Genetica, Vol. 47,3: 203-218, 1977

INTERSPECIFIC COMPETITION BETWEEN DROSOPHILA MELANOGASTER AND DROSOPHILA SIMULANS: EFFECTS OF ADULT DENSITY ON ADULT VIABILITY

Jeffrey J. MOTH & J.S.F. BARKER

Department of Animal Husbandry, University of Sydney, Sydney, NSW 2006, Australia

Received April 4, 1976 / Accepted September 9, 1976

Survival (viability) of newly eclosed adults of *D. simulans st* and *D. melanogaster* Or R-C, which had excess quantities of dead yeast available throughout life, was measured daily for 7 days in an experiment where adult density (6 levels), species frequency (6 levels) and ^{32}P tissue content (2 levels) were varied factorially. A separate experiment comparing viability in different types of experimental unit also was done. Similar experiments were done for *D. simulans st* competing against *D. melanogaster y w*. The entire data were subjected to a least-squares (unbalanced, missing-plot) analysis of variance.

D. melanogaster Or R-C had a higher average viability than *D. melanogaster y w* or *D. simulans st*, which were equal. The competing strain of *D. melanogaster* influenced *D. simulans st* viability – viability being higher when *y w* was the competitor. Viability decreased over the seven day period but at different rates for the three strains. Increasing density reduced viability for all three strains, but species frequency effects, although significant, were generally not consistent. Females had higher viability than males in both *D. melanogaster* strains, but the reverse was true in *D. simulans st*. ^{32}P lowered viability and experimental unit type altered viability. Numerous interactions were significant.

Adult density was shown to have a delayed effect on viability – the delay before the appearance of the effect (an increase in death rate) being decreased as density rose. The term 'variably delayed density dependent' has been adopted to describe the fitness component, adult viability. Some high density populations showed a readjustment (a decrease) in their death rate as a reaction to the effect of reduced density caused by high early mortality.

Introduction

To reproduce a population must survive. Yet effects of changes in viability on the intrinsic rate of increase (absolute fitness) of a population are difficult to interpret because of the rather complex relationship between l_x, m_x and r_m, viz.

$$\Sigma e^{-r_m x} l_x m_x = 1,$$

where,

r_m = intrinsic rate of increase,

x = age,

l_x = probability of survival to age x, and

m_x = number of live female births/female aged x.

Certainly, a change in the value of l_x will alter r_m.

Meats (1971) studied this relationship in some detail. He concluded that (for discrete generations) 'changes in mortality are more important to r the higher the existing mortality rate or the lower the rate of population increase'. For overlapping generations, 'the relative importance of a given variation in ... mortality ... depends principally upon the existing rates ... of mortality and population increase'.

This paper continues the analytical studies of Barker & Podger (1970a,b) and presents a detailed analysis of the effects of adult density and species proportions on adult viability in *Drosophila* populations. As a by-product of fecundity estimation experiments (Moth & Barker, 1971; Moth & Barker, in preparation), the effect of ^{32}P on adult viability has also been determined. Further, comparisons were made of adult viability in a new, easily handled, card-

board experimental unit with that in the population bottles used by Barker (1973 and earlier) in his interspecific competition studies.

Apart from the work of Pearl, Miner & Parker (1927), little data is available on the effects of density on adult viability in *Drosophila* populations. No data could be found for effects of species proportion on survival in adult *Drosophila*.

Material and methods

The strains used were:

a) A wild-type (Or-R-C) strain of *D. melanogaster* maintained since September 1958 in a population cage.

b) A yellow, white (*y w*) mutant strain of *D. melanogaster* maintained as a stock culture in bottles since June 1969 in this laboratory, and previously as a stock culture in vials at the Department of Biology, University of Chicago.

c) A scarlet (*st*) mutant strain of *D. simulans* maintained since October 1958 in a population cage (see Barker, 1973).

Samples of eggs were taken from these strains over a short period and allowed to develop in uncrowded conditions. Resultant progeny were mass mated, and their progeny raised under uncrowded conditions. Emergences from this preliminary generation were collected, fed live yeast for 2-3 days, then used as parents at the rate of 25 pairs/bottle, in bottles containing either 30 ml of medium F (Claringbold & Barker, 1961) or 30 ml of medium F to which had been added 35 μCi ^{32}P (Moth & Barker, 1971). These parents were allowed to lay eggs for a period of 24 hours, after which time they were discarded. Eight days later these bottles were cleared of all early emerging progeny. Ten hours later all further emerging adults were collected. These were stored at predetermined densities in vials containing medium and live yeast. Each ^{32}P-strain-sex combination was kept separate. Flies so raised are optimally reared, and were used to initiate all experiments.

Six separate factorial experiments were done. Briefly, the factors and levels used were:

1) Density: 20, 40, 80, 160, 320, 640 individuals. Half of this number were males and half females.

2) Strain: *st*, Or-R-C, *y w*.

3) Experimental Unit: cardboard, glass. The cardboard unit has been fully described and figured by Moth (1974). The glass unit is as described by Barker (1960), except that the second glass medium bottle is replaced by an estafoam stopper. The approximate volume of this unit is 135 cc.

4) Frequency: 20, 40, 50, 60, 80, 100 per cent of a strain.

5) ^{32}P: +, – depending on whether the strain was raised on medium containing ^{32}P or not.

Each factor level was not included in every experiment. Table 1 summarises the factor levels used in

Table 1

Factor levels used in each of the six experiments, together with the number of times that each experiment was replicated

Factor	Levels used in experiment number					
	1	2	3	4	5	6
Density	20,40,80, 160,320, 640	20,40,80, 160,320, 640	20,40,80, 160,320, 640	20,40,80, 160,320, 640	20,40,80, 160,320, 640	20,40,80, 160,320, 640
Strain	*st*, Or-R-C	*st*, *y w*	*st*, Or-R-C	*st*, Or-R-C	*st*, *y w*	*st*, *y w*
Expt. unit	C, G	C, G	C	C	C	C
Frequency	100	100	20,40,60, 80,100	50,100	20,40,60, 80,100	50,100
^{32}P	–	–	+, –	+, –	+, –	+, –
Replicates	4	4	3	2	2	2

each experiment, together with the number of times that each experiment was replicated.

Each experiment was initiated when the collected flies had recovered from the effects of etherisation (i.e. at least one hour after etherisation). For each population the appropriate numbers and combinations of flies were selected from the various collection/storage groups, then tipped without re-etherisation into the required experimental unit with the aid of a small funnel. Populations were set up in random order. Each was given an excess quantity of a 1:2 dead yeast/water paste, coated on an agar-filled teaspoon. Such food was renewed every 12 hours, and in the same random order as at initiation. At every second food change (i.e. every 24 hours), all dead adults were removed from each population, to be later classified as to sex and strain. All populations were terminated in initiation random order at the end of the seventh 24-hour period. At termination, all live flies were counted as a check on the original density. The few populations in which numbers for individual strain-sex classes deviated by more than 5% from that expected were discarded from further analysis.

Stock cages, preliminary generations, and all experimental populations were kept at 25 ± 0.5°C and 65-70% relative humidity in a room lit for 12 hours a day (6 am to 6 pm).

