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Poisson distribution of male mating success in laboratory populations of *Drosophila melanogaster*

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Summary

Variation among males and females in reproductive success is a major determinant of effective population size. Most studies of male mating success in *Drosophila*, however, have been done under conditions very different from those in typical cultures. We determined the distribution of male mating success in five laboratory populations of *D. melanogaster* maintained on a 14 d, discrete generation cycle fairly representative of standard *Drosophila* cultures. Mating success was measured as the number of matings a male could achieve under conditions closely approximating a regular culture vial of these populations. Preliminary studies determined that most mating in these populations occurred within 14 h of the flies attaining sexual maturity. Consequently, individual virgin males were marked with white paint on their thorax, put into vials with varying numbers of unmarked virgin flies of both sexes, and monitored continuously for matings over a period of up to 14 h. At various times during the assay, virgin males and females were added to these vials in proportions simulating the pattern of eclosion in culture vials. The observed variation in the number of matings per male in the five populations was, by and large, consistent with a Poisson distribution, suggesting that male mating success in short-generation-time, discrete-generation laboratory cultures of *D. melanogaster* may fulfil a fundamental assumption of the Wright–Fisher model of genetic drift in finite populations.

1. Introduction

Ever since the development of the concept of the effective population size (N_e) of a population (Wright, 1931, 1938), the importance of being able to estimate the ratio of effective to actual population size (N_e/N) has been widely recognized, and our understanding of the concept itself greatly refined (Crow & Morton, 1955; Crow & Kimura, 1970; Lewontin & Krakauer, 1971; Nei & Tajima, 1981; Ewens, 1982; Crow & Denniston, 1988; Waples, 1989; Nunney, 1995). Many theoretical studies have focused on deriving expressions for the effective population sizes of populations with varying breeding systems, sex ratios, and other genetic and ecological attributes (recent reviews by Nunney & Campbell, 1993; Caballero, 1994). At the same time, many workers have tried to estimate empirically the effective population sizes of natural populations of a variety of organisms ranging from plants to insects (Krimbas & Tsakas, 1971; Begon *et al.*, 1980; Mueller *et al.*, 1985; Husband & Barrett, 1992). Although estimates of N_e/N in natural populations are of great significance in addressing a variety of evolutionary questions, in many instances the estimation of effective size of laboratory populations is also very important. Because laboratory populations of *Drosophila melanogaster* are widely used in studies of adaptive evolution, accurate estimates of N_e/N for typical *Drosophila* cultures is important, both for experimental design and for interpretation of results.

In principle, the issue of estimating effective population size can be approached from two opposing directions. One can focus on the various biological attributes of a population, such as breeding system, or the variation in family size, that cause a population to have a specific effective size and, consequently,

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experience a corresponding degree of random genetic drift (e.g. Kimura & Crow, 1963; Crow & Denniston, 1988). Alternatively, one can determine empirically the magnitude of variation in allele frequencies across generations and infer the effective size of the population from the variance in allele frequencies (e.g. Nei & Tajima, 1981; Waples, 1989). This has typically been the favoured technique for estimating N_e/N empirically, even though there are many assumptions inherent in its use that are very difficult to test (Mueller *et al*., 1985). On the other hand, if one is trying to deduce the effective population size based on the biological attributes of a population, then a major factor that must be taken into account is the distribution of family size (Wright, 1931, 1938; Crow & Kimura, 1970; Chia & Pollak, 1974; Caballero, 1994). If the distribution of family size is Poisson, then fairly accurate estimates of effective population size can be obtained from census data (Crow & Kimura, 1970). However, if the variance of family size differs significantly from the mean, estimates of effective population size based on census data need to be corrected for the effect of non-random variation among individuals in their genetic contribution to the next generation (Crow & Denniston, 1988).

The distribution of family size across males and females is ultimately dependent upon the distribution of offspring number among females and the distribution of reproductive success among males (Mueller *et al*., 1985). Some knowledge of the distribution of reproductive success of males and females is, therefore, crucial to the accurate estimation of effective population size. For a variety of species, actual population sizes and sex ratios are often easily obtained by a population census each generation. The mean reproductive success of males and females is also relatively easily obtained. However, it is usually much more difficult to estimate the variance in reproductive success under conditions closely simulating the normal environment of the population. This constraint is especially evident in the case of females, because it is difficult to assess reproductive output of one female in a group. In the case of males, there is substantial empirical evidence that considerable genetic variance for mating success exists, at least under certain types of conditions, in both field and laboratory populations (Prout, 1971; Anderson *et al*., 1979; Brittnacher, 1981; Partridge *et al*., 1985; Sharp, 1982, 1984; Hughes 1995; but see also Quezada-Diaz *et al*., 1992). These results, together with the finding that male mating success tends to be positively correlated with various indices of body size in *Drosophila* (Partridge & Farquhar, 1983; Partridge *et al*., 1987; Markow, 1988), suggest that it is likely that the distribution of male mating success in *Drosophila* will be non-Poisson, with the variance exceeding the mean.

