4

Evolution of *Drosophila* Mating Systems

THERESE ANN MARKOW

INTRODUCTION

Animal mating systems are typically described and classified by the number of mates acquired by members of the species, monogamy to polygyny and polyandry, and by the strategies animals employ in acquiring mates and investing in progeny (Emlen and Orring, 1977). Interspecific variation in mating systems is extensive, even among closely related species. Explaining the origins and maintenance of this variation remains an important problem in evolutionary biology. Furthermore, mating system differences have important consequences for the genetic structure of populations as well as for their evolutionary potential.

A number of studies have sought to link resource ecology with mating system variation in several unrelated species of animals (reviewed in Thornhill and Alcock, 1983). Within these traditional categories of mating systems are additional distinctions, such as resource-defense polygyny, distinctions that reflect the ecological factors assumed to have shaped the differences in mating systems. However, a complete understanding of mating system evolution awaits our ability to understand the interplay between both long-term phylogenetic histories and more recent ecological constraints in the patterns we see. For many species, this remains problematic, because the requisite phylogenetic and ecological data are not simultaneously available.

Species of the genus *Drosophila* are characterized by an enormous amount of variation in their mating systems. In fact, recent studies of *Drosophila* have

THERESE ANN MARKOW • Department of Zoology, Arizona State University, Tempe, Arizona 85287-1501.

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revealed some of the most extreme reproductive phenotypes observed in the animal kingdom (Pitnick and Markow, 1994*b*; Pitnick *et al.*, 1995*a*,*b*). Early work on reproductive behavior of *Drosophila* species was largely ethological (Spieth, 1952). Investigations in subsequent decades were frequently conducted with a different orientation than were mating system studies on non-*Drosophila* species, in that the former focused on genetics of specific behaviors (Bastock, 1956; Manning, 1965). This emphasis reflects the fact that the extensive genetic work on *Drosophila melanogaster* made flies of this genus appear to be more of a laboratory tool than organisms with their own natural history. Indeed, when the field of behavior genetics started, *D. melanogaster* was as popular as the laboratory mouse for dissecting the genetic basis of behavior (Benzer, 1973), much of which was reproductive behavior (Hall, 1977).

Investigations taking an evolutionary approach to *Drosophila* reproductive behavior often focus on sexual isolation and speciation, cleverly employing the genetic tools of *Drosophila* to study the speciation process (Coyne, 1989, 1993; Coyne *et al.*, 1994). However, as the number of *Drosophila* species studied for sexual isolation increases, it is becoming clear that interspecific variation in *Drosophila* reproductive biology is extensive, with implications beyond speciation. The enormous interspecific differences in male and female reproductive morphology described by Throckmorton (1962), which have been invaluable for systematic studies, foreshadowed an even greater degree of variation in other aspects of *Drosophila* mating systems, including behavior.

A large number of *Drosophila* mating system features now have been examined in many species. These characters are listed in Table I. In this chapter, the extent of interspecific variation in these characters will be described. The evolutionary significance of this variation will then be examined in several ways. First, the association of characters within one sex will be evaluated for the existence of suites of characters. Then, the extent to which male and female characters are associated will be explored to assess the existence of mating system types or reproductive strategies. Potential relationships between phylogeny and ecology in explaining mating system variation will be discussed.

FEATURES OF *DROSOPHILA* MATING SYSTEMS

Age at Reproductive Maturity

Reproductive maturity can be assessed in several ways. One measure, presence of mature gametes, can give a different result from a behaviorally based measure such as copulation. However, copulation is not always the best evidence of reproductive maturity since immature males may copulate but not pass sperm

TABLE I. Mating System Characters in the Genus *Drosophila*

Age at reproductive maturity
Gamete size
Gamete number
Seminal nutrition
Remating frequency
Body size
Copulation duration

and females may be sexually receptive before they have mature eggs (Aspi *et al.*, 1993). Therefore, studies using different assays may not always yield strictly comparable results. A standard measure is the earliest age at which 80% of virgin flies will copulate when exposed to mature flies of the opposite sex for two hours (Markow, 1982; Pitnick *et al.*, 1995*b*). For males, this method is strongly correlated with the age at which mature sperm are found in the seminal vesicles. This measure assumes that sperm in the vesicles are fully capable of being transferred. Some investigations, however, use sperm transfer as the criterion of maturity (Pitnick, 1993).

Table II presents ages of reproductive maturity expressed as the time at which 80% of flies mate, for females and males of 42 species. Other investigations also have been concerned with female reproductive maturity (Lachaise, 1983), but the methods were not described clearly enough to include in this review. Males are first to mature in 13 species, and sexes mature at the same age in four of the species. In the majority of the 42 species studied, females mature first. In very few species do we find the majority of flies of either sex being ready to reproduce immediately upon eclosion. These relationships are consistent, regardless of the methods employed. Delayed male maturity has reached extremes in three species, *D. hydei, D. pachea,* and *D. kanekoi,* requiring a week to two longer than conspecific females. Such disparity in maturation times raises a number of issues. Clearly, the longer the period until an animal can reproduce, the greater its chance of dying before reproducing, suggesting the existence of a significant trade-off for this delay. While in the majority of cases in which there is sexual bimaturism, the last to mature is the male, in a sizable number it is the female. It is unclear if bimaturism accomplishes the same outcome or is driven by the same evolutionary forces when it is the female rather than the male that exhibits the delay in maturity. In none of the cases of delayed female maturation has an equally extreme delay for males been observed, and in species where males are severely delayed, females may mature very early, suggesting that selection for reproductive age acts independently in each sex.

It is difficult to identify the factors favoring evolution of such extreme sexual

TABLE II. The Ages at Which Males and Females Reach Reproductive Maturity

Species	Females	Males	Species	Females	Males
D. acanthoptera	6	6[e]	*D. micromelanica*	4	4[f]
D. affinis	4	2[g]	*D. micromettleri*	3	1[f]
D. americana	4	6[f]			
D. anceps	5	3[f]	*D. mojavensis*	3	7[b,i]
D. arizonae	3	4[f]		3	5[f]
D. bifurca	7	17[f]			
D. borealis	4	9[f]	*D. montana*	4	8[f]
D. busckii	2	0[f]	*D. nannoptera*	4	8[f]
D. eohydei	3	7[f]	*D. navajoa*	4	6[f]
D. eremophila	3	0[f]	*D. nigrospiracula*	4	5[f]
D. ezoana	7	14[f]	*D. novamexicana*	4	6[f]
D. flavomontana	5	9[f]	*D. pachea*	3	14[d]
D. guttifera	5	5[f]	*D. parisiena*	5	6[f]
			D. persimilis	4	0[g]
D. hydei	3	10[c,i]	*D. pseudoobscura*	3	1[h]
	3	9[e]	*D. recens*	5	4[f]
			D. robusta	6	10[f]
D. kanekoi	4	19[f]	*D. simulans*	3	1[f]
D. lacicola	3	3[f]	*D. straubae*	6	7[f]
D. littoralis	4	10[f]	*D. subpalustris*	4	3[f]
D. lummei	6	7[f]	*D. silvestris*	21	6[a,i]
D. mayaguana	8	6[f]	*D. texana*	4	6[f]
D. melanica	1	0[f]	*D. virilis*	3	6[f]
D. melanogaster	4	2[f]	*D. wassermani*	4	12[e]
D. mettleri	2	2[f]			

[a]Boake and Adkins (1994).
[b]Markow (1982).
[c]Markow (1985).
[d]Pitnick (1993).
[e]Pitnick and Markow (1994*b*).
[f]Pitnick *et al.* (1995*b*).
[g]Snook (1995).
[h]Snook *et al.* (1994).
[i]Sperm transfer.

bimaturism without considering other mating system features and knowing considerably more about the ecology of all of these species. One prospective outcome of delayed maturation, however, given what is known of dispersal in the genus, is that bimaturism is certain to promote outcrossing. In those species in which dispersal has been estimated (Dobzhansky and Wright, 1943; Johnston and Heed, 1976; Coyne *et al.*, 1982), it has involved long distances. Coupled with a difference in the age at which males and their sisters mature, inbreeding becomes unlikely. Another outcome of an extreme delay in maturity for one sex is the

potential to greatly bias the operational sex ratio and subsequently the intensity of sexual selection. These aspects will be considered later, in the context of identifying mating system patterns.

While the focus of this chapter is interspecific variation in mating systems, for many mating system features there are noteworthy levels of intraspecific variation. Such is the case with age at reproductive maturity. For example, a small number of *D. mojavensis* females will mate at 1 day of age (Markow, 1982), and a small number of *D. melanogaster* are receptive within 12 hr of eclosing. In addition to these interindividual differences, the nature of the social environment in which matings are orchestrated, i.e., groups of flies in mating chambers versus single pairs in vials, will alter the proportion of flies mating. We have found in our laboratory that while these conditions may influence the proportion of flies mating in any *Drosophila* species, the overall interspecific and sex differences in maturation are maintained under different test conditions. Thus it is important for investigators designing studies of intraspecific mating success in lesser-studied *Drosophila* to be aware of the general differences between species and sexes in ages at reproductive maturity.

Gamete Size

Female gamete sizes (Table III) can be measured in a number of ways: egg length, egg width, egg volume, and egg weight, measures that will obviously be correlated. To facilitate comparison, only egg lengths and widths are presented. Egg lengths vary by more than two times, from 0.44 to 1.09 mm. However, the magnitude of this variation is minor compared to male gamete size variation.

Male gamete sizes are reported as sperm lengths. Lengths are reported separately for species producing monomorphic (Table IV) and heteromorphic (Table V) sperm. Among species in which males produce one size class of sperm, there is tremendous (51 ×) variation in length. *Drosophila simulans* males (1.14 mm) make the shortest sperm and male *D. bifurca* (58.29 mm) produce the largest. In fact, *D. bifurca* sperm are the longest sperm known for any organism. The biological significance of such long sperm is likely to be complicated, as in some giant sperm species the entire sperm enters the egg while in others it does not (T. Karr and S. Pitnick, 1996).