From the raw data (i.e. number dead at each age), life-tables were constructed for each strain-sex class within each population according to the procedures outlined by Southwood (1966). Calculated l_x values (i.e. proportion alive at age x, or probability of surviving to age x) for each of the seven ages (days 1-7) from all life-tables and experiments were pooled to form data for a factorial of design:

6 (Density: 20, 40, 80, 160, 320, 640 individuals)
x6 (Frequency: 20, 40, 50, 60, 80, 100 per cent)
x3 (Strain: *st*, Or-R-C, *y w*)
x2 (Experimental Unit: cardboard, glass)
x2 (^{32}P: +, −)
x2 (Sex: male, female)
x7 (Age: 1, 2, 3, 4, 5, 6, 7 days).

Class 1 of the strain factor was partitioned into four subclasses to detect effects of competing *D. melanogaster* strain on *D. simulans st* viability. The four subclasses were:

a) *st* v. Or-R-C in Experiment 1
b) *st* v. *y w* in Experiment 2
c) *st* v. Or-R-C in Experiments 3 and 4
d) *st* v. *y w* in Experiments 5 and 6.

The above design is highly unbalanced, having many missing plots and differing amounts of replication for each cell, so a least-squares analysis of variance on the untransformed l_x values was done by computer according to the procedures outlined by Harvey (1968). Untransformed data was used, even though treatment variances were heterogeneous, because none of the transformations attempted removed that heterogeneity.

Table 2

Least-squares analysis of variance of the proportion of adults surviving (l_x)

Source	df	Mean square
Replicate	4	1.209***
Strain (S)	2	5.475***
Simulans Strain (ST)	3	1.916***
Sex (SX)	1	1.418***
Density (D)	5	3.978***
Frequency (F)	5	0.213***
Age (A)	6	7.058***
Expt. Unit (U)	1	4.206***
Phosphorus-32 (P)	1	0.115**
S x SX	2	5.471***
S x D	10	0.768***
S x F	10	0.111***
S x A	12	2.592***
S x U	2	0.141***
S x P	2	0.023
ST x SX	3	0.323***
ST x D	15	0.191***
ST x F	5	1.276***
ST x A	18	0.139***
ST x P	1	0.058
SX x D	5	0.415***
SX x F	5	0.071***
SX x A	6	0.543***
SX x U	1	0.080*
SX x P	1	0.032
D x F	25	0.080***
D x A	30	1.020***
D x U	5	0.622***
D x P	5	0.029
F x A	30	0.043***
F x P	5	0.259***
A x U	6	0.395***
A x P	6	0.003
Error	13075	0.016

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
Note: The interactions ST x U, F x U, and U x P were unobtainable.

Strictly, to include the age factor in such an analysis is invalid, as l_x values at any age will depend on the preceding values. However, this was considered to be a minor problem as compared with the problems associated with the comparison of seven separate analyses. In any case, further analyses were done to elucidate the effects of time on viability and the methods of these are presented in a later section.

In all statistical tests the error mean square from the pooled analysis of variance was used as the estimate of S^2 – the average sample variance.

Frequent use was made of regression analysis to describe trends in adult viability with increasing density, frequency or age. For any particular set of data, linear, quadratic and cubic equations were fitted, but unless the fitting of quadratic or cubic equations significantly improved the regression sum of squares, only the linear equations are discussed.

Results

Effects of imposed treatments

The least-squares analysis of variance for the pooled factorial is presented in Table 2. Sixty-four per cent of the total observed variation in l_x was accounted for by the factors and interactions that were included in the analysis. Variation between replicates was highly significant. All main effects, as well as the nested effect (*simulans* strain), were highly significant. Except for the SX x U interaction, and most of those that involved ^{32}P, all interactions were highly significant.

Table 3a (Column 4) gives the least-squares mean viability for each strain, and for *D. simulans st* when it competes against each *D. melanogaster* strain. The effect of competing *D. melanogaster* strain on *st* viability is presented only for the latter two subclasses of strain class 1 (see Methods), because the former two subclasses had an experimental unit effect confounded in them. These former subclasses will, because of that confounding, not be included in any further analyses or discussion. Pairwise comparisons indicated that Or-R-C viability (0.949) was significantly higher ($P < 0.001$) than that of *st* (0.803) or *y w* (0.806), but these latter were not significantly different ($P > 0.2$). When *st* competed against Or-R-C, its viability was only 0.789 as compared with 0.841 for competition against *y w* (significantly different – $P < 0.001$).

Mean survival of males (0.828) was significantly lower ($P < 0.001$) than that of females (0.871).

Within a strain, male and female viability were always significantly different (Tab. 3a). For both *D. melanogaster* strains, females had higher viability; the reverse was true for *D. simulans st*. However, viability in *D. simulans st*, as determined by sex, also depended on the competing strain of *D. melanogaster*. When Or-R-C was the competitor, male *st* had higher viability. When *y w* was the competitor, the sexes were not significantly different, although female *st* had a slightly higher viability.

Strain viability comparisons within sexes (Tab. 3b), show that each strain had a characteristic

Table 3(a)

Male, female, and average adult viability for each strain, and for *D. simulans st* when competing against each *D. melanogaster* strain, together with *t* values for between sex comparisons

Strain	Viability			Between sex
	Male	Female	Average	*t*
D. simulans st	0.813	0.792	0.803	7.000***
D. melanogaster Or-R-C	0.933	0.947	0.940	3.500***
D. melanogaster y w	0.737	0.875	0.806	27.600***
D. simulans st[1]	0.800	0.779	0.789	4.200***
D. simulans st[2]	0.836	0.845	0.841	1.800

Table 3(b)

t values for between strain and between *simulans* strain comparisons of the within sex adult viability

Comparison	Within sex *t*	
	Male	Female
st v. Or-R-C	30.000***	38.750***
st v. *y w*	19.000***	20.750***
Or-R-C v. *y w*	49.000***	18.000***
st[1] v. *st*[2]	7.200***	13.200***

[1] Competing against *D. melanogaster* Or-R-C in experiments 3 and 4.

[2] Competing against *D. melanogaster y w* in experiments and 6.

*** $P < 0.001$.

viability in each sex, which was significantly different from that of the same sex in all other strains. For male flies, the order of decreasing viability was Or-R-C, *st* then *y w*, but in females the order was Or-R-C, *y w* then *st*. The effect of the competing strain of *D. melanogaster* on *D. simulans st* viability was consistent over sexes, i.e. both sexes of *st* had significantly higher viability when *y w* was the competitor, as compared with when Or-R-C was the competitor (Tab. 3b).

As density increased, viability decreased; each density producing a viability that was significantly different ($P < 0.05$) from that produced by any other density, when tested by Tukey's *w*-procedure (Steel & Torrie, 1960; p. 109).

Adult density affected the viability of each strain to a different degree (Fig. 1). For all three strains, viability fell as density rose. The linear equations for density determination of viability are:

st: $Y = 0.9167 - 0.0005X$
Or-R-C: $Y = 0.9941 - 0.0003X$
y w: $Y = 0.8729 - 0.0003X$

All three equations have slopes that are significantly different from zero ($P < 0.01$). The Or-R-C and *y w* equation slopes are not significantly different ($P > 0.10$), but the *st* equation slope is significantly greater ($P < 0.01$) than either the Or-R-C or *y w* equation slopes. That is, the Or-R-C and *y w* strains of *D. melanogaster* were affected to the same extent by a given increase in density, but *D. simulans st* was more severely affected. The competing strain of *D. melanogaster* did not alter the relationship between density and viability for *D. simulans st*, as *st* competing against Or-R-C and *st* competing against *y w* had equations of similar slope ($b = -0.0006$ and -0.0004 respectively), and neither slope was different ($P > 0.02$) from the *st* strain equation slope (above).

