

Sexual selection and the evolution of mating systems in flies

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I. Introduction

Flies are a model group for the study of mating systems because of their extensive evolutionary diversification and ecological variation (see Yeates and Wiegmann, this volume). For example, adults of some species feed on nectar, pollen or other exudates, others suck blood from vertebrate hosts or are exclusively predaceous, and a few are even kleptoparasitic (Sivinski 1999). Larvae of many species are detritivores in semiaquatic or aquatic environments, but parasitic larval forms have also evolved repeatedly, as have plant-feeding forms. Based on classical mating system theory (Emlen & Oring 1977), one would expect that the ecological diversity exhibited by adult flies would influence the form of the mating system. As will become clear from this chapter, to a large extent this prediction is supported. Second, many species of flies in two families, Tephritidae and Drosophilidae, have received extensive study due to their economic importance and use as model organisms respectively (we reworded this, Drosophilidae have no medical or economic importance). The scope, detail and diversity of studies on these groups provide a fertile ground to identify patterns and establish causal mechanisms. Finally, due in part to the development of *Drosophila melanogaster* as a model system for the study of genetics, Mendelian markers have been available for many years and have been used to quantify parentage and sperm precedence. The combination of genetically confirmed parentage with detailed anatomical, physiological and molecular studies on the factors influencing fertilization success have allowed for unprecedented insight into the mechanisms of sperm competition in *Drosophila* and a few other species of flies.

In this review we adopt the perspective that an animal's mating system encompasses all activities of males, females and their gametes that influence zygote formation. Thus, after briefly reviewing recent theoretical work on sexual selection, we have organized this chapter according to the events that transpire before, during or after mating that influence fertilization success. We divide these events into four categories: obtaining mates, precopulatory activity, copulation, and postcopulatory activity. We further assume that if variation in morphological, behavioral, physiological, or biochemical factors influences fertilization success, then sexual selection will

occur and, if genetic variation is present, result in evolutionary change. Throughout the chapter we use this evolutionary logic to interpret variation within and among species of flies.

Our goal in this chapter is to review what is known about the mating systems of flies and identify where additional study would be particularly fruitful to advance mating system theory. We also consider the degree to which ecological factors have influenced pre- and post-copulatory activity by identifying traits that have likely undergone rapid evolutionary change. Ideally, this chapter would contain a number of comparative analyses in which, for example, the evolution of mating systems and related traits were inferred from parsimonious reconstruction of character change on a phylogenetic tree of all Diptera. We could then test the role of ecology and other factors by examining the significance of correlated change among phylogenetically independent contrasts (Felsenstein 1985). Unfortunately, relationships among many groups of flies remain controversial (Yeates & Wiegmann 1999), so such analyses across all Diptera would be premature. However, to facilitate discussion of possible evolutionary patterns, we use lower and higher Diptera to indicate the groups of families conventionally referred to as the Nematocera and Brachycera, respectively (Yeates & Wiegmann 1999). Moreover, we do discuss and present trait evolution for a few groups of flies where phylogenetic information is available.

II, Sexual selection and sexual conflict

One of the earliest experimental studies demonstrating the process of sexual selection was conducted using flies. By combining three males and three females each with recognizable dominant mutations into replicate vials, Bateman (1948) showed that the variance in reproductive success among male *Drosophila melanogaster* was greater than among females. He inferred from these results that traits which enhance male mating ability will be favored by sexual selection. In contrast, reproductive success of females is limited more by egg production than by the number of mates. Thus in flies, as well as in other animals, male and female reproductive interests rarely coincide because relative investment in gamete size and number differ (Trivers 1972). When males produce many small gametes, they are expected to expend

energy searching and competing for mates. In contrast, females that produce few large gametes are expected to base mating decisions on their ability to acquire resources and produce offspring. These ideas have been used successfully to explain how variation in the distribution of resources influences the distribution of females, which in turn influence how males distribute themselves to obtain matings, both among animals in general (Emlen & Oring 1977) and insects in particular (Parker 1978).

Sexual selection can lead to the evolution of sexual dimorphism if a trait influences a male's ability to acquire and defend resources or to attract females. For example, males often have larger bodies than females when there is resource defense (e.g. Borgia 1980; Dodson 1986; Day et al. 1987; Ding & Blanckenhorn 2002). Sexually dimorphic traits also can be the result of sexual selection acting via female choice. While females may prefer larger males (Gilburn et al. 1992) or males with head modifications that facilitate fighting or assessment (Wilkinson & Dodson 1997), females of some species choose males on the basis of traits not associated with fighting. In flies, such traits include food, pheromones, courtship behaviors, or morphological features. For example, the midlegs of the culicid mosquito, *Sabethes cyaneus*, form large, iridescent paddles that males wave while hanging to attract females (Hancock et al. 1990).

Considerable controversy revolves around what females gain from choosing males. In some cases, benefits are direct because female fecundity is enhanced. For example, in several dance fly species (family Empididae) females choose males that offer them prey (Cumming 1994). In many other cases, however, females receive only sperm from their mates so benefits can only be indirect in the sense that offspring, rather than the female, benefit. One possibility is that offspring of choosy females simply inherit genes for attractive male traits and for preferences. Such a scenario leads to rapid coevolution of trait and preference in a runaway process until natural selection acts against the ornament (Fisher 1958; Lande 1981; Kirkpatrick 1982). Alternatively, offspring might inherit genetic factors that increase their survival (Pomiankowski 1988) or otherwise enhance the propagation of their genes, such as by distorting the sex ratio (Lande & Wilkinson 1999). Distinguishing between direct and indirect benefits (Tregenza &

Wedell 2000; Bussiere 2002; Wedell et al. 2002), as well as between runaway and good genes models of sexual selection (e.g. Jones et al. 1998; Wilkinson et al. 1998; Gilburn & Day 1999), is an especially active area of research for which many species of flies undoubtedly will continue to provide valuable information.

Sexual conflict is exemplified not only by differences in courtship and mating behavior between males and females but also can occur after the termination of copulation (Alexander et al. 1997; Partridge & Hurst 1998; Birkhead & Pizzari 2002). For example, studies on *Drosophila melanogaster* have shown that males may attempt to influence female fertilization and oviposition decisions by transferring small peptides, referred to as accessory proteins, during mating. These proteins typically decrease female receptivity and increase egg-laying rate (Simmons 2001), but also can reduce female lifespan (Chapman et al. 1995) and alter the relative fertilizing ability of sperm (Wolfner 1997; Price et al. 1999; Chapman 2001). These interactions are not confined to *Drosophila* (Chapman et al. 1998), and have been reported for many species of Diptera (reviewed in Simmons 2001).

Sexual conflict is important because it can lead to a dynamic evolutionary process in which each player is continually under selection to compete with an opponent. Such an arms race need not lead to a static equilibrium, as predicted by early game theory models of mate searching (Parker 1978). Instead, male and female traits should exhibit correlated patterns of evolution, as have been observed across species for sperm length and size of female sperm storage organs in *Drosophila* (Pitnick et al. 1999) and Diopsid stalk-eyed flies (Presgraves et al. 1999) and between testes size and female accessory gland size in yellow dung flies, *Scatophaga stercoraria* (Hosken et al. 2001). Similarly, the rapid molecular evolution of accessory protein genes among *Drosophila* species is best explained by positive selection mediated by sexual conflict (Begun et al. 2000). Evolutionary change in male accessory proteins can also occur within species, as has been demonstrated by experiments using *Drosophila melanogaster* in which chromosomes have been allowed to undergo evolution only in males (Rice 1996).

It is important to realize, though, that sexual conflict is not inevitable. Sexual conflict is not expected when the reproductive interests of males and females coincide. Among vertebrates, sexual conflict is not expected in genetically monogamous species with biparental care because investment by both the male and the female is necessary for successful reproduction. Such a mating system is unknown for flies. However, sexual conflict would also not be expected in species with very short adult life spans if males and females only mate a single time. Some chironomid midges, for example, may approach this situation (Armitage 1995).

