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The evolutionary history of *Drosophila buzzatii*. XVII. Double mating and sperm predominance

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Summary – Sperm predominance in males and double mating in females have been studied in 2 stocks of the cactophilic species *Drosophila buzzatii*. The relationship between double mating and total productivity of females was also ascertained. Our results show high values of sperm predominance and double mating. Moreover, female productivity is increased with a second mate. These results are discussed in relation to the mating strategy of this species.

Drosophila buzzatii / sperm predominance / double mating / mate strategy / total productivity

Résumé – Histoire évolutive de *Drosophila buzzatii*. XVII. Accouplement double et prédominance du sperme. On a étudié la prédominance du sperme chez les mâles et le double accouplement chez les femelles dans 2 souches de l'espèce cactophile *Drosophila buzzatii*. La relation entre le double accouplement et la productivité totale des femelles a été aussi recherchée. Nos résultats montrent des valeurs élevées pour la prédominance du sperme et pour le double accouplement. De plus, on constate que la productivité des femelles est augmentée par un deuxième accouplement. Ces résultats sont discutés par rapport à la stratégie d'accouplement de cette espèce.

Drosophila buzzatii / prédominance du sperme / accouplement double / stratégie d'accouplement / productivité totale

INTRODUCTION

Multiple mating is a widespread phenomenon among insect females (Thornhill and Alcock, 1983; Smith, 1984; Ridley, 1988). If the sperm of the first male is not exhausted before female remating, then sperm competition occurs in the storage organs of the female between the sperms of different origin (Parker, 1970, 1984). Sperm predominance, usually that of the last mated male, is the general result of

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this competition. When there are genetic differences in the degree of predominance, sperm predominance may result in sexual selection. Prout and Bundgaard (1977) showed theoretically how this type of selection could maintain a population in stable equilibrium for 2 alleles. The mating strategies of many species are determined by the importance of sperm predominance in males and multiple mating in females (Smith, 1984).

Several experimental studies have been carried out to ascertain the degree of sperm predominance in *Drosophila* (Gromko *et al*, 1984). In the present work, we have studied sperm predominance in the cactophilic species *Drosophila buzzatii*. In addition, the frequency of double mating and its influence on the total female productivity were determined. *D. buzzatii* belongs to the *repleta* group of *Drosophila* and several aspects of its ecology and mating behaviour have been extensively studied in our laboratory (Ruiz *et al*, 1986, Santos *et al*, 1988, 1989).

MATERIALS AND METHODS

Two stocks were used in this experiment. One, the wild type stock, was derived from a natural population collected at Carboneras, Almeria (SE Spain), in May 1986. The other stock was homozygous for the sex-linked recessive *white* mutant which arose spontaneously and was subsequently isolated in our laboratory in April 1983. Since no attempt was made to randomize the genetic background of the 2 stocks, they might differ at many loci and the mutant *white* was merely a genetic marker.

The experimental procedure was similar to that of Turner and Anderson (1984). The crosses performed are shown in table I. The w/+ females and the w/Y males were the hybrid offspring from the 2 parental stocks. The experiment began with the first cross. Five to 6-d old virgin females were crossed individually with 2 males of the same age. The crosses were carried out in 2 × 8 cm vials with *ca* 8 cm³ of food medium. After 24 h the males were discarded. Two d later the second cross was made, also with 2 males per female. After 2 d, the males were discarded and each female was transferred daily for 11 consecutive days without etherization into vials with fresh food. Thereafter, new transfers were made at 2-d intervals.

Table I. Mating types performed, classified according to the female genotype and the sequence of male genotypes. *n* = No of females.

Group	Female	1st male	2nd male	n
1a	w/w	w/Y	+/Y	50
1b	w/w	+/Y	w/Y	50
2a	w/+	w/Y	+/Y	50
2b	w/+	+/Y	w/Y	50
3a (control)	w/w	w/Y		25
3b (control)	w/w	+/Y		25
4a (control)	w/+	w/Y		25
4b (control)	w/+	+/Y		25

All the individuals were grown at nearly optimal density (4-5 larvae per cm³ of medium). A modified formula of David's killed-yeast *Drosophila* medium (David, 1962) was used as food. The flies were kept at 23°C. The offspring of each female was classified by sex and phenotype. Statistical analysis were conducted with the BMDP Statistical Software which was implemented on the VAX Operating System.