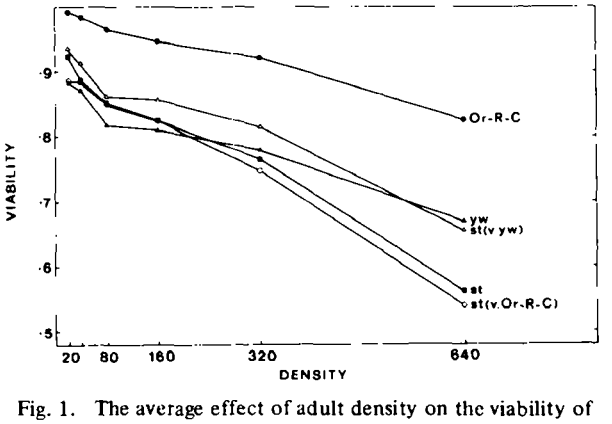

Fig. 1. The average effect of adult density on the viability of adults of each strain, and on the viability of *D. simulans st* adults competing against each *D. melanogaster* strain.

Viability at each frequency is given in Table 4. Differences in viability between frequency levels were detected by means of Tukey's *w*-procedure. At the frequencies of 50 and 100 per cent, viability was not significantly different ($P > 0.05$), but was significantly lower ($P < 0.05$) than at frequencies of 20, 40, 60 and 80 per cent. Viability at these four latter frequencies was not significantly different ($P > 0.05$). Confounding, due to the combined analysis of two different types of experiment (with respect to frequency levels), probably accounts for the observed significant differences. Indeed, viability in experiments 3 and 5(frequency levels 20, 40, 60, 80, 100%) was 0.892, but that in experiments 4 and 6 (frequency levels 50, 100%) was only 0.872. These are significantly different ($P < 0.01$) and indicate that overall viability was lower in the second design of experiment.

Because viability differed between types of experiment, no realistic effect of frequency on viability can be ascertained when the entire range of frequencies is used. However, experiments 3 and 5 examined a wide range of frequencies, and provided useful information in themselves. Thus, Figure 2 shows the mean viability of each strain (and of *D. simulans st* when it competes against each *D. melanogaster* strain) at each of the frequency levels used in these experiments. The linear equations for prediction of viability according to frequency level are, for each strain:

st: $Y = 0.8494 - 0.0001X$
Or-R-C: $Y = 0.9687 + 0.0001X$
y w: $Y = 0.8622 + 0.0003X$

Table 4

Average adult viability at each frequency*

Frequency (%)	40	60	80	20	100	50
Viability	0.864	0.855	0.854	0.853	0.838	0.833

* Averages that are not significantly different ($P > 0.05$) are underlined.

The slope of each regression is not significantly different from zero ($P > 0.05$), and the slopes are not different from each other ($P > 0.1$), indicating no effect of frequency and no difference between strains. The *simulans* strain equation slopes (*st* v. Or-R-C: 0.0003; *st* v. *y w*: -0.0007) are significantly different from each other ($P < 0.02$), and *st* v. *y w* is significantly different from zero ($P < 0.02$). For *st* viability, the effect of an Or-R-C adult is greater (but not significantly so) than that of an *st* adult, which is in turn greater (significantly) than that of a *y w* adult.

Viability decreased with increasing age; viability at any day of age being significantly different from that at any other day of age (Tukey's *w*-procedure; $P < 0.05$).

Viability of adults at each day of age is shown in Figure 3 for all three strains, together with viabilities for *D. simulans st* competing against each *D. melanogaster* strain. All strains showed reductions in viability with increases in age. The linear equations to predict viability, given the age (in days) of a population, are, for the three strains:

st: $Y = 1.0954 - 0.0732X$
Or-R-C: $Y = 1.0274 - 0.0218X$
y w: $Y = 1.1319 - 0.0815X$

All three equations have slopes that are significantly different from zero ($P < 0.01$). The slopes of the *st* and *y w* equations are not significantly different ($P > 0.3$), but are both significantly greater than the slope of the Or-R-C equation ($P < 0.001$). This would indicate that the *st* and *y w* populations were dying at a similar rate, but that that rate was much higher than that operating in the Or-R-C populations. However, because the *y w* data are best described by a quadratic equation ($Y = 1.0274 - 0.0119X - 0.0087X^2$), and the *st* data by the above linear equation, it is not true that adults in both populations were dying at similar rates. In fact, the *y w* populations had a lower rate over the first few days of life, followed by a higher rate to day 7, resulting in a similar viability (to *st*) at day 7. The competing strain of *D. melanogaster* did not alter the general relationship between age (in days) and viability of *D. simulans st* adults (above), because both *simulans* strains (i.e. *st* v. Or-R-C and *st* v. *y w*) had equations of similar slope ($P > 0.2$), neither of which was different ($P > 0.3$) from the *st* strain equation slope.

The type of experimental unit greatly influenced adult viability. In the glass unit, adult viability was only 0.812, significantly less ($P < 0.001$) than 0.887 in the cardboard unit.

Viability was significantly lower in the glass experimental unit for all strains (Tab. 5a). In each type of experimental unit, each strain had a mean viability that was significantly different from that of the other two strains (Tab. 5b). In the cardboard experimental unit, Or-R-C had highest viability, followed by *y w*, then *st*. However, in the glass experimental unit, the

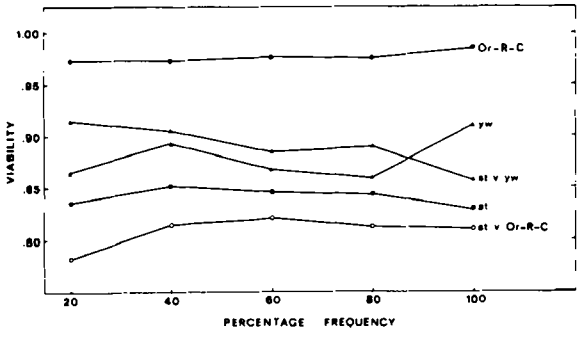

Fig. 2. The average effect (over experiments 3 and 5 only) of adult frequency on the viability of adults of each strain, and on the viability of *D. simulans st* adults competing against each *D. melanogaster* strain.

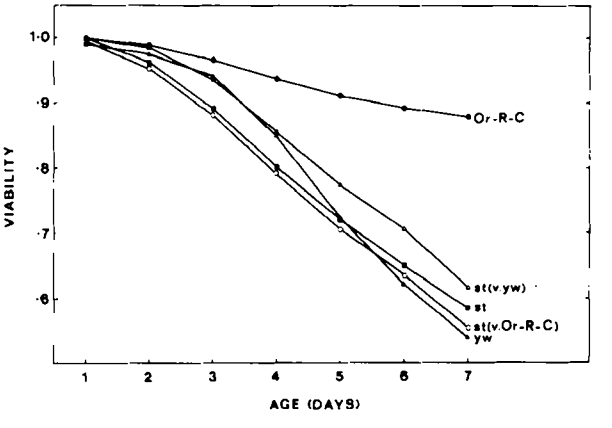

Fig. 3. The average effect of age on the viability of adults of each strain, and on the viability of *D. simulans st* adults competing against each *D. melanogaster* strain.

order of mean viabilities was changed; it being Or-R-C, *st* then *y w*. The *simulans* strain (i.e. *D. simulans st* competing against diffefent *D. melanogaster* strains) x experimental unit interaction was unobtainable due to missing plots in the combined analysis.

