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PERSPECTIVE:
FEMALE REMATING, OPERATIONAL SEX RATIO, AND THE ARENA OF SEXUAL
SELECTION IN *DROSOPHILA* SPECIES

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Abstract.—As commonly observed among closely related species within a variety of taxa, *Drosophila* species differ considerably in whether they exhibit sexual dimorphism in coloration or morphology. Those *Drosophila* species in which male external sexual characters are minimal or absent tend, instead, to have exaggerated ejaculate traits such as sperm gigantism or seminal nutrient donations. Underlying explanations for the interspecific differences in the presence of external morphological sexual dimorphism versus exaggerated ejaculate traits are addressed here by examining the opportunity for sexual selection on males to occur before versus after mating in 21 species of *Drosophila*. Female remating frequency, an important component of the operational sex ratio, differs widely among *Drosophila* species and appears to dictate whether the arena of sexual selection is prior to, as opposed to after, copulation. Infrequent female mating results in fewer mating opportunities for males and thus stronger competition for receptive females that favors the evolution of male characters that maximize mating success. On the other hand, rapid female remating results in overlapping ejaculates in the female reproductive tract, such that ejaculate traits which enhance fertilization success are favored. The strong association between female remating frequency in a given species and the presence of sexually selected external versus internal male characters indicates that the relationship be examined in other taxa as well.

Key words.—*Drosophila*, ejaculate, female remating, sexual dimorphism, sexual selection.

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Regardless of the underlying mechanisms, the elaboration of male traits (sexual dimorphism, secondary sexual characters, and ornamentation) is considered to reflect the action of sexual selection. Yet in many species, males and females are indistinguishable, or nearly so, presumably because sexual selection is less intense. In most species, however, the intensity of sexual selection has not been measured, nor has the possibility been examined that other kinds of traits, less obvious to the investigators, have experienced undetected sexual selection.

Species of the genus *Drosophila* vary widely with respect to whether or not they are sexually dimorphic. Beyond a slight sex difference in body size, males being slightly smaller than females in most species, gender in many *Drosophila* can confidently be determined only under a microscope. Despite their utility for studies of sexual selection, popularized by Bateman (1948), males of most *Drosophila* species appear, at least superficially, to be devoid of obvious sexually se-

lected external traits (Wheeler 1981; Spieth 1974). Among those *Drosophila* species that do exhibit obvious sexual dimorphism, character variability is quite diverse, including dimorphic color patterns as well as morphology and behavior (Spieth 1952). However, such sexually dimorphic species tend to be restricted to certain lineages, for example, to the melanogaster and obscura species groups and in certain Hawaiian endemic species. In addition to the male structures and color differences observed in these dimorphic species, specialized mating tactics have arisen in certain species. For example, in species of the obscura group, males regurgitate a nutritive drop which is consumed by females during courtship (Steele 1986a, b). Males of *D. melanogaster* and *D. simulans* patrol emergence sites, mating with teneral females that are unable to perform rejection behaviors or escape unwanted copulations (Markow 2000). All of the external sexual differences as well as the male mating tactics involve processes that occur prior to copulation, whether they involve intrasexual or intersexual interactions.

Recently, surprising interspecific variation for internal male reproductive characters, such as sperm length and ejaculate donation, has been reported in *Drosophila* (reviewed in

¹ This paper is dedicated to Dr. Charles M. Woolf, Professor Emeritus in the Department of Biology, Arizona State University, Tempe, Arizona, on the occasion of his 77th birthday.

Markow 1996). Among animals, sperm length has reached its most exaggerated expression in *Drosophila*, where in several species male gametes are many times the length of the males that produce them (Pitnick et al. 1995a, b). Sperm gigantism has arisen multiple times in different evolutionary lineages of *Drosophila* (Pitnick et al. 1995a) and interspecific sperm length variation appears to have been influenced by selection (Snook 1997). Another unusual feature of the *Drosophila* ejaculate, is the fate of nonsperm components in mated females of certain species. Using radioactive amino acid precursors, a number of investigations have demonstrated the uptake and incorporation of large amounts of ejaculatory substances into female somatic tissues and ovaries (Markow and Ankney 1984, 1988; Bownes and Partridge 1987; Pitnick et al. 1991, 1997). In species exhibiting "ejaculatory donations" to females, males pass an exaggerated quantity of seminal fluid to females. A phylogenetic component to the observed interspecific variation in male seminal fluid donations has been detected (Pitnick et al. 1997). Ejaculatory donations have been measured in most of the same species as examined for sperm gigantism and with few exceptions the two phenomena tend not to attain extreme expression simultaneously in the same species (Markow 1996).

Explanations for the existence of such exaggerated ejaculates, typically invoking fertilization success, remain largely untested. Curiously, however, species displaying exaggerated ejaculates tend not to be the same species in which males exhibit external sexual dimorphism, raising questions as to the phylogenetic patterns for external versus internal male sexual characters and what such patterns may reveal regarding the forces underlying their evolution.

It is easily envisioned how intense competition for females could lead to the evolution of male traits that enhance mating success, whether through male-male interactions or female choice. Such is the case when the operational sex ratio (OSR), or the proportion of sexually mature males to receptive females (Emlen and Oring 1977), is biased toward males. On the other hand, if the OSR is less male biased or even biased toward females, such that competition for females is not strong, less sexual dimorphism should be seen. One way in which the OSR could become less male biased is if females of a species remate frequently. In insects such as *Drosophila*, where females can store sperm, multiple female mating gives rise to overlapping ejaculates, moving the arena of intermale competition to inside the female rather than prior to mating. Thus, depending upon whether the arena of sexual selection for a given species is most intense before or during courtship itself, male characters useful in premating interactions are predicted to be favored, as opposed to the case in which intermale competition occurs inside the female, where ejaculate characters useful in postmating competition would be exposed to selection.