III. Obtaining a mate

As a consequence of low expenditure on gametes, males are expected to maximize encounter rates with females by searching, displaying or defending resources (Parker 1978). Among flies, males may wait for females to visit them in an aerial swarm or while they display from a substrate, which may or may not be near a feeding or oviposition site. In some species, males search for eclosing females and inseminate them immediately after they emerge from their puparia. Cross species comparisons suggest that the encounter site convention adopted by a species is strongly influenced by the distribution of defensible resources required by females. How males encounter females influences, in turn, the degree to which males fight and exhibit courtship behavior. Below we describe these patterns and discuss how sexual selection has influenced the morphology and behavior of males and females that adopt each encounter site convention.

A. Aerial swarming

Aerial swarms are the most common mating site convention in the lower Diptera (Downes 1969; Sullivan 1980; Sivinski & Petersson 1997), but are also found in species throughout the higher Diptera (Table 1). This phylogenetic distribution is consistent with aerial swarming being the ancestral mating system of the Diptera. Mating swarms typically contain 10 to 1000 individuals (Sullivan 1980; Shelly & Whittier 1997) which hover within a few cubic meters over a

conspicuous landmark, such as a ridge, hill, tree top, fence post, church steeple, or even plume of smoke (Chandler 2001). One species of chironomid midge, *Abiskomyia virgo*, even swarms on the surface of the ocean (Armitage 1995). Aerial swarms usually consist predominantly of males, although there are a few noteworthy cases in which swarm composition is strongly female-biased, e.g. the chironomid *Palpomyia brachialis* (Sivinski & Petersson 1997), the phorid *Megaselia aurea* (Sivinski 1988) and several species of empidid dance flies, genus *Rhamphomyia* (Cumming 1994; Funk & Tallamy 2000).

Aerial swarming is thought to be the most efficient strategy for obtaining a mate when the distribution of resources is homogeneous or unpredictable (Sivinski & Petersson 1997) or when population density is low (Kon 1987). In either case, males cannot reliably encounter females by defending or waiting near resources. Instead, they aggregate near conspicuous features at locations where females pass. Swarm sites, such as hilltops, sunbeams, or branch tips, have been hypothesized to be landmarks used for navigation (Sullivan 1980). Females appear to have limited opportunity to select males within a swarm and most species only mate once. Male courtship behavior has not been reported for any swarming species. Rather than display, males quickly converge on any approaching target that resembles a female. In some lower Diptera, males detect and respond to the frequency of wing vibration produced by females (Wishart & Riordan 1959; Armitage 1995). Copulation occurs in the air in some species while others copulate on the ground after males intercept females (Armitage 1995; Chandler 2001). Those that copulate in the air transfer sperm rapidly in a few seconds while those that copulate on the ground may take several minutes or in the case of the lovebug, *Plecia nearctica*, up to 3 days (Thornhill 1976; Hieber & Cohen 1983).

Aerial swarms are well known to attract predators, such as dragonflies, nighthawks and bats (Sullivan 1980). Consequently, the risks associated with revisiting a swarm likely outweigh the benefits to females of remating. Moreover, a short life span, low density, and weak flying ability all favor mating in the first swarm encountered. If predators do not recruit to swarms and they do not last long, then the per capita risk of predation should decline with swarm size as a

consequence of predator dilution. Consequently, females should prefer to mate in larger swarms, which has been observed in *Chironomus plumosus* (Neems et al. 1992) and in *Empis borealis* (Svensson & Petersson 1992).

In many swarm forming species the morphology of the compound eye exhibits sexual dimorphism. The eyes of males are typically larger than those of females and converge or join at the top of the head (Wenk 1987). Furthermore, the size of the ommatidia on the dorsal surface of male eyes is often larger and the rhabdomere is longer, which permits increased resolution and light sensitivity (Kirschfeld & Wenk 1976). These modifications allow males to detect moving objects flying overhead at greater distances than females (Zeil 1983). Body size typically does not differ between males that do or do not acquire mates, although in the b
ibionid lovebug, *Plecia nearctica*, larger males hover lower in the swarm and are first to intercept and mate with newly eclosed females that enter the swarm (Thornhill 1980). Some investigators have also argued that successful males are more maneuverable and therefore smaller males are more successful than larger males (McLachlan & Allen 1987). Males of species of *Calotarsa*, family Platypezidae, have highly enlarged hind tarsi with silver markings that flash in the sunlight, presumably to attract females (Sivinski 1997; Chandler 2001).

Female-biased aggregations occur in species that exhibit sex role reversal and reversed sexual dimorphism. For example, in the empidid *Rhamphomyia longicauda* females have larger wings, inflatable sacs on the abdomen and pinnate scales along the lateral margins of their hind legs. These traits give a flying female a saucer appearance. In this species, males capture and carry prey to a female swarm at dusk, approach a female, and exchange the prey item while copulating in the air. In this and related empidids, males provide the only protein source for adult females (Cumming 1994). Experiments with model females show that males preferentially approach females with larger abdomens (Funk & Tallamy 2000). Because abdomen size predicts egg size in a species without eversible sacs, but not in *R. longicauda*, Funk and Tallamy (2000) argue that inflated abdomens of female *R. longicauda* represents a deceptive signal to males. Alternatively, this modified morphology could decrease the energetic cost of hovering flight.

Sex role reversal has also been reported for *Empis borealis*. In this species, the distal margins of female wings are expanded and males prefer to mate with the largest females (Svensson et al. 1989), which is consistent with the flight performance hypothesis.

B. Mate searching

Another common method of locating mates is for males to search for females at potential encounter sites resulting in scramble competition polygyny. This mate searching strategy is typical of species where males mate with recently eclosed females, which is often associated with 1) harsh environments not conducive to aerial swarming, 2) early receptivity of females, and 3) either single mating or first male sperm precedence (Thornhill & Alcock 1983). These situations do not favor high overlap of female home ranges (Wickman & Rutowski 1999). For example, male Himalayan wingless glacier midges search for females near emergence sites and then mate on the snow (Kohshima 1984). Similarly, males in the chironomid midge, *Diamesa japonica*, mate with newly emerged females on the surface of fast-flowing streams (Kon 1987). In the marine genus *Clunio*, males skate over intertidal pools searching for wingless, vermiform females. Copulation occurs either on shore at low tide or on the water surface (Armitage 1995). In two species of culicid mosquitos that either develop in crab holes, *Deinocerites cancer*, or rock pools, *Opifex fuscus*, males search the water surface for pupae containing females, grab them by a pupal horn, and hold them until eclosion in order to mate with them upon emergence (Provost & Haeger 1967). In the phorid fly, *Puliciphora borinquensis*, males search for and mate with wingless females (Miller 1984). In the parasitic streblid fly, *Ascodipteron jonesi*, adult females embed into the wings of bats, lose all appendages, undergo tremendous abdominal swelling and then drop mature larvae to the floor of a cave (Oldroyd 1964). Presumably, males, which retain wings and legs, search out females for mating.

Males search for females when females must visit an unpredictable or mobile food source. For example, in seaweed flies, e.g. *Coelopa frigida*, males search for females on wracks of seaweed that have recently washed ashore. Males are larger than females due to the ability of

larger individuals to withstand female rejection attempts and resist takeovers by smaller males (Day & Gilburn 1997). Similarly, in many species of flies in which females feed on blood, males will follow potential vertebrate hosts and intercept females coming for a blood meal. Such mate finding behavior has been reported for some ceratopogonid midges (Wirth 1952), *Aedes* mosquitoes (Downes 1958), several species of blackflies (Wenk 1987), several species of hippoboscid flies (Bequaert 1953), and tsetse flies, *Glossina* (Wall & Langley 1993; Leak 1999). In tsetse flies, females can mate multiple times (Dame & Ford 1968). Similarly, males of some parasitoid phorid flies, *Megaselia* spp. and *Phalacrotophora* spp. approach and mate with females just before they oviposit into beetle pupae or prepupae (Disney 1994).