Flies that had been raised on medium containing ^{32}P had an average viability of 0.846, which was significantly lower ($P < 0.01$) than the average viability of 0.853 for flies raised on an identical medium, except that ^{32}P was absent.

The average viability of adults of each sex is plotted in Figure 4 for all density levels. Male viability was lower than female viability at all densities. Increasing the density of adults from 20 to 640 individuals reduced average viability by 31.25 per cent for males, but by only 21.96 per cent for females. The following linear equations predict the viability of each sex, as determined by density,

male: $Y = 0.9177 - 0.0004X$
female: $Y = 0.9381 - 0.0003X$

Both equations have slopes that are significantly different from zero ($P < 0.001$), but that are not different from each other ($P > 0.02$). The lesser slope of the female equation accounts for the lower reduction in viability with an increase in density, and also for the significant sex x density interaction of Table 2.

Both sex regressions of adult viability as determined by frequency had slopes that were not different from zero ($P > 0.1$), or from each other ($P > 0.1$). The female sex showed higher viability at all frequency levels.

The average effect of age on viability of adults of each sex is shown in Figure 5. Regardless of age, females again showed higher viability. For both sexes, the cubic equation significantly improved the regression sum of squares from that obtained by fitting either quadratic or linear equations. Notwithstanding this improvement, the linear equations, being easier to interpret, were used as they accounted for a considerable portion (97.4 and 99.0% respectively for the male and female sexes) of the observed variation.

Table 5(a)

Average adult viability of each strain in each type of experimental unit, together with *t* values for between experimental unit comparisons

Strain	Experimental unit		Between experimental unit *t*
	Cardboard	Glass	
D. simulans st	0.830	0.775	11.000***
D. melanogaster Or-R-C	0.975	0.905	10.000***
D. melanogaster y w	0.857	0.755	14.571***

Table 5(b)

t values for between strain comparisons of the within experimental unit adult viability

Comparison	Within experimental unit *t*	
	Cardboard	Glass
st v. Or-R-C	48.333***	14.444***
st v. *y w*	9.000***	2.778**
Or-R-C v. *y w*	39.333***	15.000***

** $P < 0.01$; *** $P < 0.001$.

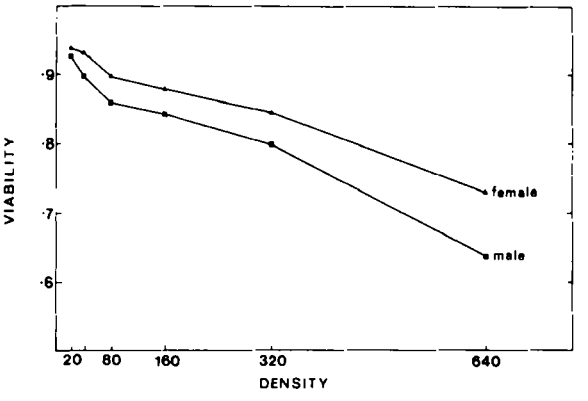

Fig. 4. The average effect of adult density on the viability of adults of each sex.

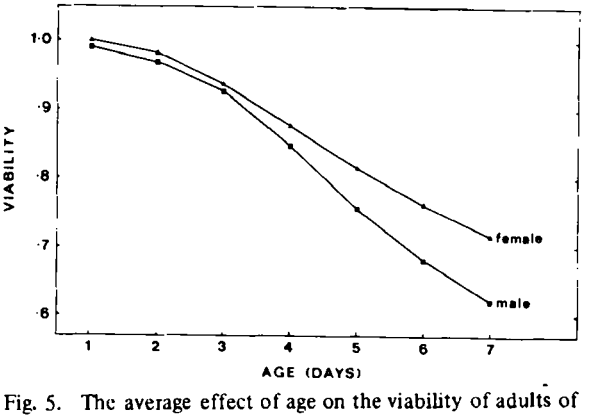

Fig. 5. The average effect of age on the viability of adults of each sex.

These linear equations, predicting viability of each sex, given age in days, are:

male: $Y = 1.0923 - 0.0661X$
female: $Y = 1.0774 - 0.0516X$

The slope of each equation is significantly different from zero ($P < 0.001$). Because both equations have similar slopes ($P > 0.02$), the significant sex x age interaction (Tab. 2) was not anticipated. The significance of this interaction must be a result of differences in cubic equation coefficients.

Table 6 gives the mean viability of each sex in experimental units of each type. *t* tests to detect differences between sexes within experimental units and between experimental units within sexes indicated that females had significantly higher viability than males in both types of unit, and that for both sexes tha cardboard experimental unit allowed significantly higher viability than the glass unit.

Table 6

Average viability of adults of each sex in each type of experimental unit, together with *t* values for between sex within unit and between unit within sex comparisons

Experimental Unit	Sex		Between sex
	Male	Female	
Cardboard	0.870	0.904	4.857***
Glass	0.785	0.838	7.571***
Between experimental unit *t*	17.000***	13.200***	

***P < 0.001

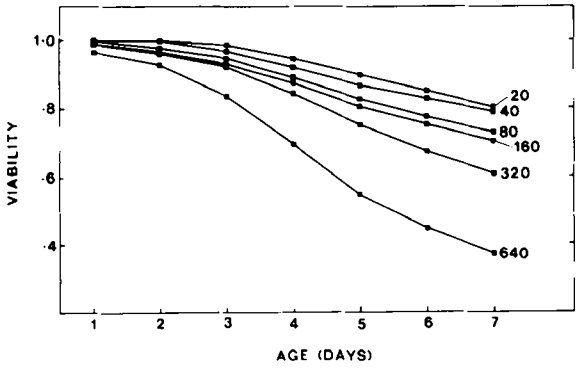

Fig. 6. The average effect of age on the viability of adults living at different densities.

Although the density x frequency interaction was highly significant (Tab. 2), at no density did viability show significant trends with frequency, nor did any linear regression coefficient differ from any other when tested by the *t* test.

For each of the six population densities, Figure 6 shows adult viability at each day of age. Viability fell as populations aged -- the linear regressions being significantly different from zero for all densities ($P < 0.001$). The regression coefficient of each density equation was compared, by means of the *t* test, with the regression coefficients of all other density equations (Tab. 7). Increasing initial population density resulted in a concomitant increase in the rate of dying (i.e. as initial population density increased, regression coefficients became more negative).

Increasing adult density reduced viability differently in each type of experimental unit (Fig. 7). Linear regression equations predicting viability, given density, are, for each type of unit:

Table 7

t values for between density comparisons of the linear regression coefficients estimated from the curves in Figure 6

Density	Density				
	20	40	80	160	320
40	0.386				
80	2.174	2.065			
160	2.671*	2.617*	0.492		
320	5.160***	5.218***	3.439**	3.047*	
640	9.702***	9.850***	8.319***	8.004***	5.045***

* P < 0.05; ** P < 0.01; *** P < 0.001.

