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Effective population size: the effects of sex, genotype, and density on the mean and variance of offspring numbers in the flour beetle, *Tribolium castaneum*

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SUMMARY

In this paper I present the results of an experimental study of the effects of genotype and density on the mean and variance of offspring numbers in both sexes of the flour beetle, *Tribolium castaneum*. From the observed variance in offspring numbers the effective population size at several different densities is estimated using the methods of Crow & Morton (1955).

I found that both the mean and variance of offspring numbers varied with genotype and density. In general, males were more variable in offspring numbers than females and this variability increased with density. Individuals homozygous for the black body colour mutant, b/b, were less variable in offspring numbers than $+/+$ individuals, but the latter produced more offspring at most densities. As density increased, $+/+$ individuals became more variable in offspring numbers whereas b/b individuals were less sensitive in this regard. These findings are discussed in relation to the ecology of selection at the black and closely linked loci.

1. INTRODUCTION

In the experiment reported below, the effective population size of the flour beetle, *Tribolium castaneum*, was estimated for a wide range of initial adult densities. Chance fluctuations in gene frequency occur in finite populations owing to variance in the sampling of gametes. The magnitude of these fluctuations in gene frequency can be represented by the variance of the binomial distribution, $p(1-p)/N_e$, where p is the frequency of the allele in question and N_e is the 'effective population size' (Wright, 1931; Crow & Kimura, 1970). The effective population size is equal to the number of breeding adults only when a randomly sampled gamete has an equal probability of coming from any breeding adult. In natural and laboratory populations, the apparent population size will generally be greater than the effective population size because variance in the numbers of progeny per parent will contribute to the variance in the sampling of gametes. In this study the effect of genotype and density on the variance in the numbers of offspring was measured for both sexes.

The relationship between the variance in offpsring numbers and density is of general interest because in sexual diploids males often have a greater variance in

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offspring numbers than females (Bateman, 1948; Crow & Morton, 1955; Wade, 1979; Wade & Arnold, 1980). This sexual difference in the variance arises as a result of variance among males in numbers of mates. As density increases, competition among males for mates may also increase and lead to changes in the variance of male offspring numbers, and, consequently, to changes in the gametic sampling variance.

These studies were also motivated by the experimental work of Wade (1977) and Wade & McCauley (1980, in the Press) with *Tribolium*. This work examines the rate of genetic and phenotypic divergence of local demes of varying size. A quantitative knowledge of the relationship between apparent and effective deme size is necessary for interpreting the results of those studies.

Although the variance in reproductive success is of primary interest, genotypic differences in mean offspring numbers are also of some interest. The beetles used in the present study were the descendants of a cross between two laboratory strains; the b/b strain, homozygous for the autosomal semi-dominant black body-colour mutant, called 'Chicago-black' (Sokoloff, Slatis & Stanley, 1960) and the wild type, $+/+$, strain of the Purdue University Foundation Stock. These strains were first crossed by Sokal & Sonleitner (1968) and allowed to mate freely and hybridize for approximately 45 generations, during which the genetic backgrounds of both founder strains were 'amalgamated'. b/b and $+/+$ offspring of $b/+$ heterozygotes from this amalgamated stock were used in the present study. Only genes within 0·02 map units of the black locus would be expected to remain in substantial linkage disequilibrium after 45 generations of random mating. For this reason, differences in the mean numbers of offspring produced by the different genotypes are of interest in regard to the ecology of selection at the black and closely linked loci.

2. MATERIALS AND METHODS

A total of approximately 200 $b/+$ virgin males and females were isolated at generation 45 from the amalgamated stock. These 200 beetles were mass mated and allowed to oviposit for one week in 250 grams of yeast-supplemented whole wheat flour. From the offspring of these $b/+$ heterozygotes, 200 b/b and 200 $+/+$ virgin adults were taken and two b/b and two $+/+$ cultures were established, each with 50 males and 50 females in 250 g of flour-yeast medium (95% by weight stone ground whole wheat flour and 5% by weight dried brewer's yeast). These adults were removed after ovipositing for one week and their offspring were used in the following experiment.