Species in which males wait, but do not fight, for females typically exhibit reverse sexual dimorphism, i.e. females are larger than males. As female fecundity is usually a function of adult body size, larger females are able to produce more offspring than smaller females. In some species, males emit signals to attract females. For example, in the gall-forming chloropid fly, *Lipara lucens*, males search for dispersed females on reeds and emit vibrations when they land. Receptive females respond by vibrating (Mook & Bruggemann 1968).

C. Resource defense

Resources that are predictable and sufficiently uncommon that they attract multiple females, such as oviposition or feeding sites, provide another location for mating encounters to occur. Typically, females will mate multiple times and exhibit last male sperm precedence (Simmons 2001). Mating occurs at oviposition sites in many resource defense systems (Table 1), such as the dung fly, *Scatophaga stercoraria* (Parker 1970a), several species of antler flies, *Phytalmia* spp. (Dodson 1997), the moose antler fly, *Protophila litigata* (Bonduriansky & Brooks 1999b), the neriid cactus fly, *Odontoloxozus longicornis* (Mangan 1979), many fungi feeding *Drosophila*, such as *D. melanderi* (Spieth & Heed 1975), and some syrphid flies, such as *Somula decora* (Maier & Waldbauer 1979). Mating occurs at feeding sites in several species of blackflies where males wait on flowers for females to visit (Wenk 1987) and in many species of *Drosophila*

(Markow 1996). In the diopsids, *Cyrtodiopsis whitei* (Lorch et al. 1993) and *C. dalmanni* (Wilkinson & Reillo 1994), mating occurs primarily at nocturnal roosting sites which occur on rootlets underneath stream embankments.

Resource defense mating systems are typically associated with little or no precopulatory courtship activity (Prokopy 1980; Burk 1981). Where male signalling occurs, it often consists solely of repetitive actions in a single sensory modality. Examples of this type of behavior include waving patterned wings in tephritid flies (Burk 1981) and producing wing vibrations in *Drosophila* (Ewing 1977). Resource defense mating systems are also often associated with male fighting. Among *Rhagoletis* fruit flies, males defend food or oviposition sites by wing-waving, charging, foreleg kicking, and boxing (Prokopy & Papaj 2000). One consequence of such male-male competition is that sexual selection favors larger male body size. Larger males typically monopolize territorial sites (Borgia 1980; Wilkinson & Dodson 1997). Moreover, in flies that exhibit resource defense mating systems, males often have larger body sizes than females (Dodson 1986; Dodson 1987; Alcock 1990).

Males of some species that engage in resource defense also possess conspicuous sexually dimorphic structures. Such traits include eye stalks, enlarged mouthparts or bristles on the head, and antler-like projections of the head (Grimaldi & Fenster 1989; Sivinski & Dodson 1992; Sivinski 1997; Wilkinson & Dodson 1997; Dodson 2000; Han 2000). In most cases, flies use these projections in direct male combat (McAlpine 1975; McAlpine 1979; Dodson 1987; Burla 1990; Dodson 2000; Han 2000). For example, in *Phytalmia mouldsi*. (Dodson 1997) males guard scattered oviposition sites and compete with each other by engaging in pushing matches using head projections. However, in some cases, such as diopsid stalk-eyed flies and some *Phytalmia* species, the dimorphic trait is only used for size assessment during confrontations (Wilkinson & Dodson 1997; Panhuis & Wilkinson 1999).

D. Substrate leks

Lek mating refers to a mating system in which males aggregate at traditional sites and display to females (Bradbury & Gibson 1980; Höglund & Alatalo 1995). Typically, few males at a lek successfully copulate with any females. Because females come to males and males avoid or fight with each other, females are presumed to choose their mating partner. Vertebrate leks are characteristically disassociated from food or other limiting resources. Among flies, aggregations of displaying males can occur on and away from food or oviposition sites at different times. Similar switching between resource defense and lek mating has been described for fallow deer (Clutton-Brock et al. 1988) and has been attributed to differences in the amount and distribution of food over time. Substrate lek mating has been described for one family of lower Diptera and 10 families of higher Diptera (Table 1), with the greatest number of examples occurring in the Tephritidae and Drosophilidae (Spieth 1974; Shelly & Whittier 1997; Aluja & Norrbom 2000; Sivinski et al. 2000).

Leks have been hypothesized to occur when resources are relatively abundant and homogeneously distributed (Bradbury & Gibson 1980; Höglund & Alatalo 1995). Consequently, resource defense by males is unlikely to be a profitable method of encountering females. Thus, as with aerial swarms, males are thought to form aggregations in areas where females are likely to pass. For example, Bombyliid flies of the genus *Comptosia* lek on a hilltop location (Dodson and Yeates 1990; Yeates and Dodson 1990). In some species, males gather on a substrate, such as above or below the surface of a leaf. Because these locations are relatively cryptic, males typically produce signals to attract females. Moreover, aggregation size is typically smaller for substrate leks than for aerial swarms (Shelly & Whittier 1997). Consequently, females are likely to have more freedom to choose mates in substrate leks than in aerial swarms.

Among tephritid flies, two general mating patterns have been described that are associated with differences in feeding and oviposition behavior. Monophagous species, which are typically temperate in distribution, usually mate on the host plant where oviposition occurs. In contrast, polyphagous species, most of which are tropical, usually mate in aggregations that form on the

surface of leaves on or near a host plant (Prokopy 1980; Burk 1981). Although some exceptions to this pattern have been described, e.g. lekking occurs in some monophagous species (Headrick & Goeden 1994), they likely represent cases in which the host is sufficiently abundant that male defense is unlikely to be profitable (Sivinski et al. 2000). Resource dispersion has also been implicated in the occurrence of substrate leks among Hawaiian *Drosophila* (Droney 1996).

In a few species, lek mating alternates with resource defense at different times of the day or year. For example, Carib flies (*Anastrepha suspensa*) court on fruit in the morning and signal from the underside of leaves in the late afternoon by emitting pheromones and producing wing vibrations. In this species, most (85%) matings occur on leaves (Burk 1983). In the medfly, *Ceratitis capitata*, males signal from leaves between mid-morning and mid-afternoon, and court on fruit earlier and later. Over twice as many successful copulations occurred under leaves than on fruit (Prokopy & Hendrichs 1979). However, calling leads to higher predation (Hendrichs & Hendrichs 1998), which provides an explanation for the occurrence of alternative mating strategies. In the monophagous fruit fly, *Rhagoletis pomonella*, male mating strategy changes seasonally, with males mating on apple tree foliage early in the season and on fruit later in the season. Mating attempts on the two locations were equally successful (Smith & Prokopy 1980).

The factors favoring lek mating have received considerable study in the medfly, *Ceratitis capitata* (Yuval & Hendrichs 2000). As is typical of other lekking species, only a small fraction of males in an aggregation mate successfully (Arita & Kaneshiro 1985; Whittier et al. 1994). In experimental chambers, longer wing length predicted male mating success (Hunt et al. 2002). However, other studies have failed to find any effect of body size on male mating success (Whittier et al. 1994; Whittier & Kaneshiro 1995). Aggregations of males in trees may form in locations that increase encounter rates with females (Field et al. 2002), a process known as the hotspot effect (Bradbury & Gibson 1980). The hotspot does not, however, provide a compelling explanation for why 2-8 males gather together to display. Some evidence indicates that females actively choose larger aggregations (Shelly 2001b), perhaps to reduce potential predation (Field

et al. 2002). Little evidence supports an alternative possibility that males gather around a preferred mate, referred to as a hotshot (Beehler & Foster 1988).

