DENSITY-DEPENDENT FERTILITY SELECTION IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The effects of larval density on components of fertility fitness were investigated with two mutant lines of *Drosophila melanogaster*. The differences in adult body weight, wing length, larval survivorship and development time verified that flies reared at high density were resource limited. Experimental results indicate that: (1) relative fecundities of both sexes show density-dependent effects, (2) there is a strong density effect on male and female mating success, and (3) in general, there is a reduction in fecundity differences between genotypes at high density. These results imply that it may be important to consider fertility in models of density-dependent natural selection.

THE field of theoretical ecological genetics came into being with the nearly simultaneous appearance of papers by ANDERSON (1971), ROUGHGARDEN (1971), CLARKE (1972) and CHARLESWORTH (1971). These papers all presented models in which the fitness of the three genotypes at one diallelic locus are decreasing functions of density and the change in population size is determined by the mean fitness. The fitness of each genotype is determined by two parameters that describe the "intrinsic growth rate" at low density, and the "carrying capacity" of the environment for each genotype alone. These models put selection between the zygote stage and the adult mating stage, so that density-dependent selection occurs only in the viability component. More recently, POULSEN (1979) developed a model more closely to simulate the life cycle of Drosophila; however, density-dependent selection occurs only in viability in his model as well.

Experimental studies with *Drosophila* have shown biological effects of crowding at virtually all stages of the life cycle. PEARL (1932) showed that females raised under crowded conditions have lowered egg production. POWSNER (1935) demonstrated a lengthening in developmental period in high-density populations. The laboratory ecology of Drosophila was investigated by SANG (1949), who demonstrated that rearing larvae under crowded conditions results in increased larval and pupal mortality and decreased body weight. Reduced population productivity under crowded conditions was demonstrated by ROBERTSON and SANG (1944), SAMEOTO and MILLER (1966) and by MUELLER and AYALA (1981). In

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addition, SOKOLOFF (1955) found an increase in variability in developmental rate as larval density increased. While studying aspects of interspecific competition, BARKER (1973) and BARKER and PODGER (1970a,b) observed the effects of larval crowding on fecundity, egg hatchability, viability and developmental rate. Most studies have been concerned with larval crowding, but MOTH (1974) and ECK-STRAND and SEIGER (1975) showed that adult crowding may affect mating rate, while BIRCH (1955) failed to find an effect of adult crowding.

In order to demonstrate density-mediated evolution, different genotypes must be shown to respond differently to crowding. For the most part, studies that claimed to demonstrate density-dependent selection concentrated on relative larval viabilities, inferring density dependence by differences in progeny ratios from the same parental composition at different densities (SOKAL and HUBER 1963; SOKAL and KARTEN 1964; SOKAL and SULLIVAN 1963; LEWONTIN 1955; LEWONTIN and MATSUO 1963; MOTH and BARKER 1977; BHALLA and SOKAL 1964; DRUGER and NICKERSON 1972; MOREE and KING 1961). Since larval viability is not a reliable predictor of net fitness (PROUT 1965), DEBENEDICTIS (1977) argued that these studies failed to reject the null hypothesis of density-independent selection. BIRCH (1955) clearly demonstrated density dependence of net fitness, but it remains to be shown that resource limitation can induce genotype-specific changes in components of fitness other than viability. Experimental work of SVED (1971), SVED and AYALA (1970), CURTSINGER and FELD-MAN (1979), ØSTERGAARD and CHRISTIANSEN (1980) and CLARK and FELDMAN (1981) demonstrated that differences in fertility can account for a large portion of the total fitness differences between genotypes. Since it is known that density affects components of fertility (PEARL 1932, BARKER 1973, BARKER and PODGER 1970b), it is likely that different genotypes respond differently to crowding.

In this paper, we present evidence that larval crowding has genotype-specific effects on aspects of fecundity and mating success in laboratory stocks of *Drosophila melanogaster*. These results are discussed in light of the theory that considers only viability to be density dependent.

MATERIALS AND METHODS

All experiments were performed with stocks of *D. melanogaster* bearing the chromosome *4* markers *sparkling-poliert* (*spa^{pol}*, abbreviated *pol*) or *eyeless* (*ey^2*), obtained from Carolina Biological Supply. Both markers produce readily identifiable abnormal eye phenotypes when homozygous, with virtually complete penetrance (LINDSLEY and GRELL 1968). Tests were also performed with F$_1$ flies obtained from these two lines, whose phenotype was wild type. F$_1$ flies were designated *ey^2/pol* or *pol/ey^2*, where the first chromosome was of maternal origin. No recombinants were observed between these loci, as was expected for chromosome *4*. The term "cyto-genotype" was used to distinguish both genotypes and maternal cytoplasm.

Assessment of resource limitation: Before the effects of density on fertility were assessed, tests were performed to define rearing methods that put flies in varying levels of resource limitation. The first test involved placing a series of 2, 5, 10, 30 and 50 pairs of virgin 4-day-old flies in half-pint bottles with 12 g Carolina 4-24 Instant Drosophila medium, 35 ml water and live yeast. Oviposition was permitted for 4 days, and on day 16 adult progeny were scored. Five replicates of each density of each of the four crosses (*ey^2 × ey^2, ey^2 × pol, pol × ey^2* and *pol × pol*) were performed. Based on these tests, the following procedure was adopted for rearing flies at low and high larval density. Low-density stocks were initiated with 10 pairs of adults in half-

pint bottles with 12 g of medium (dry weight), while high-density stocks had 50 pairs of adults on 6 g of medium. In both cases, females oviposited for 4 days, and progeny were harvested on days 11 through 14. Flies used to initiate the high- and low-density stocks were themselves raised at low larval density. These were the only two densities at which larvae were raised. All flies were maintained in an incubator at 25 ± 1°, with a 12-hour photoperiod.

The degree to which the two densities resulted in biologically significant resource limitation was assessed by measuring five aspects of morphology and development. Wing length and wet body weight of 1-day-old adults harvested on day 10 of the 4 cyto-genotypes raised at both densities were measured, and two-way analysis of variance was used to assess genotypic and environmental effects and their interaction. Egg-to-adult survivorship was measured by counting eggs and the emergent adults for the two density treatments. The number of adult progeny that emerged each day from low- and high-density stock bottles yielded information about the effect of density on egg-to-adult development time.

Fertility assessment: To determine the effects of larval and early adult crowding on aspects of fertility, 3- and 4-day-old virgins of the 4 cytogenotypes from the two densities were used. Fifty males and 50 females of each of two cyto-genotypes were placed into a half-pint bottle, which served as a mating chamber. Two hours was sufficient time for most females to be inseminated; yet, double inseminations were virtually absent (BUNGAARD and CHRISTIANSEN 1972). Females were then isolated in 95 mm shell vials with Carolina 4–24 medium and allowed to lay eggs for four days. On the 16th day after mating, adult progeny were scored and the paternal type was inferred from the progeny. The fecundity of a mating was defined as this progeny count.