In this paper, we report results from a study in which the mean and variance of male mating success in five outbred populations of *D. melanogaster* was measured under conditions very similar to those experienced by the flies in their culture vials. Most previous studies on components of male mating success in *Drosophila* used experimental protocols involving conditions very different from the environment in a typical culture vial. Many studies have used mutant flies (Barker, 1962; Prout, 1971; Harshman & Prout, 1994) or inbred populations (Parsons, 1964; Averhoff & Richardson, 1974; Brittnacher, 1981; Sharp, 1984; Partridge *et al*., 1985; Miller & Hedrick, 1993; Hughes, 1995), neither of which are representative of a typical, outbred *Drosophila* population. Moreover, measurements of components of male mating success have often been made in small mating chambers, under non-competitive conditions, or at densities and sex ratios that have little in common with conditions in culture vials when the population is being maintained on discrete generations with a relatively short generation time such as 10–14 d (Manning, 1961; Parsons, 1964; Spiess & Langer, 1964; Sharp, 1982, 1984; Partridge *et al*., 1985; Service, 1993; Hughes, 1995). Such experimental protocols, though very useful for a variety of specific investigations into the genetic control of components of male reproductive success, do not provide any information about the distribution of mating success in typical culture vials that would be useful in the estimation of the effective size of outbred laboratory populations of *Drosophila*.

2. Materials and methods

(i) *Experimental populations*

We measured the mean and variance of male mating success in the five B-populations of Rose (1984). The B-populations serve as short-generation-time controls to populations selected for postponed senescence, and are maintained on a discrete 2 week generation cycle, which is fairly typical of *Drosophila* laboratory maintenance regimes. Approximately 80 fourteen-day-old adults are allowed to lay eggs into an 8 dram vial (2·4 cm diameter × 9·5 cm height), containing 5 ml of banana–molasses food, until a density of about 80 eggs per vial is reached. The adults are then discarded and the vials incubated at 25 °C under continuous light. A total of 20 vials are collected per population. Adults begin to eclose 7–8 d after egg laying and remain in the vials until d 14, whereupon flies from all 20 vials of a population are mixed and then distributed into 20 fresh vials for egg laying, thus initiating the next generation. It is important to note that these populations are maintained at very moderate, and controlled, larval densities, thus reducing the extent of

possible adult size variation as a consequence of larval crowding in the culture vials.

(ii) *Marking male flies*

To be able to distinguish one male among others so as to monitor its mating success, we marked males on the dorsal surface of the thorax with a speck of white water-based acrylic paint. When applying the paint, we attempted to maximize the painted area while minimizing the thickness of the coat layer. After being marked with the paint, the painted males were held in vials overnight before being used in mating assays in order to give the paint sufficient time to dry fully. This technique of marking males for mating assays is similar to that used by Service (1993).

(iii) *Female mating choice assay*

The female mating choice assay was done to determine whether painted and unpainted males differed in their ability to compete for matings with a single female. We set up 120 vials, each containing 1 virgin female, 1 painted virgin male and 1 unpainted virgin male; all virgins were 2–3 d old. The rivals were monitored continuously for 2 h, and the number of painted and unpainted males that were the first to mate in their vials was recorded. All flies used in this preliminary assay came from one of the B-populations (B-2).

(iv) *Female mating profile assay*

To be able to construct an assay environment that simulated the conditions of B-culture vials as closely as possible, the pattern of female mating activity was studied in two B-populations (B-2, B-4), from the day of eclosion to d 14, the day at which eggs are collected to initiate the next generation in these populations. Virgin females were collected and kept individually in 8 dram vials, isolated from other adults for 24 h. Every 12 h thereafter, 3 males were added to each of the vials containing a single female. The males remained in the vials for 2 h, after which they were removed. Any mating that occurred during those 2 h was noted. The same males and females were reused for every 12-hourly observation period of 2 h. These data were used to determine whether mating in B-culture vials is likely to be spread out over the entire 5–7 d of adult life or whether there are shorter periods of time during which the majority of matings occur.