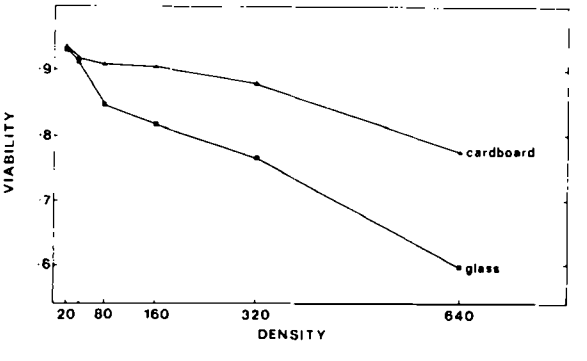

Fig. 7. The average effect of adult density on the viability of adults in different types of experimental unit.

cardboard: $Y = 0.9377 - 0.0002X$
glass: $Y = 0.9182 - 0.0005X$

Each has a slope that is significantly different from zero ($P < 0.001$), and the slopes are different from each other ($P < 0.001$). The effect of density was more severe in the glass experimented unit.

Each age regression of adult viability as determined by frequency had a slope that was not different from zero ($P > 0.2$). No slope was different from any other when tested by the *t* test, although there was a trend for slopes to become increasingly negative with age.

Slopes of fitted linear regressions (estimating viability in each ^{32}P treatment, as determined by frequency) were not different from zero ($P > 0.02$), nor were they different from each other ($P > 0.3$). These tests indicate that there were no consistent effects of the phosphorus-32 treatment at any frequency level, even though the F x P interaction was highly significant (Tab. 2).

Figure 8 shows viability at each of the seven ages for adults living in the two types of experimental unit. Linear regression equations, predicting viability as dependent on age, are for each unit type:

cardboard: $Y = 1.0710 - 0.0460X$
glass: $Y = 1.0989 - 0.0718X$

Both have slopes that are different from zero ($P < 0.001$), and that are different from each other ($P < 0.001$). Difference in slope accounts for the significant age x experimental unit interaction in Table 2. Difference in slope also means that adults living in the glass unit were dying at a faster rate than those living in the cardboard unit.

Specific action of adult density

In an attempt to determine how, and when, the effects of density on adult viability were asserted, further analyses were done after determining and removing the effect of adult age on viability.

First, for each of the three strains, viability at the 100 per cent frequency level was plotted by adult age (measured in days) for each density (Fig. 9). Then, with the intention of pooling within strain viability curves for densities that were not significantly different, the Kholmogorov-Smirnov test for differences in viability (described and used by H. Levene in a paper

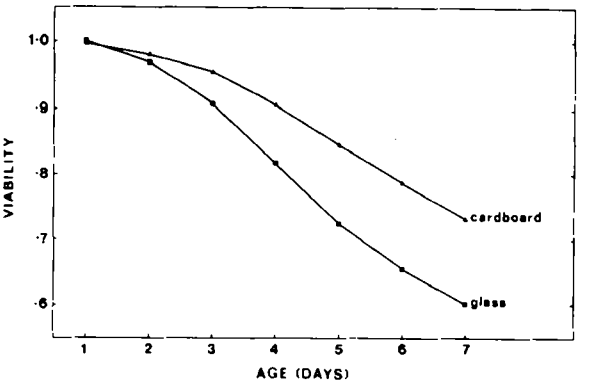

Fig. 8. The average effect of age on the viability of adults in different types of experimental unit.

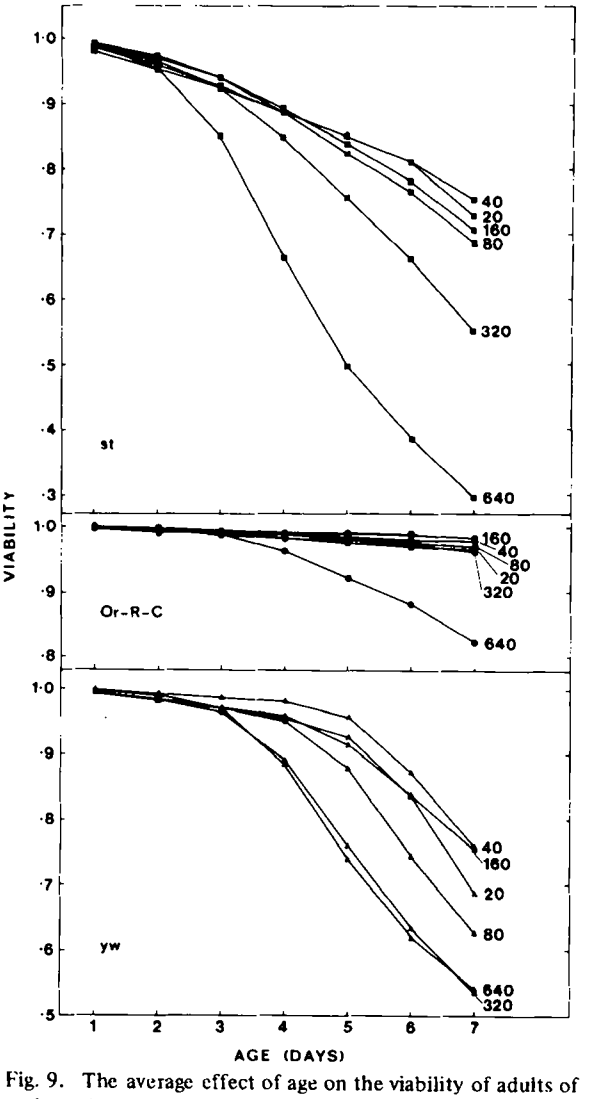

Fig. 9. The average effect of age on the viability of adults of each strain in single-strain populations of different adult density.

by Vetukhiv, 1957) was applied to this data. Indications were that for the *st* strain, three density groups were needed (20-160, 320, and 640), while for the Or-R-C and *y w* strains only two were required (Or-R-C: 20-320, and 640; *y w*: 20-160, and 320, 640). Pooled viability curves which show the proportion of the day 0 (initiation) adults that were alive on any day thereafter are plotted in Figure 10.

Figure 11 shows the same curves as plotted in Figure 10 but replotted after making the proportion of adults alive at the end of any day a proportion of the proportion of adults alive at the beginning of the same day. This removed one effect of adult age by making the proportions surviving independent of previous deaths.

Table 8a was constructed from the data of Figure 11 by subtracting the proportion surviving on a particular day from the proportion surviving on the previous day, then multiplying by 100 for standardisation. This gave, for any day of age, the standardised increase in death rate (Δq_x) over and above the rate for the previous day. (q_x is defined as the number of individuals dying in a particular age interval divided by the number alive at the start of the age interval). As an example of the use of Table 8a, take the 320 *st* stock. This had an initial death rate (q_x) of 1.0 per cent on day 1, and on day 2, Δq_x (the increase in death rate) was 1.3 per cent, therefore the operating death rate on day 2 was 2.3 per cent.

The date of Table 8a are difficult to interpret as such, presumably because of random variations in Δq_x. To overcome this difficulty each row was partitioned, independently of other rows, into sections which contained Δq_x values of approximately equal magnitude. The Δq_x values within a section were then averaged and the resultant smoothed data are presented in Table 8b. Viability curves produced from the data of Table 8b are not significantly different ($P > 0.01$, Kholmogorov-Smirnov test) from the actual viability curves plotted in Figure 10, so the Δq_x values therein are realistic.