As if this extensive variation is not enough, flies of the *D. obscura* group simultaneously produce more than one size class of sperm in the same testis (Table IV), a phenomenon first reported by Beatty and Burgoyne (1971) and termed *polymegaly*. Technical difficulties in accurately distinguishing between size classes are reflected in the different estimates reported by different investigators. In fact, these investigators initially suggested that certain *obscura* group species produced three sperm morphs (Beatty and Burgoyne, 1971), but im-

TABLE III. Egg Sizes for *Drosophila* Species

Species	Length (mm)	Width (mm)	Species	Length (mm)	Width (mm)
D. adiastola	0.820	0.230[b]	*D. murphyi*	0.910	0.210[b]
D. aldrichi	0.540	0.170[c]	*D. nigribasis*	1.040	0.240[b]
D. attigua	0.810	0.220[b]			
D. buskii	0.470	0.160[c]	*D. nigrospiracula*	0.409	**[e]
D. cardini	0.560	0.200[c]		0.483	**[a]
D. castanea	0.650	0.210[c]	*D. ochracea*	1.090	0.210[b]
D. clavisetae	0.990	0.310[b]	*D. pachea*	0.416	**[d]
D. crucigera	0.810	0.210[b]	*D. paramelanica*	0.660	0.230[c]
D. disticha	0.900	0.260[b]	*D. pavani*	0.570	0.190[c]
D. engyochracea	0.870	0.230[b]	*D. pectinitarsus*	0.690	0.220[b]
D. fasciculisetae	0.770	0.220[b]	*D. picticornis*	0.810	0.240[b]
D. fulvalineata	0.660	0.200[c]	*D. pilemana*	0.750	0.220[b]
D. funebris	0.640	0.210[c]	*D. primaeva*	0.830	0.230[b]
D. gibberosa	0.820	0.290[c]	*D. pseudoobscura*	0.550	0.200[c]
D. guaramanu	0.540	0.190[c]	*D. punalua*	0.920	0.230[b]
D. hydei	0.620	0.220[c]	*D. repleta*	0.600	0.190[c]
D. immigrans	0.650	0.200[c]	*D. robusta*	0.600	0.210[c]
D. inibula	0.600	0.240[c]	*D. sejuncta*	0.970	0.260[b]
D. kambysellisi	0.790	0.230[b]	*D. setosimentum*	0.860	0.230[b]
D. mimica	0.740	0.220[b]	*D. silvestris*	0.940	0.250[b]
D. melanocephala	0.900	0.220[b]	*D. sproati*	0.870	0.190[b]
D. mettleri	0.442	**[a]	*D. truncipenna*	0.960	0.250[b]
D. mojavensis (Baja)	0.442	**[a]	*D. villosipedis*	0.870	0.220[b]
D. montana	0.690	0.200[c]	*D. virilis*	0.870	0.230[c]
D. mulleri	0.660	0.210[c]			

[a]Heed and Mangan (1986).
[b]Kambysellis and Heed (1971).
[c]Kambysellis (1968).
[d]Markow *et al.* (1996*b*).
[e]Polak (unpublished).

proved staining techniques have shown clearly the existence of only two discrete size classes (Snook *et al.*, 1994). Within each size class there is considerable variation. Long sperm range from 0.14 (*D. obscura*) to 1.48 mm (*D. athabasca*), while short sperm range from 0.056 (*D. pseudoobscura*) to 0.24 mm (*D. subobscura*). Lengths of sperm in the two classes appear uncorrelated, illustrated by the fact that *D. athabasca* makes the longest long sperm but not the longest short sperm.

Sperm heteromorphism is known also among the lepidoptera, in which males of some species produce both eupyrene (nucleate) and apyrene (anucleate) sperm (Osanai *et al.*, 1989). In this case, it is clear that only one type can fertilize eggs. But in the case of *Drosophila*, both sperm types have chromatin-positive nuclei, making the functional capabilities of the two types an important question.

TABLE IV. Sperm Lengths in Sperm Monomorphic *Drosophila* Species

Species	Sperm length (mm)	Species	Sperm length (mm)
D. acanthoptera	5.83 ± 0.09[c]	*D. melanica*	4.93 ± 0.09[e]
D. americana	5.22 ± 0.02[e]	*D. melanogaster*	1.91 ± 0.01[e]
D. anceps	1.53 ± 0.01[e]	*D. mettleri*	2.79 ± 0.01[e]
D. arizonae	1.52 ± 0.01[e]	*D. micromelanica*	1.41 ± 0.01[e]
D. bifurca	58.29 ± 0.67[e]	*D. micromettleri*	2.22 ± 0.01[e]
D. borealis	7.54 ± 0.05[e]	*D. mojavensis*	1.90 ± 0.04[e]
D. busckii	1.18 ± 0.01[e]	*D. montana*	3.34 ± 0.02[e]
D. eohydei	18.11 ± 0.27[e]	*D. nannoptera*	15.69 ± 0.30[c]
D. eremophila	2.81 ± 0.04[e]	*D. navajoa*	1.88 ± 0.06[e]
D. ezoana	15.33 ± 0.19[e]	*D. nigrospiracula*	6.30 ± 0.06[e]
D. flavomontana	5.53 ± 0.01[e]	*D. novamexicana*	6.72 ± 0.15[e]
D. guttifera	10.29 ± 0.14[e]	*D. pachea*	16.63 ± 0.29[c]
D. hydei	6.60[b]	*D. parisiena*	2.10 ± 0.01[e]
	23.32 ± 0.51[d]	*D. recens*	7.55 ± 0.21[e]
	>10.00[a]	*D. robusta*	6.63 ± 0.09[e]
		D. simulans	1.14 ± 0.01[e]
D. kanekoi	24.29 ± 0.18[e]	*D. straubae*	2.46 ± 0.00[e]
D. lacicola	2.52 ± 0.00[e]	*D. subpaulstris*	5.96 ± 0.15[e]
D. littoralis	7.72 ± 0.08[e]	*D. texana*	5.08 ± 0.04[e]
D. lummei	7.79 ± 0.02[e]	*D. virilis*	5.70 ± 0.16[e]
D. mayaguana	1.90 ± 0.00[e]	*D. wassermani*	4.52 ± 0.03[e]

[a]Hennig and Kramer (1990).
[b]Hess and Meyer (1968).
[c]Pitnick and Markow (1994*a*).
[d]Pitnick and Markow (1994*b*).
[e]Pitnick *et al.* (1995*b*).

This issue has been resolved by the demonstration, using fluorescent and confocal microscopy, that only long sperm are found inside fertilized eggs in *D. pseudoobscura* (Snook *et al.*, 1994). A number of potential explanations exist for the significance of producing short, nonfertilizing sperm, such as control of female remating, sperm competition, or ejaculatory donations to females. Snook (1995) presents evidence showing that when *D. pseudoobscura* females remate, short sperm from the second male function to remove sperm of the first male from the sperm storage organs of the female. However, this may not be the case for all *obscura* group species. For example, *D. subobscura* females do not remate very quickly, and the sperm of the first male may have been used up by the time short sperm of the second male is present.

Gamete Numbers

The number of gametes an organism makes will depend on several of factors, such as gonad size, age at reproduction, longevity, nutritional condition,

TABLE V. Sperm Lengths in Sperm Polymorphic Species of *Drosophila*

Species	Mean (short) ± SE (mm)	Mean (long) ± SE (mm)	Reference
D. affinis	0.112 ± 0.000	0.424 ± 0.009	Joly and Lachaise (1994)
	0.130 ± 0.001	0.510 ± 0.002	Snook (1995)
D. algonquin	0.150 ± 0.002	0.894 ± 0.020	Snook (1995)
	0.120 ± 0.010	0.520 ± 0.030	Sanger and Miller (1973)
	0.130 ± 0.010	0.500 ± 0.020	Sanger and Miller (1973)
D. athabasca	0.118 ± 0.001	1.527 ± 0.047	Snook (1995)
D. ambigua			
USA	0.102 ± 0.003	0.313 ± 0.002	Snook (1995)
Europe	0.086 ± 0.002	0.310 ± 0.001	Snook (1995)
D. azteca	0.174 ± 0.002	1.433 ± 0.046	Snook (1995)
	0.143 ± 0.002	0.925 ± 0.112	Joly and Lachaise (1994)
D. bifasciata	0.083 ± 0.002	0.228 ± 0.002	Joly and Lachaise (1994)
D. guanache	0.131 ± 0.001	0.273 ± 0.003	Joly and Lachaise (1994)
D. helvetica	0.100 ± 0.001	0.223 ± 0.002	Joly and Lachaise (1994)
D. kitumensis	0.870 ± 0.001	0.248 ± 0.005	Joly and Lachaise (1994)
D. maderiensis	0.137 ± 0.001	0.218 ± 0.003	Joly and Lachaise (1994)
D. microlabis	0.068 ± 0.001	0.196 ± 0.003	Joly and Lachaise (1994)
D. miranda	0.087 ± 0.002	0.309 ± 0.001	Snook (1995)
D. obscura	0.076 ± 0.002	0.139 ± 0.003	Joly and Lachaise (1994)
	0.096 ± 0.006	0.230 ± 0.004	Snook (1995)
D. persimilis	0.067 ± 0.002	0.244 ± 0.004	Joly and Lachaise (1994)
	0.077 ± 0.001	0.325 ± 0.002	Snook (1995)
D. pseudoobscura	0.056 ± 0.002	0.263 ± 0.003	Joly and Lachaise (1994)
	0.092 ± 0.002	0.363 ± 0.002	Snook *et al.* (1994)
D. subobscura	0.085 ± 0.001	0.199 ± 0.002	Joly and Lachaise (1994)
USA	0.218 ± 0.002	0.488 ± 0.005	Snook (1995)
Europe	0.197 ± 0.001	0.327	Snook (1995)
D. tristis	0.112 ± 0.001	0.235 ± 0.003	Joly and Lachaise (1994)

as well as the measure used in its estimation (e.g., daily production or lifetime production). For *Drosophila,* there are few species in which we have enough information to precisely estimate numbers of gametes produced. However, we can compare the capacity for gamete production, especially in females, in that ovariole number can be accurately counted (Table VI). Species vary in ovariole number from 9 to 101. Ovariole number is strongly correlated with fitness (David, 1970). If ovariole number was the sole determinant of the number of eggs a female produced, then species with the greatest ovariole number would be the most productive. However, Kambysellis (1968) showed that oogenesis pro-

TABLE VI. Ovariole Number in Females of *Drosophila* Species

Species	Ovariole number	Species	Ovariole number
D. adiastola	45.92[e]	*D. murphyi*	41.57[e]
D. aldrichi	36.00[a]	*D. nigribasis*	53.33[e]
D. attigua	43.00[e]	*D. nigrospiracula*	39.81[d,g]
D. busckii	44.00[a]		39.72[d,h]
D. cardini	40.00[a]		
D. castanea	44.00[a]	*D. ochracea*	38.00[e]
D. clavisetae	38.17[e]	*D. pachea*	27.96[i]
D. crucigera	40.00[e]	*D. paramelanica*	24.00[a]
D. disticha	11.79[e]	*D. pectinitarsus*	12.42[e]
D. engyochracea	59.73[e]	*D. petalopeza*	17.75[e]
D. fasciculisetae	47.22[e]	*D. pilimana*	45.00[e]
D. fulvalineata	44.00[a]	*D. picticornis*	27.44[e]
D. funebris	42.00[a]	*D. primaeva*	101.33[e]
D. gibberosa	34.00[a]	*D. prodita*	9.00[e]
	41.38[d,f]	*D. punalua*	34.00[e]
		D. repleta	36.00[a]
D. guarumunu	38.00[a]	*D. robusta*	50.00[a]
D. hydei	46.00[a]	*D. sechellia*	16.40[b]
	49.00[i]	*D. sejuncta*	56.83[e]
D. immigrans	64.00[a]	*D. setosimentum*	35.61[e]
D. kambysellisi	15.00[e]	*D. silvestris*	52.38[e]
D. melanocephala	86.60[e]	*D. simulans*	34.85[b]
D. melanogaster	42.6[c]	*D. spenceri*	44.81[d,f]
D. mettleri	36.21[d,g]	*D. sproati*	65.55[e]
D. mimica	23.85[e]	*D. trichetosa*	10.33[e]
D. mojavensis	25.46[d,g]	*D. truncipenna*	48.00[e]
	25.86[d,h]	*D. victoria*	60.00[a]
	33.14[i]	*D. villosipedis*	47.00[e]
		D. virilis	34.00[a]
D. montana	38.00[a]		
D. mulleri	40.00[a]		

[a]Kambysellis (1968).
[b]Coyne *et al.* (1991).
[c]Robertson (1957).
[d]Heed and Mangan (1986).
[e]Kambysellis and Heed (1971).
[f]Laboratory foods.
[g]Laboratory cactus.
[h]Field.
[i]Markow *et al.* (1996b).