(v) *Development time assays*

For each population we set up five vials, each with exactly 40 freshly hatched larvae. Eclosing adults were collected every 6 h and the numbers of males and females were recorded. The adults were then discarded. Data from different vials were standardized to a common starting point by treating the time of first eclosion as hour 0. From these data, the fraction of males and females eclosing during each 6 h interval was determined in order to assess changes in sex ratio over time. A second development time assay was subsequently conducted, in exactly the same manner as the first one, except that the vials contained approximately 80 eggs, as is the case in the regular B-population culture vials. In the second assay, therefore, egg and larval densities were potentially variable across vials and more accurately duplicated the conditions of the B-population maintenance regime.

(vi) *Male mating success assay*

The protocol for the male mating success assay incorporated the findings of the female mating profile assay, and the exact density development time assay, in an attempt to determine the mating success of male flies from the B populations in conditions similar to those in their culture vials. In this assay, three series of 25 vials each were set up per population; due to handling mishaps during virgin collections, the number of vials set up for 4 of the 15 population × series combinations was only 12–14. The sequence in which varying numbers of males and females were added to the vials was planned to simulate the pattern of temporal change in sex ratio among eclosing flies that was observed in the exact density development time assay (see Section 3; Fig. 3*A*). Initially all vials contained 2 virgin males and 5 virgin females. After 4 h, another 20 virgin males and 20 virgin females were added to each vial. After a further 4 h, another 8 virgin males and 5 virgin females were added, bringing the total number of flies per vial to 60. One painted male was added to each vial at either hour 0 (series A vials), hour 4 (series B vials) or hour 8 (series C vials). Each time CO_2-anaesthetized flies were added to a vial, the flies in the vial were also lightly anaesthetized; all flies typically recovered in 2–3 min after anaesthesia. The vials were continuously monitored from the time the painted male was introduced through to hour 14. Any mating by the painted male in a vial was recorded. Only bouts of copulation lasting more than 5 min were considered to be successful matings, because shorter mating times are not sufficient for significant sperm transfer to occur (Harshman & Prout, 1994). The three different series of vials were set up in order to see whether males eclosing at different times in B-culture vials could be expected to differ in the distribution of mating success, as a consequence of varying sex ratios. All virgin flies used in the mating success assay were about 2–3 days old.

Data on number of matings per male pooled over the three series in each population, as well as from each of the 15 individual population × series combinations, were examined for goodness-of-fit to a Poisson distribution, using the sample mean number of matings per male as an estimate of the Poisson parameter. Goodness-of-fit was assessed by log likelihood ratio tests (*G*-tests) (Sokal & Rohlf, 1981), with mating categories pooled as necessary to ensure expected and observed frequencies of 1 or more in all cells. The approximation of G to χ^2 in less close for such low values of expected frequency, but the more standard procedure of pooling to ensure expected frequencies of 3 or more per cell would reduce the number of categories to a point entailing a great loss of statistical power due to shrinking degrees of freedom (Sokal & Rohlf, 1981). To partly compensate for this problem, we decided not to use Williams' (1976) correction for estimates of *G*. This correction tends to reduce the value of *G*, leading to a more conservative (less powerful) test, thus make it less likely that a departure from the expected frequencies will be detected (Sokal & Rohlf, 1981). Since our observation of a Poisson distribution of male mating success was somewhat counter-intuitive, we wanted to keep the test for departures from the Poisson expectation as powerful as possible so as to be able to have greater confidence in the observation that the distribution of male mating success did not significantly differ from a Poisson distribution.

(vii) *Assaying time to sexual maturity in males and females*

To examine whether there were differences between males and females in the time required from eclosion until the point at which they were willing to mate (henceforth referred to as 'maturation time'), we collected freshly eclosed virgin flies from all five populations and observed them continuously for the first 26 h of adult life. Freshly eclosed virgin males and females were placed individually into vials with three 2-day-old virgin flies of the opposite sex. From each B-population, 25 males and 25 females were assayed; thus, a total of 50 vials was set up per population. Maturation time for each fly was recorded as the time elapsed from the mid-point of the 4 h span over which virgins were initially collected until the time that individual mated. An analysis of variance (ANOVA) was performed on the maturation time data, treating population as a random factor and sex as a fixed factor. This assay, and the second development time assay, were conducted after the mating success assay in order to address possible explanations for some of the results seen in the mating success assay (see Section 4).

