seen from the side). (C) *Prochyliza xanthostoma*, copulating pair (male on top) viewed from the side. (D) A male *Parapiophila* sp. 1 performing the “beckoning” display, viewed from the side. (E) *Parapiophila* sp. 1, copulating pair (male on the right) engaged in a struggle. (F) A male *Parapiophila* sp. 2 (on the right) courting a female, viewed from below. (G) *Parapiophila* sp. 2, copulating pair (male on the right) prior to male–female struggle (see the text for details).



interactions include various combinations of premounting courtship, postmounting palpation, male–female struggle, and other components (see below). Because performance tended

to vary considerably among males, as indicated by large CVs, a number of these male–male and male–female interactions appear to represent sexual-selection mechanisms. These mul-

multiple selective mechanisms appear to occur in somewhat predictable combinations. They also tend to operate in a rough sequence — as layers of sexual selection — in each species, a pattern that may have important evolutionary consequences.

Sexual-selection mechanisms

Male scramble and combat competition

Scramble competition can be defined as variation among males in the ability to detect and intercept females. Scramble competition was observed in all seven species. Although the degree of variation among males in scramble performance is not known, the traits involved (i.e., running, jumping, flying, endurance, visual or olfactory acuity) are likely to contribute to variation in male mating success. Scramble competition may be especially important in *Parapiophila* spp. males because they do not engage in intense combat. Combat (i.e., male–male interactions involving physical contact) may serve to exclude rivals from prime mate-searching territories or prevent rivals from courting or intercepting a female (e.g., see Bonduriansky and Brooks 1999b). The frequency and intensity of combat varied considerably among species (see Table 2).

Premounting courtship

Premounting courtship is a sequence of behavioural signals conveyed to potential mates prior to an attempt to copulate. Darwin (1874) noted that males often “sedulously display their charms before females; and the victors transmit their superiority to their male offspring”. Possible functions of courtship include advertisement of species identity (Spieth 1968; Barrass 1979) or quality (Bradbury and Gibson 1983; Eberhard 1994) to potential mates, and or attempts to coerce them into mating by some form of sensory exploitation (Holland and Rice 1998). Intrasexual advertisement of quality or condition (Borgia 1979; Berglund et al. 1996) cannot be a function of male courtship in these species because courtship was directed only at females. Premounting courtship occurred in three species (Table 2). It was entirely absent in the other four species, where males simply stalked or charged and leaped onto any female they encountered, a pattern that appears to be plesiomorphic in the Diptera (see Spieth 1968).

Postmounting palpation

I included under “palpation” any sequences of rubbing or tapping delivered by the male after he mounted the female (see Table 2). Such behaviours may function as “copulatory courtship” (Eberhard 1991, 1994) if females engage in “cryptic” mate choice, discriminating among sperm received from different males (Thornhill 1983). Opportunities for cryptic female mate choice exist in most of these species through differential expulsion of ejaculates following copulation (see Table 1). However, variation among males appeared to be subtle (CVs < 0.5; Table 2), perhaps providing females with little opportunity for mate assessment. If these palpation behaviours do not function as copulatory courtship, there appears to be little opportunity for sexual selection on males through male–female interactions in species lacking both premounting courtship and male–female struggle (see Table 2). Alternatively, palpation behaviours may function as

female-assessment mechanisms involved in cryptic male mate choice (Bonduriansky 2001). For example, males may obtain information on the number of mature eggs carried by females (Bonduriansky and Brooks 1998b) and allocate more sperm to more fecund mates.

