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A comparative investigation of the mating system of *Drosophila hydei*

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Abstract. *Drosophila hydei* mating system characteristics were studied and compared to what has been observed for two other *Drosophila* species. *Hydei* females will copulate when they are 3 days old, while males do not exhibit courtship behaviour until they are 9 days old. Unlike any other *Drosophila, D. hydei* females will re-mate as often as four times in one morning. However, re-mating in the same morning does not increase the number of progeny a female produces. Male *D. hydei* appear to deal with the continual receptivity of females and the pressures of sperm competition by passing less material to any given female but maintaining a constant level of fertility across numerous successive copulations. *D. hydei* is a cosmopolitan species which utilizes a wide variety of resources. As in *D. melanogaster*, another cosmopolitan species which is not closely related to it, male success appears to be dependent upon genetic quality only. This pattern differs from that observed in its relative *D. mojavensis*, a cactiphilic species endemic to the Sonoran desert, in which males contribute material benefits to females in their ejaculate.

One of the major tasks confronting biologists interested in sexual selection is explaining the observable variation in mating systems. In order to do this, we need both to understand how different mating system patterns influence individual reproductive success and to define the conditions which cause the patterns we observe (Borgia 1979).

The comparative approach should be particularly useful in achieving both of these goals. Our success in employing this approach to ask how different mating patterns influence reproductive success depends upon our ability to measure fitness under controlled conditions. Field observations on a variable, such as the number of matings any given animal obtains, using a certain strategy are desirable. But it is also desirable to be able to obtain data experimentally, and in order to do so, it is preferable to work with an organism which is easily reared in the laboratory. Many species in the genus *Drosophila* are easy to culture and provide a new generation every 2–3 weeks. Furthermore, the availability of genetic markers facilitates the study of sperm competition.

Drosophila flies also provide a unique opportunity to define the conditions under which various types of mating systems evolve. The nearly 2000 species in this genus span a wide range of ecological niches. Some species are cosmopolitan, while others are endemic to small regions and are restricted to single specific resources. In addition, more is known of the phylogenetic relationships within *Drosophila* than in any other genus or organisms (Patterson & Stone 1952). These factors make it possible to evaluate the relative roles of long-term evolutionary history and of recent ecological adaptions in producing the variability in mating systems.

It is not surprising that the mating system of the well-known cosmopolitan species *D. melanogaster* has been more extensively described than that of other *Drosophila* species (see Gromko et al. 1984). It was not until recently that a systematic study of another species, the Sonoran desert endemic *D. mojavensis*, was undertaken (Markow 1982; Markow & Ankney 1984). These two species, classified in separate subgenera and differing dramatically in their ecology, were found to exhibit striking contrasts to each other in their mating patterns. What conditions are responsible for these differences? To what degree are these differences the result of ecological adaptation and to what extent do they reflect the species' taxonomic positions? I undertook a comparative study, using a third species, *D. hydei*, to answer these questions. *Drosophila hydei* is a cosmpolitan species, like *D. melanogaster*, but is in the *repleta* species group along with *D. mojavensis*. Below, I report on a comprehensive laboratory investigation of male and female reproductive behaviours and their consequences for individual fitness in *D. hydei*. Interestingly, *D. hydei* is similar to *D. melanogaster* in certain mating behaviours, to *D. mojavensis* in others, and is unique in still other ways. The possible evolutionary explanations for these observations are discussed.

METHODS

The wild-type strain of *D. hydei* employed in this study was derived from a multi-female collection ($N=31$) made at an abandoned citrus grove in Tempe, Arizona in May 1981. The culture, designated TM3, was maintained in a 12-food-cup population cage during the experimental period. Data were obtained within six generations of the collection. A laboratory strain bearing the autosomal recessive eye colour mutant cinnabar (*cn*), was obtained from Dr Winifred Doane and crossed into the wild strain three times prior to being used in sperm competition studies. All flies were reared on standard cornmeal molasses agar medium at 24 ± 1°C. Virgin males and females were separated under light ether anaesthesia and stored at 10 flies/32-ml vial until being used in the experiments.