3. Results

(i) *Female mating choice assay*

In the female mating choice assay, painted males outcompeted their non-painted counterparts, and were successful at being the first to mate in 60 out of a total of 109 vials in which a mating occurred during the 2 h of the assay. Treating the number of successful painted males to be a binomial random variable, this corresponded to a 55% success rate with a 95% confidence interval ranging from 45·3% to 64·7%, calculated as described by Zar (1984). The results were taken as an indication that the females displayed no significant mating bias either for or against painted males.

(ii) *Female mating profile assay*

In the female mating profile assay, the majority of females mated only once or twice during the several days over which the assay was conducted. A few females (~ 5%) did not mate at all, while very few mated more than twice (Fig. 1). Moreover, 70–75% of all matings occurred during the first two of the 12-hourly observation periods when the females were exposed to males for a 2 h-span (Fig. 2), suggesting that the majority of matings in the culture vials of the B-populations probably occur during the first 12–14 h of the flies attaining sexual maturity.

(iii) *Development time assays*

The results from the development time assays conducted at exact and variable density, respectively, were fairly different (Fig. 3), indicating that increased variation in larval density in the vials has a pronounced effect on the pattern of male and female eclosion over

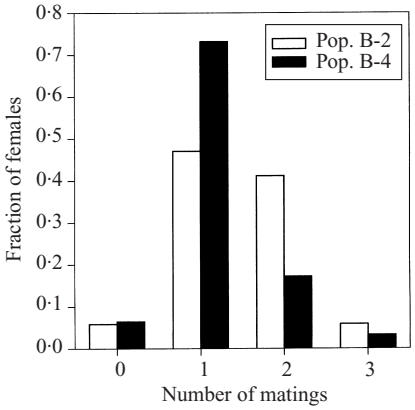

Fig. 1. Frequency distribution of the number of matings per female observed in the female mating profile assay. Only two of the five B-populations were assayed in this preliminary study.

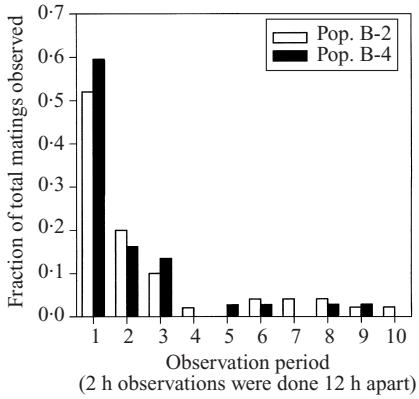

Fig. 2. Frequency distribution of the number of matings observed during each 2 h observation period in the female mating profile assay. Every 12 h, 3 males were added to vials containing a single virgin female and the number of matings that occurred during 2 h was recorded. Only two of the five B-populations were assayed in this preliminary study.

time. In the assay done at exact larval density of 40 larvae per vial, females tended to eclose earlier than males, causing a temporal pattern of changing sex ratios that was fairly consistent across vials (Fig. 3*A*). During the first period of eclosion in a given vial (arbitrarily considered to be hour 0), about 5% of the males and 15% of the females emerged. Between hours 0 and 6, approximately 25% of both males and females had emerged. By hour 12, the majority of flies in each vial had eclosed, and every 6-hourly observation thereafter showed decreasing numbers of adults eclosing. The male:female sex ratio during eclosion changed from about 2:5 at hour 0 to 1:1 at hours 6 and 12 to 8:5 at hour 18. Subsequent eclosions maintained a 1:1 sex ratio. The pattern of changing sex ratios at those specific times provided information used in deciding the protocol for the mating success assay.

In the second development time assay, conducted on vials with a more variable larval density corresponding to approximately 60–80 eggs per vial, eclosion was spread over twice as long a duration as in the first assay (Fig. 3*B*). More significantly, there was no consistent pattern of changing sex ratios over time, among either vials or populations. On average, the sex ratio at each 6-hourly observation was approximately 1:1. Since the variable larval density of the vials in this assay actually duplicated the conditions in typical B-culture vials, this suggested that there is no consistent temporal pattern of sex ratio change in the vials in which the B-populations are routinely maintained.