Male–female struggle

If females struggle with males even when they are (ultimately) “receptive”, the struggle may represent a female’s attempt to avoid mating (Arnqvist et al. 1996; Rowe and Arnqvist 1996; Rowe et al. 1994), or females’ adaptation to increase mate quality by filtering out low-quality males (Cordero and Eberhard 2003). Struggles occurred in four species, with considerable variation in struggle duration within each species (Table 2). Interestingly, in *P. latipes*, struggles occurred only at the start of male–female interactions, and thus appeared to affect only the probability of initiating copulation. In contrast, in the *Parapiophila* species and *S. nigriceps*, struggles also occurred towards the end of copulation, and thus also appeared to affect copulation duration. Although struggles near the end of copulation appear to reflect male–female conflict, the nature of this conflict is not clear. Male tenacity may represent mate guarding to prevent sperm displacement during a critical phase of sperm storage, in which case male–female conflict may be over the optimal mating rate, or at least optimal time allocation. This explanation seems unlikely, however, because *S. nigriceps* and *Parapiophila* sp. 2 females were never observed to mate more than once, whereas *Parapiophila* sp. 1 females often mated two or three times within the same pairing. More likely, the struggle over copulation duration may reflect male–female conflict over optimal ejaculate size: a larger ejaculate may increase male fertilization success (e.g., see Parker and Simmons 1994) but have deleterious long-term effects on the female (e.g., see Rice 1996; Chapman 2001). In either case, male–female struggle has the potential to increase the condition-dependence of male fertilization success in each of these species.

Revealing obstacles

In two species, aspects of male–female interaction did not fit the definition of any previously described sexual-selection mechanism. In these species, females appeared to possess behavioural or morphological adaptations that exposed variation among males without the assessment of male courtship or struggle with males. I refer to these female adaptations as revealing obstacles. A revealing obstacle can be defined generally as any female adaptation that increases female “resistance” (i.e., reduces females’ mating rate or enhances mate quality), but without a difference in the behavioural response to different male phenotypes. Revealing obstacles simply “force” all males to perform a difficult task, so that only some (high quality?) males are able to mate. Revealing obstacles differ from female preferences in that the female behavioural response is constant across all male phenotypes. Revealing obstacles thus represent a type of indirect female mate choice (Wiley and Poston 1996). Although female struggle may fit the above definition if females struggle equally against all males, I describe (below) two apparent examples of revealing obstacles that do not involve struggle.

Table 2. Potential sexual-selection mechanisms based on male–male and male–female interactions in seven piophilid species (see the text).

	Male–male scramble?	Male–male combat ^d			Male–female courtship (premounting)	Male–female palpation (postmounting)	Male–female struggle	Revealing obstacles
		Frequency (no./min)	Duration(s)	Duration(s)				
<i>Protophila litigata</i>	Yes	2	30	None	Rubbing (0.45) ^{b,c}	None	?	
<i>Protophila latipes</i>	Yes	1	15	None	Rubbing (0.41) ^b	Female shakes male (2.68) ^b	?	
<i>Stearibia nigriceps</i>	Yes	0.2	3	None	Rubbing (0.46) ^b	Female pummels male's head (2.22) ^b	?	
<i>Liopiophila varipes</i>	Yes	1	15	None	Occasional rubbing	None	Precopulatory leap (2.27) ^d	
<i>Prochyliza xanthostoma</i>	Yes	1	120	Zigzag dance (0.47) ^e	None	None	Precopulatory leap (1.07) ^d	
<i>Parapiophila</i> sp. 1	Yes	<0.1	1	Foreleg display (1.37) ^e	None	Female kicks, drags male (0.84) ^b	?	
<i>Parapiophila</i> sp. 2	Yes	<0.1	1 ^f	Foreleg display (0.44) ^b	None	Female kicks, drags male (0.78) ^b	?	

Note: Numbers in parentheses are CVs (where available) for the sexual behaviours (including pairings in which copulation did and did not occur).

^aData indicate maximum frequencies and durations observed in the field.

^bCV for the duration of the behaviour.

