

Variance-effective population number: the effects of sex ratio and density on the mean and variance of offspring numbers in the flour beetle, *Tribolium castaneum*

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SUMMARY

I report the results of an experimental study of the effects of sex ratio and density on the mean and variance in offspring numbers in both sexes of the flour beetle, *Tribolium castaneum*. The variance-effective number is estimated from the observed variance in offspring numbers using the methods of Crow & Morton (1955).

Both the mean and the variance in offspring numbers were found to vary with sex ratio and density; as was found in previous studies (Wade, 1980), males were generally more variable in offspring numbers than females. The ratio of variance to mean progeny numbers in each sex was approximately unity at all sex ratios.

1. INTRODUCTION

Random fluctuations in gene frequency occur in finite populations owing to sampling in the transmission of gametes from one generation to the next. The expected magnitude of the gene frequency drift is described by the variance in the binomial distribution, $p(1-p)/2N_e$, where p is the gene frequency and N_e is the 'effective population number' (Wright, 1931; Latter, 1959; Moran & Watterson, 1959; Cockerham, 1969; Kimura & Crow, 1963; Crow & Kimura, 1970; note that the factor one-half was omitted in Wade (1980)). The concept of effective population number was introduced by Wright (1931) to describe the amount of random genetic drift experienced by a natural population of size N in terms of that expected in an idealized population of size N_e . In natural and laboratory populations, the apparent population size, N , is generally greater than the effective size, N_e , because temporal variations in the size of breeding populations, variations among parents in the numbers of offspring, and variations in the sex ratio of breeding adults, tend to increase the variance in the sampling of gametes.

The relationship between the variance in offspring numbers and the sex ratio is of general interest because in some organisms the numbers of breeding males are usually less than those of females (Hamilton, 1967) and in small populations of any sexual diploid organism random variations in the relative numbers of the two sexes would be expected even if the primary sex ratio were unity.

Experimental investigations of population structure by Wade (1977*a*, 1980, 1982), Wade & McCauley (1980), and McCauley & Wade (1981) using *Tribolium* also

motivated the present research. These earlier studies examined the influence of the numbers of breeding adults in local demes and patterns of interdemic migration on the rate of genetic differentiation of demes. Knowledge of the effective numbers of breeding adults in relation to the apparent numbers is necessary for interpreting the results of those studies.

The effects of sex, genotype, and density on the variance in offspring numbers in *T. castaneum* have been studied in experimental populations where the sex ratio, expressed as the ratio of the numbers of breeding males to females, was fixed at unity (Wade, 1980). The present study differs in that the sex ratio was varied from treatment to treatment by design and a different genetic strain of *T. castaneum* was employed as the experimental organism.

2. MATERIALS AND METHODS

The experimental organisms were *T. castaneum* adults descended from the cSM $+/+$ and cSM b/b laboratory stocks derived in 1973 by mass mating equal numbers of virgin male and female adults from each of the four *T. castaneum* laboratory strains of Dr Thomas Park (Park, Mertz & Petruszewicz, 1961; see Wade, 1977*a*, for further details). This culture has since been maintained by transferring large numbers of adults (> 500) to fresh medium at approximately 3-month intervals. It is conservatively estimated that between 35 and 50 generations have elapsed since its foundation. Individuals from the cSM b/b stock are homozygous for an autosomal semi-dominant black body-colour mutant which has been shown to be allelic to the 'Chicago black' mutation (D. E. McCauley, pers. comm.). This mutation arose spontaneously in the cSM stock in 1977 and was isolated from several heterozygous adults.

Approximately 200 adults were randomly chosen from each of the cSM $+/+$ and cSM b/b stocks and each group was permitted to oviposit for 1 week on 250 g of flour-yeast medium (95% by weight whole wheat flour and 5% by weight dried brewer's yeast). Pupae were collected from both cultures, identified as to sex on the basis of the external genitalia, and separated into single sex cultures, one each for $+/+$ males, b/b males, $+/+$ females, and b/b females. Twenty days post eclosion, virgin adults from these cultures were used to establish the experimental populations described below.