(iv) *Male mating success assay*

In the male mating success assay, each vial was initially set up with 2 males and 5 females. After 4 h, 20 males and 20 females were added to each vial and after a further 4 h, another 8 males and 5 females were added. One painted male, that was subsequently monitored for the number of times it mated, was added to each vial at either hour 0 (series A), hour 4 (series B) or hour 8 (series C). The differences in the pattern of male mating success in the series A versus the series B and C vials were broadly consistent across all five replicate populations (Table 1), with series B

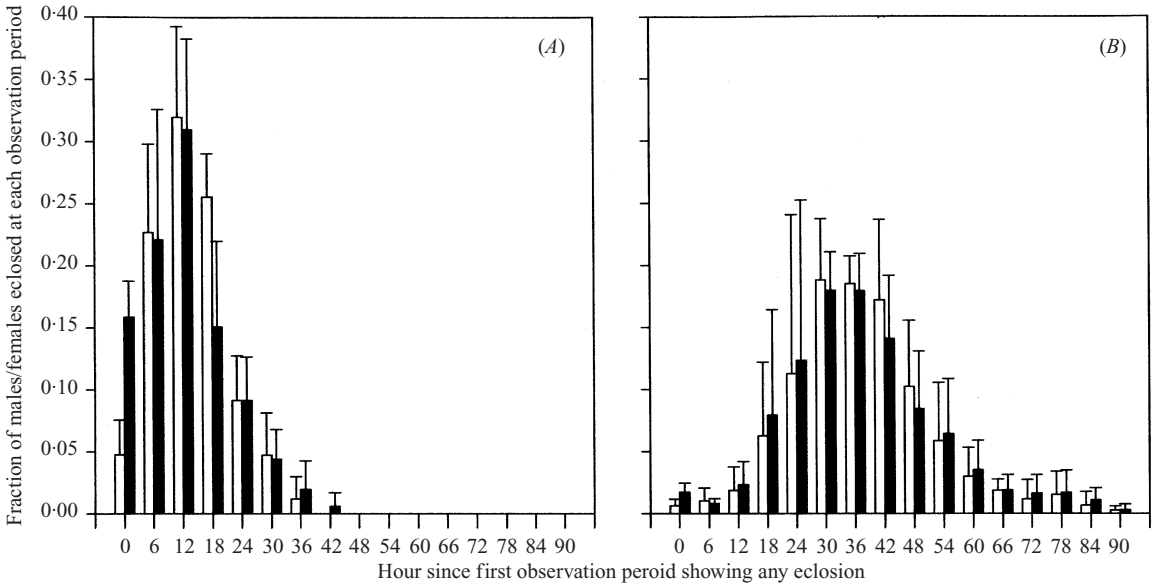

Fig. 3. Patterns of eclosion of males and females in the two development time assays. Error bars are 95% confidence intervals about the means for the five B-populations. (*A*) First assay, done on vials set up with an exact density of 40 larvae per vial. (*B*) Second assay, done on vials set up as in the B-cultures, with approximately 60–80 eggs per vial.

Table 1. *Results of the analysis of goodness of fit* (*assessed by* G-*tests*) *of the observed distributions of male mating success in the five B-populations to a Poisson distribution for series A, B and C vials separately, as well as for the pooled data from all three series of vials*