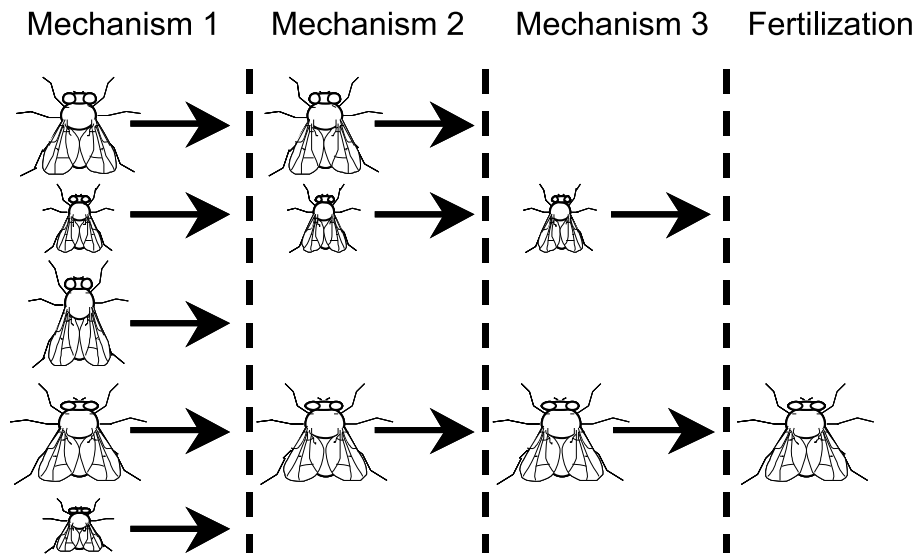
^cFrom Bonduriansky and Brooks (1998a).

^dCV for the number of unsuccessful leaps performed prior to achieving copulation.

^eCV for the frequency of the behaviour.

^fBased on laboratory observations.

Fig. 3. Layered sexual selection in piophilid flies. Selective mechanisms operate in a rough sequence (left to right), and each mechanism may reduce the subset of individuals that are able to compete in the next mechanism, thus limiting the variation among individuals exposed to selection at each stage (see the text).



Once they are accepted by a female following a bout of courtship, male *P. xanthostoma* performed a leap and 180° turn (“precopulatory leap”). Many males apparently failed in this task, either missing or falling off the female. Male *P. xanthostoma* may be “forced” to perform this leap because of a subtle combination of female behaviours. The female’s acceptance signal (i.e., reaching out to the male with her forelegs) is accompanied by a slight extension of her abdomen tip, which appears to be necessary for the male to achieve genital lock. The abdomen tip is extended and withdrawn in synchrony with the forelegs, giving the male a brief window of opportunity to achieve copulation. Indeed, if they were not immediately successful, male *P. xanthostoma* almost never persisted in attempting to achieve genital lock for longer than ~1 s, but dismounted and resumed courtship. Because, in the wild, a male is not likely to get many second chances to leap (the female may fly away or the male may be attacked by a rival), leaping ability may have important effects on male mating success. However, the precopulatory leap is not premounting courtship, since it follows acceptance by the female, and male leaping performance is unaffected by female receptivity (Bonduriansky and Rowe 2003). Nor is it a male–female struggle, since females usually offer no discernible resistance. A somewhat similar sequence characterizes the male–female interactions in *L. varipes*, where the male “stalks” the female, apparently attempting to achieve an optimal position, then leaps from behind. This behaviour is associated with the ability of female *L. varipes* to dislodge males very rapidly, without struggle, through a combination of subtle leg and abdomen movements (see Results). Thus, in *P. xanthostoma* and *L. varipes*, some combination of female behavioural traits appeared to constitute an “obstacle” to mating for males. These obstacles may “reveal” male condition by exposing variation in agility, coordination, strength, or visual acuity. Revealing obstacles may also serve to reduce females’ mating rate.

Revealing obstacles may be quite common in insects and other invertebrates. For example, it is possible that mating in

flight evolved in some insects through selection on females to avoid low-quality males. Moreover, the rotation of the male genitalia during dipteran evolution (McAlpine 1981, pp. 56–59) may represent a sequence of male co-adaptations to revealing obstacles. Revealing obstacles may also help to explain how premounting courtship (such as that of *P. xanthostoma*) evolves from the plesiomorphic “charge-and-mount” behaviour typical of Diptera (see Spieth 1968). Courtship may initially evolve as a male adaptation that mitigates the severity of a revealing obstacle by inducing females to modify their behaviour (e.g., stop walking or orient to the male). Selection on females for increased resistance, and on males for increased effectiveness, may then result in the elaboration of male behaviour into a complex “display” sequence.