RESULTS

The results are presented in table II. We have estimated some population parameters that characterize sperm predominance in double matings. P_2 is the proportion of second male offspring after remating (Boorman and Parker, 1976). P_2^w is the weighted mean of P_2 , equivalent to the mean of progeny proportions per female weighted by the female's total productivity. P_1 is the fraction of offspring sired by the first male and P' is the proportion of the first male's sperm that is used by a female before she remates (Gromko *et al.*, 1984). P' was estimated from the control crosses as the weighted proportion of 1st-3-day offspring over total female productivity. Both P_1 and P_2 were estimated from the female offspring, since the male offspring did not allow ascertainment of the sperm origin. The estimates of P_2^w have been corrected for viability differences between the offspring of the 2 male genotypes (detected by the 3-way ANOVA; see below). In all cases, the values of P_2^w were high. Groups 1b and 2b (2nd male w/Y) showed the lowest values, 0.91 and 0.92 respectively. The other 2 values (groups 1a and 2a) were close to 1. Moreover, only on the 1st d after the 2nd cross did we find offspring of the 1st male. On the other hand, some of these descendants might have been produced prior to the time when the 2nd mating occurred. This would indicate that the actual P_2 values may be larger. The values of P' , the fraction of sperm effectively used by the female before she remates, indicate the presence of at least 25-50% of sperm of the 1st male when the 2nd cross occurs. These values are underestimates, since after the 2nd cross was started females may lay eggs before remating. Given the high values of P_2 , the bias of these underestimates is insignificant. The large values of P_2 suggest that the remaining sperm is not used in the following fertilizations. Since the P_2 's variances are not equal, we used the Brown-Forsythe test (Dixon, 1985) to compare the P_2 values. The P_2 angular mean was used as the dependent variable. Differences were statistically significant between groups 1a, 2a and 1b, 2b (change in the order of males, $P < 0.001$), but not between groups 1a, 1b and 2a, 2b (change in the female genotype, $P = 0.33$). So the variation in P_2 is a function of the male genotype. We find, therefore, a high degree of predominance of the last mated male, as well as possible selective differences in this component.

A possible source of error in the estimation of the P_2 values must be now considered. If during the time in which the 2nd cross occurs a female remates more than once, then our P_2 values will be spurious, since they will correspond to $P_{2,3,\dots,n}$, where n is the number of times a female has remated from the beginning of the experiment. Patterson and Stone (1952) found that the time between matings was > 135 h in this species. This, however, is not consistent with our results, since the percentage of females that remated was practically 100% (only 3 fertile females did not produce offspring from the 2nd mate). Therefore, the time to remate in our population would be somewhat lower, but we do not know how much lower.

Table II. Total female progeny and estimated values of parameters P_2^w , P_1 and P' for the groups described in table I. See text for their definitions. n = No of females that produced progeny.

<i>Double-mated females</i>				
<i>Group</i>	<i>Total productivity</i> $\bar{x} \pm SE$	P_2^w	P_1	n
1a	139.3 \pm 11.7	0.97	0.44	37
1b	122.5 \pm 5.9	0.91	0.62	35
2a	382.9 \pm 18.8	0.99	0.34	49
2b	348.8 \pm 18.4	0.92	0.44	46

<i>Single-mated females</i>			
<i>Group</i>	<i>Total productivity</i> $\bar{x} \pm SE$	P'	n
3a	62.9 \pm 9.1	0.77	21
3b	103.8 \pm 14.9	0.65	24
4a	221.3 \pm 21.8	0.50	22
4b	229.4 \pm 17.1	0.66	25

Wheeler (1947) found that *D buzzatii* presents a high degree of expression of the insemination reaction. Patterson and Stone (1952) and Markow (1985) suggest that the insemination reaction works as a mechanism to preclude remating in females. Accordingly, *D buzzatii* females would present a long refractory period before period remating, which would diminish the chances of a remating during the period of the 2nd cross. In this sense, *D buzzatii* would be analogous to *D mojavensis*, which delays additional matings (Markow, 1985). On the other hand, we have estimated the P_2 values in the case of females being remated 2 or 3 times. We have supposed that the proportion between P_i and P_{i-1} is 1:2. This assumption is based on the values of P_2 and P_3 found in *D hydei* by Markow (1985). For females that remate twice the estimated values of P_2 for each group are: $P_{1a,2} = 0.94$, $P_{1b,2} = 0.84$, $P_{2a,2} = 0.98$ and $P_{2b,2} = 0.94$. For 3 times remated females they are: $P_{1a,2} = 0.93$, $P_{1b,2} = 0.81$, $P_{2a,2} = 0.98$ and $P_{2b,2} = 0.83$. These values are still large. So, we think that the P_2 values may be biased upwards, but not so much as to invalidate the conclusion that sperm predominance is high. However, the differences found in P_2 between male's genotypes may be due to differential mating success rather than to selection for sperm predominance.