The six cyto-genotype pairs were $(ey^2, ey^2/pol)$, $(ey^2, pol/ey^2)$, $(ey^2, pol/ey^2)$, (ey^2, pol), $(ey^2/pol, pol/ey^2)$, $(ey^2/pol, pol)$ and $(pol/ey^2, pol)$. This set of 6 experiments was replicated 3 times with low-density stocks and twice with high-density stocks. In addition, crosses between flies within each strain reared at high and low density were carried out to quantify further the sex × density interactions. In the $ey^2/pol \times pol/ey^2$ experiment, the four mating types could not be inferred from the progeny; therefore, these cyto-genotypes were not mixed in the mating chamber. Instead each of the four crosses was made with 25 females and 25 males of the respective types, and individual females were isolated in shell vials as above.

Fertility data were analyzed in three ways. Preliminary tests were performed to verify that fecundities of each mating type were normally distributed and that the variances were homogeneous. Two-way analysis of variance was separately applied to each of the 6 experiments at both densities to assess the significance of female effects, male effects and male × female interactions. Second, after it was determined that it was appropriate to pool data, a factorial three-way analysis of variance was applied to the data table consisting of 4 female × 4 male × 2 density effects. Finally, data tables in the format of Table 1 were constructed, where each cell represented the mean productivity of the respective mating type. These tables were fitted to additive and multiplicative fecundity models in order to split the fecundity of mating types into separate male and female contributions.

As Table 1 indicates, the additive model assumes that the productivity of each mating is the sum of male and female fecundities, while the multiplicative model determines each mating productivity by multiplying male and female effects. The six parameters of each model were determined by searching the parameter space for the maximum likelihood. Differences in fecundity parameters at the two densities were assessed with the heterogeneity G statistic.

Sexual selection: Another important aspect of fertility selection is mating success. Differential mating success was detected by comparing mating frequencies to the initial adult pool in the 6 pairwise experiments already described. Since all of these tests had the same initial number of the two cytogenotypes being tested, the index of sexual selection of, for example, the ey^2 male relative to the *pol* male is simply $(n_{11} + n_{21})/(n_{12} + n_{22})$, where the numbers of each of the four mating types are:

$$
\begin{array}{c}
\text{Male} \\
\begin{array}{ccc}
 & ey^2 & pol \\
\text{Female} \quad \begin{array}{c} ey^2 \\ pol \end{array} & \left[\begin{array}{c} n_{11} \\ n_{21} \end{array}\right. & \left.\begin{array}{c} n_{12} \\ n_{22} \end{array}\right]
\end{array}
\end{array}
$$

TABLE 1

Additive and multiplicative fecundity model parameters

Additive model:		Male genotype			
		ey^2	ey^2/pol	pol/ey^2	pol
Female genotype	ey^2	$1-f_1-m_1$	$1-f_1-m_2$	$1-f_1-m_3$	$1-f_1$
	ey^2/pol	$1-f_2-m_1$	$1-f_2-m_2$	$1-f_2-m_3$	$1-f_2$
	pol/ey^2	$1-f_3-m_1$	$1-f_3-m_2$	$1-f_3-m_3$	$1-f_3$
	pol	$1-m_1$	$1-m_2$	$1-m_3$	1

Multiplicative model:		Male genotype			
		ey^2	ey^2/pol	pol/ey^2	pol
Female genotype	ey_2	f_1m_1	f_1m_2	f_1m_3	f_1
	ey^2/pol	f_2m_1	f_2m_2	f_2m_3	f_2
	pol/ey^2	f_3m_1	f_3m_2	f_3m_3	f_3
	pol	m_1	m_2	m_3	1

Fecundities are normalized to the *pol* $\times$ *pol* cross.

The sexual selection index is simply the ratio of numbers of matings by each type of male. Variances of sexual selection indices were calculated based on the assumption of binomial sampling, where $p = 1 - q$ is the fraction of mated females who mated with (in this case) ey^2 males. The assumption of binomial distribution was in part verified by chi square tests for randomness of mating. Using the approximation of KENDALL and STUART (1958, p. 233)

$$\text{var } (p/q) = (1/N)(p/q)^2[(q/p)+(p/q)+2],$$

where $N = \sum_i \sum_j n_{ij}$. Additional replicates of the six experiments were scored for mating-type frequencies to bring the total to five replicates at each density. These methods of analyzing fertility and sexual selection data are discussed in more detail by CLARK and FELDMAN (1981).

RESULTS

Assessment of resource limitation: Figure 1 gives the relation between the mean number of progeny produced in a half-pint bottle and the number of parental pairs. The fact that these curves level off suggests that a resource (medium or pupariation space) becomes limiting, and that there is a maximum number of progeny that a bottle can produce in a given period of time. More striking is the fact that the maximum productivity seems to be determined by the maternal type. Both *pol* $\times$ *ey²* and *ey²* $\times$ *pol* crosses produce only heterozygotes, so that it would appear that the maternal type is important in determining the population productivity. It is not possible from these experiments to say whether this is caused by differences in the number of eggs laid, the egg hatchability or the larval survivorship, but it seems unlikely that the number of eggs laid plateaus with 20 parental pairs.
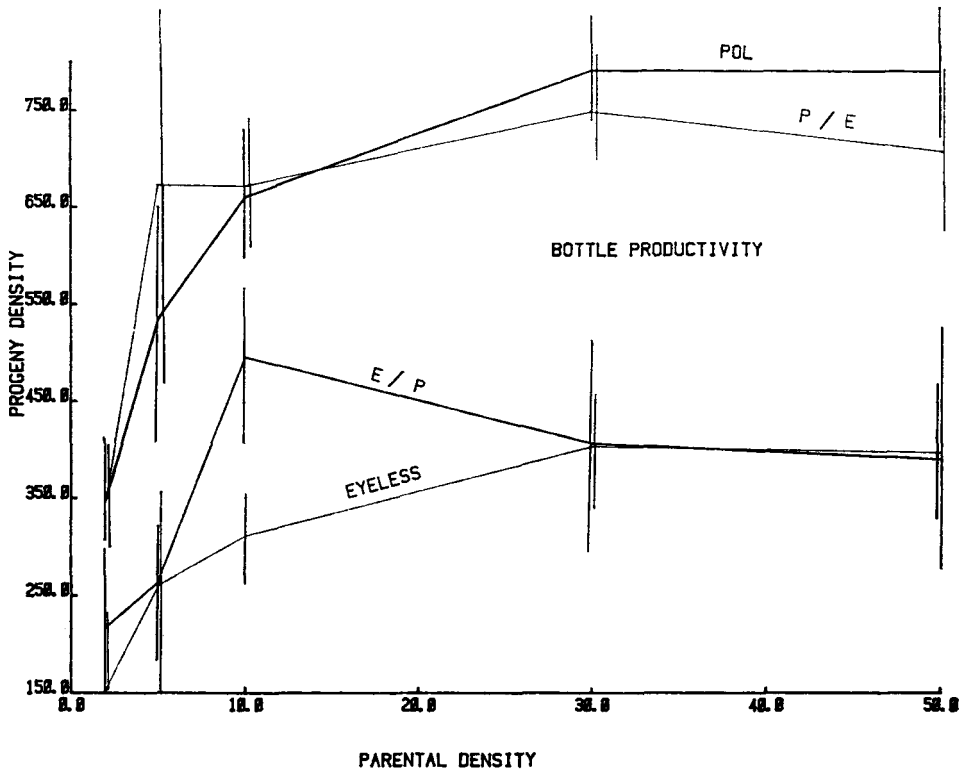
FIGURE 1.—Number of adult progeny produced in half-pint bottles by different numbers of parental pairs. Parents were allowed to lay eggs for 4 days and progeny were scored on day 16. Means ± 1 standard error of 5 replicates are plotted.