Lek mating species exhibit the most elaborate and complex courtship behaviors that have been described for flies. For example, some male tephritids produce visual (Sivinski et al. 2000), chemical (Heath et al. 2000) and acoustic signals (Webb et al. 1984; Aluja 1993; Aluja et al. 2000) to attract females. In the lekking tephritid, *Batrocerca dorsalis*, males provided with either synthetic methyl eugenol or with flowers containing methyl eugenol-like compounds have higher visitation rates and mating success in field and lab experiments (Shelly 2001a). Both males and females recruit to this substance and males almost certainly use metabolites of it in their pheromones. Males aggregate on the surface of leaves in several species of phorids and either fan their wings (*Dohnniphora maddisoni*), drum on the leaf with their hind coxae (*Borophaga incrassata*), or perform complex wing and leg movements (Disney 1994). Four species of Hawaiian *Drosophila* in the planitibia subgroup, *D. differens*, *D. planitibia*, *D. silvestris*, and *D. heteroneura*, exhibit lek mating. Males perch on tree fern fronds and perform complicated courtship movements, including waving patterned wings, purring, and emitting wing vibrations (Spieth 1978; Hoy et al. 1988; Hoikkala et al. 1994; Hoikkala & Welbergen 1995) to attract females.

Mating success on leks can be influenced by diet, presumably because diet influences male courtship activity and, therefore, attractiveness to females. For example, a high protein diet increased courtship display and mating success of *Drosophila grimshawi* in experimental leks (Droney 1996). However, in medflies diet did not affect male display activity even though males fed protein mated more successfully than males fed sugar (Shelly et al. 2002). Females are, though, more likely to remate after mating with a male fed sugar versus a male fed protein (Yuval et al. 2002) suggesting that males may transfer some proteinaceous material to females during mating in this species.

IV. Copulation and Postcopulatory Activity

Over the past 30 years, considerable evidence has revealed that fertilization success is not determined solely by copulation success. In flies, sperm is first transferred to female storage organs and then subsequently allowed to move to the micropyles, which are holes in the egg shell, or chorion, through which sperm must pass to reach the vitelline membrane and effect fertilization. The number of sperm transferred by a male can vary depending on the duration of a copulation and the presence of a spermatophore. If females remate, then subsequent males can attempt to displace or interfere with previously deposited sperm. Females can also eject spermatophores or utilize stored sperm selectively. Sperm size, sperm number, and female storage organs have undergone tremendous diversification among species of flies. While the functional significance of much of this behavioral and morphological diversity remains to be elucidated, a number of experimental studies using flies, especially involving species of *Drosophila* and the dung fly, *Scatophaga stercoraria*, have begun to shed light on the evolutionary processes responsible for the variation that has been described.

A. Female remating

Whether females mate once or multiple times depends, in part, on what males provide to them during a copulation. For example, in some species of *Drosophila* males transfer nutrients during mating and females mate repeatedly. In others, males transfer harmful substances and females mate infrequently (Markow 2002). Multiple mating by females is expected when gamete incompatibility or genomic conflict is possible (Haig & Bergstrom 1995; Zeh & Zeh 1996; Tregenza & Wedell 2000). Support for this hypothesis comes from studies showing conspecific sperm precedence in *Drosophila* (Markow 1997; Price 1997; Price et al. 2000) and from studies of mating rate and meiotic drive frequency among populations of diopsid stalk-eyed flies (Wilkinson et al. 2003). Nevertheless, for many species of flies the most likely benefit to females for remating is increased offspring production (Ridley 1988; Arnqvist & Nilsson 2000; Baker et al. 2001). This conclusion seems surprising given the conventional assumption that

males pass more sperm per copulation than needed. However, this assumption is clearly false for some species of flies (Pitnick 1996).

The frequency of female remating can influence the intensity of both precopulatory and postcopulatory sexual selection. In species where females remate frequently, the operational sex ratio should be less male-biased, which should reduce precopulatory competition for mates but increase postcopulatory competition for fertilizations. Comparisons among taxa suggest that female remating does influence pre- vs. postcopulatory competition among males. Species of *Drosophila*, for example, can be split into two groups: species where females are likely to mate more than once per day, and species where females are not. Frequently remating species are less likely to show secondary sexual dimorphism or special courtship tactics, such as nuptial feeding, than species that mate infrequently. However, frequently remating species are much more likely to have exaggerated male ejaculate traits, such as giant sperm and ejaculate donations (Markow 2002). The frequency of remating also influences whether females can store or sort sperm. In the dung fly, *Scatophaga stercoraria*, a week between copulations affords females the opportunity to sort sperm among three spermathecae, allowing females to control which males' sperm fertilizes eggs (Ward & Hauschteck-Jungen 1993).

Remating by females can also be influenced by the behavior of males. For example, males can transfer material that either hinders access to sperm storage organs by subsequent mates (Lorch et al. 1993) or directly alters female behavior. In some ceratopogonid flies, males sacrifice part of their abdomen to block female genital openings (Downes 1978). In some species of *Drosophila*, mating induces a post insemination reaction which causes females to become reluctant to mate (Markow & Ankney 1988). An alternative strategy to reduce remating is for the male to remain in contact or near the female even after copulation has terminated. This behavior is typically described as mate guarding (Alcock 1994) and has been observed in a nereid fly (Mangan 1979) and the dung fly, *Scatophaga stercoraria* (Parker 1970b). Finally, in some species copulations persist for periods exceeding the time necessary for sperm transfer.

For example, prolonged copulations by *Plecia nearctica* have been interpreted as mate guarding (Thornhill 1976).

B. Copulation duration

Among species of flies copulation duration varies enormously and independently from how males encounter females. For example, the chironomid, *Glyptotendipes paripes*, copulates for 5 seconds (Nielsen 1959) while the bibionid, *Plecia nearctica*, copulates for up to 56 hours (Hieber & Cohen 1983). Among species of *Drosophila*, copulations can last from 30 seconds in *D. robusta* to over 2 hours in *D. acanthoptera* (Markow 1996). Reconstruction of copulation duration on phylogenies for three groups of flies (Fig. 1) reveals that short copulations have been derived recently in each group. Moreover, copulation duration has repeatedly increased and decreased among closely related species. These patterns suggest that copulation duration can evolve rapidly and must, therefore, be exposed to strong selection pressure.

Some of the variation in copulation duration can be attributed to differences in the amount of sperm and seminal material transferred from the male to the female (see Sperm Transfer and Spermatophores below). For example, in most diopsid stalk eyed flies, males transfer sperm in a spermatophore and the size of the spermatophore correlates with copulation duration across species (Kotrba 1996). Similarly, copulation duration correlates with nuptial prey size in *Empis borealis* (Svensson et al. 1990), presumably to enable successful males to transfer more sperm. In some species, copulation duration depends on whether females have mated previously. In these cases, longer copulations may reflect an increase in seminal material that will either dilute or displace previously stored sperm (Snook & Karr 1998).

In some species copulation duration varies due to male behaviors that have been referred to as copulatory courtship (Eberhard 1991). For example, males may tap or rub the abdomen of the female while in copula (Kotrba 1996; Bonduriansky & Brooks 1998). Eberhard (1996) has championed the view that females base their selection of sperm on these behaviors. These behaviors may, however, also provide information to males about the number of eggs carried by

a female or her mating history. The best evidence that such behavior influences fertilization success comes from studies on *Dryomyza anilis*. In this species, the male taps the female's genitalia with his claspers while squeezing her abdomen with his hind legs (Otronen 1990). The number of tapping sequences made by a male during copulation correlates strongly with fertilization success. Some of this effect can be attributed to body size since larger males tap more and have higher fertilization success (Otronen 1994; Otronen 1997). In addition, males with more asymmetrical and shorter small claspers have higher fertilization success (Otronen 1998).

