RESEARCH PAPER

Fly 6:2, 1-4; April/May/June 2012; © 2012 Landes Bioscience

The wild side of life
Drosophila reproduction in nature

Therese Ann Markow,[1,*] Stephen Beall[2] and Sergio Castrezana[1]

[1]Division of Biological Sciences; University of California at San Diego; La Jolla, CA USA; [2]Department of Ecology and Evolutionary Biology; University of Arizona; Tucson, AZ USA

Keywords: drosophila, species, reproduction, nature, copulation

Drosophila species vary in the rates at which females remate and the number of sperm they receive in the laboratory. In species such as *D. melanogaster* and *D. pseudoobscura,* in which females receive thousands of sperm and remate infrequently compared with species such as *D. hydei* and *D. nigrospiracula*, where females receive only a few hundred sperm and remate many times in a day, wild caught females should produce far more progeny. We tested this prediction by collecting, directly from nature, females of six species whose remating rates and number of sperm received vary from high to low and assessing the proportion of females with sperm and the number of progeny females produce. Over 95% of *D. pseudoobscura* and *D. melanogaster* females were inseminated while far fewer of the other species contained any sperm. In addition, *D. pseudoobscura* females produced progeny for over two weeks, *D. melanogaster* for over a week, while *D. hydei* and *D. nigrospiracula* females ran out of sperm after 1–2 d. These observations suggest extreme sperm limitation in these latter species.

Introduction

Remating by female Drosophila varies by species when measured in the laboratory.[1] In some species females have been observed to remate up to four times in a morning while in others females may not remate for a week or more. Laboratory studies also reveal that the number of progeny produced by a single mating varies as well. Those species in which females remate very frequently (FR) in the laboratory are the same species that produce few progeny per mating compared with females of infrequently remating (IR) species.[2]

In *D. melanogaster* and *D. simulans* natural populations, courted females that are willing to remate appear to have significantly reduced sperm loads: they produce very few progeny compared with courted conspecific females collected at the same time but which had refused to mate.[3] This observation is consistent with earlier reports in reference 4–6, that after the initial effects of copulation itself, it's the presence of sperm that controls whether or not females of these species will remate.

Given the extensive evolutionary interpretations of laboratory data on Drosophila reproduction, especially in terms of sexual selection, it is critical to know how accurately laboratory observations reflect the reproductive biology of Drosophila in nature. If laboratory observations on female remating rates and productivity mirror nature, several testable predictions can be made: (1) females of FR species, collected at random in nature, should produce fewer total progeny than females similarly collected IR species females; (2) wild-caught FR females also should exhibit a higher frequency of "spermless" females in nature relative to females of IR species. Spermless females will either be virgins or females that have run out of sperm.

We utilized the species listed in **Table 1** to test these predictions. We chose these species because they were easily collected in nature and because we already had information on female remating frequency and progeny production from single copulations in laboratory studies. Of the larger number of species for which laboratory data are available, these six species were accessible to us for field collections and follow-up studies of productivity of collected females. The actual number of sperm passed during copulation is difficult to measure in Drosophila, but in those few species for which these data are available (**Table 1**), Females in the FR species clearly receive fewer sperm per mating than in the IR species. We predicted, therefore, that randomly collected wild *D. melanogaster* and *D. pseudoobscura* females will produce more progeny than will wild females of *D. nigrospiracula* and *D. hydei*. We also predicted that a higher frequency of spermless females would be observed in the latter two species.

Results

Insemination frequencies and progeny production. Proportions of wild caught females found to be inseminated, as determined by inspection of dissected sperm storage organs, were nearly 100% in *D. melanogaster* and *D. pseudoobscura*, but only 60% in *D. nigrospiracula* and *D. mettleri* (**Table 2**).