TABLE VII. Number of Sperm Transferred and Number of Progeny from a Single Mating

Species	Serm number	Progeny number
D. acanthoptera	1,023[c]	
D. hydei	126[d]	55[f]
D. melanogaster	4,600[a]	500[a]
D. nannoptera	81[c]	
D. pachea	44[c]	5[g]
D. pseudoobscura	25,000[b]	350[e]
D. wassermani	274[c]	

[a]Gilbert (1981).
[b]Snook (1995).
[c]Pitnick and Markow (1994*a*).
[d]Pitnick and Markow (1994[b]).
[e]Snook (1995).
[f]Markow (1985).

ceeds at different rates in different species, suggesting that caution be used in equating ovariole number with gamete number.

Measuring male gamete numbers or production must be achieved by different means. While sperm mature in bundles, their dissection and counting are more time-consuming than ovariole counts and thus have not been done for many species. Also time-consuming, but probably more relevant to the question of mating system evolution, is the number of sperm transferred during a single mating. Species for which this has been estimated are presented in Table VII. Once again, the interspecific variation is staggering, from 25,000 in *D. pseudoobscura* to 44 in *D. pachea*. Variation in male gamete production is far greater than for females.

Seminal Nutrition

Sperm are transferred to females in an ejaculate produced by the accessory glands of males. While the *Drosophila* ejaculate contains carbohydrates (Chen *et al.*, 1977; Baumann, 1974*a*,*b*) and lipids (Bairati, 1986; Brieger and Butterworth, 1970), the accessory gland proteins (ACPs) number more than 200 (Whalen and Wilson, 1986) and have received the greatest amount of attention. It is well known that in certain species, specific male proteins that control oviposition and sexual receptivity pass from the female reproductive tract to somatic and ovarian tissues. The ACPs are also highly variable compared to other proteins in *Drosophila* (Coulthart and Singh, 1988; Thomas and Singh, 1992), suggesting a role for these proteins in postmating isolation.

In a number of species, an enormous amount of male-derived material has been detected as having been incorporated into somatic tissues and ovarian oocytes of females (Boggs and Gilbert, 1979). These observations are typically made by rearing males on media prepared with radiolabeled amino acids and mating them to females reared on standard food. Females are then dissected at various times after mating, their tissues separated and washed, and then digested for scintillation counting. Since the initial observation that the ejaculate of *D. mojavensis* males rapidly made its way into female tissues and oocytes (Markow and Ankney, 1984), a large number of species have been examined for seminally derived nutrition (Table VIII). Studies have varied with respect to their ability to detect low levels of label in females of certain species, such as *D. melanogaster* (Markow and Ankney, 1984, 1988; Pitnick *et al.*, 1991; Bownes and Partridge, 1987). A number of factors may influence detection of label, such as specific activity, the particular isotope (^{14}C, ^{35}S, or ^{3}H), amount of label in the medium, mating status of the flies, and so on. All studies include a background and unlabeled control count. In *D. melanogaster,* some small amount of male-derived label in the form of the sex peptide, should be detectable in female soma (Chen *et al.*, 1988).

The striking observation from these studies is the enormous uptake and incorporation of male-derived material in certain species of *Drosophila,* primarily flies in the *mulleri* complex of the *repleta* species group, and to a somewhat lesser extent, in flies of the *quinaria* group. It has been possible to estimate, using whole-body radiolabel counts from males, the proportion of a male's label transferred to females. For *D. mojavensis,* this is $2.86 \pm 0.002\%$ (Markow *et al.*, 1990). While this is relatively small compared to the orthopterans (Gwynne, 1983), *Drosophila* males of these species may mate several times in one morning, further increasing the relative proportion of their body materials transferred during reproduction.

The function and evolution of such significant incorporation of male-derived products is uncertain. Proof that such material is in fact nutritive and scant. In *D. mojavensis,* these seminal nutrients have been suggested to increase female reproductive output (Markow *et al.*, 1990), but other species showing significant female incorporation of male ejaculate have not yet been studied in the same way.

I suggest that female uptake of specific male proteins to control oviposition is likely to have preceded the more massive transport seen in the *mulleri* complex species. The action of the sex peptide, which induces oviposition and suppresses remating (Chen *et al.*, 1988), is at least somewhat beneficial to both sexes. Females will not waste eggs if there are no sperm, and males can avoid sperm competition. Whatever the mechanism of transport of these peptides, it subsequently could have been exploited by females as a means of obtaining additional nutrients from seminal fluid. The precise mechanisms by which substances leave

TABLE VIII. Ejaculatory Contributions to Female Somatic Tissues and Ovaries as Determined by Radiolabel Experiments

Species	Soma[f]	Ovaries	Species	Soma	Ovaries
D. acanthopera	0	0[b]	*D. mojavensis*	2	2[b]
D. americana	1	0[a]		2	2[c]
D. arizonae	1	2[a]		2	2[e]
	1	2[e]	*D. montana*	0	0[a]
D. bifurca	2	1[a]	*D. nannoptera*	0	0[b]
D. borealis	0	0[a]	*D. navojoa*	2	1[a]
D. busckii	0	0[a]	*D. nigrospiracula*	0	0[a]
D. eohydei	1	0[a]		0	0[e]
D. ezoana	0	0[a]	*D. novamexicana*	0	0[a]
D. guttifera	2	1[a]	*D. pachea*	0	0[b]
D. hydei	0	0[e]	*D. parisiena*	2	2[a]
D. kanekoi	0	0[a]	*D. persimilis*	1	0[e]
D. lacicola	0	0[a]	*D. pseudoobscura*	1	0[e]
D. littoralis	1	0[a]		1	1[d]
D. lummei	1	0[a]	*D. putrida*	2	0[a]
D. mayaguana	2	2[a]	*D. recens*	2	1[a]
D. melanica	1	0[a]	*D. repleta*	0	0[a]
D. melanogaster	0	0[c]	*D. straubae*	2	2[a]
	0	0[e]	*D. subpalustris*	2	0[a]
	0	0[b]	*D. texana*	0	0[a]
	1	1[d]	*D. virilis*	0	0[a]
D. mettleri	1	0[a]	*D. wassermani*	0	0[b]
D. micromelanica	0	0[a]			

[a]Pitnick *et al.* (1996*c*).
[b]Pitnick *et al.* (1991).
[c]Markow and Ankney (1984).
[d]Bownes and Partridge (1987).
[e]Markow and Ankney (1988).
[f]Data are presented as relative contributions, since protocols differed between experiments; 0 no significant incorporation; 1, significant but minor incorporation; 2, large amount of incorporation.

the female tract for the soma and then enter the ovaries are completely unknown. Also unknown is whether the observed incorporation reflects selective uptake of particular products or if the incorporated material is the same in all species. We do know that, in *D. mojavensis,* male-derived products are found in oviposited eggs, showing that in at least one of these species males are directly contributing to their progeny.

Patterns of incorporation among species examined thus far show that more species exhibit uptake into soma than into ovaries. All species in which ovaries incorporate male-derived materials show significant incorporation into the soma. Only in one species, *D. arizonae,* is more label detected in ovaries than in soma.

The overall pattern is consistent with a scenario in which somatic uptake and incorporation of male substances was a predecessor of ovarian uptake.

Another form of nuptial feeding has been reported for *D. subobscura*, in which males provide a salivary drop to females (Steele, 1986*a*,*b*). Female consumption of the drop appears to have nutritional benefit.

Remating Incidence

One of the defining parameters for mating systems is the number of mates an individual has in its lifetime. For insects, it is usually impossible to get a definite count of mates for individuals in natural populations. While it has not been feasible to measure the strength of sexual selection in natural populations of *Drosophila*, the potential for sexual selection can be inferred from the frequency with which members of each sex mate. Among *Drosophila*, multiple insemination has been demonstrated by the recovery of genetic variation in the progeny of wild-caught females in a number of species (Milkman and Zeitler, 1974; Cobbs, 1977; Gromko *et al.*, 1980; Loukas *et al.*, 1981; Stalker, 1976; Craddock and Johnson, 1978).

Remating incidence, or rate, has not been examined in the laboratory in as many species as have the other mating system features I have been discussing, probably because of the more complicated logistics of assessing it. It usually has been reported as either the average time interval before females mate a second time or the cumulative number of females mating a second time. These two measures are strongly correlated: Species in which cumulative proportion remating rapidly reaches 100% also have the shortest remating interval. Because of this correlation and because of the heterogeneity of techniques for assessing remating among investigations, I have chosen to present remating data in categories (Table IX): (A) species in which females remate within an hour as well as several times during an observation period, (B) species in which females remate within 1 to 2 days, (C) species in which females remate after 3 to 5 days, and (D) those species in which females rarely or never remate. The boundaries of these

TABLE IX. Remating Incidence in Female *Drosophila*[a]

A	B	C	D
D. hydei	*D. affinis*	*D. funebris*	*D. acanthopera*
D. nigrospiracula	*D. arizonae*	*D. melanogaster*	*D. differens*
D. mettleri	*D. mojavensis*	*D. simulans*	*D. heteroneura*
D. nannoptera	*D. persimilis*		*D. subobscura*
d. pachea	*D. pseudoobscura*		*D. sylvestris*
	D. wassermani		

[a]The most frequent remating is in group A, the least frequent in group D.

categories are undoubtedly sensitive to experimental design. For example, studies may differ as to whether flies were allowed the chance to remate during a single daily observation period or during two daily observation periods. Observation periods may have been 1 or 2 hr. However, the overall pattern, I believe, will prove to be robust.

Factors controlling remating have been exhaustively studied in *D. melanogaster* by Gromko and his associates. From these studies, it is clear that there is a strong genetic component to remating propensity: selection for remating produces a rapid response (Pyle and Gromko, 1981; Gromko and Newport, 1988). However, sperm load also plays an important role (Letsinger and Gromko, 1985; Harshman *et al.*, 1988; Gromko and Markow, 1993), as have male accessory gland proteins (Richmond and Senior, 1981; Chen *et al.*, 1988).