Considerable theory has been developed to predict the optimal copulation duration in the dung fly, *Scatophaga stercoraria*, when there is sperm competition (reviewed in Simmons 2001). Females of this species store sperm in three spherical, sclerotized spermathecae. Sperm counts from previously unmated females have revealed that sperm number increases asymptotically over time as the spermathecae fill. Maximum storage capacity appears to be about 950 sperm, which can be transferred by one male in a single copulation. Males gather on freshly deposited dung and wait to intercept and mate with females who arrive to oviposit. Since most arriving females have already mated and have full spermathecae, males must displace previously stored sperm. Because sperm mixes as displacement occurs, males that continue to transfer sperm will displace their own sperm. The marginal value theorem can predict the optimal copulation time that maximizes male fitness (Parker & Stuart 1976). Males should stop copulating when the marginal gain from mating with the current female drops below that expected from finding and copulating with another female. By this logic, Parker and Stuart (1976) predicted that males should copulate for 42 min, which is reasonably close to the observed average copulation duration of 35 min.

Including additional factors, such as phenotypic differences in males and females, refines such estimates. For example, larger males displace sperm faster than smaller males and, therefore, have shorter copulation times (Simmons & Parker 1992). In addition, larger males are more likely to take over females from smaller males, which both reduces large males' search

times and also leads to shorter copulation durations (Parker & Simmons 1994). Assuming that females mediate displacement by allowing sperm to move from the bursa copulatrix to the spermathecae allows an even better fit between observed and predicted copulation durations (Parker & Simmons 2000). Finally, copula duration increases with female size primarily because larger females carry more eggs, which increases the fertilization benefit to the male (Parker et al. 1999).

Much of the theoretical work on copulation duration assumes that males control mating. This assumption provides a reasonable fit to the data for yellow dung flies but may not be appropriate for other species. For example, in the dung fly, *Sepsis cynipsea*, females are reluctant to mate and attempt to dislodge mounted males by vigorous shaking (Blanckenhorn et al. 2000). Experiments have revealed that male genitalia injure females internally during copulation and these injuries increase with mating frequency (Blanckenhorn et al. 2002). Mated females show higher mortality than unmated females. Thus, copula duration seems to be another example of sexual conflict. Which sex controls mating may depend on relative body size, densities of each sex, predation rates, and other ecological factors.

C. Sperm transfer and spermatophores

Many flies have closed copulatory systems where males pump free sperm, often from bifid or trifold penes, directly to spermathecal duct openings. However, in many species of lower Diptera and a few species of higher Diptera, sperm is transferred in spermatophores, packages of accessory gland secretions that enclose the sperm mass (Pollack 1972; Kotrba 1996). The use of spermatophores is ambiguous in some taxa, including species of Culicidae, Phoridae, and Spheropteridae (Kotrba 1996), where clear gelatinous material surrounds the sperm mass after sperm transfer to the female. Whether flies employ spermatophores is not clear even in some *Drosophila* species, including *Drosophila melanogaster*, which have varying reports of a 'sperm sac' (DeVries 1964; Gromko & Gilbert 1984; Alonso-Pimentel & Tolbert 1994).

Why males in some fly taxa package sperm in spermatophores while others pump free sperm into their mates seems to depend on several selection pressures. Spermatophores might provide a competitive advantage to males that mate repeatedly (Gerber 1970). Mating aggregations allow the opportunity to mate several times in rapid succession and provide selective pressure for small, rapidly deployed spermatophores. Males that package and deposit their sperm quickly are free to copulate with other females. Among flies, some evidence bears out this hypothesis. Several taxa that form mating aggregations also transfer spermatophores, including chironomid midges (Nielsen 1959), black flies (Wenk 1987), tsetse flies, *Glossina austeni* (Pollack 1970), and mosquitos, *Anopheles gambiae* (Giglioli & Mason 1966).

Spermatophores do not always permit rapid sperm deployment, however. To allow males to mate quickly, spermatophores need only be small, simple sacks packed with sperm. In contrast, diopsid stalk-eyed fly spermatophores range from small sacs in the genus *Sphyracephala*, to larger and elongate (*Cyrtodiopsis*), club-shaped (*Diasemopsis*), or tear-shaped (*Teleopsis*) structures that fill the bursa (Kotrba 1996). Nor do all flies that transfer spermatophores mate quickly. Flies that transfer large spermatophores form them inside the females' vaginal cavity during copulation. Such behavior takes time. For example, male dung flies, *Sepsis cynipsea*, and stalk-eyed flies in the genus *Teleopsis*, may remain in copula for more than 40 minutes while transferring spermatophores (Kotrba 1996; Martin & Hosken 2002). Moreover, male lovebugs, *Plecia nearctica*, both form mating swarms and transfer spermatophores (Pollack 1972), but remain in copula for days (Thornhill 1976). Thus spermatophores do not function solely for rapid sperm transfer in Diptera. Large spermatophores may evolve in response to sperm competition where males do not have the opportunity to remate quickly (Pollack 1972; Kotrba 1996). In some taxa, like the dungfly, *Sepsis cynipsea*, males mate longer and transfer more sperm to females likely not to be virgins (Martin & Hosken 2002). Thus, males of this species can adjust their spermatophore size to account for the risk of sperm competition.

Spermatophores have other effects, potentially, than rapid deployment. In diopsids and *Anopheles gambiae*, spermatophores can act as mating plugs, preventing other males from

inserting sperm into the same spermathecal duct (Giglioli & Mason 1966; Lorch et al. 1993). However, packaging sperm has drawbacks. Males may displace previously deposited spermatophores (Kotrba 1996) and females can reject spermatophores and consequently fertilize eggs selectively with sperm from some males over others. The secondary reduction and loss of spermatophores in the stalk-eyed fly genus *Diopsis* could, for example, be a response to displacement of whole spermatophores (Kotrba 1996).

D. Sperm length

In addition to mode of transfer, sperm morphology varies enormously among fly species and can influence fertilization success. Sperm length varies 60-fold among Diopsidae (Presgraves et al. 1999) and 200-fold within *Drosophila* (Pitnick et al. 1995). For example, *Drosophila bifurca* have sperm almost 58 μm long, or twenty times their body length. Not surprisingly, in *Drosophila*, longer testes and longer sperm evolve together (cf. Fig. 2, Pitnick 1996). In experimental lines alleviated from sexual selection through random, monogamous mating, males had fewer sperm and shorter testes (Pitnick et al. 2001). Thus, long sperm seem to have evolved for postcopulatory competition. This notion is at odds with the usual evolutionary theories of anisogamy in which ejaculates of many tiny sperm evolved to compete against each other to fertilize eggs (Parker 1982). Sperm size should come at the expense of sperm number, and across *Drosophila*, species that produce long sperm tend not to produce as many (Pitnick 1996; Snook & Markow 2001). Females in species of *Drosophila* with long sperm tend to remate frequently (Markow 2002), yet display lesser degrees of second-male sperm precedence than species with small sperm (Simmons 2001). The reason for this pattern is not obvious, although a numbers game may be involved. In species with longer sperm, ejaculates may not contain enough sperm for high second-male paternity patterns. However, these are conditions that should favor ejaculates of many small sperm (Parker 1982). Clearly something else is involved (see Female Reproductive Anatomy below).