Testing for a Male Nutrient Contribution from the Ejaculate

The possibility that ejaculate-derived nutrients contribute to female somatic maintenance and oogenesis was tested by mating radiolabelled males to unlabelled females and then assaying for the presence of radioactivity later in various female body parts (Markow & Ankney 1984). Fifty second-instar larvae were placed in 1·5 g of culture medium made up with 70 Ci of a mixture of ^{3}H amino acids (ICN 20063). Labelled males, aged 1 week, were mated to unlabelled females. Females were dissected either immediately after mating or 24 h after mating. Female parts were placed in 100 μl of Scintigest (Fisher) tissue solubilizer at 50°C. After 24 h, 2·5 μl of glacial acetic acid was added to neutralize the solution. Then, 0·5 ml of Scintiverse I (Fisher) scintillation fluid was added and each vial was allowed to sit for an additional 24 h prior to being placed in the scintillation counter. The female parts were: head, thorax, abdomen minus reproductive tracts, ovarian eggs, and reproductive tracts (uterus, vagina, ventral receptacle and spermathecae).

Age at Reproductive Maturity

The age at which flies engage in copulation was determined separately for each sex. Individual males, aged 0–12 days, were paired with mature virgin females in 32-ml shell vials for 24 h. Females were dissected and examined for evidence of insemination. In another group of experiments, mature males were paired with females 0–6 days of age and these females were dissected after 24 h.

Incidence of Female Re-mating

Individual virgin females, 6 days old, were mated to 10-day-old males in shell vials. Mated females were then aspirated into new shell vials containing virgin 10-day-old males, and the number re-mating was scored. Females that re-mated were transferred to new vials with virgin males. Females not re-mating within 1 h were retested the following morning.

Influence of Female Re-mating on Female Fertility

Two experiments were conducted. In experiment A, females were allowed to mate once, twice or three times in a given morning. In experiment B, females were allowed to mate three times, once on each of three consecutive mornings. Each female was then placed in her own food vial. Females were transferred to fresh vials every 24 h for 2 weeks, and the progeny emerging from each vial were counted.

Incidence of Male Re-mating and its Influence on Male Fertility

Ten-day-old virgin males were paired with 6-day-old virgin females in vials. Mated males were transferred repeatedly to vials containing new virgin females during a 2-h period. The number of times each male mated was recorded. Females from each mating were supplied with fresh food daily and the progeny produced by each were counted, to compare the number of progeny from each male on each of his first six matings.

Sperm Utilization by Multiply Mated Females

Three sets of sperm-competition experiments were conducted. The females in all of these experiments were always homozygous *cn*. Males were either wild-type or homozygous *cn*. In the first group of experments, females were mated to a wild-type male and then a *cn* male within 1 h, or to a *cn* male first and then to a wild-type male. In the second set of experiments, females were mated twice consecutively to the same type of male, either *cn* or wild-type, and then a third time to a male of the other genotype. The third group of experiments consisted of mating females to males of one

genotype on one day and to males of the other genotype 24 h later. Females were transferred daily to fresh culture vials. The relative numbers of mutant and wild type progeny produced after the last mating of each female was determined. This information was used in calculating 'P_2' and 'P_3' values, that is, the number of progeny sired by the second and third male, respectively, after the second or third mating.

Male Discrimination of Re-mating Females

Virgin 10-day-old males were placed individually into shell vials containing two 6-day-old females: one of these was a virgin and the other had mated once, within the previous hour. Females were marked with microdust to distinguish virgins from non-virgins (Markow 1981, 1982). The first female courted by the male and the first female to mate were both recorded.

Male Body Size and Courtship Success

A 6-day-old virgin wild-type female was placed with two randomly chosen virgin 10-day-old males. Males were distinguished with coloured dust. The colour of the first male to court and the first male to mate were recorded. After copulating, the mated female was replaced with a virgin female and the colour of the male that then mated was recorded. This procedure was repeated to give each pair of 'contestant' males three opportunities to mate. After the third female mated, all males were anaesthetized and their thoraces were measured.