*Correspondence to: Therese Ann Markow; Email: tmarkow@ucsd.edu
Submitted: 11/06/11; Revised: 01/21/12; Accepted: 01/31/12
http://dx.doi.org/10.4161/fly.19552

Table 1. Species of drosophila utilized in the field studies of female reproduction

Species	Female remating frequency-laboratory	Number of sperm transferred per copulation [Reference]
D. melanogaster	5 d	4,000[15,25,27]
D. pseudoobscura	3–4 d	25,000[11]
D. arizonae	daily	NA
D. hydei	4X day	126 + 10[26]
D. nigrospiracula	4X day	544[28]
D. mettleri	2X day	NA

Female remating frequency is from studies in which females were allowed opportunities to remate during a 2 h period every morning, one female in a vial with two males.[1] The number of sperm transferred was based on cytological observations in earlier investigations.

Table 2. Number of dissected wild-caught females with sperm in their reproductive tracts, the number of offspring produced by wild-caught females, number of days females were fertile after being captured

SPECIES	% females (n) with sperm	Mean ± SE (n) Offspring/Fertile female	Days fertile
D. melanogaster	98.4% (63)	182.1 ± 27.9 (21)	7.9 ± 0.8
D. pseudoobscura	96.5% (57)	225.2 ± 17.8 (36)	14.9 ± 0.8
D. arizonae	73.9% (69)	98.5 ± 10.6 (25)	5.6 ± 0.7
D. hydei	78.3% (23)	28.5 ± 4.8 (24)	1.3 ± 0.1
D. nigrospiracula	62.8% (35)	22.0 ± 2.1 (25)	1.2 ± 0.1
D. mettleri	59.1% (44)	17.9 ± 1.9 (27)	1.1 ± 0.1

Table 3. ANOVA of species differences in offspring of inseminated wild-caught Drosophila females

Source	DF	SS	F ratio	P
Species	5	1167776.2	44.2	5.50E-28
Error	152	803069.7		
Total	157	1970845.9		

Average progeny production by wild-caught females (**Table 2**) varied significantly (**Table 3**) among species. Distributions of progeny produced by individual females from each species were highly variable (**Fig. 1**). Even the most fully inseminated females of species such as *D. hydei*, did not approach the average progeny number produced by females of *D. melanogaster* and *D. pseudoobscura*. Although they lived for many weeks afterwards (data not shown), female *D. hydei*, *D. nigrospiracula* and *D. mettleri* stopped producing progeny within 1 or 2 d of collection. At the other extreme, female *D. pseudoobscura* continued producing progeny for about 2 weeks. Average progeny production was highest in those species, *D. melanogaster* and *D. pseudoobscura,* in which the majority of collected females also was found to be inseminated when dissected.

Reproductive potential of males from nature. Because the mean number of progeny produced by females of each species is less than the numbers reported from laboratory studies, we asked how many progeny males randomly selected from nature produce when mated to laboratory reared virgin females. The average number of offspring from these single matings to female *D. melanogaster* was 106.7 ± 5.0 (n = 10), to *D. hydei*, 20.5 ± 1.9 (n = 13), and to *D. arizonae* 57.6 ± 4.0 (n = 8), in each case lower than the values reported in laboratory studies (**Table 4**).

The relatively lower numbers of progeny produced by females collected from nature was not a function of their having fewer ovarioles. Ovariole numbers per female for *D. melanogaster* were 42.0 ± 1.66 (n = 12), for *D. hydei* 48.35 ± 2.02 (n = 9) and 32.72 ± 1.01 (n = 19) for *D. arizonae*. These values are well within the ranges observed for laboratory stocks of these same species.