The interpretation of the data in Table 8b is very interesting. For the Or-R-C strain, the densities of 20-320 produced a Δq_x of 0.1 per cent for every day of age, i.e. the increase in q_x was linear and consistent. This can be taken as the effect of time on survival (viability) if we assume that density had no effect. However, at the density of 640, Or-R-C showed a similar Δq_x (0.2%) on days 1 to 3, but then Δq_x jumped suddenly to 1.5 per cent on day 4, and retained that value for each day thereafter. On comparison with the lower densities this is interpreted to mean that the effect of density on survival did not appear till day 4, i.e. it was delayed. With the

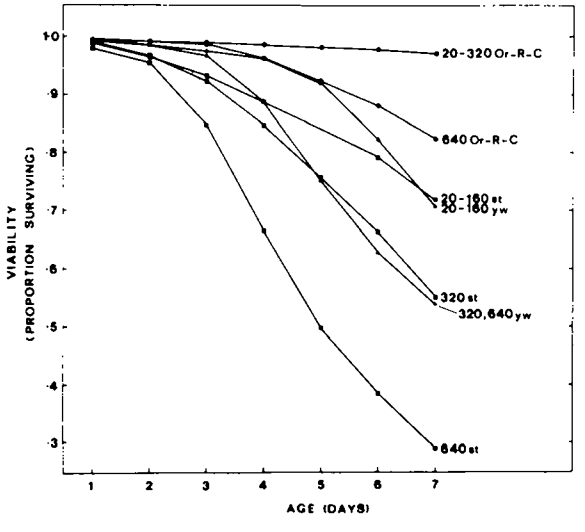

Fig. 10. The average effect of age on the viability of adults of each strain in single-strain populations of different adult density. Within strain densities that were not significantly different have been pooled.

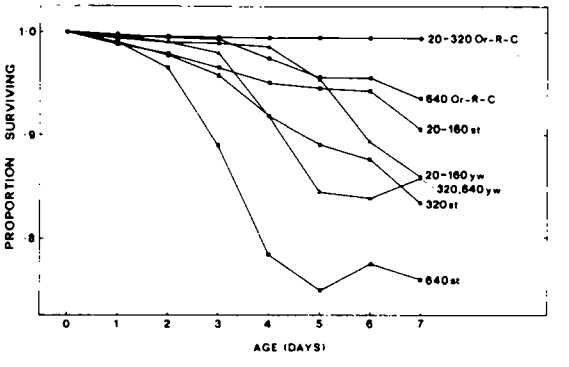

Fig. 11. The average proportion of adults alive at the beginning of a specified age interval that survive to the end of the age interval. Populations of different strain and adult density combination are plotted separately except for within strain densities that were not significantly different, which have been pooled.

Table 8(a)

Standardised increase in death rate for each day of age for adults of the indicated strain-density combination

Strain-density combination	Day of age						
	1	2	3	4	5	6	7
20-320 Or-R-C	.3	.0	.0	.1	.0	.1	.1
640 Or-R-C	.2	.2	.2	1.9	1.8	.1	2.1
20-160 *st*	1.2	1.0	1.1	1.5	.6	.3	3.8
320 *st*	1.0	1.3	1.9	4.1	2.5	1.6	4.3
640 *st*	1.0	2.4	7.7	10.5	3.5	−2.5*	1.5
20-160 *y w*	0.6	.2	.2	.3	3.2	6.2	3.3
320, 640 *y w*	.4	.5	.9	6.5	7.2	.7	−1.9*

Table 8(b)

Smoothed standardised increase in death rate for each day of age for adults of the indicated strain-density combination

Strain-density combination	Day of age						
	1	2	3	4	5	6	7
20-320 Or-R-C	.1	.1	.1	.1	.1	.1	.1
640 Or-R-C	.2	.2	.2	1.5	1.5	1.5	1.5
20-160 *st*	1.1	1.1	1.1	1.6	1.6	1.6	1.6
320 *st*	1.2	1.2	2.9	2.9	2.9	2.9	2.9
640 *st*	1.0	6.0	6.0	6.0	6.0	−0.5*	−0.5*
20-160 *y w*	.3	.3	.3	.3	4.2	4.2	4.2
320, 640 *y w*	.6	.6	.6	6.9	6.9	−0.6*	−0.6*

* Negative values indicate that survival was higher than for the previous day.

st strain, the lowest densities (20-160) did not show consistent and linear increases in q_x with time, Δq_x being 1.1 per cent on days 1 to 3 then 1.6 per cent each day thereafter. This could be either the pattern of survival of *st* with time when density had no effect, or it might be that even at the density of 20, *st* was showing a density effect (i.e. delayed till day 4, as in Or-R-C). In the latter case the 'normal' Δq_x would be approximately 1.0 per cent at some density below 20. At a density of 320, *st* had the same Δq_x (1.2%) for days 1 and 2 as at the lower densities, but then Δq_x jumped to 2.9 per cent at day 3, and remained at that level thereafter. Thus, the effect of density was first evident at day 3 (i.e. it was delayed as in Or-R-C). At density 640, *st* had a Δq_x of 1.0 per cent at day 1, 6.0 per cent on days 2-5, then -0.5 per cent on days 6 and 7. Again, the effect of density was delayed and first appeared on day 2. A new effect was also evident on days 6 and 7, with the appearance of a negative Δq_x. This is interpreted to mean that the population was readjusting its Δq_x for the decrease in density that had occurred. The low densities of *y w* (20-160) had a constant Δq_x (0.3%) for days 1 to 4, which then rose to a constant 4.2 per cent on day 5 and thereafter. Failure of the low *y w* densities to give linear and consistent Δq_x, as in 20-320 Or-R-C, could have been due to either of the two reasons given earlier for 20-160 *st* failure. At higher *y w* densities (320, 640), Δq_x was approximately the same on days 1-3 (0.6%) as that in the low densities, but at day 4-5 Δq_x rose to 6.9 per cent, when the delayed effect of density appeared, then fell to -0.6 per cent on days 6 and 7 as the population readjusted to decreased density. Thus, two effects of density should be noted. These are (a) the period of delay before the effect of density was registered decreased as density rose, and (b) the magnitude of Δq_x at, and after, the registration of the density effect increased as density increased.

Discussion

Significant replicate variability was not unexpected. Barker & Podger (1970a) had previously observed variation between replicates when measuring the fitness components larval viability, developmental time and adult body weight. Biological systems are intrinsically complex, and in that and the present study, unmeasurable changes in any one of many 'constant' factors could easily have resulted in the random, but significant, small differences between replicates.

Adult viability (survival) differences between species have been recorded by Barker & Podger (1970b) and Tantawy & El-Wakil (1970) for *Drosophila*, and by Lloyd & Park (1962), Mertz, Park & Youden (1965), and Park, Mertz & Petrusewicz (1961) for *Tribolium*. Differences within species were reported by Heidenthal, Nelson & Clark (1972) for *Habrobracon*; by Crovello & Hacker (1972) for *Aedes aegypti*; by Mertz, Park & Youden (1965) and Park, Mertz & Petrusewicz (1961) for *Tribolium*; and by Birch, Dobzhansky, Elliot & Lewontin (1963), Buzzati-Traverso (1955), Clark & Gould (1970), Maynard Smith (1958a) and Tantawy (1961) for *Drosophila*. However, Tantawy & El-Helw (1970) failed to show strain

differences in *D. melanogaster*. In the present study, *D. melanogaster* Or-R-C and *D. simulans st* were shown to have different viabilities to day 7 of adult life. Also, *D. melanogaster* Or-R-C was different from *D. melanogaster y w*. Such differences are expected a priori, as each species (strain) would have its own particular genetic make-up, and thus ecological strategy, which enables it to exploit its environment to the fullest extent.