Table I. Male-derived radioactivity found in female body parts immediately after mating and 24 h later

	Mean DPM*	
	Time after mating	
Female body part	0 h	24 h
Head	21·23	22·51
Thorax	25·02	20·55
Abdomen (minus reproductive tract)	25·11	22·83
Reproductive tract	160·87†	30·23
Ovarian eggs	28·0	29·08

* Counts per minute were converted to decompositions per minute (DPM) following a standard quench. The amount of male-derived radioactivity, expressed as DPM, for each body part at any time point is an average for six females.

† The amount of radioactivity found in reproductive tracts immediately after matings was significantly higher ($P < 0{\cdot}01$) than in a control experiment (females mated to unlabelled males). All other body parts at 0 and 24 h were not different from controls.

RESULTS

Testing for Male Contribution Using Radioisotopes

A significant amount of radioactivity was found in female reproductive tracts immediately following copulation (Table I). Twenty-four hours later, the amount of label in the tracts had decreased to the level of unlabelled controls. No other body parts showed significant radioactivity at either time after mating. The data do not give any evidence of male contribution to female somatic tissues or to developing oocytes.

Age at Reproductive Maturity

The ages at which males and females were capable of copulation, as diagnosed by sperm transfer, are reported in Table II. A large difference exists between males and females for the time at which reproductive maturity is reached. The majority of males failed to inseminate mature females until they were about 10 days old, while most females were inseminated by 3 days of age by mature males. Behavioural observations suggest that the absence of insemination does in fact indicate the absence of mating. Young males were not observed to court females.

Incidence of Female Re-mating

Of 97 mated females, 88 re-mated within 1 h. When these 88 females were immediately provided with another opportunity to re-mate, 61 of them copulated again within 1 h of their second mating. Nineteen of these females were observed to mate a fourth time in the same morning. The 9 females that did not re-mate the same day were placed with males 24 h later: six of them re-mated. At least 5 each of once-, twice-, and three-times-mated females were dissected immediately after mating and the location and distribution of sperm was

Table II. Relative ages at which males and females become reproductively mature, measured by proportion of flies inseminated at any given age

(a) Number of mature females inseminated by males of ages 3–12 days

Male age (days)	Number of females inseminated	%
3	0/31	0
4	0/28	0
5	0/45	0
6	0/34	0
7	1/29	3·4
8	5/32	15·6
9	16/30	53·3
10	24/28	85·7
11	35/37	94·6
12	19/20	95·0

(b) Number of 0–5-day-old females inseminated by mature males

Female age (days)	Number of females inseminated	%
0	0/26	0
1	0/16	0
2	9/37	24·3
3	24/27	88·9
4	21/23	91·3
5	31/33	93·9

examined. After a single mating, sperm were found in the spermathecae and in the very proximal part of the ventral receptacle. Sperm from additional matings went to the ventral receptacle and was observed to fill this organ to about one half of its length.

Influence of Female Re-mating on Female Fertility

The total number of progeny produced by females that mated once, twice, and three times in one morning is shown in Table III. No increase in productivity was observed with a second or third mating. On the other hand, when females mated once daily for 3 days, more offspring were produced. It is interesting that females continued to oviposit almost daily until their death (about 3–4 weeks after mating), although after 2–3 days none of the eggs was fertilized. Five females were dissected after 3 weeks to see if they had simply run out of sperm. To my surprise, all of them contained large quantities of motile sperm, but this was always confined to the most distal portions of the receptacles: the spermathecae were usually empty.

Incidence of Male Re-mating and its Influence upon Male Fertility

Males were observed to re-mate up to 10 times when continually supplied with virgin females during a 2-h period ($N = 31$). Females of each of six successive matings by 12 males were saved and their progeny counted. Table IV shows that males sired as many progeny on their sixth mating as on their first.