There were 7 sex-ratio treatments and 2 density treatments with 6 replicate populations per treatment (7 sex-ratio treatments \times 2 density treatments \times 6 replicates per treatment = 84 total populations). Each population consisted of an 8 dr shell vial with 8 g of acclimatized flour-yeast medium and either 10 or 20 adults depending on the treatment density. The sex ratio of the initial adults was varied by setting the proportion of males at 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, or 0.8. In 3 of the 6 replicates at each sex ratio, one of the males was homozygous, b/b , while all other males and females were homozygous, $+/+$. The other 3 replicates were similar except that one of the females was homozygous, b/b . For example, consider the treatment with 0.7 males at an initial density of 20 adults per 8 g. Three of the replicates in this treatment were established with a starting configuration of 1 b/b male, 13 $+/+$ males, and 6 $+/+$ females while the other three replicates had a starting configuration of 14 $+/+$ males, 1 b/b female, and 5 $+/+$ females.

All populations were positioned at random in a darkened incubator at 29 °C and 70 % relative humidity. After a 50-day period, the populations were censused for adults and the adults were scored for genotype. The heterozygous $+/b$ offspring of the single b/b parent could readily be distinguished by body colour from the homozygous $+/+$ offspring of all other matings. The variance in offspring numbers of the b/b males and females was used to analyse the effective population number following the methods of Crow & Morton (1955) and Wade (1980).

The influence of population density and sex ratio on the sex differences in mean-offspring numbers, u_k , will be considered first before analysing the variance in offspring numbers, V_k , and its relation to effective-population number. When analysing the mean-offspring numbers, the proportion of homozygous b/b individuals in the appropriate sex will be used as the expected number of offspring. For example, with a starting configuration of 1 b/b male, 3 $+/+$ males, and 6 $+/+$ females, we would expect the b/b male to sire a proportion 0.25 of the total offspring produced, i.e. one quarter of the offspring are expected to be $+/b$ heterozygotes.

3. RESULTS

(i) *Mean offspring numbers.* The means and the standard errors of the mean for the total numbers of offspring produced in each treatment are presented in Table 1. Two-way analysis of variance of the log-transformed data reveals significant effects of sex ratio ($P < 0.0001$) and density ($P < 0.025$) as well as an interaction between sex ratio and density that approaches significance ($P = 0.06$). As is clear in Table 1, decreasing the proportion of initial males and increasing the proportion of initial females tends to increase the total numbers of offspring produced by a population; this increase is greater, the higher the initial density.

In Table 2, the mean proportion of $+/b$ offspring produced by b/b males in a sex-ratio configuration of (X -males, Y -females) is compared with that produced by b/b females in a sex-ratio configuration of (Y -males, X -females). Although the total numbers of offspring produced in these two sex-ratio configurations differ as shown above, the expected proportion of $+/b$ offspring is the same in each case, ($1/X$).

In only 8 of 84 cases (9.5 %) did the numbers of offspring produced by the b/b individual deviate significantly from expectation (chi-square test, $P < 0.025$) and there were equal numbers of positive and negative deviations. A chi-square test of the data pooled over replicates within treatments reveals four instances in which the observed proportion of $+/b$ heterozygotes for a treatment as a whole differed significantly from expectation (Table 2). All four cases occurred at the lower density and three of the four occurred at the extreme sex ratios.

Comparing the mean proportion of $+/b$ offspring produced by b/b males in a sex ratio configuration of (X -males, Y -females) with that of the b/b females in a configuration of (Y -males, X -females), we find only four cases of significant heterogeneity ($P < 0.010$), all occurring at the lower density. Males in the sex-ratio configurations (2, 8), (4, 8), and (7, 3) produced a significantly greater proportion of heterozygous progeny than did their respective female counterparts. Females in a configuration of (2, 8), however, produced a greater proportion of heterozygous offspring than males in a configuration of (8, 2).