The competing strain of *D. melanogaster* altered *D. simulans st* viability markedly (Tab. 3a); viability with *D. melanogaster y w* being much higher than that with *D. melanogaster* Or-R-C. Polnik (1960) has suggested that attempts by *T. confusum* males to mate with *Latheticus oryzae* females might result in fatal damage. Lloyd & Park (1962) have shown that the longevity of *T. castaneum* females is considerably shortened by *T. confusum* males because the aedeagus of the male causes physical damage to *T. castaneum* females. Shorey & Bartell (1970) showed that wild-type *D. melanogaster* could not detect sex or species differences until the touching and/or orientation phase of courtship. It is therefore possible that the increased death rate in *D. simulans st* when it competes with Or-R-C is associated with interspecific courtship, and if such is the case, is most probably due to some type of physical damage caused by Or-R-C. Inspection of Table 3a partially confirms a damage theory for *D. simulans*, because *D. simulans st* females have a lower viability than males when with Or-R-C, yet all other stocks (i.e. Or-R-C, *y w*, and *st* when with *y w*) have higher viability in the female sex.

Although the two *D. melanogaster* strains are not isogenic, and therefore no specific effects of the *y* or *w* genes can be ascertained, it is possible to get some indication of the involvement of the X-chromosome in viability determination. In Or-R-C, male viability is 98.5 per cent that of the female, while in *y w* it is only 84.2 per cent. Expressed differently, the *y w* female has a viability equal to 92.4 per cent that of the Or-R-C female, while the *y w* male's viability is only 79.0 per cent that of the Or-R-C male. This indicates that the *y w* male fares relatively poorer, or the *y w* female relatively better than the equivalent sex in Or-R-C. Such differences must be due to genes on the X-chromosome (not necessarily *y* or *w*), unless one of the strains contains sex-limited viability genes.

Increasing density significantly reduced adult viability. This was true for all strains, but *D. simulans st* was much more severely affected than was *D. melanogaster* Or-R-C or *D. melanogaster y w* (Fig. 1). Reductions in viability with increasing density were observed by Hodjat (1969) in the locust *Dysdercus*; by Davis (1945) for male *Trogoderma*; and by Yoshida (1966) for male *Callosobruchus chinensis*. However, reduced viability with increasing density is by no means a universal result, as is evidenced by the data of Frank (1952) for *Daphnia* and *Simocephalus*; of Frank, Boll & Kelly (1957) for *Daphnia pulex*; of Pearl, Miner & Parker (1927) for *D. melanogaster*; and of Tawfik (1969) for the bedbug *Cimex*, all of whom found that longevity increased up to a certain density, thereafter falling rapidly as density increased further. In the study being reported here there were no effects of undercrowding on viability, even at the lowest densities. The alternate possibilities of no change or increased viability with increasing density also have been observed. Mertz (1969) working with *T. castaneum* and Yoshida (1966) using *C. chinensis* females found increased viability with increasing density. Ebeling & Reierson (1970) working with the roach *Blattella germanica*, Davis (1945) using *Trogoderma* males and Yoshida (1966) using *C. maculatus* all found no effect of increased density.

Little information has been published regarding the effects of interspecific association on adult viability. In *Tribolium* Lloyd & Park (1962) found that *T. confusum* lowered the lifespan of both male and female *T. castaneum*. Yoshida (1966) states that *C. maculatus* reduces the lifespan of *C. chinensis* females by 8 per cent at intermediate and high density, and that *C. chinensis* reduces the lifespan of *C. maculatus* females at all densities. Neither of these authors varied the species proportions and no data appear to be available for *Drosophila*. In the present study, no consistent effect of a systematic change in strain (species) frequency was observed, although frequency effects were significant (Tab. 2). Similarly, the interactions of frequency with strain, sex, density, age and ^{32}P were all significant, but no consistent trends were detectable. There was, however, a significant decrease in viability of *st* adults in the *st/y w* competing populations when the frequency of *st* was increased. It is concluded that strain frequency has no effect per se on adult viability in Or-R-C or *y w* populations under the conditions tested, although hitherto unknown factors may cause significant deviations to be ob-

served between different frequency levels. In *st* populations frequency effects may be observed when the competitor strain is of a radically different fitness. Predicting adult viability in interspecific populations may prove unreliable.

References to sex differences in adult viability are widespread throughout the literature. Park, Mertz & Petrusewicz (1961) found that *T. castaneum* females had a higher median longevity than did males, and that for *T. confusum* both sexes had equal median longevity. Lloyd & Park (1962) found that the female sex had longest lifespan in both *T. castaneum* and *T. confusum* but Mertz, Park & Youden (1965) found that either sex could have longest lifespan and that this was dependent on the strain of *T. castaneum* or *T. confusum*. Miller & Thomas (1958) showed that males of *D. melanogaster* had longer lifespans than females, but that the male had a higher death rate in the first 15-30 days of adult life. Maynard Smith (1958a) concluded that sex-limited genes were partly responsible for differences in viability between sexes in *D. subobscura* and Hollingsworth (1966, 1969) showed for *D. subobscura* and *D. melanogaster* respectively, that progeny from reciprocal crosses of inbred lines differed in viability, indicating X-chromosome involvement. In the present study, *D. melanogaster* Or-R-C and *D. melanogaster y w* both had higher viability in the female sex, but *D. simulans st* had higher viability in the male sex. Viability in *D. simulans st* was, as already noted, influenced by the competing *D. melanogaster* strain. Against *y w* both sexes of *st* had equal viability, although the female sex was slightly higher. Disregarding *st* competing against Or-R-C, which appears to be a special case, all strains had higher death rates in the male sex in the first seven days of life – a similar result to that of Miller & Thomas (1958).

Several authors have investigated the effects of egg production on female viability in *Drosophila*. In *D. subobscura* Lamb (1964) showed that an increase in longevity was correlated with a decrease in fecundity. For the same species, Maynard Smith (1958a, b) found that unmated, ovary-less or heat treated (regressed ovary) females all lived longer than mated controls. Malick & Kidwell (1966) found that *D. melanogaster* females lived 6 days longer when unmated, but Lints & Lints (1971) are emphatic that lifespan, at least in their strains of *D. melanogaster*, does not depend on fecundity. Moth (1974) found in experiments similar to those here that the percentage of fertilised *D. simulans st* females decreased to 53 per cent at the highest density tested (viz. 640). If unmatedness increases viability in *D. simulans*, then the decrease due to density should be at least partly counterbalanced at the higher densities. Such an event might be detected by examining the slopes of the density regression in two parts, viz. densities 20-80 and densities 160-640. The slopes of these two regressions ($b = -0.0011$ and -0.0006 respectively), while not significantly different ($P > 0.10$), lend support to the theory that unmatedness increases viability of *D. simulans st* females.