Here I explore whether different "arenas" of sexual selection (prematuring vs. postmating) could account for the observations described above, namely that males of different *Drosophila* species tend to exhibit either external, sexually dimorphic traits or exaggerated ejaculates, but not both. First, I describe an approach to estimating the opportunity for premating versus postmating sexual selection by quantifying female remating, an important component of the OSR, in 21

TABLE 1. Female remating frequencies 24 h following initial mating. Females were assigned to rapid versus infrequent remating groups based upon whether more than half of females remate within a 24-h period. Sexual dimorphism for body size, expressed as the ratio of the female to male thorax length, is given in the last column.

Species	% Remating in 24 h (n matings in 24 h)	Remating category (rapid vs. infrequent)	Female/male thorax length
<i>D. nigrospiracula</i> ^a	100% (4)	rapid	1.12
<i>D. hydei</i> ^b	100% (4)	rapid	1.08
<i>D. montana</i> ^c	100% (3)	rapid	1.05
<i>D. littoralis</i> ^c	100% (3)	rapid	1.04
<i>D. mettleri</i> ^d	100% (1.5)	rapid	1.08
<i>D. recens</i> ^e	100% (1)	rapid	1.11
<i>D. guttifera</i> ^e	100% (1)	rapid	1.05
<i>D. nanoptera</i> ^{d,f}	0.96	rapid	1.09
<i>D. pachea</i> ^{d,f}	0.96	rapid	1.09
<i>D. mojavensis</i> ^d	0.95	rapid	1.08
<i>D. arizonae</i> ^d	0.95	rapid	1.08
<i>D. wassermani</i> ^{d,f}	0.68	rapid	1.08
<i>D. pseudoobscura</i> ^g	0.05	infrequent	1.08
<i>D. affinis</i> ^g	0.05	infrequent	1.17
<i>D. persimilis</i> ^g	0.05	infrequent	1.14
<i>D. melanogaster</i> ^h	0.02	infrequent	1.12
<i>D. simulans</i>	0.02	infrequent	1.11
<i>D. acanthoptera</i> ^{d,f}	0.0	infrequent	1.07
<i>D. subobscura</i> ⁱ	0.0	infrequent	1.14
<i>D. silvestris</i> ^j	0.0	infrequent	1.01
<i>D. heteroneura</i> ^j	0.0	infrequent	1.00

^a Markow 1988; ^b Markow 1985; ^c Aspi and Lankinen 1992; ^d Markow 1982; ^e Jaenike, pers. comm.; ^f Pitnick et al 1991; ^g Snook 1995; ^h Pyle and Gromko 1981; ⁱ Maynard Smith 1956; ^j Schwartz and Boake 1992; Wisotsky 1987.

Drosophila species. These particular 21 species were chosen because sufficient data are available for them from which to rank not only remating frequency, but also to categorize them according to both their internal and external male characters. I then used the resulting two groups of species, those with frequent versus infrequent female remating, to test the prediction that infrequent female remating, through increased competition for females, is associated with the presence of sexual dimorphism and external secondary sexual characters. I also tested the prediction that when females mate frequently, the arena for sexual selection moves to the female's reproductive tract where the evolution of unusual or exaggerated ejaculates is favored. The well-documented evolutionary relationships of *Drosophila* species permits these predictions to be examined in the context of their phylogenetic relationships.

METHODS

Female Remating Frequency

Table 1 presents the remating frequencies for females of 21 species reported by Markow (1996) for which other relevant mating system data also were available. In order to dichotomize remating frequency for subsequent analyses, the 21 species were divided into two groups: one in which at least half the females remate daily or more frequently and the other in which less than 50% of females remate within a day. The first group is designated as the "rapid remating" group, the second as the "infrequent remating" group.

External morphological characters.—Three aspects of external morphology were examined for sexual dimorphism:

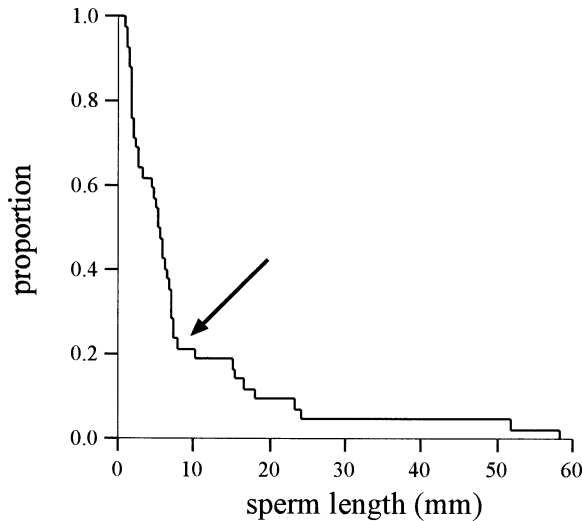


FIG. 1. Distribution of sperm lengths (from Pitnick et al. 1995b) for 42 species of *Drosophila*. Arrow indicates break in distribution at 6 mm in length of sperm.

body size, body color, and secondary sexual characters. *Drosophila* body size typically is measured by thorax length. The ratios of female to male thorax lengths, in mm (Markow 1996), for the 21 species are also given in Table 1. Species descriptions and visual inspection of live flies obtained from my own laboratory and from the *Drosophila* species stock center, located at that time at Bowling Green State University, Bowling Green, Ohio, were used in determining the presence of secondary sexual characters and for sex differences in body color.

Special behavioral tactics.—Two behaviors were identified as representing “special tactics” used in mating. These were the provision of a nuptial gift during courtship (Steele 1986a, b) and the forced copulation of teneral females (Markow 2000).