The interspecific variability in female remating frequency has important implications for evolutionary biology. In species in which females remate frequently, the operational sex ratio will be biased toward females. This bias should translate into more frequent mating opportunities for males and less intense competition between males for access to females. On the other hand, frequent female remating leads to the potential for competition between ejaculates within the female reproductive tract and predicts that mechanisms assuring paternity are under intense selection in these species.

Male remating frequency has received less attention. In those few studies in which males have been provided with a continual supply of receptive females and observed until they were no longer willing or able to mate, some variation has emerged. Males of *D. melanogaster* (Lefevre and Jonsson, 1962; Markow *et al.*, 1978) have been observed to mate about three to five times before refusing to court. *Drosophila hydei* males will mate as many as 10 times in a day (Markow, 1985; Pitnick and Markow, 1994*b*), while *D. mojavensis, D. arizonae, D. pachea, D. wassermani,* and *D. nannoptera* will mate from five to eight times daily (Markow, 1982; Markow *et al.*, 1990; Pitnick and Markow, 1994*a*). The lowest frequency of male remating was observed in *D. acanthoptera,* in which males will only mate once or twice in a day (Pitnick and Markow, 1994*a*). These were laboratory studies in which males were supplied with an ad libitum supply of receptive females, a situation unlikely to exist often in natural populations. However, this approach reveals differences in male remating potential, and it is this potential that may reflect the history of selection pressures on males of different species.

Body Size

Drosophila adult body size is most often reported as thorax length because this measure is unchanged by posteclosion nutrition or hydration. Thorax lengths

of females and males are summarized in Table X. Male flies are typically smaller than females of the same species. Body size is included in this chapter as a mating system feature for two reasons. One is that it is generally considered to be sexually selected in insects, with larger males enjoying an advantage (Thornhill and Alcock, 1983). It is also a life history character for which investigators usually seek a wide range of correlations. These aspects of body size will be explored later, in the section on male reproductive strategies.

Copulation Duration

The length of time a pair stays *in copula* cannot be considered to be the property of one or the other sex. Copulation duration varies from 30 sec in *D. mulleri* to over 2 hr in *D. acanthoptera* (Table XI). Despite this large range, the majority of species mate for 10 min or less. A fair number mate for 10 to 20 min. Extremely long durations are only found in a few species. Investigations are not always consistent in the durations obtained for a species. For example, in *D. melanogaster,* durations of 15.4 min (Pitnick *et al.,* 1991), 18.35 min (Grant, 1983), and 18.14 min (Spieth, 1952) were found; in *D. mojavensis,* 4.26 min (Spieth, 1952) and 2.5 min (Pitnick *et al.,* 1991) were reported. These may reflect biological differences in the strains examined or the conditions under which flies were mating. For example, Gromko and Markow (1993), observing mating pairs of *D. simulans* and *D. melanogaster* in nature, recorded copulation durations of 39 min and 27 min, respectively, far longer than observed in any laboratory study. Gromko *et al.* (1991) estimated that in *D. melanogaster,* copulation duration has a heritability of 0.23, and subsequently showed that it responds rapidly to selection in either direction.

FEMALE REPRODUCTIVE STRATEGIES

Many female characters exhibit extensive variation, but remating incidence and ovariole number appear to have the most significance with respect to mating system evolution. Remating frequency defines the number of mates an individual will have and is thus a critical feature of mating systems. Remating will dictate the potential for sperm competition as well as influence the operational sex ratio, while ovariole number reflects the amount of energy a female may allocate to egg production.

There are several possible reasons for remating in female *Drosophila*. One is to maintain adequate sperm supply for fertilization (see Ridley, 1988). Another is to obtain some material benefit from males. Females also will alter the genetic

TABLE X. Thorax Lengths of Females and Males in *Drosophila* Species

Species	Thorax length (mm)		Species	Thorax length (mm)	
	Females	Males		Females	Males
D. adiastola	2.41 ± 0.920	**[b]	*D. eremophila*	1.01 ± 0.010	0.87 ± 0.00[a]
D. acanthoptera	1.21 ± 0.010	1.13 ± 0.00[a]	*D. ezoana*	1.41 ± 0.010	1.37 ± 0.00[a]
D. affinis	1.08 ± 0.010	0.92 ± 0.00[a]	*D. fasciculisetae*	2.65 ± 0.222	**[b]
D. americana	1.40 ± 0.000	1.38 ± 0.00[a]	*D. flavomontana*	1.38 ± 0.010	1.35 ± 0.01[a]
D. anceps	1.28 ± 0.010	1.16 ± 0.01[a]	*D. guttifera*	0.99 ± 0.010	0.94 ± 0.01[a]
D. arizonae	1.03 ± 0.000	0.95 ± 0.00[a]	*D. hydei*	1.44 ± 0.010	1.33 ± 0.01[a]
D. attigua	2.67 ± 0.063	**[b]	*D. kambysellisi*	1.51 ± 0.076	**[b]
D. bifurca	1.65 ± 0.000	1.60 ± 0.00[a]	*D. kanekoi*	1.62 ± 0.010	1.59 ± 0.00[a]
D. borealis	1.31 ± 0.010	1.28 ± 0.00[a]	*D. lacicola*	1.27 ± 0.010	1.21 ± 0.01[a]
D. busckii	0.90 ± 0.010	0.82 ± 0.01[a]	*D. littoralis*	1.44 ± 0.010	1.38 ± 0.01[a]
D. clavisetae	2.71 ± 0.114	**[b]	*D. lummei*	1.40 ± 0.000	1.34 ± 0.00[a]
D. crucigera	2.09 ± 0.188	**[b]	*D. mayaguana*	0.98 ± 0.010	0.94 ± 0.00[a]
D. disticha	1.45 ± 0.101	**[b]	*D. melanica*	1.23 ± 0.010	1.12 ± 0.01[a]
D. engyochracea	2.48 ± 0.115	**[b]	*D. melanocephala*	3.31 ± 0.342	**[b]
D. eohydei	1.50 ± 0.000	1.39 ± 0.00[a]	*D. melanogaster*	0.99 ± 0.010	0.88 ± 0.01[a]

D. mettleri	1.27 ± 0.001	1.17 ± 0.01[a]	*D. primaeva*	3.00 ± 0.124	**[b]
D. micromettleri	1.06 ± 0.000	0.95 ± 0.00[a]	*D. prodita*	1.37 ± 0.138	**[b]
D. micromelanica	1.11 ± 0.010	0.98 ± 0.01[a]	*D. pseudoobscura*	1.09 ± 0.010	1.01 ± 0.01[a]
D. mimica	1.78 ± 0.184	**[b]	*D. punalua*	2.30 ± 0.136	**[b]
D. mojavensis	0.96 ± 0.010	1.13 ± 0.00[a]	*D. recens*	1.09 ± 0.010	0.98 ± 0.01[a]
D. montana	1.48 ± 0.010	1.41 ± 0.01[a]	*D. robusta*	1.52 ± 0.010	1.44 ± 0.01[a]
D. murphyi	2.43 ± 0.119	**[b]	*D. sejuncta*	2.24 ± 0.212	**[b]
D. nannoptera	1.08 ± 0.010	0.99 ± 0.01[a]	*D. setosimentum*	2.13 ± 0.086	**[b]
D. navojoa	1.09 ± 0.000	0.99 ± 0.01[a]	*D. silvestris*	3.16 ± 0.129	**[b]
D. nigribasis	2.98 ± 0.305	**[b]	*D. simulans*	0.97 ± 0.010	0.87 ± 0.01[a]
D. nigrospiracula	1.42 ± 0.010	1.27 ± 0.01[a]	*D. sproati*	2.78 ± 0.149	**[b]
D. novamexicana	1.30 ± 0.010	1.28 ± 0.01[a]	*D. straubae*	1.06 ± 0.000	1.00 ± 0.01[a]
D. ochracea	2.40 ± 0.096	**[b]	*D. subpalustris*	1.29 ± 0.020	1.23 ± 0.02[a]
D. pachea	1.12 ± 0.010	1.02 ± 0.01[a]	*D. texana*	1.36 ± 0.010	1.29 ± 0.01[a]
D. parisiena	1.12 ± 0.010	1.06 ± 0.01[a]	*D. trichetosa*	1.35 ± 0.096	**[b]
D. pectinitarsus	1.26 ± 0.028	**[b]	*D. truncipenna*	3.22 ± 0.129	**[b]
D. persimilis	1.06 ± 0.010	0.93 ± 0.01[a]	*D. villosipedis*	2.09 ± 0.144	**[b]
D. petalopeza	1.75 ± 0.072	**[b]	*D. virilis*	1.33 ± 0.000	1.27 ± 0.01[a]
D. pilimana	2.19 ± 0.167	**[b]	*D. wassermani*	1.16 ± 0.020	1.07 ± 0.01[a]
D. picticornis	1.77 ± 0.980	**[b]			

[a]Pitnick *et al.* (1995*a*).
[b]Kambysellis and Heed (1971).

TABLE XI. Duration of Copulation in *Drosophila* Species

Species	Mean duration (minutes:seconds)	Species	Mean duration (minutes:seconds)
D. acanthoptera	137:07[b]	*D. melanogaster*	18:14[a]
D. affinis	01:46[a]		15:04[d]
	01:17[c]		18:35[c]
D. aldrichi	01:37[a]	*D. melanopalpa*	01:46[c]
	02:30[c]	*D. mercatorum*	02:02[a]
D. algonquin	05:28[a]		01:54[c]
D. americana	02:30[a]	*D. micromelanica*	06:45[a]
	02:28[c]		07:03[c]
D. ananassae	04:11[a]	*D. miranda*	08:46[a]
	04:03[c]	*D. mojavensis*	04:26[a]
D. arizonensis	01:37[a]		02:05[b]
D. arizonae	01:16[c]		03:14[c]
D. athabasca	07:46[a]	*D. montana*	04:17[a]
D. auraria	06:37[a]	(1218.8d)	01:58[a]
D. azteca	05:17[a]	*D. montana*	03:20[c]
	02:16[c]	(1862.2a)	
D. baeomyia	03:10[a]	*D. montium*	03:46[a]
D. busckii	01:29[a]	*D. mulleri*	00:29[a]
	01:02[c]		00:37[d]
D. buzzatti	01:46[a]	*D. munda*	08:47[a]
	02:22[c]	*D. nannoptera*	05:07[a]
D. canapalpa	02:18[a]		07:45[e]
D. capricorni	09:55[a]	*D. nebulosa*	01:37[a]
D. duncani	11:49[a]		02:11[c]
D. equinoxialis	15:55[a]	*D. neocardini*	21:20[a]
	21:04[c]	*D. nigrohydei*	07:25[a]
D. funebris	16:52[a]	*D. nigromelanica*	01:01[c]
	18:16[c]	*D. novamexicana*	02:44[a]
D. fumipennis	04:20[a]	*D. occidentalis*	07:41[a]
D. gibberosa	06:43[a]	*D. pachea*	39:31[b]
D. guttifera	07:49[a]	*D. palustris*	07:16[a]
	06:37[c]	*D. paramelanica*	04:43[a]
D. hamatofila	09:26[a]	*D. paulistorum*	15:25[a]
D. hydei	02:13[a]	*D. peninsularis*	01:37[a]
	02:22[c]		02:56[c]
D. immigrans	47:47[a]	*D. persimilis*	05:46[a]
	40:00[c]		06:02[c]
D. lacicola	03:10[a]	*D. polychaeta*	00:25[a]
D. lebanonensis	00:47[a]		00:58[c]
D. limpiensis	38:56[a]	*D. prosaltans*	21:08[a]
D. longicornis	02:21[a]		11:34[c]
D. macrospina	46:09[a]	*D. pseudoobscura*	07:01[a]
	32:49[c]		05:43[c]
D. melanica	07:03[c]		