Figure 2 depicts the same data scaled to show the productivity per female. All genotypes show decreasing productivity per female with increased crowding, contrary to some previous work (BARKER 1973; SANG 1949) that indicated an increase in productivity with density at low densities (an "Allee" effect). This discrepancy can be explained in part by the fact that mold was a problem in the 2 and 5 parental-pair bottles, and any moldy bottle was discarded, biasing the estimates of productivity up (see also MUELLER and AYALA 1981).

The wing lengths of females and males of the four cyto-genotypes at the two densities are plotted in Figures 3 and 4. Two-way analysis of variance indicated that there were significant genotype and density effects, but neither sex showed genotype × density interactions. In every case, the flies raised under higher larval densities had shorter wings, and the lack of interactions implied that the crowding had approximately the same effect on all genotypes.

Figures 5 and 6 represent the adult body wet-weights when larvae were raised under the two levels of crowding. In the females, the body weights of the different genotypes were fairly similar at low density, but the heterozygotes are the heaviest at high density. The pol males seem to be markedly smaller than other
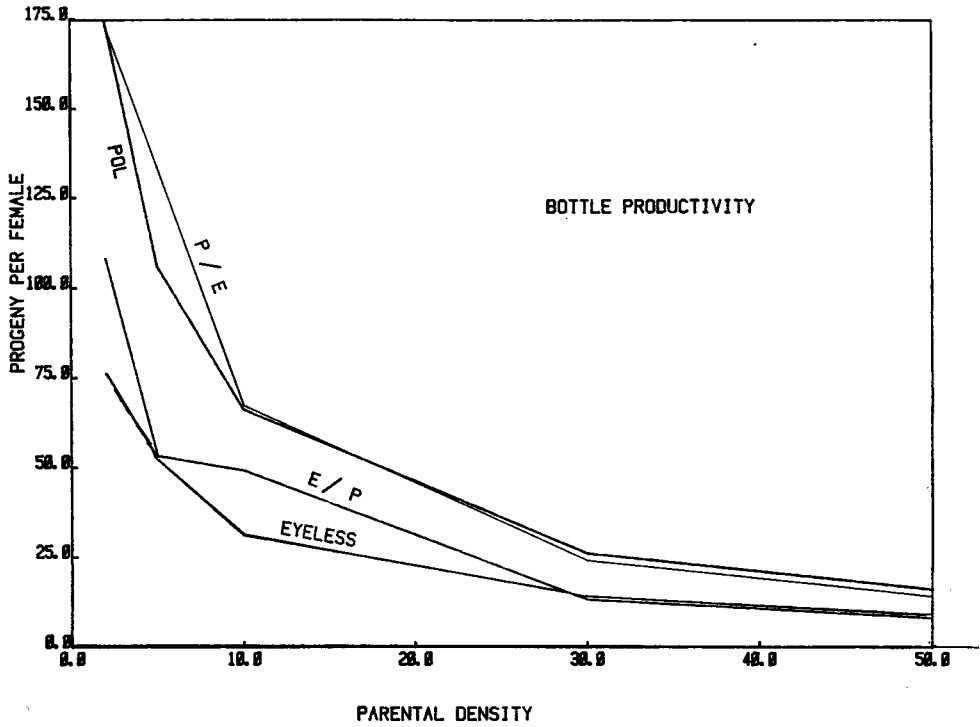
FIGURE 2.—Productivity per female in half-pint bottles at different parental densities. (Data plotted are the same as those in Figure 1.)
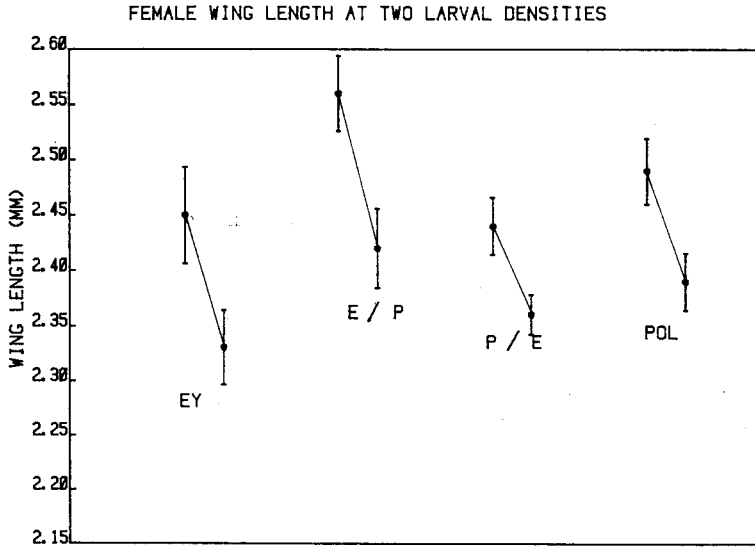

FIGURE 3.—Mean female wing length ± 1 standard error at two levels of larval crowding. Above each genotype label, the left-hand bar represents flies raised at low density and the right-hand bar represents flies raised at high density. Sample size of each type is 25.
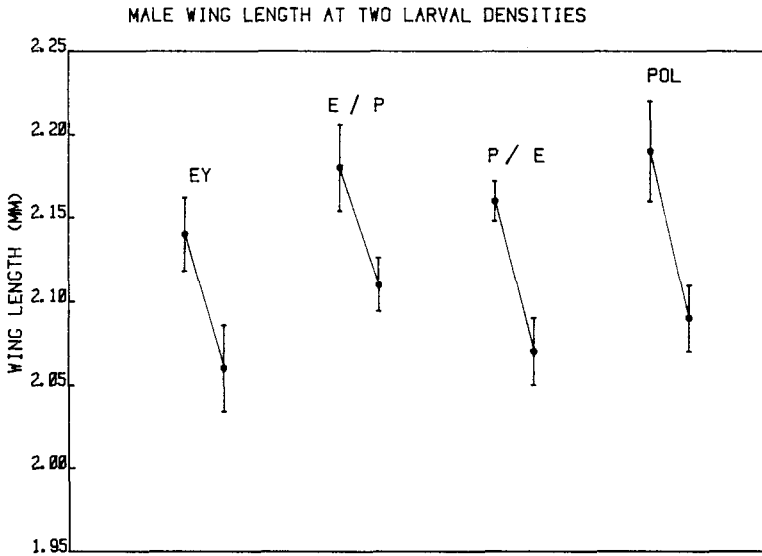
MALE WING LENGTH AT TWO LARVAL DENSITIES

FIGURE 4.—Mean male wing length ± 1 standard error under two levels of larval crowding. Sample size of each type is 25.

types at high density. Two-way ANOVA on body-weight data indicated significant density and genotype effects, as well as genotype × density interactions in both sexes.