Ejaculate features.—The ejaculate is comprised of both sperm and seminal fluid. Here, the term “exaggerated ejaculate” is used to refer to cases of sperm gigantism (Pitnick et al. 1995a) and to cases in which radiolabeled amino acids or proteins are incorporated into developing oocytes by females (Pitnick et al. 1997). In the species examined here, sperm length range from 0.325 mm in *D. persimilis* (Snook 1997) to 58.29 mm in *D. bifurca*. To dichotomize sperm length for analysis, sperm lengths of all 42 species *Drosophila* species in which sperm have been measured (Pitnick et al. 1997) were plotted, and a natural break in the distribution was identified (Fig. 1). Thus, in the group of 21 species for which remating data are available, those that have sperm shorter than 6 mm were assigned to the “short” sperm group. The remainder was placed in the “gigantic” sperm group. With respect to the seminal fluid, species were easily assigned either to the “ejaculate donation” or “no ejaculate donation” group based upon previous reports (Pitnick et al. 1997; Markow and Ankney 1984, 1988) of the incorporation of male-derived amino acids into ovaries.

Phylogenetic analyses.—A number of sources were utilized in compiling the phylogeny. Morphological (Throckmorton 1975; Grimaldi 1992) and molecular (Beverly and Wilson

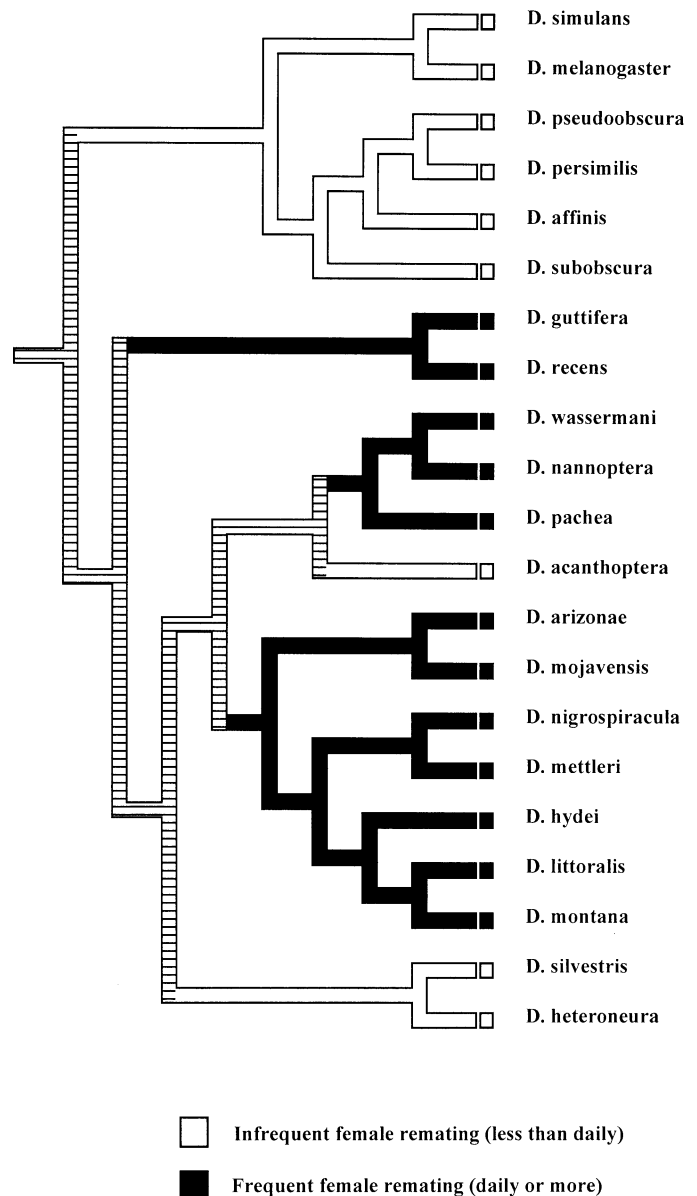


FIG. 2. Female remating mapped onto a phylogenetic tree of 21 *Drosophila* species. Females were assigned to one of two remating groups. In group one the majority of females do not remate for at least 24 h. In the second group, females remate at least every 24 h, as given in Table 1.

1982; Sullivan et al. 1990; Caccone et al. 1992; De Salle 1992; Pelandakis and Solinac 1993; Kwiatowski et al. 1994; Powell and DeSalle 1995) datasets were used to infer higher order relationships. Lower level relationships were reported previously (Pitnick et al. 1997, 1999). The concentrated changes test (Maddison 1990; Maddison and Maddison 1997) was used to examine whether female remating exhibited significant associations with external versus internal male traits.

RESULTS

Female Remating Frequency

When rapid female remating is mapped onto the phylogeny of the 21 species used in this study, it is clear that it is