Discussion

While we could not determine whether those wild females that lacked sperm were virgins or had run out of sperm, we feel the latter is more likely. On one hand, among *D. arizonae*, *D. hydei* and *D. nigrospiracula,* delays in male relative to female sexual maturity could argue for an abundance of virgin females in a population, and encounter rates with mature males therefore may be expected to be relatively low in nature for those species. However, all wild males that we collected and paired with virgin females were sexually mature and produced progeny, arguing against any deficiency of sexually mature males in the field. High dispersal rates[8] make it likely that wild populations contain many sexually mature males from a range a localities. Furthermore, the reproductive biology of *D. mettleri* suggests that the interspecific differences are not explained entirely by delayed male maturity. Male *D. mettleri* are sexually mature within a day of emergence while females attain maturity a day or two later.[1,9] Yet, a relatively high proportion of *D. mettleri* females in nature lack sperm and inseminated females run out of sperm quickly, consistent with frequent female remating in this species.[10] Elevated female remating, if it does occur in nature as well as in the laboratory, clearly is critical to maintain female fertility in *D. hydei*, *D. arizonae*, *D. nigrospiracula* and *D. mettleri*. In light of what is likely a less male-biased sex ratio in the FR species, the difference in the variance of female reproductive success in the two types of species would be of interest to compare.

It is puzzling, given the large numbers of sperm transferred by male *D. pseudoobscura* and *D. melanogaster* during a single copulation in the laboratory, that wild females of these species carry as few sperm as they do. Laboratory studies report that female *D. pseudoobscura* receive 25,000 sperm[11] and that female *D. melanogaster* receive 4,000–5,000[12-15] on a given copulation. Furthermore, in nature *D. pseudoobscura* females are known to mate with up to four males[16,17] and *D. melanogaster* females with up to six.[18] If the numbers of sperm transferred in laboratory matings is reflective of what occurs in nature, females receive enormous numbers of sperm relative to what they carry at any given time. Ovariole numbers in *D. pseudoobscura* and *D. melanogaster* are similar to those of species like *D. hydei* and *D. nigrospiracula*,

making it unlikely that females of the former two species are using sperm faster to fertilize eggs.[2]

Although sperm dumping by females[19] could contribute to the lower than expected sperm loads of some females, our data suggest that females in nature actually receive fewer sperm than they do in laboratory studies. Why might this be the case? Field caught males are unlikely to be virgins and thus most matings or rematings are unlikely to be with virgin males. On the other hand laboratory matings typically employ flies that have been held as virgins for a number of days, during which sperm numbers build up in the seminal vesicles, likely resulting in the passage of atypically large sperm numbers. Our data for matings of wild-caught males to virgin females show that in nature *D. melanogaster* females appear to receive many fewer sperm per copulation than they do in the laboratory. They also may receive lower amounts of the accessory gland proteins that accompany the sperm and influence female reproductive behavior. Our observations are consistent with (1) the need to remate frequently to avoid sperm limitation and (2) with the evidence that wild-caught females of all Drosophila species examined to date carry sperm from many males.[15-17,20]

In addition to maintenance of their fertility, are there additional consequences for females from frequent mating? In *D. melanogaster*, laboratory mating has been shown to decrease female lifespan, as a result of proteins in the male ejaculate.[21] Yet female *D. melanogaster* obviously live long enough in the wild, however, to mate with many males.[18] Given that nature is likely to be more stressful than the laboratory, it seems surprising that *D. melanogaster* females would not experience even more "harm" when mating in the wild. If however, females in nature receive fewer ejaculatory components along with fewer sperm, the costs of mating observed in the laboratory would be different in the wild. As expected, however, mating and remating have no effect on laboratory lifespan in frequently remating species such as *D. nigrospiracula* and *D. mettleri*.[21] Either ejaculates or female responses to ejaculates differ in these species compared with those of *D. melanogaster* such that mating does not shorten female lifespan. In fact, in *D. mojavensis*, the sister species of *D. arizonae*, mated females actually experience increased resistance to desiccation,[22] suggesting that at least in some species females derive benefits from frequent mating in addition to fertility. Costs of mating in *D. melanogaster,* with respect to lifespan,[23] would appear to differ in nature compared with the laboratory. Potential benefits of multiple mating may include increased success of progeny[24] and increased opportunity for postcopulatory female control of reproduction.