Figure 7 clearly shows that, at high larval density, larval survivorship is much lower. It also indicates that larval survivorship is somewhat higher in the *pol/ey²*

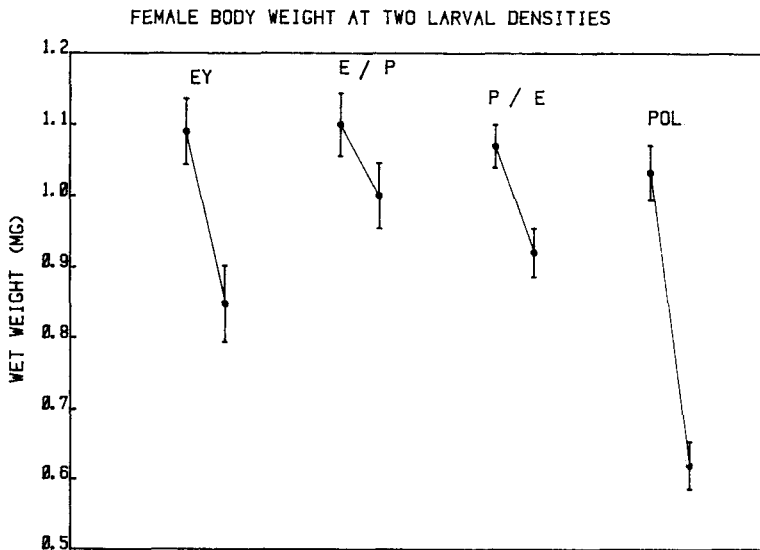FEMALE BODY WEIGHT AT TWO LARVAL DENSITIES

FIGURE 5.—Mean female wet body weight ± 1 standard error under two levels of larval crowding. Sample size of each type is 25.
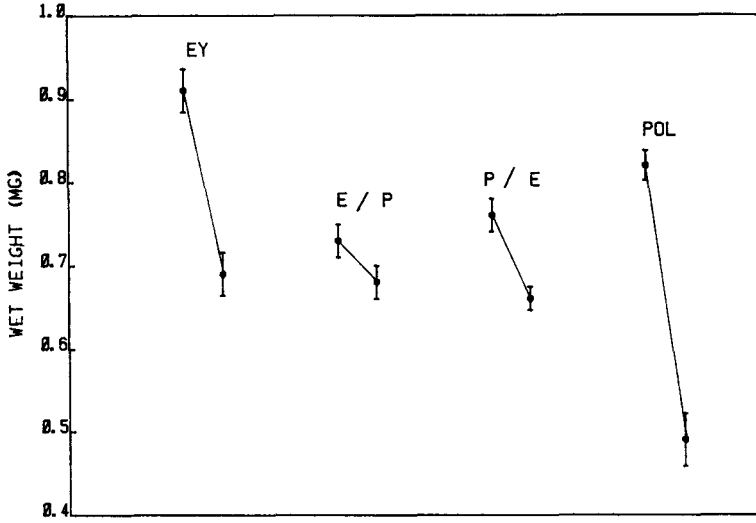
MALE BODY WEIGHT AT TWO LARVAL DENSITIES

FIGURE 6.—Mean male wet body weight $\pm$ 1 standard error under two levels of larval crowding. Sample size of each type is 25.
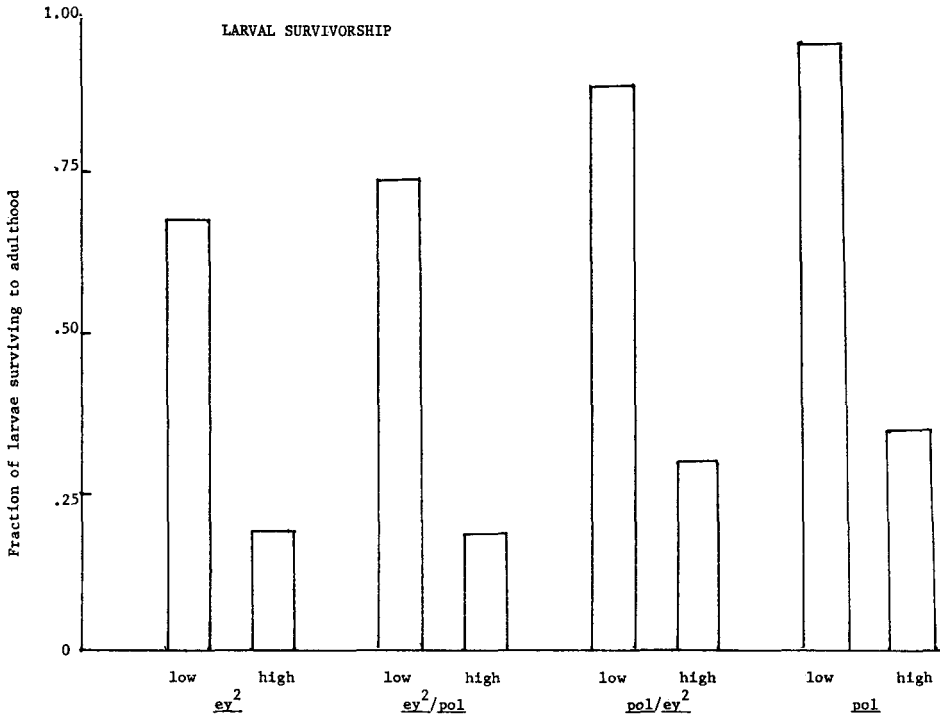

FIGURE 7.—Larval survivorship at two levels of larval crowding. Bars represent mean survivorship to day 16 over 3 bottle replicates.

TABLE 2

Mean and confidence intervals of egg-to-adult development period at two larval densities

Stock	Low density	High density
ey²/ey²	10.70 ± 0.18	12.64 ± 0.17
ey²/pol	10.68 ± 0.18	12.42 ± 0.12
pol/ey²	10.24 ± 0.11	12.36 ± 0.11
pol/pol	10.06 ± 0.10	12.59 ± 0.10

and *pol* stocks; a result consistent with the higher carrying capacity in these two cultures.

Figure 8 is a plot of the mean over 5 replicates of the number of adults emerging on days 8 through 16 after egg-laying. The mean and confidence interval in emergence time for the 4 cyto-genotypes are listed in Table 2. Clearly, increased larval density slows development rate and there are differences between genotypes. Qualitatively, the results of larval crowding reported here are consistent with previous studies (SANG 1949; SOKOLOFF 1955; POWSNER 1935; BARKER and PODGER 1970b), and they provide convincing evidence that our particular rearing methods present the flies with different levels of resource limitation.
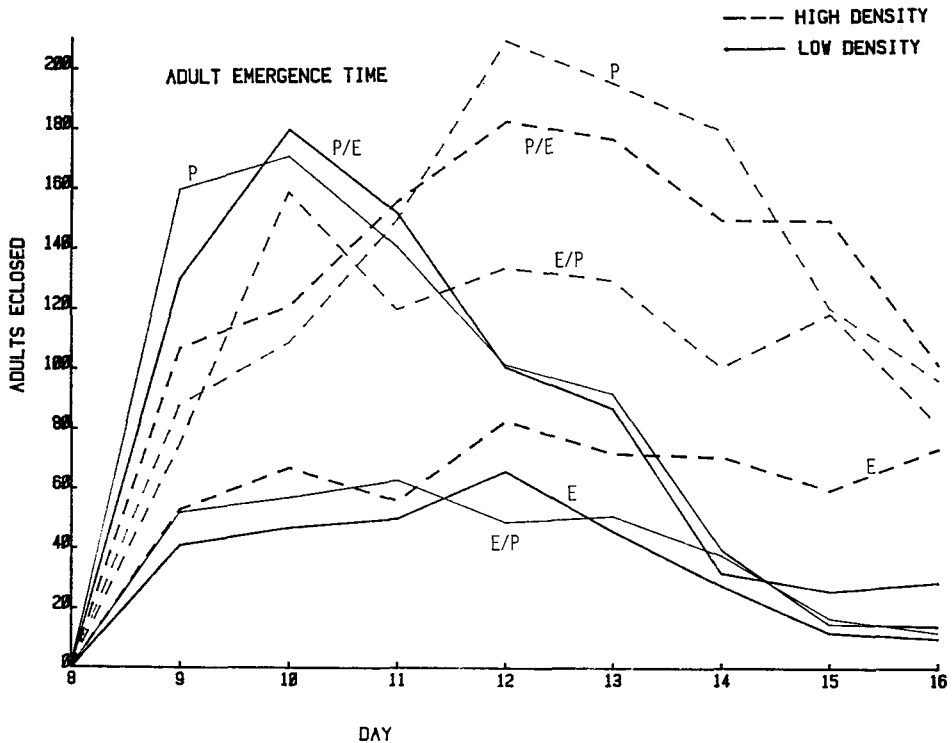