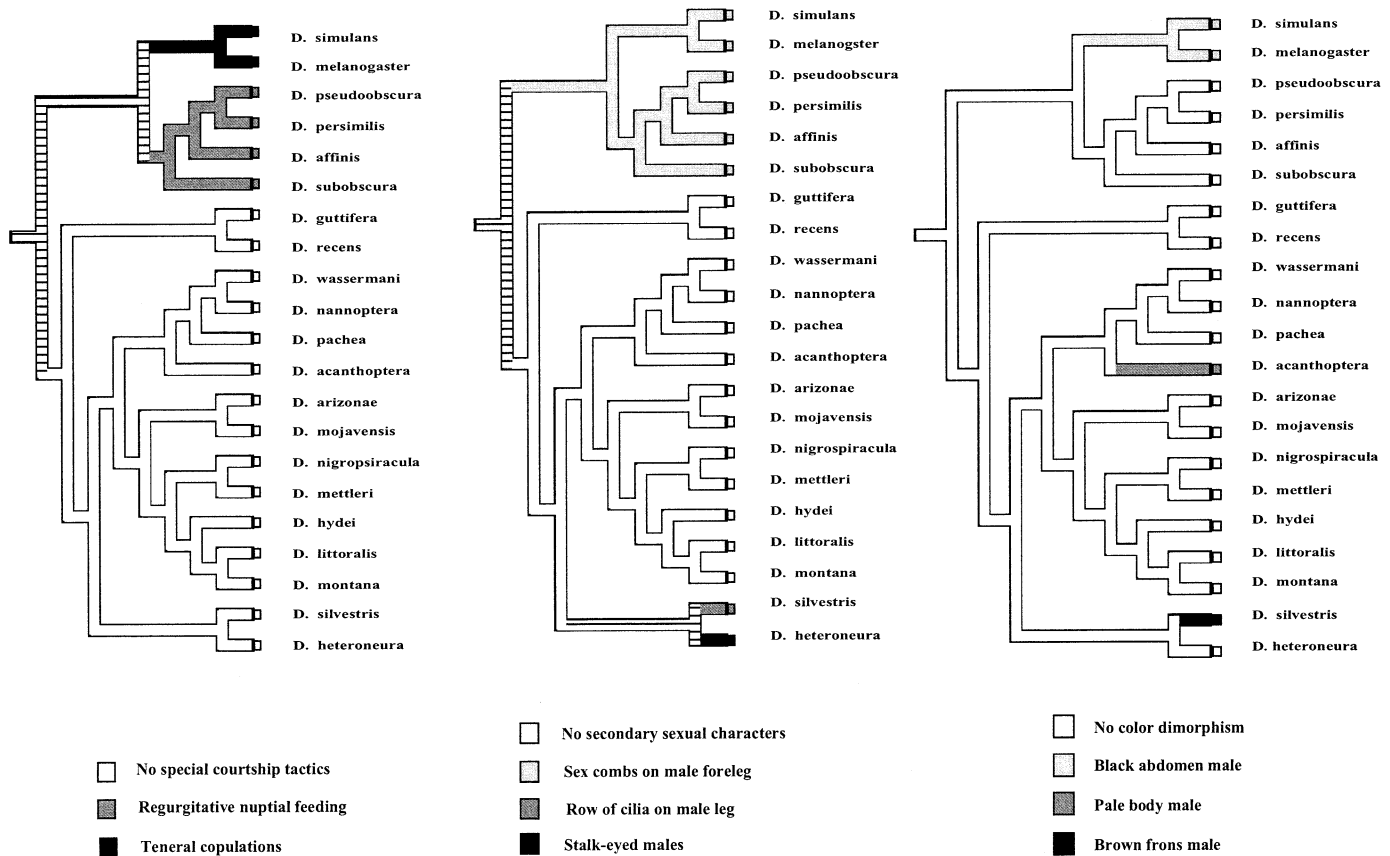


FIG. 3. Phylogenetic relationships of sexually selected external traits in *Drosophila* males. (a) Behavioral strategies: males of *D. pseudoobscura*, *D. persimilis*, *D. subobscura*, and *D. affinis* offer a regurgitative drop of food to females during courtship, whereas *D. melanogaster* and *D. simulans* males patrol emergence sites, mating with teneral females. (b) Secondary sexual traits: sex combs occur on the forelegs of male *D. melanogaster*, *D. simulans*, *D. pseudoobscura*, *D. persimilis*, *D. subobscura*, and *D. affinis*; *D. silvestris* males have a row of cilia on their tibiae; and *D. heteroneura* males have very broad heads, bordering on a stalk-eyed appearance. (c) Color dimorphism: males of *D. melanogaster* and *D. simulans* have black abdomens; males of *D. acanthoptera* have pale yellow bodies; and *D. silvestris* males have black frons. References for characters appear in text.

influenced by evolutionary history (Fig. 2). Species in the melanogaster and obscura groups remate infrequently, as do the Hawaiian species, *D. silvestris* and *D. heteroneura*. *Drosophila acanthoptera* is the only member of the nannoptera group in which females rarely or never remate.

External morphological characters.—Some slight degree of sexual dimorphism for size is usually observed. The ratio of female to male thorax lengths, in mm, taken from Markow (1996) were compared between species assigned to the high and low remating groups (Table 1). Pitnick et al. (1995b) showed previously that body size in these species is not constrained by phylogeny. Males tend to be smaller than females in most species for which data have been summarized. If infrequent female remating promotes sexual size dimorphism, the two groups are predicted to differ for this character. Although the sexual dimorphism for body size is greater in the infrequent remating, male-biased OSR group ($\bar{x} = 1.092 \pm 0.018$) compared to the frequent remating group ($\bar{x} = 1.079 \pm 0.007$), the difference is not significant ($t = -0.67$, $P = 0.51$).

Body color dimorphism, when it occurs, involves different characters in different species. Color dimorphism is shown in Figure 3a. In *D. melanogaster* and *D. simulans*, males have

black abdomens as opposed to the striped patterns seen in females. Color dimorphisms involving different characters in other species also are observed. For example, males of *D. acanthoptera*, a nannoptera group species, have pale translucent bodies compared to the pigmented females. Females of the Hawaiian *D. silvestris*, have yellow frons, compared to the black frons of conspecific males. Morphological dimorphisms also are found (Fig. 3b). Sex combs occur on the forelegs of male flies of the *Sophophoran* subgenus species belonging to the melanogaster and obscura groups (Markow et al. 1996). Among Hawaiian species, *D. silvestris* males have a row of cilia on the tibia of their forelegs, and males of *D. heteroneura*, its sibling species, have very broad heads, bordering on a stalk-eyed appearance (Carson et al. 1994).

Unusual behavioral tactics associated with mating are shown in Figure 3c. Males of several species of the obscura group regurgitate a nutritive drop that is consumed by females during courtship (Steele 1986a, b), and in *D. subobscura*, males offering such a drop enjoy a mating advantage. In *D. melanogaster* and *D. simulans* (Markow 2000), males patrol emergence sites, mating with teneral females incapable of rejecting them.