Sperm length also can vary among males within a species. For example, male *Drosophila melanogaster* and dung flies, *Scatophaga stercoraria*, show heritable variation in sperm length independent of body size (Ward 2000b; Miller & Pitnick 2002). Longer sperm in dung flies give males a postcopulatory advantage because long sperm are more likely to enter spermathecal ducts, although this effect is influenced by other factors, such as male size and sperm number (Ward & Hauschteckjungen 1993; Otronen et al. 1997). Sperm length can vary within individuals, as well. Many insects have heteromorphic sperm (Swallow & Wilkinson 2002). In Diptera, both the long and short sperm in sperm-heteromorphic species are nucleated, although only the long sperm morph have been found to fertilize eggs (Snook & Karr 1998). As a general rule, sperm in heteromorphic species are shorter than sperm in monomorphic species (Swallow & Wilkinson 2002). For example, Markow (2002) makes a case that "giant" sperm in *Drosophila* are over 6 mm long, and the longest sperm for heteromorphic species of *Drosophila* are 1.8 mm in *D. azteca* (Bircher & Hauschteck-Jungen 1997). The role short sperm play is not entirely clear, although they may function to eliminate the sperm of rival males from female spermathecae (see Female Reproductive Anatomy below). Conditions that would favor the evolution of sperm that displace rival males' sperm and improve the odds of fertilization are generally consistent with dipteran mating systems (Swallow & Wilkinson 2002).

F. Female reproductive anatomy

Evolutionarily, female flies are not static vessels for sperm storage. Instead, recent evidence clearly indicates that female anatomy can evolve rapidly to create a dynamic battleground on which sperm must compete to fertilize eggs. Flies may store sperm in seminal receptacles, spermathecae, or both, before sperm have the opportunity to fertilize eggs. Females in some species may also use other organs for sperm storage. For example, female *Drosophila nigricruria* store sperm in paired parovaria, which are about twice as large as those of other species, as well as in the spermathecae and seminal receptacles (Pitnick et al. 1999). The relative sizes of these female reproductive structures also varies hugely among taxa. For example, just as

with sperm length, there is 200-fold variation in seminal receptacle length in *Drosophila* (Pitnick et al. 1999). Spermathecae number can vary among taxa, too. While *Drosophila* tend to have two spermathecae (Pitnick et al. 1999), most Diopsidae have three (Presgraves et al. 1999), and dung flies *Scatophaga stercorariai* usually have three, but sometimes four (Ward 2000a). Therevidae and their relatives have one, 2 or 3 spermathecae and may have an associated spermathecal sac. The function of the sac is unknown, but it does contain sperm after mating (Winterton et al. 1999).

Having multiple sperm storage organs can allow females to manipulate which sperm they use to fertilize eggs. For example, larger yellow dung fly males, *Scatophaga stercoraria*, have a postcopulatory advantage (Ward & Hauschteck-Jungen 1993), siring a greater proportion of progeny than smaller males if they are second to mate with a female. Examining which male's sperm winds up in which spermatheca suggests that females may allow sperm to distribute evenly among spermathecae during the first mating, but eject the sperm of the first male if the second male is larger (Otronen et al. 1997). Complicating this effect is sperm number and sperm length, where larger sperm tend to be less numerous but are more likely to arrive in spermathecae. Females may preferentially use sperm with different phosphoglucosyltransferase alleles to best match the environmental conditions in which larvae grow (Ward 1998).

The presence of monomorphic and heteromorphic sperm is also correlated with female reproductive tract morphology.. For example, while some species of diopsid stalk-eyed flies exhibit sperm dimorphism and three well developed spermathecae, one genus, *Diasemopsis*, has large (0.9-2.9 mm) monomorphic sperm, only two spermathecae, and in at least one species these are degenerate. Across Diopsidae, spermatheca size correlates with the length of the short rather than the long sperm morph (Presgraves et al. 1999). In sperm-heteromorphic *Drosophila* species, short sperm tend to arrive in spermathecae more quickly than long sperm (Snook & Markow 2001), and receptive females tend to have a lower proportion of short sperm in their ventral receptacle than non-receptive females (Snook 1998). These findings bolster the claim

that short sperm function in postcopulatory competition, perhaps by displacing rival males' functional sperm while stored in the spermathecae (Swallow & Wilkinson 2002).

Reproductive tract morphology, especially seminal receptacle length, correlates strongly with sperm length across taxa. In *Drosophila*, species with longer sperm also have longer seminal receptacles (cf. Fig. 2, Pitnick et al. 1999). In diopsid stalk-eyed flies, too, ventral (seminal) receptacle length correlates with sperm length (Presgraves et al. 1999). Interestingly, the *Drosophila* reported to have heteromorphic sperm do not appear to mate as frequently as *Drosophila* with giant sperm (Markow 2002). Although short sperm almost certainly function in postcopulatory competition (Presgraves et al. 1999), in *Drosophila* the conditions that favor intense postcopulatory competition, namely, rapid remating by females, tend to lead to giant rather than heteromorphic sperm (Markow 2002). Recently Miller and Pitnick (2002) selected *D. melanogaster* lines for either female seminal receptacle length or for male sperm length. They then allowed males of short- or long-sperm strains to compete for fertilizations within females of short- or long-receptacle strains. Males with long sperm sired a greater proportion of offspring when competing for fertilizations within long-seminal receptacle females. That is, males with long sperm have an advantage if female receptacles are long. Further, in lines selected for longer seminal receptacles in females, males evolved longer sperm (Miller & Pitnick 2002). Thus, longer sperm receptacles afford a competitive advantage to longer sperm. Miller and Pitnick (2002) conclude that giant sperm are the "cellular equivalent of the peacock's tail" because female reproductive tracts bias paternity for males with longer tails.

V. Conclusions

At least two conclusions can be drawn from this review. First, the link between the distribution of resources, especially food and oviposition sites, and type of mate encounter convention is strong while that between ecological factors and postmating activities is less clear and almost certainly more complex. Much of this complexity arises because we now know that flies do not fit the stereotypic image of females as passive sperm recipients and males as prolific producers

of tiny sperm. Interestingly, the tremendous morphological variation in male and female reproductive traits of *Drosophila* has been known for many years (e.g. Patterson 1943). Nevertheless, the functional significance of this variation was not appreciated until formal comparative studies revealed that male and female reproductive characters exhibit correlated evolutionary change (Pitnick et al. 1999; Presgraves et al. 1999). This result suggests that old taxonomic treatments of other dipteran groups may provide a treasure trove of information on reproductive trait morphology. Rather than rely on these traits to infer systematic relationships, reconstructing an independent hypothesis for the evolutionary relationships among species may help reveal the functional significance of structure variation and identify taxa worthy of observational and experimental study.

A second conclusion that can be drawn is that some behaviors related to mating have evolved very rapidly among species in some closely related groups of flies. For example, dramatic differences in precopulatory courtship, male fighting, and copulation duration have been reported for species of piophilid carrion flies (Bonduriansky & Brooks 1999a), *Anastrepha* fruit flies (Aluja et al. 2000), and Hawaiian *Drosophila* (Spieth 1978; Hoikkala et al. 1994; Boake et al. 2000; Boake 2002). These observations suggest that the form of the mating system may influence the rate of speciation. In particular, those species that rely on courtship behavior to choose mates may be more likely to become reproductively isolated as a consequence of small differences in courtship behaviors that might arise from genetic drift or selection. Such a scenario has long been thought to be important for the evolution and radiation of Hawaiian *Drosophila* (Ringo 1977; Boake 2000; Boake 2002). A corollary of this possibility is that those species that do not exhibit precopulatory courtship may be less likely to evolve premating isolation, but more likely to evolve postmating isolation mechanisms as a consequence of epistatic incompatibilities accumulating due to local coadaptation within isolated populations. Comparative studies of species diversity across taxa with different mating systems would be useful in assessing this possibility.