FIGURE 8.—Number of adults emerging on each day from bottles at two levels of larval density.

If sufficient eggs were laid by single females in vials to result in resource limitation of larvae, the reduced egg-to-adult viability of progeny would confound the measure of fecundity. We investigated whether resource limitation occurred in vials by scoring egg-to-adult viability (absolute viability) in vials with a range of numbers of eggs. Figures 9 and 10 show that there is no significant reduction in absolute viability at even the highest egg densities for progeny of females raised at low and high densities. The lower survivorship at low egg densities can be explained by an increased sampling variance and mold contamination.

Fecundity data: The mean fecundities of the 16 mating types and standard errors are tabulated in Tables 3 and 4 for the two densities. Generally, the female type appears to have a greater effect than the male type in determining mating-type productivity. This is especially evident when comparing fecundities of reciprocal crosses between high and low density stocks (Table 5). In all cases, the cross involving a female raised at low density had a higher fecundity than the reciprocal cross. The effects of density are seen graphically in Figure 11, where the mean fecundity at both densities is plotted for all mating types. The fact that the ordering of fecundities change in the two environments implies that there are genotype × environment interactions. Some of the details of these interactions will be addressed with analysis of variance and the fecundity models.
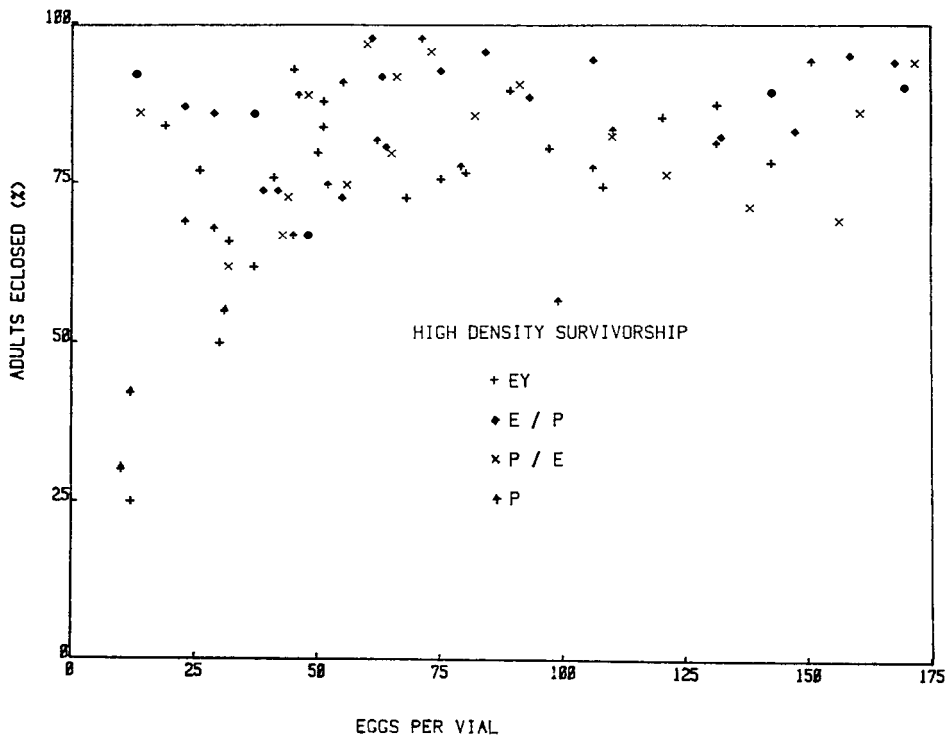

FIGURE 9.—Egg-to-adult viability of low density stocks in vials at a range of densities.
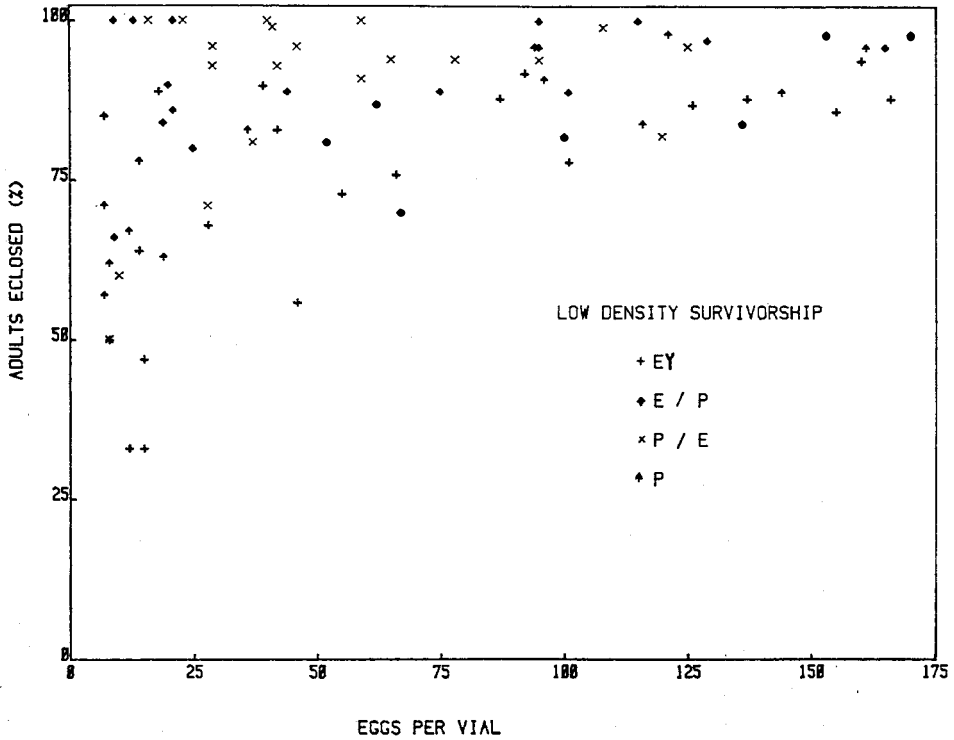
FIGURE 10.—Egg-to-adult viability of high density stocks in vials at a range of densities.