Sperm Utilization by Multiply Mated Females

In Table V, sperm-competitive abilities are expressed as P_2 or P_3 values and their arcsin transformations. P_2 is the proportion of progeny sired by the second male and P_3 is the proportion of the progeny sired by the third male. The average of the two P_2 values from experiment A is about 50%, suggesting that sperm mix. This is further sup-

Table III. Effect of re-mating on the reproductive fitness of *D. hydei* females

Experiment	Number of matings	Number of females	Mean number of progeny per female	*F*
(a) Same morning	1	12	55·29 ± 5·91	
	2	12	57·88 ± 7·14	0·0427
	3	12	55·17 ± 6·80	
(b) Successive mornings	1	15	56·01 ± 7·23	
	2	14	79·99 ± 7·58	6·715*
	3	15	96·33 ± 8·41	

* $P < 0·05$.

Table IV. The effect of male re-mating on male reproductive fitness

Number of matings	Number of males	Mean number of progeny per male
1	10	61·40±6·26
2	10	50·78+8·61
3	10	52·00+7·62
4	10	57·20±8·41
5	10	59·24±6·68
6	10	48·90+5·16

$F=1{\cdot}012$, $P>0{\cdot}80$.

Table V. Results of sperm competition experiments in *D. hydei*: (a) two males 20 min apart, (b) two males 24 h apart, (c) three males 20 min apart

	Mating order: 1st	2nd	3rd	*n*	Mean P_2*±SE	Arcsin-transformed mean P_2±SE
(a)	+/+	*cn/cn*		50	0·228±0·25	11·97±16·08
	cn/cn	+/+		50	0·722±0·31	24·35±25·27
(b)	+/+	*cn/cn*		42	1·0	
	cn/cn	+/+		41	1·0	
	1st	2nd	3rd	*n*	Mean P_3±SE	Arcsin P_3±SE
(c)	+/+	+/+	*cn/cn*	29	0·188±0·15	11·079±9·12
	cn/cn	*cn/cn*	+/+	31	0·311±0·19	18·427±11·82

* See text for explanation.

ported by the P_3 values: a third male sired either 19% or 31% subsequent progeny, depending upon whether he was mutant or wild-type respectively. Interestingly, a second mating 24 h after the first yielded P_2 values of 100%, regardless of male genotype.

Table VI. Thorax lengths of successful male contestants

Number consecutive contests	$\bar{X}$±SE thorax length (mm) Winners	Losers	F
1	1·21±0·11	1·09±0·08	0·924
2	1·26±0·10	1·01±0·08	3·306*
3	1·29±0·07	1·00±0·06	4·855*

*$P<0{\cdot}01$.

Male Discrimination of Re-mating Females

Males presented with a choice between a virgin and a recently mated (1 h) female showed no preference for virgins in terms of first female courted (virgin, $N=38$, versus mated, $N=43$), or first copulation (virgin, $N=41$, versus mated, $N=36$).

Male Body Size and Courtship Success

Thorax lengths of successful and unsucessful males are presented in Table VI. Males winning a single contest tended to be larger than losers, although mean thorax lengths did not differ significantly. When these data were analysed with a sign test, winners were larger in 80% of the contests ($P<0{\cdot}01$). Males winning two and three consecutive contests were significantly larger than their competitors by both sign tests and analysis of variance.

Table VII. Comparative mating systems of *Drosophila* species

Mating system character	*D. hydei*	*D. mojavensis*†	*D. melanogaster*‡
1. Age at reproductive maturity	Sexual dimorphism, males delayed	Sexual dimorphism, males delayed	Both sexes mature simultaneously
2. Female re-mating interval	60 min	24 h	5 days
3. Average number of progeny from a single mating	55	70	400
4. Effect of one re-mating on female productivity	No effect	No effect	Large increase in progeny numbers
5. Effect of multiple mating on male fertility	None	None	Fertility decreases
6. Oviposition pattern	Lays large quantities of unfertilized eggs	Only lays fertilized	Will lay a few unfertilized eggs
7. Relative size of successful males	Larger	No difference	Larger
8. P_2* at earliest time females will re-mate	50% (equal)	68%	95%
9. Do males preferentially court virgin females?	No	Yes	Yes
10. Male ejaculate contribution	No	Yes	No
11. Copulatory plug	No	Yes	No

* See text for explanation.
† Markow 1982.
‡ Markow 1984.