The experiment consisted of 24 treatments with 10 replicate populations per treatment. Each population consisted of an 8 dram shell vial with 8 g of acclimatized flour-yeast medium and some initial numbers of adults depending on the treatment. There were six density treatments with the initial number of adults being 2, 4, 8, 16, 24, or 48 individuals in a one to one sex ratio of males to females. For each density, four different genotypic configurations of the initial adults were used (6 densities $\times$ 4 configurations per density = 24 treatments). At the lowest

starting density of two adults, two within-strain ($b/b \times b/b$ and $+/+ \times +/+$) and two reciprocal between-strain crosses ($b/b \times +/+$ and $+/+ \times b/b$) were set up and each cross was replicated 10 times. At all other densities, the four starting genotypic configurations are given by the entries in Table 1; each was replicated 10 times. The average age of the experimental adults was 10 days and ranged from 4 to 16 d. All 240 populations were set up on the same day. For each

Table 1. *The experimental design used to establish all density (D) treatments**

		Genotypic configurations			
Adults		1	2	3	4
Males	b/b	D/2	(D/2) − 1	0	1
	$+/+$	0	1	D/2	(D/2) − 1
Females	b/b	(D/2) − 1	D/2	1	0
	$+/+$	1	0	(D/2) − 1	D/2

* D = 4, 8, 16, 24, or 48 adult beetles. There were ten replicates of each configuration.

population females were introduced to the flour prior to males. The populations were positioned at random in a darkened incubator maintained at the standard conditions of 29 °C and 70 % relative humidity. After a 48 d interval, the adult populations were censused and the genotypes of all adult progeny were recorded.

It will be seen from Table 1 that each replicate contains a single individual, the progeny of which (in all cases, $+/b$) can be distinguished from those of other matings in the same culture. The analysis is then mainly concerned with the variation in progeny number of these 'marked' individuals. Since all their progeny are heterozygotes, heterozygote advantage cannot be responsible for treatment differences. However, when the marked parent is $+/+$, its offspring compete with b/b individuals only and vice versa. Thus, differences in mean offspring numbers between $+/+$ and b/b parents are always confounded with the genotype of the competitor which their progeny have to face. Such confounding does not affect contrasts between sexes of the same genotype.

An analysis of the sex and genotypic differences in mean offspring numbers, u_k, will be presented before considering the variance in offspring numbers, V_k, and its relation to effective population size. The analysis of mean offspring numbers will be based on the χ^2 test, using an a priori expectation of offspring number proportional to initial representation. The analysis of effective population size, N_e, and V_k will follow the methods developed by Crow & Morton (1955).

3. RESULTS

(i) *Mean offspring numbers*: The four types of single pair matings do not differ significantly in numbers of offspring (Table 2). At this low density (0·25 beetles per g of flour) mean offspring number does not vary with sex or genotype. As

initial adult density increases, treatment differences in total vial productivity become apparent (Table 3). Three-way analysis of variance of the log-transformed data reveals highly significant effects of both density and genotype ($P < 0{\cdot}005$) and weaker density × genotype interactions ($P \leqslant 0{\cdot}05$). (There were seven missing replicate values in this analysis owing to death of the marked individual in four cases and technical mistakes in three cases. To correct for these missing values the analysis was conducted in two ways: (1) using the highest within-treatment value for each missing replicate, and (2) using the lowest treatment value. Only those conclusions supported by *both* analyses are reported.)

Table 2. *The mean* ($\bar{X}$) *and standard error* (S.E.) *of the mean numbers of offspring of single pair crosses made between and within genotypic strains*

Genotypes		Productivity	
Male	Female	X	S.E.
b/*b*	*b*/*b*	191·0	5·91
b/*b*	+/+	168·3	17·10
+/+	*b*/*b*	159·1	17·48
+/+	+/+	162·1	16·40

Table 3. *The mean* ($\bar{X}$) *and standard error* (S.E.) *of the mean for total numbers of offspring per vial for the five initial density treatments and the four genotypic configurations*