When all male sexually selected traits are considered and

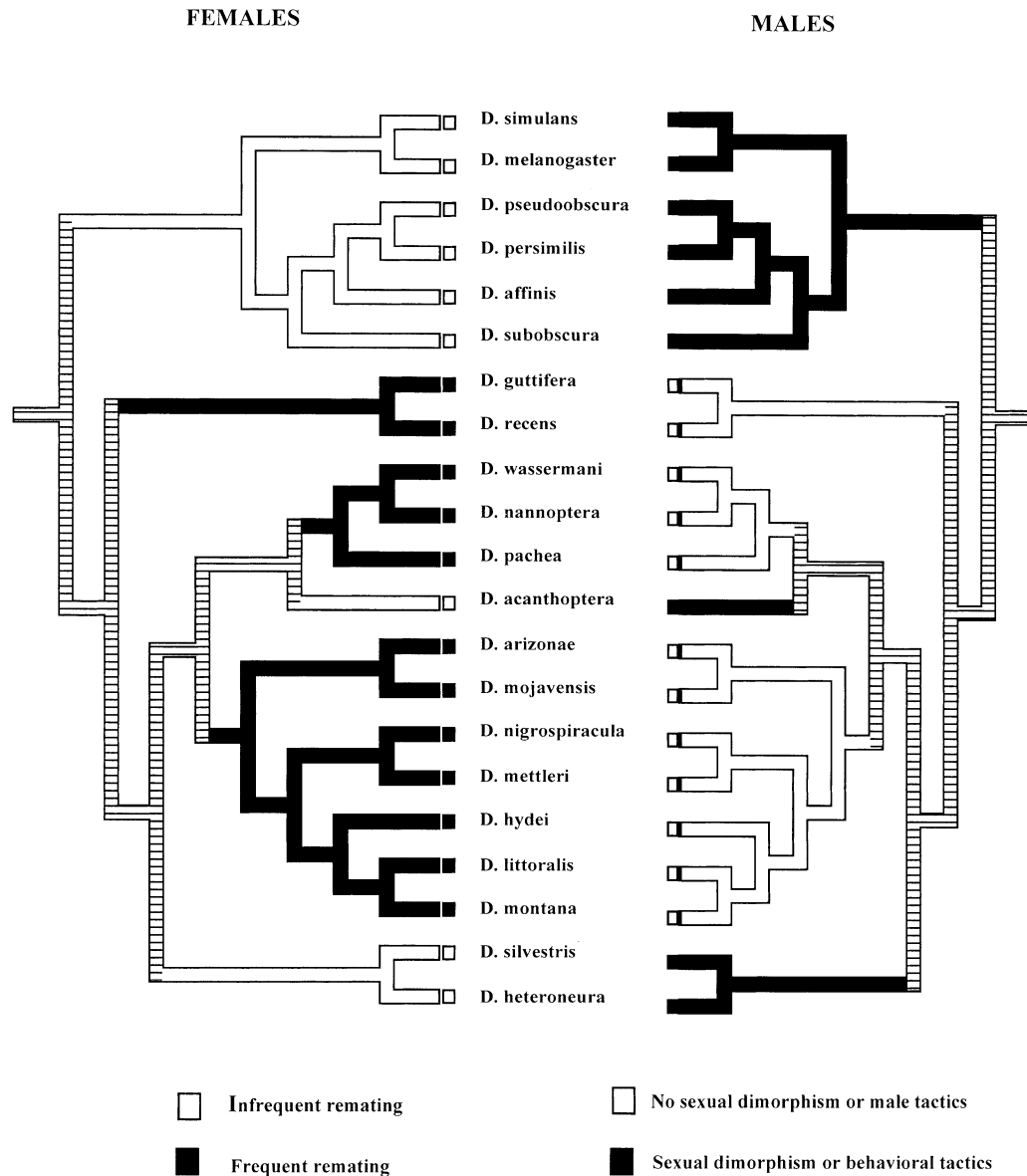


FIG. 4. A phylogenetic comparison of female remating frequency as a measure of operational sex ratio with the presence or absence of external secondary sexual characters or sexual dimorphism in conspecific males.

mapped onto the phylogeny as present or absent, the relationship between male characters and female remating is highly congruent (Fig. 4). The concentrated-changes test (Maddison 1990; Maddison and Maddison 1997) demonstrates the statistical significance ($P < 0.002$) of this association. Male secondary sexual characters appear in only those species in which females remating is less frequent and thus male mating opportunities more limited. The fact that a variety of male characters are represented here, arising independently and probably at different times, suggests that female remating frequency may have changed first and that males of different species responded to the change in different ways.

Male ejaculatory characters.—The distribution of sperm gigantism in the 21 species is shown in Figure 5a, while species in which male donation to oogenesis is observed are

shown in Figure 5b. A comparison of the two figures shows that sperm gigantism and ejaculate donation tend not to have evolved in the same species. More frequent female remating is expected to create a situation in which male-male competition is more intense at the ejaculate rather than at the population level. To ask whether the ejaculate traits, sperm gigantism or ejaculate donation, are associated with frequent female remating, the two male traits are considered together as either the presence or absence of “exaggerated” ejaculates and examined in the context of female remating rate (Fig. 6). The congruence is quite striking, such that unusual ejaculate traits appear only in species in which females remate frequently. The significant outcome ($P < 0.016$) of the concentrated-changes test (Maddison 1990; Maddison and Maddison 1997) suggests that the occurrence of these two sets of characters, female remating frequency and exaggerated