TABLE 3

Low-density fecundities

		Male genotype			
		ey^2	ey^2/pol	pol/ey^2	pol
Female genotype	ey^2	55.89 ± 1.57 (136; 7601)	61.70 ± 2.96 (54; 3332)	60.05 ± 3.99 (39; 2342)	55.38 ± 4.01 (26; 1440)
	ey^2/pol	114.0 ± 4.25 (58; 26629)	122.90 ± 2.48 (211; 25933)	117.0 ± 3.25 (59; 6961)	103.68 ± 7.04 (31; 3214)
	pol/ey^2	117.37 ± 4.19 (48; 5634)	105.79 ± 3.50 (73; 7723)	110.42 ± 2.18 (250; 27605)	94.5 ± 5.77 (36; 3402)
	pol	78.12 ± 3.03 (64; 5000)	74.00 ± 3.95 (55; 4070)	73.37 ± 3.61 (65; 4769)	57.09 ± 2.07 (121; 6909)

Each entry gives the mean number of adult progeny scored on day 16 produced per singly inseminated female isolated for 4 days in 95 mm shell vials. Mating types are inferred from female type and the progeny. Parents were raised under high-density conditions. Numbers in parentheses indicate the number of vials and number of progeny scored for each mating type.

TABLE 4

High-density fecundities

		Male genotype			
		ey^2	ey^2/pol	pol/ey^2	pol
Female genotype	ey^2	48.71 ± 2.01 (143; 6966)	38.27 ± 2.18 (59; 2258)	57.94 ± 3.79 (48; 2781)	64.10 ± 4.70 (19; 1218)
	er^2/pol	40.60 ± 4.14 (25; 1015)	67.12 ± 2.21 (154; 10336)	84.56 ± 4.61 (42; 3551)	80.27 ± 5.90 (29; 2328)
	pol/ey^2	71.09 ± 3.86 (40; 3057)	82.49 ± 4.43 (37; 3052)	74.92 ± 2.01 (143; 10714)	73.75 ± 4.30 (24; 1770)
	pol	69.57 ± 4.37 (35; 2435)	63.68 ± 3.31 (71; 4521)	72.07 ± 4.60 (40; 2883)	47.91 ± 2.38 (92; 4408)

Each entry gives the mean number of adult progeny scored on day 16 produced per singly inseminated female isolated for 4 days in 95 mm shell vials. Mating types are inferred from female type and the progeny. Parents were raised under high-density conditions. Numbers in parentheses indicate the number of vials and number of progeny scored for each mating type.

Two-way analysis of variance was used for each experiment, comparing pairs of cyto-genotypes. At low density, each of the 6 cyto-genotype pair experiments were replicated 3 times; thus, 18 analyses were performed without pooling. These revealed 14 cases of significant female effect, 6 cases of significant male effect and 6 significant male × female interactions. At high density the experiments were replicated twice, so that 12 analyses were performed. These yielded 6 cases of significant female effect, 4 cases of significant male effect and 3 cases of significant male-by-female interactions. We conclude that there are somewhat fewer cases of significant fecundity effects at higher density, indicating that the distinction between genotypes is smaller when flies are raised under crowded conditions (see DISCUSSION).

TABLE 5

Fecundities of crosses between lines raised at low and high density

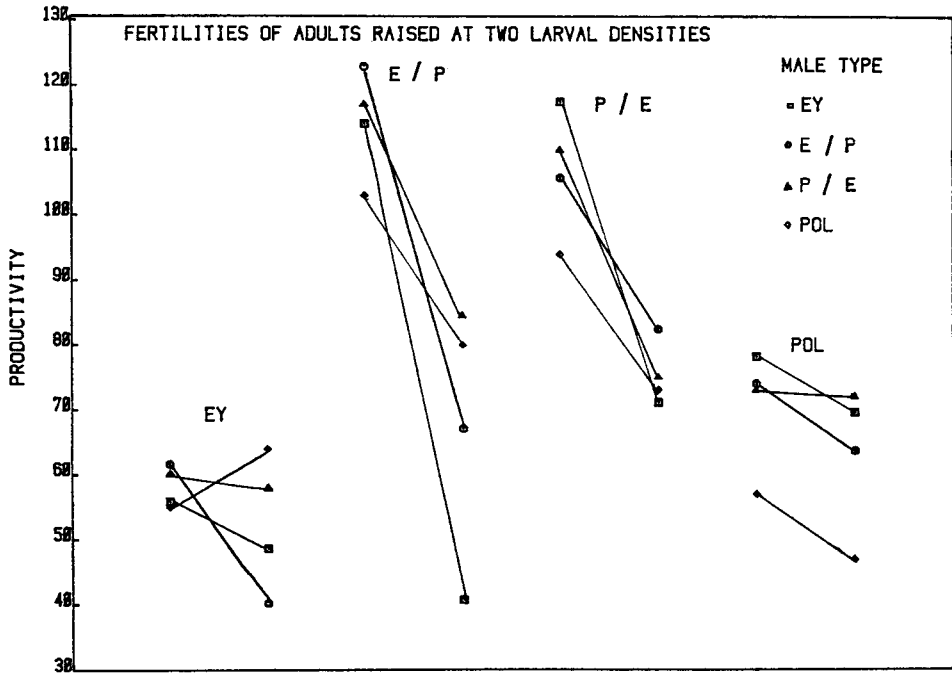| Cross	Mean ± 1 S.E.	N (crosses)	F ratio
$ey^2 \times ey^2$			
low × high	80.10 ± 5.12	39	6.63**
high × low	62.45 ± 4.58	44	
$ey^2/pol \times ey^2/pol$			
low × high	98.51 ± 6.03	43	11.24**
high × low	77.46 ± 2.25	46	
$pol/ey^2 \times pol/ey^2$			
low × high	102.22 ± 3.81	49	8.45**
high × low	85.12 ± 4.55	42	
$pol \times pol$			
low × high	63.84 ± 5.30	45	5.63*
high × low	46.33 ± 5.04	39	

FIGURE 11.—Mean fecundity of all mating types at two larval densities. Under each female genotype label are plotted the productivity of matings with the 14 male types. On the left of each set are the low density productivities, while productivities of high density stocks are plotted on the right.

The experiment is set up as a three-way factorial design with 4 female cyto-genotypes, 4 male cyto-genotypes and 2 densities. In order to simplify calculations of the three-way ANOVA, subsets of the data were considered having 20 observations per treatment. Observations were drawn at random and the analysis was performed twice to verify that representative samples were obtained. Results are presented in Table 6. Of the single effects, density has by far the most significance, but the female effect is also significant. Both two-way interactions with density and the three-way interactions are highly significant, while the female × male interaction is not. We conclude that crowding has profound and complex effects on the relative fecundities of these mating types.

The significance of cytoplasmic effects and interactions with density were assessed by three-way analysis on a $2 \times 2 \times 2$ subset of the data. The three effects were female cyto-genotype (ey^2/pol or pol/ey^2), male cyto-genotype (ey^2/pol or pol/ey^2) and density. Table 7 shows that the only significant effects are density and the three-way interaction. In addition to showing that the source of cytoplasm is not important in determining productivity of hybrids between the two lines, this result demonstrates that the X chromosomes of the two lines do not confer different male fecundities. The significant three-way interaction, however, prevents us from concluding that the cytoplasm (or the X chromosome) is irrelevant with respect to fecundity.