	Genotypic configurations							
	4		3		2		1	
Initial density	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.
4	217·6	19·9	224·0	15·6	217·0	10·1	193·4	22·6
8	260·1	38·0	295·0	25·9	286·1	15·9	308·0	32·1
16	337·5	20·9	285·6	29·1	277·4	32·1	258·4	30·3
24	309·8	13·5	260·3	27·1	229·4	25·8	219·6	33·1
48	200·4	17·7	165·9	11·3	238·3	13·1	142·5	12·1

Although there is no systematic effect of the sex of the marked individual, one a posteriori contrast stands out in this regard. At an initial density of 48 adults, total vial productivity of configuration 2 is markedly greater than that of configuration 1 (compare Table 3, row 5, columns 2 and 4). This difference cannot be attributed to differences in the genotypic environment of the maturing offspring.

The numbers of offspring produced by a marked individual are expected a priori to be equal to its proportional representation in the initial parental cohort, 1/D, times the total productivity of its replicate. I used these expectations in a series of χ^2 tests to analyse the offspring numbers of marked individuals within each vial and in tests of the heterogeneity of these offspring numbers between vials (Sokal & Rohlf, 1969, p. 581).

In 38% of all surviving replicates (74 of 193) the numbers of offspring produced by the marked individual deviated significantly from expectation ($P < 0.025$). Within all treatments, excepting configurations 1 and 3 at high density, the marked individuals were heterogeneous in their deviations from expectation ($P < 0.005$). That is, not only did some individuals produce greater or fewer offspring than expected but individuals within the same treatment differed from one another in the direction or the extent of these deviations (Table 4). The row totals of Table 4

Table 4. *The number of significant* ($P < 0.025$) *positive* (+) *and negative* (−) *deviations in the productvity of marked individuals from expectation*

Marked Individual	Initial adult density					
	4	8	16	24	48	Total
	−,+	−,+	−,+	−,+	−,+	−,+
b/b male	5,0	1,2	4,3	2,2	0,3	12,10
b/b female	3,1	2,1	1,1	1,0	0,1	7,4
+/+ male	0,4	1,3	0,6	0,7	0,5	1,25
+/+ female	0,3	0,1	0,6	0,3	0,2	0,15
Total	8,8	4,7	5,16	3,12	0,11	20,54

Table 5. *The proportion of b/+ offspring produced by the marked individuals as calculated from the pooled replicates of each treatment*

Marked Individuals	Initial adult density				
	4	8	16	24	48
b/b males	0·367***	0·263	0·118	0·081	0·052*
b/b females	0·563***	0·225**	0·130	0·085	0·040
+/+ males	0·587***	0·261	0·230***	0·190***	0·070***
+/+ females	0·583***	0·245	0·179***	0·100***	0·064***
Expected proportion	0·500	0·250	0·125	0·083	0·042

*** $P < 0.005$; ** $P < 0.010$; * $P < 0.050$.

show that +/+ males and females tend to produce greater numbers of offspring than expected (binomial test, $P < 0.005$), but *b/b* males and females exhibit no overall tendency.

For each treatment, the numbers of offspring of marked individuals can be summed over all ten replicates and compared with the a priori expectation. In this way, the heterogeneity of offspring numbers between genotypes (*b/b* males and females versus +/+ males and females) and between sexes within genotypes can be tested (Sokal & Rohlf, 1969, p. 581). The observed proportion of the total offspring per treatment produced by marked individuals is shown in Table 5. There was significant heterogeneity between genotypes in these proportions ($P < 0.005$) at all but the lowest density. Differences between sexes occurred at low densities (4 adults, $P < 0.005$; 8 adults, $P < 0.001$) for *b/b* individuals but at intermediate densities (16 adults, $P < 0.025$; 24 adults, $P < 0.005$) for +/+ individuals.

(ii) *Variance in offspring numbers*: I use the theoretical and experimental methods suggested by Crow & Morton (1955) to examine variance in offspring numbers and its relation to effective population size. Because the gene frequency changes from the parental cohort to the progeny in many instances as shown above, variance in offspring numbers is calculated around the mean observed numbers of $+/b$ progeny, $\bar{X}$, for each treatment. This procedure eliminates all but the second order effects of selection (Crow & Morton, 1955). I do this because I am primarily interested in a general estimate of effective population size in *T. castaneum* and not one associated with selection at the black locus.