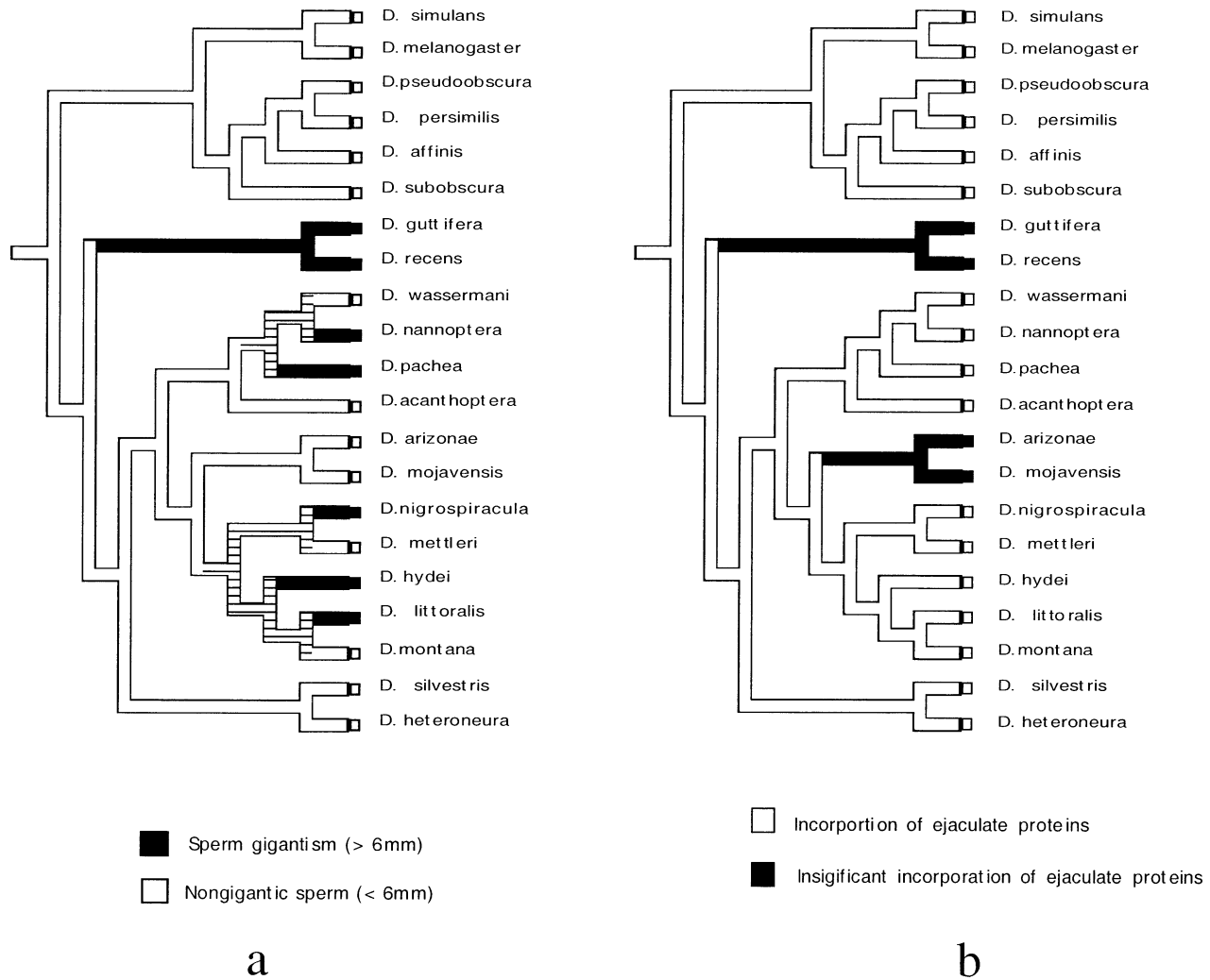


FIG. 5. Phylogenetic mapping of (a) sperm length categories (from Pitnick et al. 1995b), and (b) of ejaculate donation categories (from Pitnick et al. 1997).

ejaculates, are highly correlated. In *D. mettleri* (Markow and Ankney 1988; Pitnick et al. 1997) while that male-derived ejaculate materials may not appear in significant levels in ovarian tissue, they are incorporated into female somatic tissue. Thus *D. mettleri* could be included among those species with exaggerated ejaculates, making for an even closer association with female remating. It is at this level, of the ejaculate components, where we expect to see the evolution of internal morphological and physiological strategies that enhance reproductive success.

DISCUSSION

Clearly, the discrete categories used here have been imposed upon continuously varying traits. The associations are quite striking, however, and would be present to a significant degree, even if the categorical boundaries were drawn somewhat differently. A strong distinction exists between species with infrequent versus frequent female remating with respect to type of male characters, external or internal, that have responded to sexual selection. Many external male characters presumed to have evolved in response to sexual selection are considered

“ornaments.” The association reported here, between the arena of sexual selection and the type of male character under selection, raises the question of whether exaggerated ejaculate characters, such as giant sperm, also can be considered to be “ornaments.” If so, species in which males exhibit external ornaments or external sexual dimorphism do not, for the most part, have ornamented ejaculates, consistent with the idea that these two kinds of traits have evolved in different selective “arenas.”

The arenas of sexual selection identified here are not intended to imply that sexual selection acts exclusively on external versus internal male characters, depending upon the female remating frequencies of particular species. Arenas of sexual selection, as used here, may more accurately be considered as primary arenas. It would be unimaginable to assume, for example, that in species with considerable male mating opportunities, competition for females would be nonexistent, especially given temporal and spatial variation in operational sex ratios (Pitnick 1993). Furthermore, in species like *D. melanogaster*, where pre-mating sexual selection is intense, female remating certainly occurs and there is evidence of genotype dependence in ejaculate competitive abilities (Clark et al. 1995).