TABLE 6

Three-way ANOVA results on a random subset of fecundity data

Source	d.f.	SS	MS	F
Female	3	245510.2	81836.7	2.96*
Male	3	13760.7	4586.9	0.398
Density	1	137211.9	137211.9	221.84***
Female × male	9	30256.6	3361.84	0.074
Female × density	3	81075.0	27025.0	43.69***
Male × density	3	32723.6	10907.8	17.64***
Female × male × density	9	401487.0	44609.7	72.12***
Within	608	376053.6	618.5	
Total	639	942024.6		

Twenty matings of each of the 32 treatments were selected at random.

The 4 × 4 tables of mean fecundities listed in Tables 3 and 4 were fitted by means of maximum likelihood to additive and multiplicative fecundity models. Table 8 lists the goodness-of-fit and the estimated parameters of both models at both densities. The low density data fit both additive and multiplicative models very well, and indicate directional selection favoring ey^2 males and overdominance in female fecundity. The male parameters are generally closer to 1.0 than are the female fecundities, supporting the statement that females have a greater effect in determining the productivity of a mating. The low-density estimates indicate that differences in the two types of heterozygotes are small (*i.e.*, only slight cytoplasmic effect). At high densities, neither fecundity model adequately fit the data, so that there appear to be significant female × male interactions. This is consistent with the variance analysis, since female × male interactions at one density and not at another imply female × male × density second-order interaction.

Rather than assessing density effects by comparing individual parameter estimates, the two fecundity matrices were fitted jointly to the two models. In the

TABLE 7

Three-way ANOVA on cytoplasmic effects

Source	d.f.	SS	MS	F
Female	1	3861.3	3861.3	1.57
Male	1	1345.6	1345.6	0.57
Density	1	139712.4	139712.4	269.18***
Female × male	1	409.6	409.6	0.05
Female × density	1	1932.1	1932.1	3.72
Male × density	1	1836.1	1836.1	3.54
Female × male × density	1	81068.3	81068.3	156.19***
Within	152	78892.4	519.03	
Total	159	230165.0		

Analysis based on a random set of 20 matings of each of the 8 treatments.

TABLE 8

Additive and multiplicative fecundity estimates

	ey^2	ey^2/pol	pol/ey^2	pol
Low density				
Additive model: $G = 3.63$				
Female	0.84	1.81	1.68	1.00
Male	1.27	1.26	1.25	1.00
Multiplicative model: $G = 3.89$				
Female	0.87	1.71	1.60	1.00
Male	1.24	1.23	1.22	1.00
High density				
Additive model: $G = 37.42^*$				
Female	1.00	1.33	1.45	1.00
Male	1.08	1.13	1.33	1.00
Multiplicative model: $G = 38.90^*$				
Female	1.00	1.31	1.42	1.00
Male	1.05	1.12	1.29	1.00

* For the additive case, $1-f_i$ and $1-m_i$ are reported, while f_i and m_i are reported for the multiplicative model. $\chi^2{}_{(0.05, 9\ d.f.)} = 16.92$. Note that both male and female fecundity effects are normalized to the *pol* type.

additive case, the best joint estimate gave a total G of 95.76 (25 d.f., $p < 0.001$). Since the low and high density fecundity tables gave individual G values of 3.63 and 37.42, respectively, the difference ($95.76 - 3.63 - 37.42 = 54.71$) is the heterogeneity G. With 7 degress of freedom, this is also highly significant. In the multiplicative case $G_{total} = 142.8$ and $G_{het} = 99.69$. These results suggest that even when fecundities are normalized, the relative fecundities vary with density. This is consistent with the significant female × male × density interaction observed in the variance analysis.

Sexual selection: A 2×2 contingency chi square test was performed on each pairwise mating test to assess randomness of mating. Of 50 such tests, 7 were significant at the 5% level. In no case did more than one replicate of a given test indicate significant nonrandomness. Random mating implies that our assumption of an independent bivariate binomial distribution of matings is acceptable.

Tables 9 and 10 report the relative mating success and confidence intervals for each pair of genotypes at both densities for females and males, respectively. Significant sexual selection is inferred when a confidence interval does not contain the point 1.0. In Table 9, 4 of the 10 tests indicate significant differences in female mating success, implying either that not all females mated or that not all mated females were fertile. At low density, the ey^2 females are at a disadvantage relative to other types, and the *pol* females are at a disadvantage relative to the hybrids.

Among females raised at high density, the pattern of mating success is quite different, and 3 of the 5 comparisons indicate significant differences between the

TABLE 9

Sexual selection indices of females raised at two larval densities

	ey^2/pol	pol/ey^2	pol
ey^2	0.78 ± 0.14* (459)	0.48 ± 0.09* (473)	0.85 ± 0.18 (347)
	*	*	*
	1.09 ± 0.22 (381)	0.99 ± 0.20 (374)	1.61 ± 0.38* (289)
ey^2/pol			1.23 ± 0.24 (420)
			0.94 ± 0.20 (396)
pol/ey^2			1.35 ± 0.25* (472)
			0.97 ± 0.22 (302)

* Within each cell, the upper number indicates the sexual selection index of the row head relative to the column head, and an asterisk indicates significance. The lower range is for high-density stocks. Asterisks between high and low ranges indicate significant differences between them. Numbers in parentheses are sample sizes.

two densities in sexual selection indicies. All three cases indicate an improvement in ey^2 female mating success at high density. The pattern can be summarized by saying that both inbred strains have a greater mating success relative to the hybrids when raised at higher density.

Among males (Table 10), 9 of the 10 sexual selection indices indicate significant differences in mating success. At both densities, the hybrid types have greater mating success than either inbred line, and *pol* males have lower mating success than ey^2 males. Comparisons between low- and high-density stocks indicate that the *pol* males have a consistently lower relative mating success at high density. The 3 cases of significant differences between low- and high-density mating success indicate an improvement in the ey^2 male mating success at high density. This is identical to the situation in females.

TABLE 10

Sexual selection indices of males raised at two larval densities

	ey^2/pol	pol/ey^2	pol
ey^2	0.51 ± 0.11* (459)	0.58 ± 0.11* (473)	1.84 ± 0.40* (347)
	*	*	*
	0.83 ± 0.15* (381)	0.88 ± 0.18 (374)	2.78 ± 0.73* (289)
ey^2/pol			1.95 ± 0.39* (420)
			2.47 ± 0.57* (396)
pol/ey^2			2.33 ± 0.45* (472)
			2.44 ± 0.61* (302)

* Within each cell, the upper number indicates the sexual selection index of the row head relative to the column head, and an asterisk indicates significance. The lower range is for high-density stocks. Asterisks between high and low ranges indicate significant differences between them. Numbers in parentheses are sample sizes.

Transitivity of mating success can be tested by comparing ratios of the sexual selection indices. If mating success were transitive, we would expect the relative mating success of $ey^2 : ey^2/pol$ to equal the ratio of $ey^2 : pol$ to $ey^2/pol : pol$. The significance of these comparisons was tested by t-tests, where the standard errors of the ratios were approximated by the formula in KENDALL and STUART (1958, p. 233). Table 11 indicates that there are exceptions to transitivity, and that the two densities did not result in different patterns of transitivity.