Population:	B-1		B-2		B-3		B-4		B-5	
No. of matings:	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Series A vials										
0	—	—	—	—	—	—	—	—	—	—
1	—	—	2	2·7708	4	3·2507	—	—	1	4·0262
2	3	6·5624	2	4·1147	2	3·1909	2	5·9526	1	4·9811
3	6	5·0710	6	5·1571	3	2·8636	8	4·9325	14	5·5125
4	9	4·8681	8	4·8477	2	1·9274	9	4·8832	7	4·5753
5	6	3·7387	5	3·6454	2	1·8889	2	3·8675	2	5·8472
6	1	4·3355	1	2·2845	—	—	3	2·5525	—	—
7	—	—	1	1·8038	—	—	1	2·2328	—	—
Mean mating success:	3·84		3·76		2·69		4·00		3·32	
Variance of mating success:	1·14		2·02		3·06		1·92		0·98	
$G \sim \chi^2$ with 2 d.f.:	11·1260*		5·9696		0·4463		11·1053*		21·7623***	
Series B vials										
0	11	11·3368	11	10·0047	4	3·7368	5	3·2480	4	5·5830
1	8	8·5026	6	8·7541	2	4·3596	4	6·4961	11	8·1418
2	5	4·1606	6	3·8299	6	3·9035	5	6·4961	5	5·9368
3	—	—	1	1·4113	—	—	7	4·3307	2	2·8859
4	—	—	—	—	—	—	2	2·1654	2	1·4520
5	—	—	—	—	—	—	1	1·2580	—	—
6	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—
Mean mating success:	0·75		0·86		1·17		2·00		1·46	
Variance of mating success:	0·63		0·90		0·88		2·09		1·30	
$G \sim \chi^2$ with 2 d.f.:	0·1994		2·2513		2·5862		3·7626		2·0488	
Series C vials										
0	10	12·1688	13	13·7203	7	6·5055	8	7·9061	18	18·1537
1	13	8·7615	9	8·2322	3	4·5038	4	4·5177	6	5·8092
2	1	3·1542	3	3·0475	3	1·9908	2	1·5762	1	1·2790
3	1	0·9155	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—
Mean mating success:	0·72		0·60		0·69		0·57		0·36	
Variance of mating success:	0·54		0·50		0·73		0·57		0·49	
$G \sim \chi^2$ with 2 d.f.:	4·2123		0·1087		1·0485		0·1678		−0·4105	
Series A, B and C vials pooled										
0	21	12·6012	24	13·3011	12	8·9372	13	5·4668	23	13·4821
1	21	22·3076	17	22·8276	8	12·9354	8	13·3633	17	22·9560
2	9	19·7452	11	19·5886	11	9·3611	9	16·3329	6	19·5436
3	7	11·6515	7	11·2061	3	4·5163	15	13·3083	17	11·0923
4	9	5·1566	8	4·8080	4	2·6204	11	8·1328	9	4·7217
5	7	2·6006	7	2·4620	—	—	3	3·9760	2	2·1989
6	—	—	—	—	—	—	4	2·4810	—	—
7	—	—	—	—	—	—	—	—	—	—
$G \sim \chi^2$ with 2 d.f.:	21·5254***		21·8007***		3·8628		15·9511**		25·9353***	

Each vial was initially set up with 2 males and 5 females. After 4 h, 20 males and 20 females were added to each vial and after a further 4 h, another 8 males and 5 females were added. One painted male, that was subsequently monitored for the number of times it mated, was added to each vial at either hour 0 (series A0), hour 4 (series B) or hour 8 (series C). Mating categories (number of matings) were pooled as necessary to ensure observed and expected frequencies of 1 or more in all cells. Obs., observed; Exp., expected.

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; ** $P < 0{\cdot}001$.

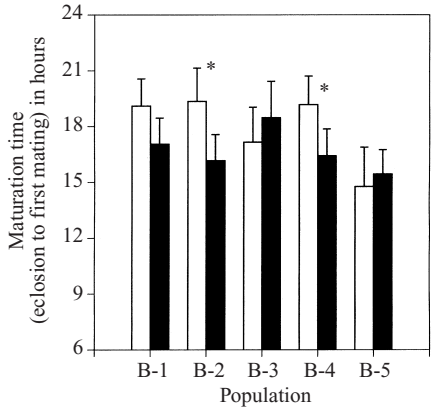

Fig. 4. Maturation time (the duration between eclosion of an individual fly and its first mating) of males and females from the five B-populations. Error bars are 95% confidence intervals about the mean based on observed variation in each population × sex combination, and should not be used for visual hypothesis testing (*significant at 0·05; **significant at 0·01, in pairwise *t*-tests based upon MS Error in the mixed model ANOVA).

and C vials in each population exhibiting similar patterns of male mating success, with a substantial fraction of males being unable to accomplish even one mating. Moreover, the ratio of the mean to the variance of numbers of matings per male in series B and C vials of all populations was close to 1 (Table 1), suggesting a Poisson distribution of mating success among these males. More formally, the data from series B and C vials in each population was consistent with a Poisson distribution of male mating success (*G*-test: *P* values ranging from 0·2 to 0·5 or more) (Table 1). The males in series A vials, where the sex ratios were skewed towards more females for the first 4 h of the assay, showed substantially higher levels of mating success (Table 1). In these vials, the variance in mating success was typically much smaller than the mean (Table 1), suggesting a distribution of mating success more uniform that would be expected if matings were completely random (the one population, B-3, in which the variance exceeded the mean, had a smaller sample size than the others; only 12 males per series were assayed, rather than 25). Formally, series A data for three of the populations showed significantly non-Poisson distribution of male mating success (*G*-test: $P < 0{\cdot}05$); data from populations B-2 and B-3 did not significantly differ from Poisson expectations (Table 1). Testing data from series A, B and C pooled for each population, the distribution of male mating success was seen to be significantly non-Poisson (*G*-test: $P < 0{\cdot}01$) for four of the five B-populations; the exception was population B-3 ($G = 3{\cdot}86$; $0{\cdot}1 < P < 0{\cdot}2$), which had reduced sample size (Table 1). The results of *G*-tests on series A, B and C data separately, however, clearly suggest that the non-Poisson distribution of mating success observed in the pooled data was basically due to the series A vials in each population (Table 1).