Viability change with increasing density was different in the two sexes (Fig. 4). As density increased the male sex was affected to a greater degree than was the female sex. This is similar to what was observed by Yoshida (1966) in *C. chinensis*, but opposite to the observations of Davis (1945), who studied *Trogoderma*, and Tauber (1968) who studied *Fannia femoralis*. Such a differential density effect might be associated with genes on the X-chromosome – the female, having two X-chromosomes, being more buffered against density and other environmental effects.

Characteristically ^{32}P emits pure β particles, the subsequent absorption of these particles by cellular material proving mutagenic, as does the transmutation of ^{32}P to ^{32}S (Oftedal, 1959). Because of this absorption and transmutation, constituent cells become damaged and, as there is no mitotic process in the *Drosophila* adult (Bozcuk, 1970 – cited by Lints & Lints, 1971) cannot be replaced. Such damage is presumably responsible for the lower viability of adults raised as immatures on media containing ^{32}P. These results support the random cell damage theory of ageing and death.

King (1954), using *D. simulans* and *D. melanogaster*, has shown that ^{32}P turnover is much faster in the female sex. King & Wilson (1955) obtained accurate measures of ^{32}P turnover in *D. melanogaster* after overcoming effects of etherisation which were present in the earlier work. They found that turnover in the female was 1.6 times faster than in the male. Despite this difference in turnover rates, our study showed that both sexes suffered similar reductions in survival when fed, as larvae, on ^{32}P. This could mean that the cellular material received its damage before the adults eclosed (i.e. in the larval or pupal stage), or that the female, despite its higher turnover rate, still

contained enough ^{32}P in its tissues to cause similar damage to that observed in the male.

Enclosing adults in either a glass or a cardboard experimental unit significantly altered their viability (Tab. 2), lower viability being obtained in the glass unit. This may be due to an effect of light. Erk & Samis (1970) and Pittendrigh and Minis (1972) showed that *D. melanogaster* longevity was shortened by continuous light although Allemand, Cohet & David (1973) found, for a different strain of *D. melanogaster*, no effect of light on longevity (apart from the complete absence of light which increased lifespan by 20-40%). If the *st*, Or-R-C or *y w* strains are sensitive to light then those adults living in a cardboard unit, which necessarily has a dark section, might be expected to have a higher viability. However, lower viability in the glass unit could also have been due to an effect of humidity. The glass unit was observed on numerous occasions to be quite moist inside, and this may have been responsible for reduced viability therein. Finally, it should also be noted that the two types of experimental unit, although of similar design, were not of equal volume (glass unit 135 cc, cardboard unit 165 cc). It might thus be that the difference in viability is a direct effect of density. It was not possible to determine conclusively which, or what combination of, factors were responsible for the observed difference. Density can probably be excluded because Or-R-C and *y w*, which were shown to be affected identically by an increase in density, were each affected to a different degree in the glass unit, and *st*, which was more sensitive to a density increase than either of the *D. melanogaster* strains, fared best of all three strains when viability in the glass unit was expressed as a proportion of that in the cardboard unit. That density is not responsible is also shown by the fact that the equations predicting viability, as determined by density, have different slopes for each type of unit. If density were entirely responsible, the slopes would be identical, but the intercept for the glass unit would be lower.

With the passage of time, the proportion of adults alive (l_x) must fall. Thus it is not surprising to find a significant effect of age on adult viability (Tab. 2), especially since viability on any day is dependent on that for the previous day. Pearl & Miner (1935) and Deevey (1947) classified life-table curves into three types, viz.

I: Negatively Skew Rectangular

II: Positively Skew Rectangular (type III of Deevey), and

III: Diagonal (type II of Deevey).

The life-table curves for each strain used in this experiment cannot be satisfactorily classified as to type, even though they were different (Fig. 3), because mortality was only recorded for the first 7 days of adult life. The Or-R-C strain showed a lower rate of decrease in viability with time than did *st* or *y w*, which were similar (Fig. 3). Differences in rates may be expected if different strains show different mean longevities, although strains with equal rates over the early period of adult life will not necessarily have equal longevities if the life-curve is of a type other than type III (type II of Deevey). The competing strain of *D. melanogaster* did not alter the rate of decrease in viability for *D. simulans st*. This is somewhat unexpected considering the marked differences in viability between the Or-R-C and *y w* strains. *D. simulans st*, when with Or-R-C, would be at a much higher density towards the end of the 7 days, compared to when with *y w*, and would be expected to suffer accordingly, especially since the density x age interaction is significant, with higher densities having higher rates of decrease in viability. Both sexes showed similar decreases in viability with increasing age, but as already mentioned, this does not necessarily mean that both sexes have equal longevity.

Change in viability with increasing adult age was also examined after deleting dependence on previous survival (Tab. 8b). This analysis proved more rewarding. Or-R-C, when density effects were absent, showed an increase in the death rate (q_x) of 0.1 per cent for every day of age. Why this should be is beyond the scope of this paper, but Pearl, Miner & Parker (1927) found that q_x increased with adult age, and Pearl & Miner (1935), who examined life-table and q_x curves for several strains of 7 different species state that 'the general trend of all q_x curves so far observed is ascending'. Similarly, the *y w* and *st* strains also had increasing q_x with increasing age, but the increase in q_x was not consistent as in Or-R-C. For *st*, the rate was 1.1 per cent for the first 3 days, then 1.6 per cent for the next 4 days, and for *y w* it was 0.3 per cent for 4 days, then 4.2 per cent for 3 days. This may be the actual trend of q_x with time for these strains, but it is more likely that the change observed is a density effect, and that at a population size where density exerts no influence, the increase in q_x is expected to

be steady at 1.1 per cent per day for *st*, and 0.3 per cent per day for *y w*.

Perhaps the most exciting part of this work has been the determination of how and when the effects of density influence adult viability. It is abundantly clear from Table 8b that the effect of density is to dramatically increase the death rate. When this increase appears is dependent on population density – the effect being observed earlier as initial density increases. Some populations were also shown to react to decreases in density by adjusting their death rate a second time. Frank (1952), using *Daphnia* and *Simocephalus*, found that a sudden increase in density always produced an immediate and full increase in the death rate, but that decreases in density produced delayed effects. These results are somewhat different to those given here. Although no populations were suddenly increased in density, it is doubtful (if they had been) that they would have adjusted their death rate immediately. Changes in death rate were observed after decreases in density, but no definite information as to whether the change was delayed could be obtained from this study. It is felt that these changes in death rate were delayed because the major changes in density came some time earlier than the change in death rate.

There is no doubt that the fitness component, adult viability, is *delayed density dependent*, but a more precise description is *variably delayed density dependent*.

We are indebted to Mr J. Mulley, Miss P. Brown and Miss N. Hardy for technical assistance. Mr L.N. Balaam, Director of Biometric Services, University of Sydney, and Dr J.W. James, School of Wool and Pastoral Sciences, University of New South Wales, provided valuable statistical advice. Dr N. Jackson, C.S.I.R.O., Division of Animal Genetics, modified Dr W.R. Harvey's computer program, LSMLGP, to handle our data. The project was supported by a grant from the Australian Research Grants Committee to J.S.F.B., while J.J.M. held a University of Sydney Research Grant Postgraduate Studentship.

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