<div align="center">DISCUSSION</div>

It is well known that limitation of resources affects many aspects of the biology of organisms. These effects are seen in several different life-cycle stages, including larval survivorship, larval development rate, adult weight, fecundity and mating success. The time of crowding or resource limitation may also be important, especially in regard to mating behavior (MOTH 1974; ECKSTRAND and SEIGER 1975; BARKER and PODGER 1970b). The critical issue is to what extent these manifold effects can be summarized as a monotonic decreasing function of net fitness with increasing density for each genotype independently.

The fecundity data presented here suggest that this approximation may not be very good. Fecundity selection behaves like viability selection only in special cases (PENROSE 1949; BODMER 1965). In general, differences in fecundity behave in a way that makes net fitness appear to be frequency dependent (PROUT 1965), and the equilibrium structure of fecundity models can be quite complex (HADELER and LIBERMAN 1975). The complexity arises because, in fecundity models, fitness is considered to be a property of a mating pair, so that the model's dimensionality is increased (in particular, genotype rather than gene frequencies are required for a complete description of fecundity selection). Different ap-

<div align="center">TABLE 11</div>

<div align="center">*Transitivity of sexual selection indices and t-tests of significance*</div>

I.		$\dfrac{ey^2 : pol}{ey^2/pol : pol}$	$\overset{?}{=}$	$ey^2 : ey^2/pol$
	Female			
	Low	0.69 ± 0.07	yes	0.78 ± 0.07
	High	1.71 ± 0.19	no	1.09 ± 0.11
	Male			
	Low	0.94 ± 0.10	no	0.51 ± 0.06
	High	1.13 ± 0.14	yes	0.83 ± 0.08
II.		$\dfrac{ey^2 : pol}{pol/ey^2 : pol}$	$\overset{?}{=}$	$ey^2 : pol/ey^2$
	Female			
	Low	0.63 ± 0.06	no	0.48 ± 0.05
	High	1.66 ± 0.19	no	0.99 ± 0.10
	Male			
	Low	0.79 ± 0.08	no	0.58 ± 0.06
	High	1.14 ± 0.15	yes	0.88 ± 0.09

parent fitnesses may be seen when the proportions of adult genotypes are varied in single-generation experiments, and this has been somewhat confusingly referred to as frequency-dependent selection (DeBENEDICTIS 1977; ALVAREZ, FARINA and FONTDEVILA 1979). These results are fully consistent with simple differences in fertility. We believe that it is more precise to avoid the use of the term frequency dependence unless the parameters of the model vary with genotype frequency. In the absence of rare-male advantage or medium-conditioning effects (i.e., true frequency dependence), constant parameter fertility models more accurately describe the mechanism of frequency effects than frequency-dependent viability models.

The highly significant female × male × density interaction in the fecundity ANOVA underscores the nonindependence of genotypic fitness at different densities. In addition, significant female × density and male × density interactions show that relative fecundities of both sexes generally decrease under crowded conditions (Tables 6 and 8). Note that a significant density effect in this analysis does not by itself imply density-dependent selection; it merely signifies a net change in absolute fecundity. True density-dependent fecundity selection is indicated by the interactions, which show that relative fecundities change with density. The significant heterogeneity statistics in the additive and multiplicative models also verify that relative fecundities change with density. These changes can be broadly summarized as a reduction in the fecundity differences between genotypes under crowded conditions. These results are consistent with MUELLER and AYALA's (1981) result that differences between isogenic lines in intrinsic growth rate decrease when populations are crowded. DYKHUIZEN and HARTL (1980) also found density-dependent selection at the 6PGD locus in chemostat populations of E. Coli K12.

Another aspect of fertility selection that differs qualitatively from viability selection (O'DONALD 1980) is differential mating success. The strong sexual selection that we observed was consistent with previous work using the eyeless phenotype (PROUT 1969; BUNGAARD and CHRISTIANSEN 1972). We observed significant changes in the pattern of mating success when flies were raised at two different densities. In both sexes, the relative mating success of ey^2 increased at high density, while the mating success of pol relative to the hybrids increased in females and decreased in males. Similarly, MOTH (1974) scored the percentage of fertile females under different levels of adult crowding and observed increased levels of sterility at high densities in D. simulàns. Our data confound female sterility and female mating success in the measure of female sexual selection, but, whichever component is affected, there are marked differences between genotypes and a strong density effect. Among females, for both fecundity and sexual selection measures, there was a relative improvement in the ey^2 type at high density, indicating a positive correlation between these components. The apparent frequency dependence that DeBENEDICTIS (1977) reported (using the pol, ci sv^n system) is also consistent with differences in mating success, although he did not see any density effect. We did not test mating success at different genotypic fre-

quencies, so that we cannot rule out the possibility of rare-male advantage (true frequency dependence).

Comparisons between progeny of reciprocal crosses should reveal any significan cytoplasmic effects in the fecundity of either sex. In addition, since pol/ey^2 and ey^2/pol males carry different X chromosomes, the observed lack of significant differences in fecundity (Table 7) suggests that the X chromosomes of the two lines do not confer different male fecundities. The analysis of variance did produce a significant three-way interaction, but the meaning of this is unclear. In the case of sexual selection, pairwise t-tests indicate no significant differences in mating success between ey^2/pol and pol/ey^2 males or females. It is noteworthy that such cytoplasmic effects would complicate selection models considerably. As the data stand, comparison of fecundity and sexual selection parameters in the two hybrid types serves as a check on the internal consistency of the fertility measures.

Two critical questions remain: (1) Can the results be summarized by a single net fitness parameter for each genotype, and is this parameter density dependent? (2) Does the observed density dependence in fertility behave differently from the classical density-dependent models? The answer to the first question is no. For a given set of adult proportions, we could calculate relative fitnesses of each genotype, but due to the nature of fecundity selection, as soon as the proportions change, the relative fitnesses will also. DeBenedictis (1977) argued that proof of density-dependent viability was not sufficient to prove that density dependence of net fitness was significant. In experiments not reported here, we found (as he did) that the segregation of adult genotypes (*i.e.*, egg-to-adult relative viability) was independent of density. For a given set of adult proportions, the net fitness must therefore be density dependent. We stress, however, that the degree to which the net fitnesses appear to be density dependent depends on the genotypic distribution. It is probably best to consider the fitness components separately, rather than to use net fitnesses. Selection experiments generally show wide variation in density from one generation to another, and this may in part explain the temporal heterogeneity observed in selection components (Prout 1969; Bungaard and Christiansen 1972; Clark 1979).

Models of density-dependent fertility selection behave like the classical density-dependent selection models only in very special cases (*i.e.*, sexually symmetric and additive). As Bodmer (1965) showed in the density-independent multiplicative fertility model, the situation is analogous to viability differences between the sexes, so that even with the simple multiplicative model, there is no longer necessarily a unique internal equilibrium. In addition, fertility models may have simultaneously stable fixation states. Some consequences of population density on sexual selection were explored by Eshel (1979), but he was more concerned with sampling effects than with density-dependent fitness. It remains an interesting theoretical problem to explore the evolutionary consequences of density-dependent differences in fertility.

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