Natural populations of Drosophila consist of flies of variable genotypes, phenotypes, ages and mating histories. Unfortunately no means exist to determine the ages of flies captured in nature and thus our sample was likely to contain flies of more than one age and mating status. Therefore the relative contributions of these variables, as well as phylogenetic constraints, to the measures made in the present study remain unknown. Nonetheless, and more importantly, these populations represent the natural milieu in which reproductive processes occur and thus provide the contexts of natural and sexual selection that have shaped the

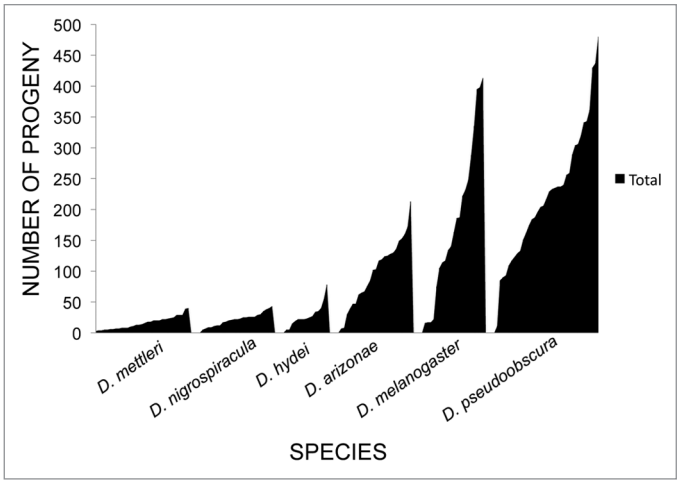

Figure 1. The total number of progeny produced by individual wild caught females of each species. Each bar for a given species represents the progeny of one female.

Table 4. Productivity following mating of wild-caught males to virgin females in comparison to the progeny produced by wild-caught females and those from earlier studies of single matings of laboratory reared males

Species	Mean ± SE number of progeny (number matings)		
	Field Females Present Study	Wild Males Present Study	Previous Lab Studies
D. melanogaster	182.1 ± 27.9 (42)	106.7 ± 5.0 (10)	130–419[27,29-33]
D. hydei	28.5 ± 4.8 (24)	20.5 ± 1.9 (13)	126[26]
D. arizonae	98.5 ± 10.6 (50)	57.6 ± 4.0 (8)	101[10]

differing reproductive biologies of these flies. Why do they differ? Why do females of some species carry so few sperm relative to others? What is the role of sperm limitation on female mate choice and ovipoisiton site selection? These are questions to address at the mechanistic, ecological and evolutionary levels.

Materials and Methods

Collection and processing of females. We aspirated flies from rotting fruits or cacti at various localities near Tucson, Arizona in the summers of 2006 and 2007. Species included *D. melanogaster*, *D. pseudoobscura*, *D. hydei*, *D. arizonae*, *D. nigrospiracula* and *D. mettleri*. With the exception of *D. pseudoobscura,* which was collected on Mount Lemmon (32° 26' 43.93''N, 110 46'23.03''W), all flies were collected and observed in the Catalina Mountain Foothills (32° 18' 16.37''N, 110 57'11.40''W).

We immediately isolated females, without anesthetization, into individual eight-dram glass food vials seeded with live yeast. We also dissected, in ringer's solution, a subset of the isolated females within two hours of their collection in order to examine their ventral receptacles and spermathecae to determine the number with sperm in their reproductive tracts. The number of progeny produced per female was used as a proxy for sperm load, rather than number of eggs laid as some oviposited eggs may

not be fertilized. Females were transferred to fresh food daily. For progeny counts, female *D. nigrospiracula* and *D. mettleri* were placed in potato-cactus medium with chunks of saguaro cactus[7] which is their optimal laboratory food for oviposition, while standard banana medium was utilized for the other four species. Once females had failed to lay eggs for three days, we dissected them. None of the females that had stopped ovipositing contained sperm.