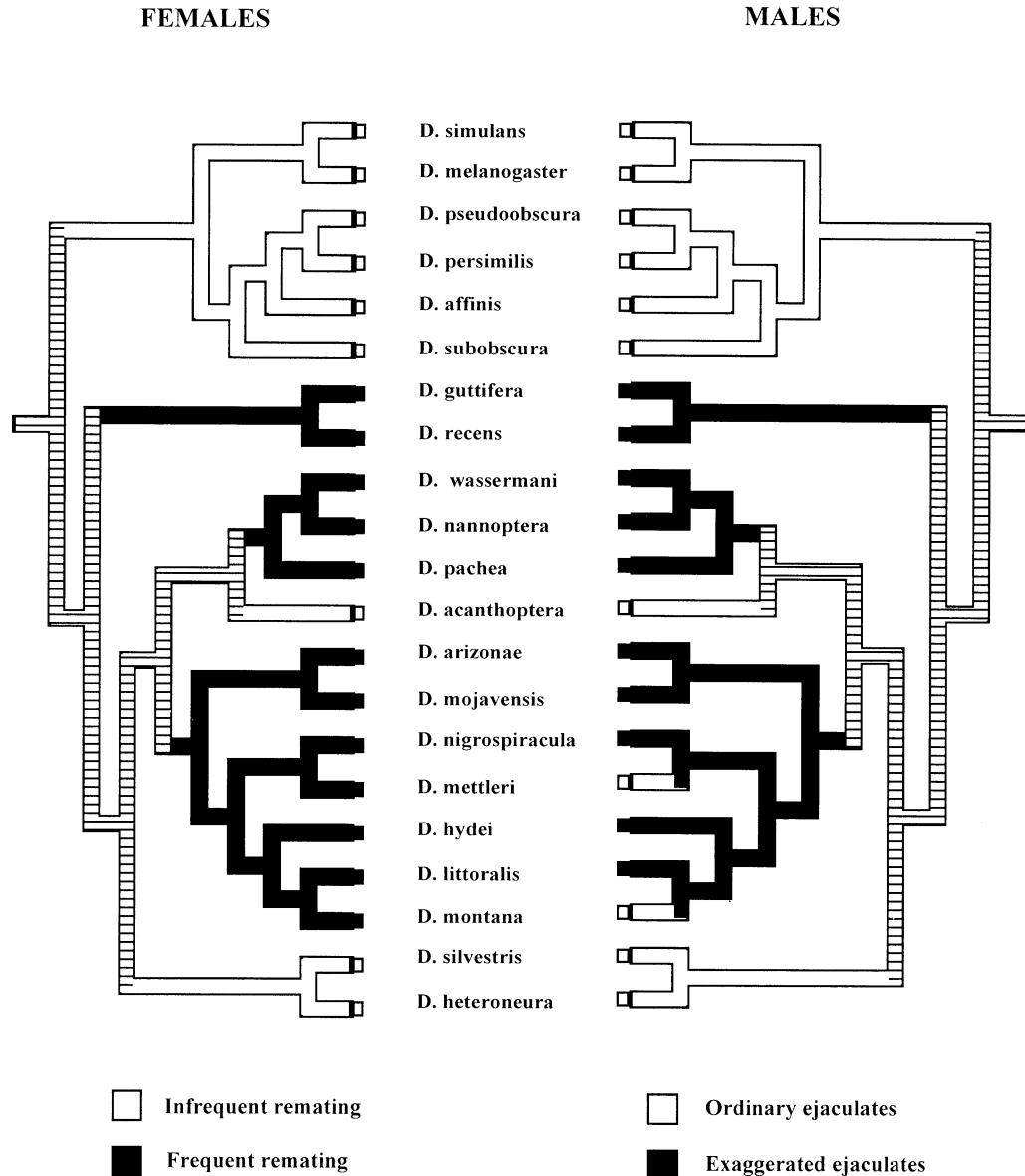


FIG. 6. A phylogenetic comparison of female remating frequency with the occurrence of exaggerated male ejaculate traits.

Morphological characters are not the only ones that potentially exhibit sexual dimorphism. Chemical traits, especially epicuticular hydrocarbons which serve as pheromones in some species, may be sexually dimorphic. Data are available for an insufficient number of species, however, to enable inclusion of chemical signals in this study. Interestingly, however, existing data are consistent with the patterns seen for morphological traits. For example, sexually dimorphic hydrocarbon molecules are found for species of the *melanogaster* (Ferveur 1997) and *obscura* groups (Noor and Coyne 1996) in which females mate less frequently. On the other hand, little or no hydrocarbon dimorphism is observed in a subset of species in which females remate frequently and in which males also exhibit no morphological or color dimorphism (Markow and Toolson 1990; Bartelt et al. 1986, 1989; Jackson and Bartelt 1986).

Female Remating, the Operational Sex Ratio, and the Primary Arenas of Sexual Selection

The OSR, or the number of receptive females relative to the number of sexually mature males, is a more general parameter assumed to underlie the intensity of sexual selection (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). Because the number of receptive females is a key component of the OSR, the frequency at which females of a given species remate will strongly influence male mating opportunities as well as the degree of overlap of ejaculates in the female reproductive tract, and, subsequently the primary arena of sexual selection.

Under highly male-biased OSRs, competition for access to receptive females is intense. Where there is strong competition for females, we expect to see the evolution of external

TABLE 2. Expected mating system characteristics under conditions ranging from male-biased to female-biased operational sex ratios.

Character	Relative degree of expression	
	Male-biased OSR	Female-biased OSR
Female remating	infrequent	frequent
Male age at maturity	before females	after females
Sexual dimorphism	present	absent
Male 2° sexual characters	present	absent
Special behavioral tactics	present	absent
Sperm gigantism	absent	present
Unusual ejaculates	absent	present
Sexual selection arena	pre mating	post mating

male characters, morphological and behavioral, that enhance the likelihood of obtaining mates. When mating opportunities are less limited, competition for mates will be less intense and selection for morphological and behavioral characters enhancing mating success should be weaker.