(v) *Maturation time assay*

There was no consistent difference across populations between male and female maturation times (Fig. 4). Moreover, the effect of sex in the ANOVA was not significant ($F_{1,4} = 1{\cdot}73$, $P > 0{\cdot}25$), although there were significant effects due to population ($F_{4,210} = 4{\cdot}61$, $P < 0{\cdot}002$) and the population × sex interaction ($F_{4,210} = 3{\cdot}21$, $P < 0{\cdot}02$) (Table 2). In two of the populations (B-2, B-4), females took significantly less time to become sexually mature than males (Fig. 4).

4. Discussion

In the male mating success assay three series of 25 vials each were set up per population with varying numbers of males and females added to the vials in a manner simulating the changing sex ratio among eclosing flies seen in the exact density development time assay (see Section 3; Fig. 3*A*). In series A vials, where the sex ratios were skewed towards more females for the first 4 h of the assay, males had relatively greater mating success than in series B or C (Table 1), and the distribution of mating success tended to differ significantly from Poisson expectations (Table 1). In the Series B and C vials, on the other hand, the male : female ratio was closer to 1 : 1, and the distribution of male mating success did not differ significantly from Poisson expectations (Table 1).

Thus, the results from the male mating success

Table 2. *Analysis of variance on maturation time* (*time in hours from eclosion to first mating*) *for males and females from the five B populations, which were treated as random blocks in the analysis*

Source	d.f.	SS	MS	*F*	*P*
Block	4	939874·9	234968·7	4·61	0·0014
Sex	1	282801·2	282801·2	1·73	> 0·25
Block × Sex	4	654152·6	163538·2	3·21	0·0100
Error	210	10713115·5	51014·8	—	—

assay indicated that when the number of flies in a vial is reasonably high, and sex ratio is close to 1:1, the number of matings accomplished by the individual males in these populations follows a Poisson distribution. The results also suggested that early-eclosing males in B-culture vials (analogous to series A males in the assay) may be able to mate with many more females than can males eclosing later on, when the sex ratio is close to 1:1. If this were true, then males in the B-cultures should have experienced considerable selection for faster development from egg to eclosion. Yet the development time of males in these populations is typically longer than that of females, at least when assayed in vials with exact low densities of 40 or 60 larvae. A possible explanation that although females eclose earlier they may take longer than males to become sexually active, was ruled out by the results of the maturation time assay (Table 2, Fig. 4). Results from the variable density development time assay, however, showed that under larval density conditions closer to the B-culture vials than the fixed densities used in the first development time assay, the sex ratio remained more or less 1:1 all though the time that eclosion occurred in the vials (Fig. 3*B*). This clearly suggests that, in fact, only the series B and C data from the mating success assay are useful indicators of mating activity in the B-culture vials. Since only the series A data provided any evidence for non-Poisson distribution of mating success (Table 1), this further strengthens the conclusion that, by and large, male mating success in these populations does appear to follow a Poisson distribution, suggesting that all males in a typical B-culture vial have the same probability of successfully mating per unit time.

The above conclusion may seem somewhat surprising in the light of substantial evidence that variation in male size is strongly correlated with measures of mating success in both laboratory and field populations of *Drosophila* (Partridge & Farquhar, 1983; Partridge *et al*., 1987; Markow, 1988; Markow & Ricker, 1992). However, it should be noted that the populations used in our study are not only laboratory populations, but are maintained at controlled and moderate densities of about 60–80 larvae per vial. The range of size variation in populations kept at controlled moderate density is very small compared with laboratory populations reared without explicit controls on larval density, e.g. by the serial transfer system or commonly used population cage designs (A. Joshi, personal observation). The range of size variation in field populations is typically quite large due, in part, to environmentally induced or magnified differences in size, and we do not doubt that size under such scenarios may be an important correlate of male mating success. Similarly, in population cage experiments the fitness of genotypes is determined partly by lifetime reproductive success, because generations overlap. In such studies there is evidence that larger males have higher lifetime reproductive success in part because of greater longevity and higher mating success at advanced ages than smaller males (Partridge & Farquhar, 1983). In *D. melanogaster*, the trade-off between adult size and fast development is well known (Partridge & Fowler, 1993; Zwaan *et al*., 1995; Nunney, 1996; Chippindale, 1997; Betran *et al*., 1998), suggesting that when rapid development is at a premium, the benefits of faster development may override those of larger size, leading to stabilizing selection on body size (Wilkinson, 1987). In populations maintained on a relatively short-generation-time, discrete-generation regime, such as the B-populations used in our study, development time is known to be under strong selection (Chippindale *et al*., 1994). Thus, we do not feel that our observation contradicts previous reports on the correlation of body size and male mating success under conditions of uncontrolled density in the field or the laboratory. What our results suggest is that in moderate-density cultures maintained on discrete and short generation cycles, on the other hand, male mating success tends to vary at random.