Reproductive potential of males from nature. We aspirated single males at random from the same sites as females and placed them individually, immediately and without anesthetization, into vials containing single virgin conspecific females that had been reared in the laboratory. These experiments were conducted with *D. melanogaster*, *D. hydei* and *D. arizonae*. All pairs mated within 20 min and produced progeny. We serially transferred mated to fresh food vials as above, until they ceased ovipositing, to later count progeny obtained from matings to wild males.

Progeny counts. Food vials were examined daily to see if females had oviposited. The labor-intense nature of transferring so many females precluded our ability to count the numbers of eggs. Females having oviposited were transferred to new food vials. Females not ovipositing were allowed to remain in the same vial for three days, after which they were transferred to fresh vials that continued to be checked for eggs. We recorded the number of offspring emerging from each vial for every female, which yielded estimates of sperm loads and revealed the number of days over which females continued to produce progeny.

Ovariole numbers in females from nature. After collecting females from the field, we dissected ovaries immediately in insect Ringers's solution, teased the ovarioles apart with insect pins and stained them with Schiff's reagent before counting them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgements

We thank Dan Lindsley for comments on the manuscript and discussions of spermatogenesis. S.B. was supported by a National Science Foundation Research Experience for Teachers (RET) award to T.A.M.

References

1. Markow TA. Evolution of Drosophila mating systems. Evol Biol 1996; 29:73-106.
2. Markow TA, O'Grady PM. Drosophila: a guide to species identification and use. Academic Press, London 2005.
3. Gromko MH, Markow TA. Courtship and remating in field populations of Drosophila. Anim Behav 1993; 45:253-62; http://dx.doi.org/10.1006/anbe.1993.1031.
4. Manning A. A sperm factor affecting the receptivity of *Drosophila melanogaster* females. Nature 1962; 194:252-3; http://dx.doi.org/10.1038/194252a0.
5. Manning A. The control of sexual receptivity in female Drosophila. Anim Behav 1967; 15:239-50; PMID:6030948; http://dx.doi.org/10.1016/0003-3472(67)90006-1.
6. Liu H, Kubli E. Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. Proc Natl Acad Sci USA 2003; 100:9929-33; PMID:12897240; http://dx.doi.org/10.1073/pnas.1631700100.
7. Castrezana S. A new recipe for rearing cactophilic Drosophila. Drosoph Inf Serv 1997; 80:92-3.
8. Markow TA, Castrezana S. Dispersal in cactophilic Drosophila. Oikos 2000; 89:378-86; http://dx.doi.org/10.1034/j.1600-0706.2000.890219.x.
9. Castrezana S. Patterns of speciation in *Drosophila mettleri*. Doctoral Dissertation University of Arizona, Tucson 2006.
10. Markow TA. Mating systems of cactophilic Drosophila. In Barker JSF, Starmer WT, (Eds). The Drosophila-Cactus-Yeast Model System; Academic Press, New York 1982; 273-87.
11. Snook RR, Markow TA, Karr TL. Functional nonequivalence of sperm in *Drosophila pseudoobscura*. Proc Natl Acad Sci USA 1994; 91:11222-6; PMID:7972038; http://dx.doi.org/10.1073/pnas.91.23.11222.
12. Kaufmann BP, Demerec M. Utilization of sperm by the female *Drosophila melanogaster*. Am Nat 1942; 76:445-69; http://dx.doi.org/10.1086/281068.
13. Lefevre G Jr, Jonsson UB. Sperm transfer, storage, displacement and utilization in *Drosophila melanogaster*. Genetics 1962; 47:1719-36; PMID:13929245.