Layered sexual selection

Of the six distinct sexual-selection mechanisms discussed above (see Table 2), three or more appeared to play some role in the sexual behaviour of each of the seven species (Table 2). Further research is likely to identify additional mechanisms, such as sperm competition, in some of these species. And, of course, some mechanisms, such as premounting courtship, are readily divisible into components (see Johnson 2000). Because multiple mechanisms appear to operate in each system, and these mechanisms tend to act in a rough chronological sequence (see Moore 1990; Andersson et al. 2002), they can be regarded as layered selection vectors, each layer preventing some males from advancing to the next layer (Fig. 3). For example, having survived to adulthood, a male *P. xanthostoma* must compete through scramble and combat interactions for opportunities to encounter females. If he is successful in these intrasexual interactions, he must court females that he encounters. Finally, if a female accepts him, he must perform the precopulatory leap successfully to achieve copulation.

Male–male interactions evolve (at least in part) in response to selection on males to maximize access to females (Parker 1978), whereas male–female interactions are likely

Fig. 4. (A) If multiple sexual-selection mechanisms tend to select for quality, each mechanism will act to increase the mean quality of males that have the opportunity to sire offspring. The plot shows a hypothetical distribution of male qualities in the population, and the arrows indicate the mean quality of males that sire offspring as a result of three layered sexual-selection mechanisms. (B) Alternatively, if multiple sexual-selection mechanisms tend to act on different traits, the evolutionary result may be the elaboration of male phenotypes (see the text).

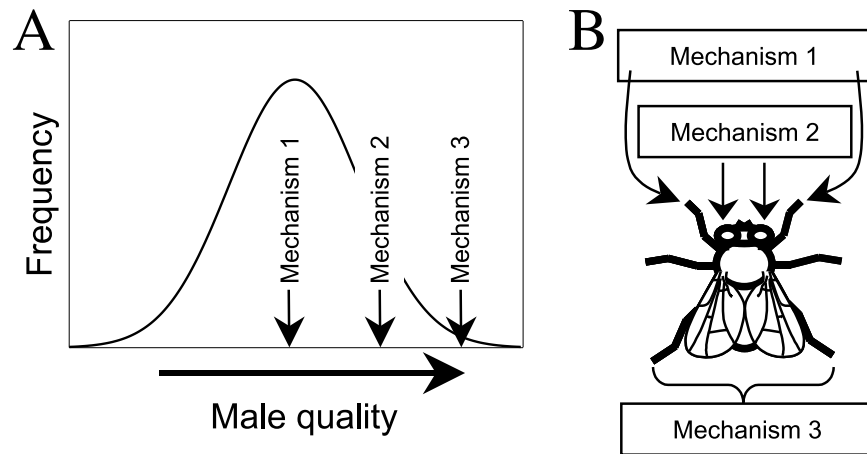
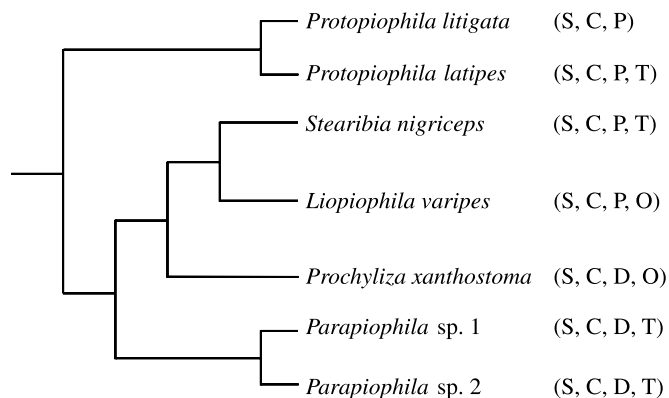


Fig. 5. A phylogeny of the subtribe Piophilina (some genera are not shown) based on morphological characters (modified from McAlpine 1977). The letters in parentheses following each species name indicate the apparent sexual-selection mechanisms which occur in that species (see Table 2): S, male–male scramble competition; C, male–male combat; D, male–female courtship; O, revealing obstacle; P, male–female palpation; T, male–female struggle.