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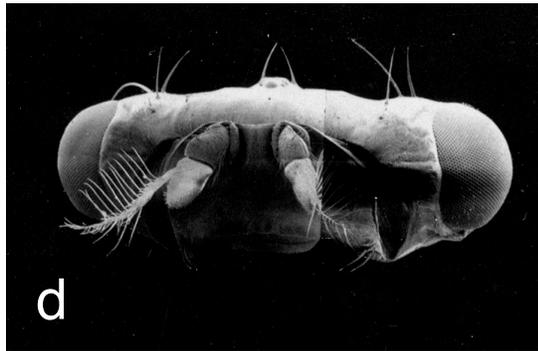
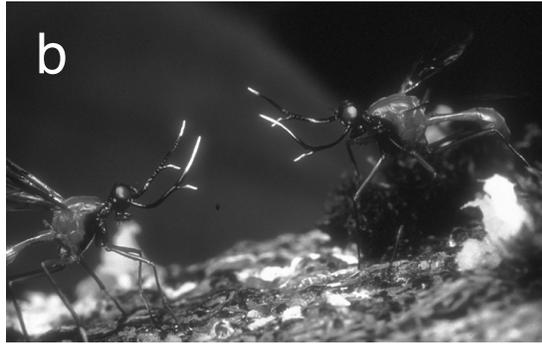
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Figure Legends

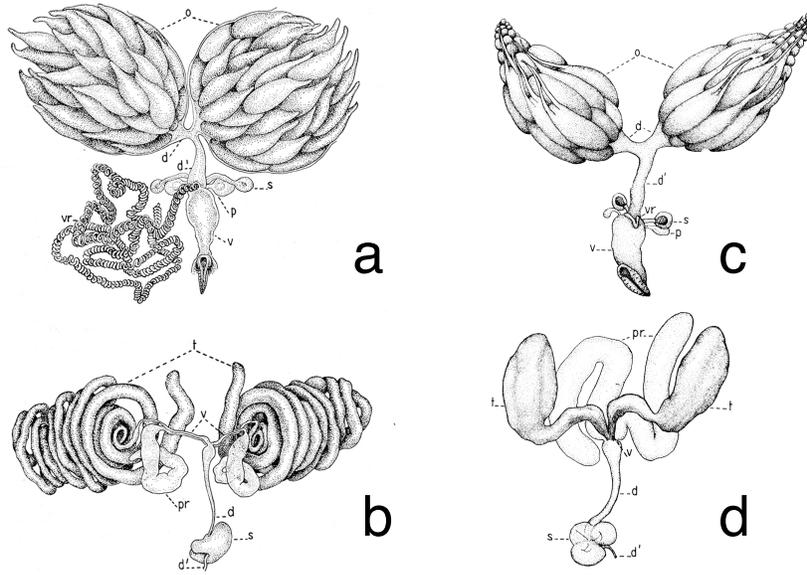
Figure 1. Examples of flies with head projections. a) *Teleopsis breviscopium* from Brunei, b) *Phytalmia cervicornis* from Papua New Guinea, c) *Zygothrica exuberans* from Ecuador, and d) *Drosophila heteroneura* from Hawaii.

Figure 2. Squared change parsimony reconstruction (Maddison & Maddison 1997) of copulation duration for fruit flies in the genus a) *Anastrepha*, family Tephritidae and b) *Drosophila*, family Drosophilidae. We illustrate the variation in copulation duration for *Anastrepha* (Aluja et al. 2000) and *Drosophila* (Markow 1996) by taking natural logarithms of minutes. The *Anastrepha* phylogeny is based on a neighbor-joining analysis of 175 phylogenetically informative sites from 16S rRNA mitochondrial DNA (McPheron et al. 2000). The *Drosophila* phylogeny is derived from a combination of morphological and molecular characters, including 2.7 Kb of nuclear sequence and 1.5 Kb of mitochondrial sequence (Pitnick et al. 1999).

Figure 3. Reproductive tracts of *Drosophila hydei* a) females and b) males, and of *D. victoria* c) females and d) males. *D. hydei* males produce sperm that are 23.3 μ m in length. Female anatomy: o, ovary; d, lateral oviduct; d', common oviduct; vr, ventral (seminal) receptacle; s, spermatheca; p, parovarium (female accessory gland); v, vagina. Male anatomy: pr, paragonium (male accessory gland); t, testis; v, vas deferens; d, testicular duct; s, sperm pump; d', posterior ejaculatory duct. Adapted with permission (pending) from Patterson (1943).



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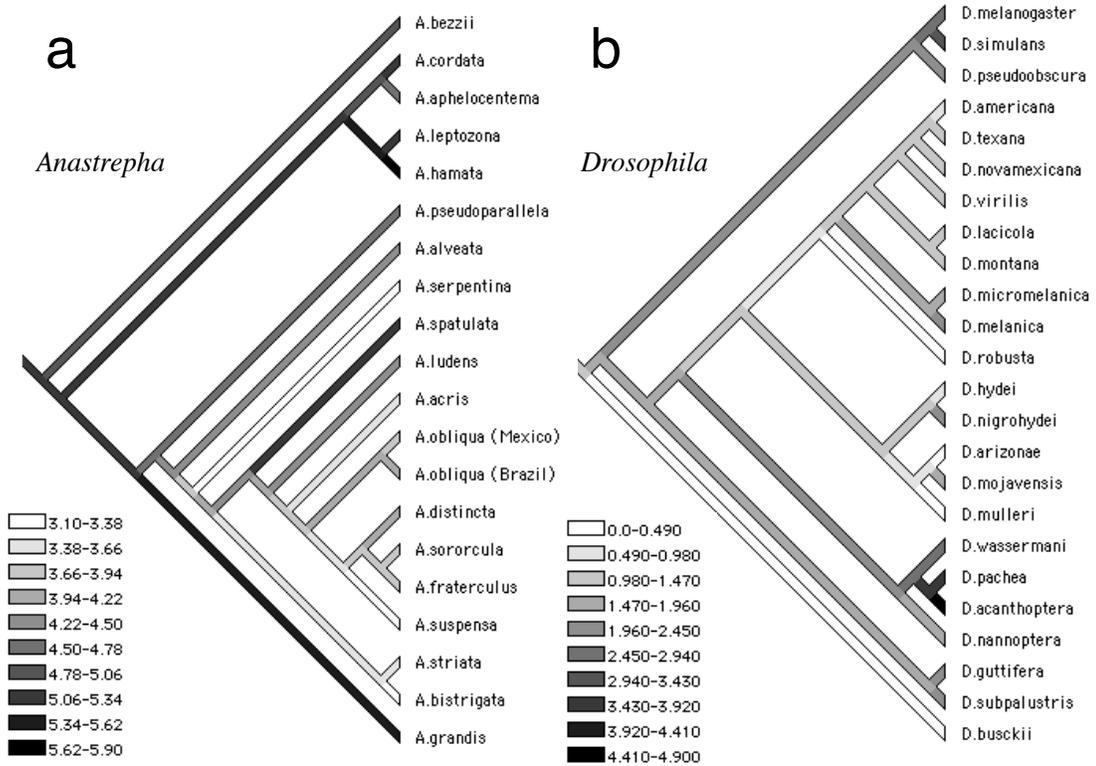


Table 1. Distribution of mate encounter strategies among families of flies.