Table XI. (*Continued*)

Species	Mean duration (minutes:seconds)	Species	Mean duration (minutes:seconds)
D. quinaria	05:30[a]	*D. sturtevanti*	12:54[c]
	07:08[c]	*D. takahashii*	17:30[a]
D. repleta	02:01[a]	*D. texana*	03:35[a]
	02:56[c]		02:43[c]
D. ritae	08:57[a]	*D. transversa*	07:11[a]
D. robusta	00:34[a]	*D. tripunctata*	33:25[a]
	00:40[c]		38:11[c]
D. rufa	20:15[a]	*D. trispina*	15:17[a]
D. simulans	16:52[a]	*D. tropicalis*	12:16[a]
	23:39[c]	*D. tumiditarsus*	24:16[a]
D. subfunebris	37:31[a]	*D. victoria*	00:33[a]
	28:39[c]	*D. virilis*	03:11[a]
D. subobscura	08:15[a]		02:05[c]
D. suboccidentalis	13:44[a]	*D. wassermani*	14:09[b]
D. subpalustris	07:11[a]	*D. willistoni*	14:41[a]
D. subquinaria	09:42[a]		17:30[c]
D. sucinea	20:33[a]		
	17:16[c]		

[a]Spieth (1952).
[b]Pitnick and Marklow (1994*a*).
[c]Grant (1983).
[d]Pitnick *et al.* (1991).

composition of their offspring with each mate. These factors are not mutually exclusive, and female remating may be driven by different forces in different species.

Unfortunately, remating frequency has not been studied in enough species of *Drosophila* to determine the degree to which it forms part of a suite of female characters. Species vary with respect to the impact of remating on female fertility. In some species there is little gain in progeny numbers, while in others the gain is significant (Markow, 1982; Pitnick, 1993; Snook, 1995). When we first found that females of certain *Drosophila* species remated very frequently, we were certain that it was to obtain nutrients from males (Markow and Ankney, 1984). It is clear from Table VII that this is not the case. In species in which females mate most frequently, males either make no ejaculate or salivary donation, or they make an insignificant one. Furthermore, remating incidence is not related to age at maturity, egg size, or ovariole number.

Drosophila females have two sperm storage organs: the ventral receptacle and the paired spermathecae. The size and shape of these structures are highly variable (Throckmorton, 1975), and species differ as to whether they use one or

both sperm storage organs (Pitnick and Markow, 1994*a*; T. A. Markow, unpublished data). There is a general positive relationship between ventral receptacle length and sperm length, even in species that use only spermathecae. A close examination of the relationship between the size and morphology of the storage organs and female remating may be more informative.

Is there any indication that female reproductive allocation reflects the existence of suites of characters? Despite the existence of ample interspecific variation in female reproductive characters, they have not yet been examined with the same scrutiny as have male characters. An obvious relationship to seek is the predicted trade-off between egg size and number (Cody, 1966; Wilbur, 1977). Two data sets exist in which both egg size and ovariole numbers are available for a number of species, one that includes a variety of species with respect to phylogeny and geographic distribution (Kambysellis, 1968), and the other consisting of Hawaiian endemics (Kambysellis and Heed, 1971), and both of which are incorporated into this review. Montague *et al.* (1981) utilized the latter to test for an interspecific correlation between egg size and number, inferring egg number from ovariole number. They found that the relationship was discontinuous and that the discontinuities were related to larval habitat (see Phylogenetic and Ecological Origins section).

Unfortunately, the remaining interspecific data are not suitable to accurately test for the existence of the predicted trade-offs. Species for which the relevant data are summarized are not always the same for all of the characters of interest. For example, 12 species include data both on the age at female sexual maturity and egg size. For ovariole number and egg size, there are ten species, and for ovariole number and age at maturity there are nine. But these measures were made under a range of environmental conditions, and a recent study demonstrated a significant effect of adult female diet on egg size (Markow *et al.*, 1996*b*). Since existing measures have not always been made using the same protocol or with similar degrees of rigor, the absence of any correlations in existing data sets would be impossible to interpret.

Ovariole number is highly variable within species as well. Robertson (1957) successfully selected for high and low ovariole number in *D. melanogaster* and obtained realized heritabilities of 0.46 ± 0.03 in the high line and 0.14 ± 0.06 in the low line. The asymmetry is obviously confounded with ceiling effects. Robertson was also interested in the relationship between ovariole number and egg number and examined this in several ways. While the high ovariole selected line produced more eggs than the low line, the difference was not significant. In other words, having more ovarioles could not significantly increase the rate at which eggs could be produced. In another, more elegant experiment, one of the two primordial gonads was surgically removed from female larvae and egg production of adult females was measured. Single-ovary females increased their per

ovary egg output by 50% over control females. Although ovariole number was unchanged, single ovaries were observed to be larger and to fill the abdominal cavities.

These experiments indicate that ovarioles are competing for a limited amount of nutrients and that the supply of nutrients places an important constraint on the output of eggs. When body size was manipulated by nutritional deprivation of larvae, a proportional reduction in ovariole number and egg production was observed (Robertson, 1957). However, there was no influence of body size on the number of eggs produced per ovariole, and body size and ovariole number were uncorrelated. On the other hand, Heed and Mangan (1986) found that ovariole number and body size were strongly correlated in species of cactophilic *Drosophila*.

The likelihood that nutrient availability within females limits egg production has important implications for the relationships among female reproductive characters as well as for the evolution of mating systems. One character related to age of reproduction, but not discussed above, is the length of time required for females to produce mature eggs, or the length of oogenesis. Kambysellis (1968) describes considerable variation among species with respect to the stage of oogenesis present at the time of eclosion. Using the classification of King *et al.* (1956), in which vitellogenesis begins at stage 8 of 14 stages, species were found to range from stage 2 to stage 7 at the time adult females eclose. Species differed also in the rates at which oogenesis proceeds, and mature eggs are finally produced from 3 to 9 days under laboratory conditions. The age at which females are sexually receptive may not exactly correspond to the age at which they have mature oocytes, but this relationship has yet to be specifically addressed in a comparative study.

If the rate of oogenesis is nutrient-limited, we can envision the advantages to females of securing materials from males, either by salivary or seminal feeding. Yet in *D. mojavensis,* where males provide a huge nutrient contribution, the majority of females do not mate until 3 days of age, and if they are nutritionally deprived, the delay in sexual receptivity is even further delayed (Markow *et al.*, 1990). Trevitt and Partridge (1991) and Chapman *et al.* (1995) suggested that mating shortens female lifespan in *D. melanogaster,* making it less surprising that females may delay mating until they are capable of reproduction. It may be that in species with seminal feeding, males are providing only a specific type of precursor that is not required in large amounts, and thus it would be of little value to females to mate early, especially given a cost in longevity. This should be the case for species whose oviposition sites may be scarce or unpredictable, making maximization of longevity an important consideration. On the other hand, females need an adequate sperm supply in the event that an oviposition site is encountered.

MALE REPRODUCTIVE STRATEGIES

Interspecific variation in *Drosophila* male reproductive characters is far more extensive than observed for females. Sperm length variation is the most extensive in nature, reproductive maturity can be delayed by 2½ weeks, and males of some species provision oocytes through their ejaculates. While many of the species reviewed here do not exhibit such extreme mating system features, unusual characters occur in enough of them to raise questions as to why they exist.

Sperm length is the most variable character described above. Male gametes have long been assumed cheap to produce because they are small (Parker, 1970). When sperm are large, it raises the question of costs. The actual cost of making a long sperm versus a short sperm cannot be directly measured. However, assuming males have a limited amount of energy for growth and reproduction, certain trade-offs of producing giant sperm are expected. These trade-offs include number of gametes produced, time required to produce them, ability to direct energy into other kinds of reproductive effort such as seminal nutrition, as well as more general life history characters such as development time, size, and longevity. It has been possible to examine the relationships between a number of these for some of the species described here.

Prior to the discovery of gigantic sperm in several *Drosophila* species, discussions in the literature concerning trade-offs between gamete size and number were approached from a female perspective, i.e., egg size versus clutch size (Cody, 1966; Wilbur, 1977). Pitnick and Markow (1994*a*) focused attention on the potential for similar trade-offs in males, suggesting that the relationship among related species of the *nannoptera* group is the same for male gametes: males making larger gametes produce far fewer of them. Reduction in the number of sperm produced is accompanied by fewer of them being transferred on a single mating (Table VIII). *Drosophila pachea* and *D. nannoptera* transfer 44 ± 6 and 81 ± 6 sperm, respectively, in a typical mating, while *D. acanthoptera* and *D. wassermani* transfer 1023 ± 48 and 274 ± 14, respectively, and their sperm are only about one third as long. This relationship was further supported by observations on *D. hydei*, in which males make a 23-mm long sperm and transfer an average of 126 sperm to females (Pitnick and Markow, 1994*b*). At the other end of the spectrum, the species with the shortest sperm, *D. pseudoobscura* and *D. melanogaster*, transfer 25,000 and about 5,000, respectively. Pitnick (1996) evaluates the relationship between sperm size and number in 11 *Drosophila* species, removing effects of phylogeny and showing this trade-off to be significant, with males producing giant sperm making a large investment in testes as well.

Production of fewer larger gametes does not mean that males exhaust their fertility earlier than males making larger numbers of shorter sperm. *Drosophila melanogaster* males transfer thousands of sperm and suffer a period of temporary sterility on or after the third or fourth consecutive copulation (Lefevre and Jonsson, 1962). This loss of fertility, in *D. melanogaster*, has been thought to reflect a depletion of accessory gland secretions as well as of sperm supply (Lefevre and Jonsson, 1962; Kaufmann and Demerec, 1942; Markow *et al.*, 1978), but this assumption has been recently challenged (Snook, 1995). *Drosophila mojavensis* and *D. hydei* provided the first indications that not all *Drosophila* species are characterized by a fertility decline after consecutive matings (Markow, 1982, 1985). Furthermore, both of these species differ from *D. melanogaster* in that a single mating with the former yields hundreds of progeny, while in *D. mojavensis* a single mating yields 51, and in *D. hydei* a single mating yields 55. In other words, unlike *D. melanogaster*, males of these species appear to partition their sperm across a series of females. The concept of male gametic strategies is discussed in Pitnick and Markow (1994*a*), who expanded these observations to the *nannoptera* species group and firmly linked allocation of ejaculates to the size of the gametes produced for most species.