to represent a co-evolutionary outcome of selection on females to increase mate quality (e.g., Parker 1983) or reduce the mating rate (e.g., Rowe et al. 1994), and selection on males to overcome female resistance (e.g., Rowe et al. 1994). Thus, selection acting simultaneously but differently on males and females (particularly in the context of sexual conflict) may often result in the simultaneous evolution of at least two sexual-selection mechanisms. Several hypotheses have been proposed to account for the elaboration of male–female interactions into multiple sexual-selection mechanisms (e.g., Møller and Pomiankowski 1993; Pomiankowski and Iwasa 1993; Iwasa and Pomiankowski 1995; Prum 1997; Holland and Rice 1998). In general, each layer may select for either overall condition (Fig. 4A) or a distinct set of male traits (Fig. 4B). The layering of sexual-selection mechanisms may have considerable evolutionary importance, since each layer

may alter the distribution of phenotypes exposed to the next layer. For example, male–male interactions and female preferences may exert conflicting sexual selection on a male trait (e.g., Moore and Moore 1999; Andersson et al. 2002; Sih et al. 2002; Bonduriansky and Rowe 2003). The layering of mechanisms, such as the tendency for male–male combat over territory to precede female mate choice (see Andersson et al. 2002), may act to reduce females' access to their preferred mate phenotypes. This would reduce the opportunity for female mate choice, and the importance of female preference as an agent of sexual selection, in comparison with male–male interactions.

The comparative analysis also suggests the existence of several other patterns. First, congeneric species exhibit broadly similar patterns, but none of the intergeneric variation can be explained by phylogeny (Tables 1 and 2, Fig. 5). For example, the sister-group to *Parapiophila* includes genera that exhibit the full range of male–male and male–female interactions. This diversity of sexual traits is especially striking when compared with the relatively slight differences in morphology and ecology. Second, each species exhibits either premounting courtship or postmounting palpation sequences, but not both. Thus, it may be that these two types of interactions represent alternative evolutionary responses to similar selective pressures. For example, females may evaluate male quality through either premounting courtship (Barrass 1979) or copulatory courtship (Eberhard 1991, 1994). However, male–female interactions of both types co-occur with male–female struggle in some species and with intense male–male combat in other species (Table 2), suggesting that these interactions evolve in response to different selection pressures. Thus, although these patterns are based on a small number of species, they suggest that multiple sexual-selection mechanisms may combine in somewhat predictable ways that call for an explanation.

Multiple layered sexual-selection mechanisms appear to be characteristic of many groups of animals (see Andersson 1994; Berglund et al. 1996). For example, male *Dryomyza anilis* search for females, defend territories, and attempt take-

overs of copulating females on carcasses (Otronen 1984). Male flies mount females without premounting courtship and perform a series of palpation ("tapping") sequences, the number of which is positively correlated with male fertilization success (Otronen 1990, 1994). Similarly, on sage grouse (*Centrocercus urophasianus*) leks (Gibson and Bradbury 1986), neighbouring males fight for positions and interfere with copulations, and females appear to choose among males on the basis of their courtship. In wood frog (*Rana sylvatica*) mating aggregations (Berven 1981), males scramble and wrestle for access to females, as well as calling to attract females, and both sexes appear to prefer large individuals as mates. In the pipefish *Nerophis ophidion* (Rosenqvist 1990), the most dominant female exhibits the highest fecundity and is preferred as a mate by males. Finally, multiple courtship elements characterize many species of dabbling ducks, and interspecific variation in courtship "repertoire size" may be related to variation in the intensity of sexual selection (Johnson 2000). These examples illustrate that multiple sexual-selection mechanisms occur in a variety of animal taxa, and typically operate in a somewhat predictable sequence, as layers of sexual selection. Understanding the nature, causes, and consequences of these patterns is an important goal of evolutionary research.

Acknowledgments

I am grateful to the staff of the Wildlife Research Station for their support. Locke Rowe and an anonymous reviewer provided many helpful comments and suggestions. Funding was provided by the Natural Sciences and Engineering Research Council of Canada through a PGS-B grant to R.B. and a research grant to Locke Rowe.