14. Fowler GL. Some aspects of the reproductive biology of Drosophila: sperm transfer, sperm storage and sperm utilization. Adv Genet 1973; 17:293-360; http://dx.doi.org/10.1016/S0065-2660(08)60173-X.
15. Gromko MH, Gilbert DG, Richmond RC. Sperm transfer and use in the multiple mating system of Drosophila. In Smith RL, (Ed.) Sperm Competition and the Evolution of Animal Mating Systems; Academic Press, New York 1984; 371-426.
16. Anderson WW. Frequent multiple insemination in a natural population of *Drosophila pseudoobscura*. Am Nat 1974; 108:709-11; http://dx.doi.org/10.1086/282949.
17. Cobbs G. Multiple insemination and male sexual selection in natural populations of *Drosophila pseudoobscura*. Am Nat 1977; 111:641-56; http://dx.doi.org/10.1086/283197.
18. Imhof M, Harr B, Brem G, Schlötterer C. Multiple mating in wild *Drosophila melanogaster* revisited by microsatellite analysis. Mol Ecol 1998; 7:915-7; PMID:9691492; http://dx.doi.org/10.1046/j.1365-294x.1998.00382.x.
19. Snook RR, Hosken DJ. Sperm death and dumping in Drosophila. Nature 2004; 428:939-41; PMID:15118726; http://dx.doi.org/10.1038/nature02455.
20. Good JM, Ross CL, Markow TA. Multiple paternity in wild-caught *Drosophila mojavensis*. Mol Ecol 2006; 15:2253-60; PMID:16780438; http://dx.doi.org/10.1111/j.1365-294X.2006.02847.x.
21. Markow TA, O'Grady PM. Evolutionary genetics of reproductive behavior in Drosophila: connecting the dots. Annu Rev Genet 2005; 39:263-91; PMID:16285861; http://dx.doi.org/10.1146/annurev.genet.39.073003.112454.
22. Knowles LL, Hernandez BB, Markow TA. Exploring the consequences of postmating-prezygotic interactions between the sexes. Proc Biol Sci 2004; 271:357-9; PMID:15504017; http://dx.doi.org/10.1098/rsbl.2004.0192.
23. Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature 1995; 373:241-4; PMID:7816137; http://dx.doi.org/10.1038/373241a0.
24. Gowaty PA, Kim YK, Rawlings J, Anderson WW. Polyandry increases offspring viability and mother productivity but does not decrease mother survival in *Drosophila pseudoobscura*. Proc Natl Acad Sci USA 2010; 107:13771-6; PMID:20643932; http://dx.doi.org/10.1073/pnas.1006174107.
25. Gilbert DG. Sperm counts and initial sperm storage in *D. melanogaster*. Drosoph Inf Serv 1981; 56:46-7.
26. Pitnick S, Markow TA. Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. Proc Natl Acad Sci USA 1994; 91:9277-81; PMID:7937755; http://dx.doi.org/10.1073/pnas.91.20.9277.
27. Lefevre G Jr, Jonsson UB. Sperm transfer, storage, displacement and utilization in *Drosophila melanogaster*. Genetics 1962; 47:1719-36; PMID:13929245.
28. Pitnick S. Investment in testes and the cost of making long sperm in Drosophila. Am Nat 1996; 148:57-80; http://dx.doi.org/10.1086/285911.
29. Duncan FN. Some observations on the biology of the male *Drosophila melanogaster*. Am Nat 1930; 64:545-51; http://dx.doi.org/10.1086/280338.
30. Gromko MH, Briot A, Jensen SC, Fukui HH. Selection on copulation duration in *Drosophila melanogaster*: Predictability of direct response versus unpredictability of correlated response. Evolution 1991; 45:69-81; http://dx.doi.org/10.2307/2409483.
31. Markow TA, Quaid M, Kerr S. Male mating experience and competitive courtship success in *Drosophila melanogaster*. Nature 1978; 276:821-2; http://dx.doi.org/10.1038/276821a0.
32. Markow TA, Sawka S. Dynamics of mating success in experimental groups of *Drosophila melanogaster* (Diptera: Drosophilidae). J Insect Behav 1992; 5:375-83; http://dx.doi.org/10.1007/BF01049845.
33. Zimmering S, Fowler GL. Progeny: sperm ratios and non-functional sperm in *Drosophila melanogaster*. Genet Res 1968; 12:359-63; PMID:5713604; http://dx.doi.org/10.1017/S0016672300011940.