In addition to female mating frequency, other factors undoubtedly influence the OSR as well. Relative ages at which conspecific males and females attain sexual maturity show striking interspecific variation. In 31% of 42 species examined, males mature earlier than females, sometimes within hours of emergence, whereas in 60% of species, males mature later (Markow 1996). For a number of species, delayed male maturity is highly exaggerated. In the most extreme case, *D. kanekoi* males become sexually mature at 19 days of age as opposed to four days in females, a fourfold increase during which males may experience predation, desiccation, parasitism, etc., and thus ultimately a reduction in the actual number of sexually mature males. On the other hand, in the Hawaiian species, *D. heteroneura* and *D. silvestris*, females mature weeks later than males. Early male maturity serves to increase male bias in OSR because more sexually mature males are present to compete for fewer receptive females. Delayed male maturity operates to reduce the OSR, making it less male biased, or even, as in the case of *D. pachea*, female biased (Pitnick 1993). Evolutionary factors underlying interspecific variation in male maturation rate may or may not be correlated with either age of female maturity or with female remating rates.

The foregoing relationships among mating system characters and their predictions for sexual selection are summarized in Table 2.

The interaction between female remating rates and male maturation age can create a dramatic range in the OSR. For example, in *D. hydei* and *D. pachea* males mature seven and eleven days, respectively, after conspecific females. At the opposite extreme are *D. heteroneura* and *D. silvestris* and, to a lesser degree, *D. subobscura* and *D. melanogaster*, in which males mature earlier than females and female remating is absent or limited. As predicted, it is in these latter species where external male sexual traits are the most obvious, and in the former species, where ejaculate traits such as sperm gigantism have reached their most extreme expression.

Those cases in which field data on mating frequencies are available support the proposed relationships among female remating frequency, the OSR, and the primary arena of sexual selection. For example, Pitnick (1993) was able to determine

directly the OSR in natural populations of *D. pachea* by placing field females or males with laboratory-reared sexually mature virgins of the opposite sex and scoring the sexual maturity or receptivity of wild caught males and females, respectively. Thus, field observations are consistent with the laboratory conclusion that this species has a less male-biased OSR, and at times is even female biased, compared to more strongly male-biased OSR species, in which females mate less frequently. In other cases, frequencies at which mating pairs are observed, relative to the total number of individuals at a typical mating site, can be used to infer mating opportunities or OSR. Thus for *D. melanogaster*, which occurs in large numbers on rotting fruits and where males are constantly courting, copulating pairs are observed only infrequently (Partridge et al. 1987; Gromko and Markow 1993), consistent with the assignment of this species to the infrequent remating group. In contrast, other cactophilic flies, such as the rapidly remating *D. nigrospiracula*, a high proportion of flies at a necrotic patch are found to be *in copula* (Markow 1988), consistent with a less strong male bias in their OSR. For the Hawaiian species of *Drosophila*, matings have not been observed in nature (Herman Spieth, pers. comm.), suggesting that they are rare events. Laboratory data suggesting low remating by *D. silvestris* and *D. heteroneura* females are consistent with observations on females from natural populations (Craddock and Johnson 1978).

A broad question of long-term interest is how to explain variation in female remating frequency (Arnold and Halliday 1992). The observation that sperm gigantism and ejaculate donations appear in several different and unrelated lineages each suggests that female remating frequency changed prior to the evolution of exaggerated ejaculates. Although this pattern appears robust, it does not suggest any specific processes by which these different evolutionary trajectories were favored. Many factors could have contributed to an initial increase in female remating. Females of certain species may have developed resistance to the proteins in the ejaculate that initially suppressed remating (Wolfner 1997), creating the need for males to respond directly to interejaculate competition rather than prevent it. Sperm limitation may also have been responsible in certain cases (Pitnick 1993). It is possible that depending upon oviposition site availability in nature, females may have needed to remate frequently to maintain a viable sperm supply. Limitation of compatible sperm is another factor. In two species, *D. mojavensis* and *D. nigrospiracula*, females tend not to use sperm of related males to fertilize their eggs (Markow 1982, 1997). In some species, especially at certain times of the year, population sizes are small and the potential for inbreeding high. Remating increases the likelihood of receiving genetically different sperm and females may have evolved a means to discount sperm based upon close relatedness.

An intriguing question is whether pre mating reproductive isolating mechanisms evolve more rapidly or are more important in species with infrequent female mating, as opposed to post mating-prezygotic mechanisms in species with more frequent female remating. For example, secondary sexual characters such as sex combs show clear differences between sister species. If secondary sexual characters vary considerably between closely related species, owing to different

selective trajectories, they are available to serve in premating species recognition. On the other hand, in sibling species lacking obvious external differences between males, weaker premating isolating barriers may create stronger selection for postmating-prezygotic isolating mechanisms (Knowles and Markow 2001). These are not issues that the present study was designed to address, although some obvious questions with testable predictions can be generated. For example, is divergence in ejaculate features such as sperm length or accessory gland proteins nonsynonymous substitution rate greater between sibling species in which the primary arena of male-male competition is inside the female reproductive tract? Nonsperm ejaculate components, especially proteins, have been extensively examined in *D. melanogaster*. Produced by the accessory glands and ejaculatory bulb, approximately 100 proteins are transferred to females at mating (Wolfner 1997). Several of these have been directly characterized with respect to their fate and function and more than one protein has been found to stimulate oviposition and decrease female receptivity to remating (Wolfner 1997). In *D. melanogaster*, and its sibling species *D. simulans*, these proteins are quite variable relative to others (Coulthart and Singh 1988) and show high divergence among related species that has been attributed to positive selection (Swanson et al. 2001; Begun et al. 2000; Tsaour et al. 1998; Thomas and Singh 1992). Furthermore, Clark et al. (1995) provide evidence that variability in these proteins plays a role in sperm displacement and resistance to displacement in multiply mated females.

Finally, the observations on *Drosophila* reported here suggest that additional taxa be examined for similar patterns. Other insect genera exhibit interspecific variation in the presence of external male sexual dimorphism. In certain groups of these insects, such as dung beetles in the genus *Onthophagus* (Emlen 1997; Emlen and Nijhout 1999), Dytiscid diving beetles (Bergsten et al. 2001), and Diopsid stalk-eyed flies (Baker and Wilkinson 2001), it is likely that enough of the morphological and mating system measures could be obtained to permit detection of similar patterns, if they exist.

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