That delayed maturation is a significant cost of giant sperm production has been revealed in both intraspecific and interspecific studies. In *D. hydei*, males produce the same size sperm regardless of their body size (Pitnick and Markow, 1994*b*). Because large testes are required to produce large sperm in *Drosophila* (Pitnick, 1995), smaller males devote a greater proportion of their growth to testes than do larger males in *D. hydei* and require significantly longer to become sexually mature (Pitnick and Markow, 1994*b*). Interspecific comparisons reveal the same trade-off. When we assessed the relationship between sperm length, age at maturity, and body size in 42 species, controlling for phylogeny by independent contrasts, delayed maturity was significantly associated with long sperm production, although this cost appears partially mitigated by large size (Pitnick *et al.*, 1995*b*).

The other form of investing heavily in reproduction, for males, is the production and transfer of large ejaculates. As described above, males in species exhibiting seminal nutrition may invest up to 3% of their body components in a single copulation, raising the question of costs of such a strategy. No correlation was found between male size and ejaculate size in *D. mojavensis* (Markow *et al.*, 1990). In species in which males contribute ejaculate proteins to ovarian production, sexual maturity is delayed significantly, although not to the same degree as in species making giant sperm (Pitnick *et al.*, 1995*c*).

Given the significant cost associated with allocation to long sperm or to ejaculate nutrition, males could potentially allocate to one or the other, but not both. While this may be the trend for species examined thus far, there are

exceptions. The species making the largest sperm, *D. bifurca,* also makes an ejaculate contribution to females. However, males of this species also exhibit an extreme delay in male reproductive maturity (Pitnick *et al.*, 1995*a–c*).

Thus several male mating strategies appear to exist in *Drosophila.* One is the production of expensive ejaculates, either in the form of giant sperm or, to a somewhat lesser degree, seminal nutrition. At the opposite extreme is the least expensive strategy: production of large numbers of tiny gametes with no ejaculate donations. This group includes species of the *obscura* group, in which males produce more than one type of sperm (Snook, 1995). A large number of species fall somewhere in between these two extremes.

Only a few of the potential trade-offs of one extreme strategy over another have been examined. The relative costs of an "expensive ejaculate" strategy versus an "inexpensive ejaculate" strategy can only be fully defined when placed in a broader biological context. For example, male fitness characters, such as longevity and lifetime reproductive success, have not been described for any of these species except *D. melanogaster.* From an energetics perspective, males from species with costly ejaculates should have less energy to invest in dispersal and in mate acquisition activities.

ARE THERE DISTINCT MATING SYSTEMS IN *DROSOPHILA?*

Male and female reproductive characters are all variable to some degree, and for the most part this variability is continuously distributed. Many species do not exhibit especially extravagant reproductive strategies. However, extreme expression of a number of male and female traits are associated in predictable ways, revealing two distinguishable patterns:

1. Pattern A. Males investing heavily in reproduction, either in large gametes or in seminal feeding, show delayed reproductive maturation and the partitioning of ejaculates across sequential matings. Females remate relative frequently. Species showing this type of mating system include *D. hydei, D. pachea, D. mojavensis, D. arizonae,* and *D. nigrospiracula.* The robustness of the apparent association between male ejaculate partitioning and frequent female remating is a critical question and should be tested in additional species in which males make a costly versus a cheap ejaculate.

2. Pattern B. At the opposite end of the spectrum are species such as *D. melanogaster* and *D. pseudoobscura,* in which males mature relatively early, produce and transfer thousands of tiny sperm per copulation, but do not appear as able to partition ejaculates between successive mates as are males of pattern A. This is likely to be related to the fact that females of these species do not remate

frequently, but whether the male strategy of passing more sperm preceded the longer latency of female remating or whether fewer mating opportunities selected for larger ejaculates is unknown.

Depending on how frequently females remate and how few mature males are present, the operational sex ratio (OSR) can become female-biased, with more mating opportunities for males. This is the case for species with a pattern A mating system. In *D. pachea,* the only pattern A species thus far examined in nature, the OSR was extremely female biased (Pitnick, 1993), allowing numerous mating opportunities for sexually mature males. In such species, there should be less competition among males to secure copulations. In fact, an extremely female-biased OSR could promote competition among females for mates.

Depending on the pattern of sperm utilization prior to female remating, competition between ejaculates may be common. The potential for sperm competition should select for any mechanism that can assure paternity. A number of reproductive characters in pattern A species have been proposed to function in this capacity. One is the association between the insemination reaction mass or copulatory plug and seminal feeding (Markow and Ankney, 1988). Another is male preference for females with whom they will maximize their fitness. In *D. mojavensis,* the time that has elapsed since the first mating has been shown to influence the proportion of progeny sired by the second male (P2), such that the longer the interval, the higher the P2 (Markow, 1982). Male *D. mojavensis* were observed to discriminate against recently mated females, even prior to courting them. Partitioning of ejaculates between females is another potential male adaptation to sperm competition. In *D. hydei,* sperm from males mating sequentially are equally represented in the progeny (Markow, 1985), but males pass few sperm to any given female, a possible form of bet hedging (Pitnick and Markow, 1994*a*). And finally, sperm gigantism itself may be advantageous in sperm competition (Pitnick *et al.*, 1995*a*).

Under conditions of less frequent female remating (pattern B), the OSR will be more male-biased, leading to selection for characters that enhance male courtship success. These may include large size and behavioral or morphological traits that give males an advantage in mating. This is not to suggest that sperm competition is unimportant, only that selection on mating ability is more intense when opportunities to mate are limited. These hypotheses have not yet been specifically addressed. However, in some pattern B species, there is rather obvious sexual dimorphism in size and coloration, as well as the presence of secondary sexual characters such as the sex combs on the foretarsi of males (Markow *et al.*, 1995*a*). Observations on natural populations of *D. melanogaster* and *D. simulans* suggest that mating opportunities for males of these species are extremely limited (Gromko and Markow, 1993). Males were observed to spend considerable time courting, which, under conditions of high density, included extensive

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male–male aggressive interactions as well as delivery of courtship to individual females. However, matings were infrequent.

Studies of male size and mating success in *Drosophila* have been inconsistent, suggesting that the relationship is a complex one. Most studies report a size advantage to large males of a variety of *Drosophila* species (Markow, 1988*b*; Markow and Sawka, 1992; Partridge *et al.*, 1987; Ruiz *et al.*, 1991; Santos *et al.*, 1988), but other studies of the same species fail to show this relationship (Markow and Ricker, 1992; Partridge *et al.*, 1987; Markow *et al.*, 1996*a*; Zamudio *et al.*, 1995; James and Jaenike, 1992). One study, of *D. montana*, revealed the advantage was held by small males (Aspi and Hoikkala, 1995). Given what is now known about delayed male maturity in certain species, it is clear that studies of male size and courtship success must include some assessment of male age in their design. Solitary males may, on the average, be smaller than mating males merely because they are younger and natural selection has not yet had the opportunity to operate.

The existence of species in which females remate frequently also provides the opportunity to examine the relationship between remating and fitness. Ridley (1988) attempted to resolve the question of why female insects remate. The focus of that review was to determine if females remate to maintain their fertility. While that study correctly pointed out the importance of controlling for paternal investment in future studies, the extensive variation in sperm size and numbers had not yet been reported. This variation may influence remating to an even greater extent than paternal investment.

In species making few sperm, gametic ratios in the species as a whole will be far less biased toward mate gametes and potentially may even be biased toward eggs. In extreme cases, sperm may even be limiting, as suggested by Pitnick (1993) for *D. pachea*. It is also clear from Table VII, though the number of species examined is small, that storage and fertilization efficiency are much greater in species making fewer, longer sperm. Sperm limitation may explain the greater efficiency of utilization in these species.

The last mating system feature that deserves mention is copulation duration. Copulation duration is a highly variable reproductive character that is the product of the behavior of both sexes. Its degree of variability is on the same level as sperm length, and for this reason its significance has intrigued many investigators (Spieth, 1952; Grant, 1983; Gromko *et al.*, 1991). Attempts to correlate copulation duration with other sexual characters have been completely unsuccessful. Species with longer copulation durations do not transfer more or less expensive ejaculates, nor is duration of copulation related to remating frequency. Grant (1983) found no relationship of duration with the formation of the copulatory reaction mass. Gromko *et al.* (1991) failed to find any consistent correlated responses in other reproductive traits to selection for copulation duration. Pairs *in copula* are assumed to be more vulnerable to predation or parasites, since they

are typically immobile, suggesting a greater cost of copulation in species like *D. acanthoptera* or *D. pachea*, compared to *D. mulleri*. That a consistent relationship between copulation during and any other reproductive character has not been found remains puzzling.

PHYLOGENETIC AND ECOLOGICAL ORIGINS OF MATING SYSTEM VARIATION

Why do we see such extreme mating strategies and what are their consequences for the genetic structure and evolutionary potential of these species? These mating systems have their roots both in the long-term phylogenetic histories and the more recent ecological pressures confronting each species. Phylogenetic patterns are already obvious for some characters. For example, sperm heteromorphy has only been described in the *obscura* group, and the production of a seminal nutrient donation is most developed in the *mulleri* complex of the *repleta* group and the *quinaria* group. On the other hand, sperm gigantism has arisen rather frequently in unrelated lineages (Pitnick *et al.*, 1995*b*). The production of two different kinds of expensive ejaculates that appear, for the most part, to be mutually exclusive suggests that these strategies arose independently and, compared to pattern B, are highly derived. Both appear to be associated with frequent female remating, raising the question as to whether one evolved in response to the other. Two interpretations—that females remate to obtain nutrients or to maintain their sperm supply—form circular arguments with why males make expensive ejaculates. As additional species become more completely characterized with respect to these reproductive characters, it should be possible to explore the evolutionary relationships among them using techniques such as character mapping.

Broadening our understanding of *Drosophila* reproductive ecology should also be useful in addressing the evolution of female mating strategies. However, with few exceptions, ecological information is restricted to knowing the feeding and breeding sites of a particular species (Heed, 1978; Pipkin *et al.*, 1966; Atkinson and Shorrocks, 1978). Breeding site specificity is useful in the study of adaptation to host chemistry (Jaenike *et al.*, 1983; Fogleman and Abril, 1990), but unless the quality and distribution of resources are documented, this knowledge will be of limited value in understanding mating system evolution. For example, females of cosmopolitan species of *Drosophila* are polyphagous, and are thus not likely to confront the same constraints as are females of monophagous species whose hosts are rare and perhaps unpredictable (Heed and Mangan, 1986; Breitmeyer, 1994).