Family	Species	Swarm	Mate searching	Resource defense	Substrate lek
Lower Diptera					
Bibionidae	<i>Plecia nearctica</i> (Thornhill 1976)	+			
Cecidomyiidae	<i>Anarete prichardi</i> (Chiang 1968)	+			
Ceratopogonidae	<i>Serromyia femorata</i> , <i>Culicoides brevitarsus</i> (Sullivan 1980)	+			
Chironomidae	<i>Abiskomyia virgo</i> , <i>Smittia extrema</i> (Armitage 1995), <i>Stictochironomus crassiforceps</i> (Shelly & Whittier 1997), <i>Chironomus plumosus</i> (Neems et al. 1992), <i>C. strenzkei</i> , <i>C. riparius</i> (Sullivan 1980), <i>Palpomyia brachialis</i> (Sivinski & Petersson 1997), <i>Allochironomus crassiforceps</i> , <i>Glyptotendipes paripes</i> , <i>Chricotopus</i> sp. (Sullivan 1980) <i>Clunio marinus</i> , <i>Diamesa japonica</i> , <i>Oliveridia hugginsi</i> , <i>Pontomyia</i> sp (Armitage 1995)	+		+	
Culicidae	<i>Aedes communis</i> , <i>A. excrucians</i> , <i>A. caspius</i> , <i>A. dorsalis</i> , <i>A. cataphylla</i> , <i>A. hexodontus</i> , <i>A. taeniorhynchus</i> , <i>Anopheles franciscanus</i> , <i>A. punctipennis</i> , <i>A. maculipennis</i> , <i>Culex pipiens</i> , <i>Mansonia fuscopennata</i> , <i>Psorophora ferox</i> (Sullivan 1980)	+			
Simuliidae	<i>Austrosimulium pestilens</i> (Shelly & Whittier 1997)	+			
Limoniidae	<i>Erioptera gemina</i> (Shelly & Whittier 1997)	+			
Psychoyidae	<i>Lutzomyia longipalpis</i> (Jarvis & Rutledge 1992; Jones & Hamilton 1998)				+
Tipulidae	<i>Pseudolimnophila inornata</i> (Sullivan 1980), <i>Erioptera taenionota</i> (Shelly & Whittier 1997)	+			
Higher Diptera					
Bombyliidae	<i>Comptosia</i> sp. (Shelly & Whittier 1997) <i>Lordotus pulcherrimus</i> (Shelly & Whittier 1997)				+
Mydidae	<i>Mydas ventralis</i> , <i>M. xanthopterus</i> (Shelly & Whittier 1997)				+

Empididae	<i>Aplomera</i> , <i>Empis</i> , <i>Hilara</i> , <i>Rhamphomyia</i> (Downes 1969; Cumming 1994)	+		
Stratiomyidae	<i>Hermetia comstocki</i> (Alcock 1990), <i>Hermetia illucens</i> (Tomberlin & Sheppard 2001)			+
	<i>Platyna hastata</i> , <i>Beris morrisii</i> (Oldroyd 1964)	+		
Streblidae	<i>Ascodipteran jonesi</i> (Oldroyd 1964)		+	
Rhagionidae	<i>Symphoromyia sackenia</i> (Shelly & Whittier 1997)	+		
Tabanidae	<i>Chrysops atlanticus</i> , <i>Tabanus bishoppi</i> (Shelly & Whittier 1997)	+		
Phoridae	<i>Megaselia aurea</i> (Sivinski 1988), (Sullivan 1980)	+		
	<i>Puliciphora borinquensis</i> (Miller 1984), <i>Phalacrotophora sp.</i> (Disney 1994)		+	
	<i>Dohrniphora maddisoni</i> , <i>Borophaga incrassata</i> (Disney 1994)			+
Platypezidae	<i>Calotarsa pallipes</i> , <i>C. calceata</i> , <i>C. insignis</i> (Sivinski & Petersson 1997; Chandler 2001)	+		
Syrphidae	<i>Syrphus ribesii</i> (Shelly & Whittier 1997)	+		
	<i>Somula decora</i> (Maier & Waldbauer 1979)			+
Coelopidae	<i>Coelopa frigida</i> (Day & Gilburn 1997), <i>C. ursina</i> (Crean & Gilburn 1998), <i>C. nebularum</i> (Weall & Gilburn 2000), <i>C. vanduzeei</i> , <i>C. pilipes</i> , <i>Gluma musgravi</i> , <i>G. nitida</i> (Crean et al. 2000)		+	
Dryomyzidae	<i>Dryomyza anilis</i> (Otronen 1984)			+
Drosophilidae	<i>Drosophila adunca</i> , <i>D. cnecopleura</i> , <i>D. comatifemora</i> , <i>D. conformis</i> , <i>D. crucigera</i> , <i>D. cyrtoloma</i> , <i>D. differens</i> , <i>D. engyochraceae</i> , <i>D. formella</i> , <i>D. grimshawi</i> , <i>D. heteroneura</i> , <i>D. imparisitae</i> , <i>D. lasiopoda</i> , <i>D. mimica</i> , <i>D. mixtura</i> , <i>D. mycetophaga</i> , <i>D. nigribasis</i> , <i>D. obscuripes</i> , <i>D. percnosoma</i> , <i>D. planitibia</i> , <i>D. polypori</i> , <i>D. pullipes</i> , <i>D. silvestris</i> (Shelly & Whittier 1997)			+
	<i>D. melanderi</i> (Spieth & Heed 1975)			+
Chloropidae	<i>Lipara lucens</i> (Mook & Bruggemann 1968)		+	
Otitidae	<i>Physiphora demandata</i> (Shelly & Whittier 1997)			+
Neriidae	<i>Odontoloxozus longicornis</i> (Mangan 1979)			+

Piophilidae	<i>Protopiophila litigata</i> (Bonduriansky & Brooks 1999b), <i>P. latipes</i> , <i>Stearibia nigriceps</i> , <i>Liopiophila varipes</i> , <i>Prochyliza xanthostoma</i> , <i>Parapiophila</i> sp. (Bonduriansky & Brooks 1999a)			+
Sepsidae	<i>Sepsis cynipsea</i> (Parker 1972)			+
Tephritidae	<i>Microsepsis armillata</i> (Eberhard 2000)			+
	<i>Anastrepha fraterculus</i> , <i>A. ludens</i> , <i>A. obliqua</i> , <i>A. pseudoparella</i> , <i>A. serpentina</i> , <i>A. sororcula</i> , <i>A. suspensa</i> , <i>Batrocerca cucurbitae</i> , <i>B. dorsalis</i> , <i>B. tryoni</i> , <i>Procecidochares</i> sp. (Shelly & Whittier 1997), <i>Ceratitidis capitata</i> (Field et al. 2002)			+
	<i>Aciurina trixa</i> , <i>Valentibulla dodsoni</i> (Dodson 1987), <i>Phytalmia mouldsi</i> , <i>P. biarmata</i> , <i>P. cervicornis</i> , <i>P. alaicornis</i> (Dodson 1997; Dodson 2000), <i>Toxotrypana curvicauda</i> (Landolt & Hendrichs 1983), <i>Anastrepha bistrigata</i> (Morgante et al. 1993), <i>Rhagoletis boycei</i> , <i>R. juglandis</i> (Papaj 1994), <i>Achias australis</i> (McAlpine 1979)			+
Lonchaeidae	<i>Lonchaea</i> sp. (McAlpine & Monroe 1968)		+	
Gasterophilidae	<i>Gasterophilus intestinalis</i> (Shelly & Whittier 1997)			+
Cuterebridae	<i>Cuterebra austeni</i> , <i>C. grisea</i> , <i>C. latifrons</i> , <i>C. lepivora</i> , <i>C. polita</i> , <i>C. tenebrosa</i> (Shelly & Whittier 1997)			+
Fanniidae	<i>Fannia canicularis</i> (Shelly & Whittier 1997)		+	
Glossinidae	<i>Glossina morsitans</i> (Wall & Langley 1993; Leak 1999).			+
Muscidae	<i>Ophyria leucostoma</i> (Shelly & Whittier 1997)		+	
Oestridae	<i>Cephenemyia apicata</i> , <i>C. jellisoni</i> , <i>Hypoderma lineatum</i> (Shelly & Whittier 1997)			+
Scathophagidae	<i>Scatophaga stercoraria</i> (Parker 1970a)			+