References

- Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, N.J.
- Andersson, S., Pryke, S.R., Ornborg, J., Lawes, M.J., and Andersson, M. 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. *Am. Nat.* **160**: 683–691.
- Arnqvist, G., Rowe, L., Krupa, J.J., and Sih, A. 1996. Assortative mating by size: a meta-analysis of mating patterns in water striders. *Evol. Ecol.* **10**: 265–284.
- Barrass, R. 1979. The survival value of courtship in insects. *In* Sexual selection and reproductive competition in insects. *Edited by* M.S. Blum and N.A. Blum. Academic Press, New York. pp. 403–416.
- Berglund, A., Bisazza, A., and Pilastro, A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linn. Soc.* **58**: 385–399.
- Berven, K.A. 1981. Mate choice in the wood frog, *Rana sylvatica*. *Evolution*, **35**: 707–722.
- Bonduriansky, R. 1995. A new Nearctic species of *Protopiophila* Duda (Diptera: Piophilidae), with notes on its behaviour and comparison with *P. latipes* (Meigen). *Can. Entomol.* **127**: 859–863.
- Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev. Camb. Philos. Soc.* **76**: 305–339.
- Bonduriansky, R., and Brooks, R.J. 1998a. Copulation and oviposition behaviour of *Protopiophila litigata* (Diptera: Piophilidae). *Can. Entomol.* **130**: 399–405.
- Bonduriansky, R., and Brooks, R.J. 1998b. Male antler flies (*Protopiophila litigata*; Diptera: Piophilidae) are more selective than females in mate choice. *Can. J. Zool.* **76**: 1277–1285.
- Bonduriansky, R., and Brooks, R.J. 1999a. Reproductive allocation and reproductive ecology of seven species of Diptera. *Ecol. Entomol.* **24**: 389–395.
- Bonduriansky, R., and Brooks, R.J. 1999b. Why do male antler flies (*Protopiophila litigata*) fight? The role of male combat in the structure of mating aggregations on moose antlers. *Ethol. Ecol. Evol.* **11**: 287–301.
- Bonduriansky, R., and Rowe, L. 2003. Interactions among mechanisms of sexual selection on male body size and head shape in a sexually dimorphic fly. *Evolution*. In press.
- Borgia, G. 1979. Sexual selection and the evolution of mating systems. *In* Sexual selection and reproductive competition in insects. *Edited by* M.S. Blum and N.A. Blum. Academic Press, New York. pp. 19–80.
- Bradbury, J.W., and Gibson, R.M. 1983. Leks and mate choice. *In* Mate choice. *Edited by* P. Bateson. Cambridge University Press, Cambridge. pp. 109–138.
- Chapman, T. 2001. Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity*, **87**: 511–521.
- Cordero, C., and Eberhard, W.G. 2003. Female choice of sexually antagonistic male adaptations: a critical review of some current research. *J. Evol. Biol.* **16**: 1–6.
- Darwin, C.R. 1874. The descent of man and selection in relation to sex. 2nd ed. Hurst and Co., New York.
- Dufour, L. 1844. Des métamorphoses et de l'anatomie du *Piophila patasioniz*. *Ann. Sci. Nat. Zool. Biol. Anim.* **3**(1): 365–388.
- Eberhard, W.G. 1991. Copulatory courtship and cryptic female choice in insects. *Biol. Rev. Camb. Philos. Soc.* **66**: 1–31.
- Eberhard, W.G. 1994. Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution*, **48**: 711–733.
- Freidberg, A. 1981. Taxonomy, natural history and immature stages of the bone-skipper, *Centrophlebomyia furcata* (Fabricius) (Diptera: Piophilidae, Thyreophorina). *Entomol. Scand.* **12**: 320–326.
- Gibson, R.M., and Bradbury, J.W. 1986. Male and female mating strategies on sage grouse leks. *In* Ecological aspects of social evolution. *Edited by* D.I. Rubenstein and R.W. Wrangham. Princeton University Press, Princeton, N.J. pp. 379–398.
- Holland, B., and Rice, W.R. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, **52**: 1–7.
- Iwasa, Y., and Pomiankowski, A. 1994. The evolution of mate preferences for multiple sexual ornaments. *Evolution*, **48**: 853–867.
- Iwasa, Y., and Pomiankowski, A. 1995. Continual change in mate preferences. *Nature (Lond.)*, **377**: 420–422.
- Johnson, K.P. 2000. The evolution of courtship display repertoire size in the dabbling ducks (Anatini). *J. Evol. Biol.* **13**: 634–644.
- McAlpine, J.F. 1977. A revised classification of the Piophilidae, including 'Neottiophilidae' and 'Thyreophoridae' (Diptera: Schizophora). *Mem. Entomol. Soc. Can.* **103**: 1–66.
- McAlpine, J.F. (Editor). 1981. Manual of Nearctic Diptera. Vol. 1 (Monogr. No. 27). Agriculture Canada Research Branch, Ottawa, Ont.
- McAlpine, J.F. (Editor). 1987. Manual of Nearctic Diptera. Vol. 2 (Monogr. No. 28). Agriculture Canada Research Branch, Ottawa, Ont.
- Moore, A.J. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual and intersexual selection. *Evolution*, **44**: 315–331.