While we are far from understanding the impact of resource variation on

Drosophila mating systems, several studies point to the potential of this approach for understanding relationships among reproductive characters. Heed and Mangan (1986) examined the relationship between female reproductive effort (the ratio of thorax length to ovariole number) and resource characteristics for cactophilic *Drosophila* endemic to the Sonoran Desert. The cactus host species of flies showing greater reproductive effort, such as *D. nigrospiracula*, provided more stable larval habitats, but were greater distances apart than the less stable host cacti of *D. mojavensis*, which showed a lower allocation to reproduction. These authors pointed out that the need to disperse greater distances between larger, more stable hosts is a factor underlying the larger size of *D. nigrospiracula*.

For Hawaiian *Drosophila*, Montague *et al.* (1981) showed that in species using temporally and spatially predictable resources of low-quality clutch sizes are significantly smaller than in species whose resources were high quality but unpredictable. Lachaise (1983) examined the correlation between delay in female reproduction and ecological breadth in African species of *Drosophila*, and concluded that more specialized species are significantly delayed over generalists in attaining reproductive maturity. He also showed that specialized species are less productive than generalists.

CONCLUSIONS

Drosophila species clearly vary greatly with respect to all features of their reproductive biology. Above, I have attempted to not only review this variation, but to propose the existence of patterns that may reflect the nature of the forces underlying the observed differences. My classification of *Drosophila* mating systems is rooted in the extent to which males invest in their ejaculates; expensive ejaculates versus inexpensive ones. This scheme is based on what information is presently available on the mating systems of these species. It is not intended to be complete or cast in stone. Hopefully the summarized interspecific mating system features as well as the categories proposed will inspire additional investigations and suggest new approaches.

Male mating strategies appear to be strongly associated with female remating incidence; but female remating, at least in species examined thus far, does not appear to show any consistent relationships to other female traits or to ecological parameters. This could be because in different species females remate for different reasons, rather than because we lack adequate interspecific data to detect the existence of patterns. Ecological conditions favoring female remating have not been established. Female reproductive allocation, on the other hand, is suggested to be associated with the quality and distribution of breeding sites, at least in those species examined.

The term *mating system* carries a different meaning for evolutionary ecologists than for geneticists. Mating systems, for geneticists, are concerned with the outcomes of types of matings, namely inbreeding versus outcrossing, for the genetic structure of populations and species. These two approaches are not as conceptually different as might appear at first glance. The incidence of remating, especially when coupled with long-range dispersal, can strongly influence the genetic structure and evolutionary potential of a species. Only in *Drosophila* do these two approaches to the study of mating systems have the potential to converge into an enormous and uniquely informative picture of evolutionary principles.

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REFERENCES

Atkinson, W., and Shorrocks, B., 1978, Breeding site specificity in the domestic species of *Drosophila*, *Oecologia* **29**:223–232.

Aspi, J., and Hoikkala, A., 1992, Sexual selection in *Drosophila littoralis* and *Drosophila montana*. I. Male mating success and survival in the field with respect to size and courtship song characters, *J. Insect Behav.* **8**:67–72.

Aspi, J., Lumme, J., Hoikkala, A., and Heikkinen, E., 1993, Reproductive ecology of the boreal riparian guild of *Drosophila*, *Ecography* **16**:65–72.

Bairati, A., 1968, Structure and ultrastructure of the male reproductive system in *Drosophila melanogaster*. Meig II. The genital duct and accesory glands, *Monit. Zool. Ital.* **2**:105–182.

Bastock, M., 1956, A gene mutation which changes a behavior pattern, *Evolution* **10**:421–439.

Baumann, H., 1974*a*, The isolation, partial characterization and biosynthesis of the paragonial substances, PS-1 and PS-2, of *Drosophila funebris*, *J. Insect Physiol.* **20**:2181–2194.

Baumann, H., 1974*b*, Biological effects of the paragonial substances, PS-1 and PS-2, in females of *Drosophila funebris*, *J. Insect Physiol.* **20:**2181–2194.

Beatty, R. A., and Burgoyne, P. S., 1971, Size classes of the head and flagellum of *Drosophila* spermatozoa, *Cytogenetics* **10:**177–181.

Benzer, S., 1973, Genetic dissection of behavior, *Sci. Am.* **229:**24–37.

Boake, C. R. B., and Adkins, E., 1994, Timing of male physiological and behavioral maturation in *Drosophila silvestris* (Diptera: Drosophilidae), J Insect Beh **7:**411–584.

Boggs, C. L., and Gilbert, L. E., 1979, Male contribution to egg production in butterflies: Evidence for transfer of nutrients at mating, *Science* **206:**83–84.

Bownes, M., and Partridge, L., 1987, Transfer of molecules from ejaculate to females in *Drosophila melanogaster* and *Drosophila pseudoobscura*, *J. Insect Physiol.* **33:**841–842.

Breitmeyer, C. M., 1994, Factors influencing population size in Sonoran Desert *Drosophila*. [MSc thesis.] Arizona State University, Tempe, Arizona.

Brieger, G., and Butterworth, F. M., 1970, *Drosophila melanogaster:* Identity of the male lipid in reproductive system, *Science* **167:**1262–1264.

Chapman, T. L., Liddle, F., Kalb, J. M., Wolfner, M. F., and Partridge, L., 1995, Cost of mating in *Drosophila melanogaster* females is mediated by male accesory gland products, *Nature* **373:**241–244.

Chen, P. S., Fales, H. M., Levenbook, L., Sokoloski, E. A., and Yeh, H. J. C., 1977, Isolation and characterization of a unique galactoside from male *Drosophila melanogaster*, *Biochemistry*, **16:**4080–4085.

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., and Bohlen, P., 1988, A male accesory gland peptide that regulates reproductive behavior of female *D. melanogaster*, *Cell* **54:**291–298.

Cobbs, G., 1977, Multiple insemination and male sexual selection in natural populations of *Drosophila pseudoobscura*, *Am Nat.* **111:**641–656.

Cody, M. L., 1966, A general theory of clutch size, *Evolution* **20:**174–184.

Coulthart, M. B., and Singh, R. S., 1988, Differing amounts of genetic polymorphism in testes and male accesory glands of *Drosophila melanogaster* and *Drosophila simulans*, *Biochem. Genet.* **26:**153–164.

Coyne, J. A., 1989, Genetics of sexual isolation between two sibling species, *Drosophila simulans* and *Drosophila mauritana*, *Proc. Natl. Acad. Sci. USA* **86:**5464–5468.

Coyne, J. A., 1993, The genetics of an isolating mechanism between two sibling species of *Drosophila*, *Evolution* **47**(3)**:**778–788.

Coyne, J. A., Boussy, I. A., Prout, T., Bryant, S. H., Jones, J. S., and Moore, J. A., 1982, Long distance migration of *Drosophila*, *Am. Nat.* **119:**589–595.

Coyne, J. A., Crittenden, A. P., and Mah, K., 1994, Genetics of a pheromonal difference contributing to reproductive isolation in *Drosophila*, *Science* **265:**1461–1464.

Coyne, J. A., Rux, J., and David, J. R., 1991. Genetics of morphological differences and hybrid sterility between *Drosophila Sechella* and its relatives, *Genet. Res.* **57:**113–122.

Craddock, E. M., and Johnson, W. E., 1978, Multiple insemination in natural populations of *Drosophila silvestris*, *Drosophila Inf. Serv.* **53:**138–139.

David, J. R., 1970, Le nombre d'ovarioles chez la *Drosophila:* Relation avec la fecondite et valeur adaptative, *Arch. Zool. Exp. Genet.* **111:**357–370.

Dobzhansky, Th., and Wright, S., 1943, Genetics of natural populations. X. Dispersion rates in *Drosophila pseudoobscura*, *Genetics* **28:**304–340.

Emlen, S. T., and Orring, L. W., 1977, Ecology, sexual selection and the evolution of mating systems, *Science* **197:**215–223.

Fogleman, J. C., and Abril, J. R., 1990, Ecological and evolutionary importance of host plant chemistry, in: *Ecological and Evolutionary Genetics of Drosophila*, (J. S. F. Barker, W. T. Starmer, and R. J. MacIntyre, eds.), pp. 121–144, Plenum Press, New York.

Gilbert, D. G., 1981, Ejaculate esterase-6 and initial sperm use by female *Drosophila melanogaster*, *J. Insect Physiol.* **27**:641–650.

Grant, B., 1983, On the relationships between average copulation duration and insemination reaction in the genus *Drosophila*, *Evolution* **37**:854–856.

Gromko, M. H., and Markow, T. A., 1993, Courtship and remating in field populations of *Drosophila*, *Anim. Behav.* **45**:253–262.

Gromko, M. H., and Newport, M. E. A., 1988, Genetic basis for remating in *Drosophila melanogaster*. II. Response to selection based on the behavior of one sex, *Behav. Genet.* **18**:621–632.

Gromko, M. H., Sheehan, K., and Richmond, R. C., 1980, Random mating in two species of *Drosophila*, *Am. Nat.* **115**:467–479.

Gromko, M. H., Briot, A., Jensen, S. C., and Fukui, H. H., 1991, Selection on copulation duration in *Drosophila melanogaster:* Predictability of direct response versus unpredictability of correlated response, *Evolution* **45**:69–81.

Gwynne, D. T., 1983, Male nutritional investment and the evolution of sexual differences in Tettigoniidae and other Orthoptera, in: *Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects* (D. T. Gwynne and G. K. Morris, eds.), pp. 337–366, Westview Press, Boulder, CO.

Hall, J. C., 1977, Portions of the central nervous system controlling reproductive behavior in *Drosophila melanogaster*, *Behav. Genet.* **7**:291–392.

Harshman, C. G., Hoffman, A. A., and Prout, T., 1988, Environmental effects on remating in *Drosophila melanogaster*, *Evolution* **42**:312–321.

Heed, W. B., 1978, Ecology and genetics of the Sonoran Desert *Drosophila*, in: *Ecological Genetics: The Interface* (P. F. Brussard, ed.), pp. 109–126, Springer-Verlag, New York.

Heed, W. B., and Mangan, R. L., 1986, Community ecology of the Sonoran Desert *Drosophila*, in: *The Genetics and Biology of Drosophila*, Vol. 3e (M. Ashburner, H. L. Carson, and J. N. Thompson Jr., eds.), pp. 311–341, Academic Press, London.

Hennig, W., and Kramer, H., 1990, Spermatogenesis of *Drosophila hydei*, *Int. Rev. Cytol.* **123**:129–175.

Hess, O., and Meyer, G. F., 1968, Genetic activities of the Y chromosome in *Drosophila* during spermatogenesis, *Adv. Genet.* **14**:171–223.

Jaenike, J., Grimaldi, D. A., Sluder, A. E., and Greenleaf, A. L., 1983, Alpha-amanitin tolerance in mycophagous *Drosophila*, *Science* **221**:165–167.