Additionally, we performed a 3-way analysis of variance to confirm the previous comparisons (table III). The log of the progeny number sired by the first male was used as a dependent variable. The goal of this analysis was to test: a), the effect of the female remating; b), the effect of the female's genotype; and c), the effect of the male's genotype on the offspring of the 1st male. We found significant fixed effects for each factor, but no interaction among them. First, the number of progeny sired by a male is significantly reduced if his partner subsequently remates ($F = 9.42$, $P < 0.01$). From this we deduce again that sperm predominance occurs.

Second, the female's genotype also influences productivity ($F = 182.32$, $P < 0.001$) very significantly. These differences are probably due to the different genetic background of both stocks and not to the genetic marker itself. From the third factor, we find genetic differences among male genotypes ($F = 10.95$, $P < 0.01$), but these cannot be attributed to selective differences in sperm predominance since the mate \times male interaction is non-significant ($F = 0.50$, $P = 0.48$). The absence of interaction indicates that the variance of this factor is negligible in relation to the variance due to other components, such as viability. It would appear, therefore, that selection for sperm predominance, if it exists, is not as important as the other components in these experiments.

Table III. A 3-way analysis of variance of the progeny number sired by the first mating male. The factors are mating type (mate), female genotype (female) and male genotype (male).

Source of variation	df	Mean square	F	Probability
Mating type (single or double)	1	0.571	9.42	0.002
Female genotype (w/w or w/+)	1	11.057	182.32	0.000
Male genotype (w/Y or +/Y)	1	0.663	10.95	0.001
Mate \times female	1	0.143	2.37	0.125
Mate \times male	1	0.030	0.50	0.478
Female \times male	1	0.072	1.19	0.276
Mate \times female \times male	1	0.039	0.65	0.420
Error	252	0.060		

Except for groups 1b and 3b, statistically significant differences were found for the total progeny between the single and double mated females. Similar results have also been obtained in *D pseudoobscura* (Turner and Anderson, 1983) and *D mojavensis* (Markow, 1982), although contrary results have been reported for *D melanogaster* (cf Boorman and Parker, 1976; Prout and Bundgaard, 1977; Gromko and Pyle, 1978; Alvarez and Fontdevila, 1981). In our case, it is clear that a single insemination is not sufficient to fertilize all the eggs a female can normally lay. This is against one assumption of the model of Prout and Bundgaard (1977), which claims that female output is independent of the number of matings.

DISCUSSION

In the face of sperm competition, the male strategies can lead to 2 different, not necessarily exclusive, ways of adaptation. One which increases P_1 , the fraction of offspring sired by the first male, either by an increase of P' (ie, through an increase in the time before female remating) or by "resisting" the predominance of the second male. The other way is to increase P_2 . Our results seem to suggest that the male mating strategy of *D buzzatii* is to maximize P_2 (ie the "remator" strategy) as well as P' (see Gwynne, 1984; p 141 for a possible selective explanation), whereas that of the female is multiple mating. In order to confirm this conclusion it would be necessary to perform different experiments with other stocks and varying the

time of the second cross, since P_2 could depend both on the stock background and on the remating time (Boormer and Parker, 1976).

Gwynne (1984) believes that the males of those species with higher predominance supply food to the female or to his offspring. Markow and Ankney (1984, 1988) found in *D mojavensis* that males transfer nutrients to females. We have indirect evidence that *D buzzatii* males transfer yeast to females during mating (Starmer *et al*, 1988). Thus, transmission of nutrients might help to explain the increases in female productivity with the number of matings. A more likely possibility to account for female multiple mating is in relation with the high total productivity found in this species (Barker and Fredline, 1985; Barbadilla, 1986). Ridley (1988) reviews extensive evidence showing that species with higher productivities are less likely to receive sufficient sperms at one mating than species with low productivity, whereby in species with a high productivity a multiple mated female will leave more offspring than a single one.

The strong sperm predominance found in *D buzzatii* has a considerable technical interest. Natural populations of this species show a moderately high inversion polymorphism in 2 autosomes. The karyotype of wild males can be ascertained by crossing them with virgin females from a laboratory stock homozygous for certain chromosome arrangements, and analyzing the salivary gland chromosomes of a number of larvae from each progeny. On the other hand, we seldom find out the karyotype of wild females, for they are usually already inseminated when collected in the field. One way to overcome this difficulty could be to remove sperm from the females by means of a low temperature treatment prior to their mating with the laboratory stock males (Anderson *et al*, 1979). This method, however, has proven to be completely ineffective in *D buzzatii* (unpublished results). The high values of P_2 suggest a simple solution to this problem. If wild females brought to the laboratory are crossed with males of known karyotypes and then transferred every other day to a new vial with fresh food, we expect that after a few days almost all the progeny will be sired by the laboratory males, allowing the correct identification of the female's karyotype. In a recent study carried out in the population of Carboneras (Ruiz *et al*, submitted) this prediction has proved to be essentially correct.

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