- Moore, A.J., and Moore, P.J. 1999. Balancing sexual selection through opposing mate choice and male competition. *Proc. R. Soc. Lond. B Biol. Sci.* **266**: 711–716.
- Murphy, C.G. 1998. Interaction-independent sexual selection and the mechanisms of sexual selection. *Evolution*, **52**: 8–18.
- Møller, A.P., and Pomiankowski, A. 1993. Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.* **32**: 167–176.
- Omland, K.E. 1996. Female mallard mating preferences for multiple male ornaments. I. Natural variation. *Behav. Ecol. Sociobiol.* **39**: 353–360.
- Otronen, M. 1984. Male contests for territories and females in the fly *Dryomyza anilis*. *Anim. Behav.* **32**: 891–898.
- Otronen, M. 1990. Mating behavior and sperm competition in the fly, *Dryomyza anilis*. *Behav. Ecol. Sociobiol.* **26**: 349–356.
- Otronen, M. 1994. Fertilization success in the fly *Dryomyza anilis* (Dryomyzidae): effects of male size and the mating situation. *Behav. Ecol. Sociobiol.* **35**: 33–38.
- Parker, G.A. 1978. Evolution of competitive mate searching. *Annu. Rev. Entomol.* **23**: 173–196.
- Parker, G.A. 1983. Mate quality and mating decisions. *In* *Mate choice*. Edited by P. Bateson. Cambridge University Press, New York. pp. 141–166.
- Parker, G.A., and Simmons, L.W. 1994. Evolution of phenotypic optima and copula duration in dungflies. *Nature (Lond.)*, **370**: 53–56.
- Pomiankowski, A., and Iwasa, Y. 1993. Evolution of multiple sexual preferences by Fisher's runaway process of sexual selection. *Proc. R. Soc. Lond. B Biol. Sci.* **253**: 173–181.
- Prum, R.O. 1997. Phylogenetic tests of alternative intersexual selection mechanisms: trait macroevolution in a polygynous clade (Aves: Pipridae). *Am. Nat.* **149**: 668–692.
- Rice, W.R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature (Lond.)*, **381**: 232–234.
- Rosenqvist, G. 1990. Male mate choice and female–female competition for mates in the pipefish *Nerophis ophidion*. *Anim. Behav.* **39**: 1110–1115.
- Rowe, L., and Arnqvist, G. 1996. Analysis of the causal components of assortative mating in water striders. *Behav. Ecol. Sociobiol.* **38**: 279–286.
- Rowe, L., Arnqvist, G., Sih, A., and Krupa, J.J. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends Ecol. Evol.* **9**: 289–293.
- Sacchi, L., Grigolo, A., and Chieppa, M. 1978. Influenza in *Piophilina casei* L. del secreto paragoniale sull' attivita sessulae dei maschi e sulla durata della copula. *Riv. Parassitol.* **39**: 11–23.
- Sih, A., Lauer, M., and Krupa, J.J. 2002. Path analysis and the relative importance of male–female conflict, female choice and male–male competition in water striders. *Anim. Behav.* **63**: 1079–1089.
- Spieth, H.T. 1968. Evolutionary implications of sexual behavior in *Drosophila*. *Evol. Biol.* **2**: 157–193.
- Swammerdam, J. 1758. *The book of nature, or, the history of insects*. C.G. Seyffert, London. [Originally published in 1669 as *Historia Insectorum Generaliz*, M. van Drennen, Utrecht, the Netherlands.]
- Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am. Nat.* **122**: 765–788.
- Wiley, R.H., and Poston, J. 1996. Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution*, **50**: 1371–1381.