James, A., and Jaenicke, J., 1992, Determinants of male mating success in *Drosophila testacea*, *Anim. Behav.* **44**:168–170.

Johnston, J. S., and Heed, W. B., 1976, Dispersal of desert-adapted *Drosophila:* The saguaro-breeding *D. nigrospiracula*, *Am Nat.* **110**:629–651.

Joly, D., and Lachaise, D., 1994, Polymorphism in the sperm heteromorphic species of the *Drosophila obscura* group, *J. Insect. Physiol.* **40**:933–938.

Kambysellis, M. P., 1968, Comparative studies of oogenesis and egg morphology among species of the genus *Drosophila*, *Univ. Texas Stud. Genet.* **6852**:71–92.

Kambysellis, M. P., and Heed, W. B., 1971, Studies of oogenesis in natural populations of Drosophilidae. I. Relation to ovarian development and ecological habitats of the Hawaiian species. *Am. Nat.* **105**:67–91.

Karr, T. L., and Pitnick, S., 1996, The ins and outs of fertilization, *Nature* **379**:405–406.

Kaufmann, B. P., and Demerec, M., 1942, Utilization of sperm by the female *Drosophila melanogaster*, *Am. Nat.* **76**:445–469.

King, R. C., Robinson, A., and Smith, R., 1956, Oogenesis in *Drosophila melanogaster*, *Growth* **20**:121–140.

Lachaise, D., 1983, Reproductive allocation in tropical Drosophilidae: Further evidence on the role of breeding-site choice, *Am. Nat.* **122**:132–146.

Lefevre, G. Jr., and Jonsson, U. B., 1962, Sperm transfer, storage displacement and utilization in *Drosophila melanogaster*, *Genetics* **47**:1719–1736.

Letsinger, J. T., and Gromko, M. H., 1985, The role of sperm numbers in sperm competition and female remating in *Drosophila melanogaster*, *Genetica* **66:**195–202.

Loukas, M., Vergini, Y., and Krimbas, C. B., 1981, The genetics of *Drosophila subobscura* populations. XVIII. Multiple insemination and sperm displacement in *Drosophila subobscura*, *Genetics* **57:**29–37.

Manning, A., 1965, *Drosophila* and the evolution of behavior, in: *Viewpoints of Biology* (J. Carthy, and C. Duddington, eds.), Butterworth, London.

Markow, T. A., 1982, Mating systems of cactophilic *Drosophila*, in: *Ecological Genetics and Evolution: The Cactus–Yeast–Drosophila Model System* (J. S. F. Barker and W. T. Starmer, eds.), pp. 273–287, Academic Press, New York.

Markow, T. A., 1985, A comparative investigation of the mating system of *Drosophila hydei*, *Anim. Behav.* **33:**775–781.

Markow, T. A., 1987, Genetics of sexual selection in *Drosophila*, in: *Genetics of Invertebrate Behavior* (H. Huettl, ed.), pp. 89–91, Plenum Press, New York.

Markow, T. A., 1988*b;* Reproductive behavior of *Drosophila* in the laboratory and in the field, *J. Comp. Physiol.* **102:**169–174.

Markow, T. A., and Ankney, P. F., 1984, *Drosophila males* contribute and oogenesis in a multiple mating species, *Science* **224:**302–303.

Markow, T. A., and Ankney, P. F., 1988, Insemination reaction in *Drosophila:* A copulatory plug in species showing male contribution to offspring, *Evolution* **42:**1097–1100.

Markow, T. A., and Ricker, J. P., 1992, Male size, development stability, and mating success in natural populations of three *Drosophila* species, *Heredity* **69:**122–127.

Markow, T. A., and Sawka, S., 1992, Dynamics of mating success in experimental groups of *Drosophila*, *J. Insect Behav.* **5:**375–383.

Markow, T. A., Quaid, M., and Kerr, S., 1978, Male mating experience and competitive courtship success in *D. melanogaster*, *Nature* **276:**821–822.

Markow, T. A., Gallagher, P. D., and Krebs, R. A., 1990, Ejaculate derived nutritional contribution and female reproductive success in *Drosophila mojavensis*, *Func. Ecol.* **4:**67–73.

Markow, T. A., Bustoz, D., and Pitnick, S., 1996*a*, Sexual selection and a secondary sexual character in two *Drosophila* species, *Anim. Behav. (in press).*

Markow, T. A., Tello, K., and Saldaña, S., 1996*b*, Ovariole number and egg size in the species of *Drosophila*, *in preparation.*

Milkman, R., and Zeitler, R. R., 1974, Concurrent multiple paternity in natural and laboratory populations of *Drosophila melanogaster*, *Genetics* **78:**1191–1193.

Montague, J. R., Mangan, R. L., and Starmer, W. T., 1981, Reproductive allocation in the Hawaiian Drosophilidae: Egg size and number, *Am. Nat.* **118:**865–871.

Osanai, M., Kusuga, H., and Aigaki, T., 1989, Isolation of eupyrene sperm bundles and apyrene spermatozoa from seminal fluid of the silkmoth, *Bombyx mori*, *J. Insect Physiol.* **35:**401–408.

Parker, G. A., 1970, Sperm competition and its evolutionary consequences in the insects, *Biol. Rev.* **45:**525–567.

Partridge, L., Hoffman, A., and Jones, S., 1987, Male size and mating success in *Drosophila melanogaster* and *Drosophila pseudoobscura* under field conditions, *Anim. Behav.* **35:**468–476.

Pipkin, S. B., Rodríguez, R. L., and León, J., 1966, Plant host specificity among flower-feeding neotropical *Drosophila* (Diptera: Drosophilidae), *Am. Nat.* **100:**135–156.

Pitnick, S., 1993, Operational sex ratios and sperm limitation in populations of *Drosophila pachea*, *Behav. Ecol. Sociobiol.* **33:**383–391.

Pitnick, S., 1996, Investment in testes and the cost of making long sperm in *Drosophila*, *Am. Nat. (in press).*

Pitnick, S., and Markow, T. A., 1994*a*, Male gametic strategies: Sperm size, testes size, and the allocation of ejaculate among successive mates to the sperm-limited fly *Drosophila pachea* and its relatives, *Am Nat.* **143:**785–819.

Pitnick, S., and Markow, T. A., 1994*b*, Large male advantage associated with cost of sperm production in *Drosophila hydei*, a species with giant sperm, *Proc. Natl. Acad. Sci. USA* **91:**9277–9281.

Pitnick, S., Markow, T. A., and Riedy, M., 1991, Transfer of ejaculate and incorporation of male-derived substances by females in the nannoptera species group (Diptera: Drosophilidae), *Evolution* **45:**774–780.

Pitnick, S., Spicer, G., and Markow, T. A., 1995*a*, How big is a giant sperm? *Nature* **375:**109.

Pitnick, S., Markow, T. A., and Spicer, G., 1995*b*, Delayed male maturity is a cost of producing large sperm in *Drosophila*, *Proc. Natl. Acad. Sci. USA* **92:**10614–10618.

Pitnick, S., Spicer, G., and Markow, T. A., 1995*c*, Male ejaculating contributions in *Drosophila* species, *Evolution* (in review).

Pyle, D. W., and Gromko, M. H., 1981, Genetics basis for repeated mating in *Drosophila melanogaster*, *Am. Nat.* **117:**133–146.

Richmond, R. C., and Senior, A., 1981, Esterase 6 of *Drosophila melanogaster:* Kinetics of transfer to females, decay in females and male recovery, *J. Insect Physiol.* **27:**849–853.

Ridley, M., 1988, Mating frequency and fecundity in insects, *Biol. Rev.* **63:**509–549.

Robertson, F. W., 1957, Studies in quantitative inheritance X. Genetic variation of ovary size in *Drosophila*, *J. Genet.* **55:**410–427.

Ruiz, A., Santos, M., Barbadilla, A., Quezada-Díaz, J. E., Hasson, E., and Fontedevilla, A., 1991, Genetic variance for body size in a natural population of *Drosophila buzzattii*, *Genetics* **128:**739–750.

Sanger, W. G., and Miller, D., 1973, Spermatosoan length in species of the *Drosophila affinis* subgroup, *Am. Mid. Nat.* **90:**485–489.

Santos, M., Ruiz, A., Barbadilla, A., Quezada-Díaz, J. E., Hasson, E., and Fotedevilla, A., 1988, The evolutionary history of *Drosophila buzzattii* XIV. Larger flies mate more often in nature, *Heredity* **61:**255–262.

Snook, R. R., 1995, *The Evolution of Sperm Polymorphism in the Drosophila obscura group*, doctoral dissertation, Arizona State University, Tempe.

Snook, R. R., Markow, T. A., and Karr, T. L., 1994, Functional nonequivalence of sperm in *Drosophila pseudoobscura*, *Proc. Natl. Acad. Sci. USA* **91:**11222–11226.

Spieth, H. T., 1952, Mating behavior within the genus *Drosophila* (Diptera), *Bull. Am. Mus. Nat. Hist.* **90:**398–474.

Stalker, H. D., 1976, Enzymes and production in natural populations in *Drosophila euronotus*, *Genetics* **84:**375–384.

Steele, R. H., 1986*a*, Courtship feeding in *Drosophila subobscura*. I. The nutritional significance of courtship feeding, *Anim. Behav.* **34:**1087–1098.

Steele, R. H., 1986*b*, Courtship feeding in *Drosophila subobscura*. II. Courtship feeding by males influences mate choice, *Anim. Behav.* **34:**1099–1108.

Thomas, S., and Singh, R. S., 1992, A comprehensive study of genic variation in natural population of *Drosophila melanogaster*. VII. Varying rates of genic divergence as revealed by two-dimensinoal electrophoresis, *Mol. Biol. Evol.* **9**(3):507–525.

Thornhill, R., and Alcock, J., 1983, *The Evolution of Insect Mating Systems*, Harvard University Press, Cambridge, MA.

Throckmorton, L. H., 1962, The problem of phylogeny in the genus *Drosophila*, *Univ. Tex.* **6205:**206–343.

Throckmorton, L. H., 1975, The phylogeny, ecology, and geography of *Drosophila*, in: *Handbook of Genetics*, Vol. 3 (R. C. King, ed.), pp. 421–469, Plenum Press, New York.

Trevitt, S., and Partridge, L., 1991, The cost of receiving sperm in the female fruitfly *Drosophila melanogaster, J. Insect Physiol.* **37:**714–723.

Whalen, M., and Wilson, T. G., 1986, Variation and genomic localization of genes encoding *Drosophila melanogaster* male accesory gland proteins separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis, *Genetics* **114:**77–92.

Wilbur, H. M., 1977, Propagule size, number, and dispersion pattern in *Ambystoma* and *Asclipias, Am. Nat.* **111:**43–68.

Zamudio, K. R., Huey, R. B., and Crill, W. D., 1995, Bigger isn't always better: Body size, developmentsal and parental temperature and male territorial success in *Drosophila melanogaster, Anim. Behav.* **49:**671–677.