In addition, within treatments, the number of offspring of a marked individual was often correlated with the total offspring numbers of the same replicate; the product-moment correlation (r) exceeded 0·59 in eight of the 20 treatments ($P < 0{\cdot}05$). For example, in configuration 2 at a density of eight adults ($r = 0{\cdot}672$), the least productive replicate produced 57 offspring while the most productive had 369. Nevertheless, the proportion of $+/b$ heterozygotes among the offspring was 0·211 in the first case and 0·209 in the latter. Events which influence replicate productivity are evidently experienced in the same way by all individuals within the replicate. Thus, the between-replicate variance in the actual numbers of progeny of marked individuals does not contribute to the gene frequency variance within replicates. For this reason, the proportion, p_i, of $+/b$ individuals in each replicate, i ($i = 1, 2, \ldots, 10$), of a treatment was calculated and the sampling variance, σ^2, for the treatment was taken around the mean proportion, $\bar{p}$. The values of u_k and V_k were then obtained by multiplying $\bar{X}$ by $\bar{p}$ and σ^2 by $\bar{X}^2$.

Crow & Morton (1955) proposed that the ratio, V_k/u_k, be called the 'Index of Variability'. When values of the index are less than one, the actual population size is greater than the effective population size, and when values are greater than one, the actual population size is smaller than the effective population size. They noted that values of this ratio are highly dependent on the mean number of progeny as is clearly the case here. When population size is constant, the value of the measured index should be adjusted to a mean productivity of 2. The appropriate method of adjustment depends on the nature of the mortality factors which reduce the excess productivity. Crow and Morton considered two models of suvivorship: (1) random survival and (2) within-family correlations in survivorship. In the work of Wade (1977) and Wade & McCauley (1980, in the Press), populations were reduced to the original numbers of parental adults at the start of every generation by choosing a sample of adults at random from within progeny groups similar to those of this study. The random survival model is, therefore, the appropriate one. With this model the adjusted variance, V_a, for a mean of two progeny per individual is

$$V_a = s(1-s)u_k + s^2 V_k, \tag{1}$$

where s is the probability of survivorship (see equation 13 of Crow & Morton, 1955). In the present work, s is equal to the initial number of adults, D, divided by the average number of progeny produced, $\bar{X}$.

The total variance, V_{kt}, in the sampling of gametes from male and female parents, assuming random mating and equal mean productivities, is

$$V_{kt} = 0{\cdot}25\ (V_{k\delta} + V_{k\varphi}). \tag{2}$$

Using the mean over both sexes of u_k and s, the effective population size, N_e, is determined as

$$N_e = 2N/(1 + (V_a/u_a)), \tag{3}$$

where u_a is 2.

The values of u_k, V_k/u_k, V_a/u_a and N_e/N as determined from the variance in offspring numbers of each sex separately and for males and females combined are given in Table 6.

The range of V_a/u_a values exhibited in Table 6 is greater than the range observed by Crow & Morton (1955) for *Drosophila*, *Lymnaea* (snails), and man. That is, *T. castaneum* manifests a wider range of values for the adjusted index of variability over several densities than has been observed over several species. It is also clear that males are generally more variable than females in terms of offspring numbers and give, consequently, lower estimates of N_e. This result agrees with the observarions of Bateman (1948) and others (Crow & Morton, 1955; Wade, 1979; Wade & Arnold, 1980) that males generally have a greater variance in offspring numbers because males can vary in their numbers of mates.

Estimates of N_e from $+/+$ individuals tend to be lower than those from b/b individuals. This genotypic difference is difficult to interpret because of the differing genotypic environments experienced by the offspring of $+/+$ and b/b individuals. The $+/+$ individuals in b/b groups usually produce more offspring than their b/b counterparts and this difference alone would tend to lower the index of variability and raise estimates of N_e. The observed trend runs counter to this expectation.