Male reproductive fitness in *Drosophila* has also been seen to have substantial genetic variation compared to several other components of fitness (Prout, 1971; Anderson *et al*., 1979; Brittnacher, 1981; Kosuda, 1983; Miller & Hedrick, 1993; Hughes, 1995). In two of these studies, the genotypes screened were either morphological mutants (Prout, 1971) or karyotypes (Anderson *et al*., 1979), which in both cases were known to be associated with major fitness effects, making a direct comparison with our populations difficult. In the other four studies, lines rendered homozygous for entire chromosomes were shown to undergo inbreeding depression for various measures of male mating success (Brittnacher, 1981; Kosuda, 1983; Miller & Hedrick, 1993; Hughes, 1995). In most of these studies it is not clear whether larval density of populations and experimental flies was deliberately controlled at moderate levels or not, once again making a direct comparison with our results difficult. In *Drosophila*, larval density has a profound effect on many fitness components, and on correlations between them (Mueller, 1990; Joshi, 1997; Santos *et al*., 1997; Borash *et al*., 1998), and often differences in results can be due to inadvertent differences in culture densities (see Discussion in Chippindale *et al*., 1994).

In studies of male mating success in *D. melanogaster*, it has often been seen that genetic variation in mating success is detected only at high (male-biased) operative sex ratios (Sharp, 1982, 1984; Partridge *et al*., 1985). This has been thought to be significant because it is considered likely that in field populations, and in laboratory populations where there is an opportunity

for males to sire offspring for a major part of their adult life (e.g. an overlapping-generation culture or a discrete-generation culture with really long generation time), the operative sex ratio will tend to be male-biased because females, but not males, undergo a refractory period after mating (Prout & Bundgaard, 1977; Gromko & Pyle, 1978; Markow *et al*., 1978). Once again, we do not doubt that this is quite likely true for populations with overlapping generations, or those where reproductive success can be attained over a major period of the life of an individual. Nevertheless, our results, especially those from the variable density development time assay and the male mating success assay taken together, suggest that the operative sex ratio in short-generation-time, discrete-generation cultures is not very different from 1:1 during the first 12–14 h after eclosion, which is the period in which most mating activity occurs in these cultures. The point we wish to stress is that the pattern of mating seen by us need not necessarily be generalizable to other types of cultures, especially when generations are overlapping or when generation times are relatively large. The corollary to this point is that we do not see our results as being contradictory to different results obtained with overlapping-generation *Drosophila* cultures or with field populations.

It is also known that in the presence of actively mating flies, virgin *D. melanogaster* males and females become more efficient at obtaining matings (Stanic & Marinkovic, 1990, 1992; Marinkovic & Stanic, 1995). Consequently, it is likely that this kind of learning may tend to equalize the likelihood of a given male mating when the flies are kept in large groups in a vial, as opposed to single males being assayed individually for mating success, as has been done in many previous studies.

The overall conclusion we draw from this study is that the likelihood of male mating success having a Poisson distribution in moderate-density, discrete-generation laboratory cultures of *Drosophila* is quite high. Our results are, consequently, of significance to laboratory researchers using *Drosophila* to study evolutionary problems because they suggest that the simple techniques for estimating effective population size based on census information may provide fairly accurate results for many typical laboratory populations of *Drosophila*. We should, nevertheless, also point out that in this study we ignored the potential role that sperm displacement may play in affecting the relationship between male mating success and fertilization success. Sperm displacement, wherein offspring of a female mated with two males in succession are largely produced using sperm from the second male, is known to occur in *D. melanogaster* (Lefevre & Johnson, 1962; Prout & Bundgaard, 1977; Newport & Gromko, 1984; Harshman & Prout, 1994; Clark *et al*., 1995). However, empirically studying the extent to which sperm displacement plays a role in determining reproductive success under typical culture conditions is likely to prove a technically daunting task.

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