DISCUSSION

What is the influence of the *D. hydei* male mating behaviour on individual reproductive fitness? Males take longer than females to become sexually mature. Whether any amount of selection on males occurs during this time is unknown. When males do become sexually mature they are faced with females who are continually receptive. *D. hydei* females store sperm, and because no copulatory plug is formed, and sperm mixing occurs, males cannot be assured of any high level of paternity. It would appear that males have adjusted to this problem by not giving up too many gametes to any given female, and instead, maintaining a consistent level of fertility across a large number of consecutive matings. Since a male's probability of siring offspring is the same whether he was the first or second male to mate with a female, it is not surprising that *D. hydei* males do not discriminate between virgin and mated females.

What is the influence of the *D. hydei* mating pattern on female fitness? Females mature earlier than males and in doing so they probably decrease the probability of mating with a sibling. The actual importance of a decrease in sibling mating is hard to evaluate in the absence of a detailed knowledge of population structure. It is clear that females maintain their fertility over time by re-mating daily. However, several matings in one morning do not appear to increase female productivity over the long term. Multiple matings do, nevertheless, enable the sperm of several males to compete within the female reproductive tract, and may result in progeny of higher quality. Unlike females of the Sonoran desert endemid *D. mojavensis, D. hydei* females obtain no nutrients from male ejaculate.

In Table VII, the mating system of *D. hydei* is compared to that of *D. melanogaster* and *D. mojavensis*. What conditions have caused the variability we observe among these three species? *D. hydei* and *D. mojavensis* are both members of the *repleta* group of the subgenus *Drosophila* (Wasserman 1960). Frequent female re-mating and delayed male maturation are both characteristic of this species group (Markow 1982). However, of the two species, only *D. mojavensis* males make a nutrient

contribution through their ejaculate and only *D. mojavensis* females exhibit the copulatory plug following mating. The hypothesis that male investment is associated with living in a harsh environment on specific host plants can be tested by examining other desert endemics and close relatives of *D. mojavensis*. Male contribution is clearly not associated with a cosmopolitan existence.

Cosmopolitan species such as *D. melanogaster* and *D. hydei* utilize a broad range of resources, including fruits and decaying vegetable and animal matter. The dispersion of these resources differs from that of the necrotic cacti utilized by *D. mojavensis* (Heed 1978). In the case of the cosmopolitan species, males do not control resources, nor do they provide any material benefits to females through their ejaculate. Sexual selection theory would predict that under these conditions, female choice should operate on the basis of genetic benefits provided by males. The actual indicators of genetic superiority are often difficult to determine; however, in the case of *D. melanogaster* and *D. hydei*, it is possible to differentiate between successful and unsuccessful males on the basis of their relative size. Successful males are significantly larger and less variable in size than unsuccessful males. Furthermore, successful males court sooner and more persistently than unsuccessful males. Markow (in press) provides evidence that in *D. melanogaster* the larger size and more vigorous courtship of successful males are a function of heterosis, a possibility raised previously by Borgia (1979).

In *D. mojavensis*, unsuccessful males are no larger but they are less variable in size than the unsuccessful ones. Males of this species do provide a material benefit to females. However, females are not limited by this contribution, since they can mature several batches of oocytes without ever mating. *D. mojavensis* may represent the situation proposed by Borgia (1979) in which male success depends upon both genetic and material benefits. The role of body size in the mating success of *D. mojavensis* males is difficult to interpret without knowing the degree of correlation, if any, between the genetic and material benefits that males provide.

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