Finally, there is a slight tendency for the N_e/N ratio to decrease as initial adult density increases and this tendency is most pronounced in males. The N_e/N ratios based on the combined male and female data, however, declined only 12–14 % as density was increased twelve-fold from 0·5 to 6 beetles per gram of medium. I conclude that changes in density do not greatly affect the variance in the sampling of gametes.

4. DISCUSSION

The range of N_e/N values estimated for *T. castaneum* compares favourably with those measured in other studies of other organisms (Crow & Morton, 1955; Prout, 1954; Kerr & Wright, 1954; Dobzhansky & Wright, 1941) being on average somewhat higher (0·71 to 0·95 versus 0·70 to 0·80). It should be noted, however, that, in addition to variation in offspring numbers, many other factors such as selection and migration affect the genic sampling variance. In this study, as in the others mentioned, the influence of other factors was eliminated as far as possible by design in order to study the effects of variation in offspring numbers alone.

The tendency of N_e to decrease as density increased was slight. The total sam-

Table 6. *Estimates of the mean numbers of offspring (u_k), the unadjusted (V_k/u_k) and adjusted (V_a/u_a) indices of variability, and the ratio of effective to apparent population size (N_e/N), for males, females and both sexes combined*

Density and genotype	Males				Females				Both sexes combined			
	u_k	V_k/u_k	V_a/u_a	N_e/N	u_k	V_k/u_k	V_a/u_a	N_e/N	u_k	V_k/u_k	V_a/u_a	N_e/N
4, *b/b*	78·7	18·9	0·95	1·03	104·1	7·5	1·04	0·98	91·4	6·2	0·09	1·05
4, +/+	127·9	5·4	1·28	0·88	115·3	8·2	1·39	0·92	121·6	3·4	1·27	0·88
8, *b/b*	68·7	5·0	1·20	0·91	66·4	4·6	0·99	1·01	67·5	2·4	1·02	0·99
8, +/+	76·1	5·0	1·18	0·92	84·7	2·1	1·13	0·94	80·4	1·7	1·11	0·95
16, *b/b*	40·5	9·0	1·31	0·87	38·0	2·4	1·15	0·93	39·3	2·9	1·12	0·94
16, +/+	57·2	2·5	1·81	0·71	46·6	2·8	1·60	0·77	51·9	1·3	1·59	0·77
24, *b/b*	24·8	4·7	1·24	0·89	23·2	1·8	1·14	0·93	24·0	1·6	1·07	0·97
24, +/+	42·1	5·4	1·92	0·69	23·2	1·0	1·27	0·88	32·6	1·9	1·57	0·78
48, *b/b*	10·1	2·7	1·71	0·74	7·7	1·5	1·26	0·89	8·9	0·7	1·21	0·91
48, +/+	17·0	1·6	1·90	0·69	9·6	1·1	1·66	0·75	13·3	1·1	1·64	0·76

pling variance in gene frequency is not greatly influenced by density in *T. castaneum*. However, estimates based solely on the males show a more pronounced decline than estimates based on females. This is evidence that the intensity of competition among males for mates does increase with density, increasing the disparity between successful and unsuccessful males. Females, on the other hand, were not demonstrably heterogeneous in their offspring numbers at the highest density studied.

In addition to these differences in variance, differences in mean number of offspring were also observed between genotypes and between sexes within genotypes. These differences persist despite 45 generations of free random mating with a stock population maintained with relatively large numbers of adults (300–500). It is possible that strong selection on the black and closely linked loci continues within the stock population. However, the studies of Sokal & Sonleitner (1968) on the same stock over generations 1 through 19 do not support this possibility. It is more likely that change in the conditions going from stock to vial husbandry alters the ecology of selection at these loci. This change would appear to be small relative to the types of environmental variation, both biotic and abiotic, occurring among populations in nature. The effects of density-related changes in the ecology of selection in nature remain to be evaluated, but they appear repeatedly in experimental studies (Lewontin, 1955; Lewontin & Matsuo, 1963; Sokal & Huber, 1963; Sokal & Karten, 1964; Sullivan & Sokal, 1963; Sokal & Sullivan, 1963; McCauley & Sokal, 1977).

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