Table 1. *The mean (X) and standard error (S.E.) of the mean numbers of total offspring per replicate for the seven sex ratio treatments at both densities*

Proportion of males	Sex of <i>b/b</i> individual	Initial density of adults			
		10 adults		20 adults	
		<i>X</i>	S.E.	<i>X</i>	S.E.
0.2	Male	86.3	2.19	110.0	9.87
0.2	Female	75.7	9.35	103.0	7.81
0.3	Male	74.3	6.89	80.7	4.18
0.3	Female	67.3	15.20	91.0	6.25
0.4	Male	68.7	7.06	90.3	3.71
0.4	Female	87.7	4.91	77.0	5.86
0.5	Male	66.0	3.28	89.5	2.48
0.5	Female	71.7	9.29	83.0	6.08
0.6	Male	67.7	4.10	66.7	6.12
0.6	Female	64.7	5.04	60.0	1.53
0.7	Male	70.3	3.93	60.0	6.25
0.7	Female	73.7	8.19	68.0	4.51
0.8	Male	51.7	3.48	54.3	9.77
0.8	Female	50.3	7.62	60.0	6.56

Table 2. *The number of significant ($P < 0.025$) positive (+) and negative (-) deviations in the offspring numbers of *b/b* individuals from expectation and the observed (*O*) and expected (*E*) proportions of +/*b* offspring for the seven sex ratio treatments at both initial densities*

Proportion of males	Sex of <i>b/b</i> individual	Initial density of adults					
		10 adults			20 adults		
		+, -	<i>O</i>	<i>E</i>	+, -	<i>O</i>	<i>E</i>
0.20	Male	1, 0	0.641	0.500	0, 0	0.227	0.250
0.20	Female	0, 0	0.178	0.125	0, 0	0.052	0.063
0.30	Male	1, 0	0.404	0.333	0, 0	0.190	0.167
0.30	Female	0, 0	0.074	0.143	0, 0	0.066	0.071
0.40	Male	0, 0	0.272	0.250	0, 1	0.144	0.125
0.40	Female	1, 0	0.190	0.167	0, 0	0.043	0.083
0.50	Male	0, 0	0.147	0.200	1, 1	0.123	0.100
0.50	Female	0, 0	0.205	0.200	0, 0	0.104	0.100
0.60	Male	0, 0	0.133	0.167	0, 0	0.090	0.083
0.60	Female	0, 1	0.160	0.250	0, 0	0.122	0.125
0.70	Male	0, 0	0.180	0.143	0, 0	0.067	0.071
0.70	Female	0, 0	0.317	0.333	0, 0	0.147	0.167
0.80	Male	0, 1	0.065	0.125	0, 0	0.080	0.063
0.80	Female	0, 0	0.497	0.500	0, 0	0.228	0.250

(ii) *Variance in offspring numbers.* Because the number of +/b offspring in some treatments was correlated with the total numbers but the proportion of +/b offspring was not, the proportion of +/b offspring, p_i , in each replicate i ($i = 1, 2, \text{ or } 3$) was calculated. The sampling variance of p , S_p^2 , for each treatment was calculated about the mean proportion, p . The values of mean-offspring numbers, u_k , and variance in offspring numbers, V_k , were then obtained as Xp and $X^2S_p^2$ where X is the mean number of total offspring (see Wade, 1980). This procedure eliminates all but the second order effects of selection (Crow & Morton, 1955).

Because the size of the breeding populations in the work of Wade (1977a, 1982) and Wade & McCauley (1980) was held constant by experimental design, observed values of u_k were adjusted to a mean value of $(1/m)$ for males and $(1/(1-m))$ for females where m is the proportion of males in the initial group of adults. The random survival model of Crow & Morton (1955) was used for this adjustment. With this model, the adjusted variance, V_a , is given by

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

where s is the probability of survivorship (see equation 13 of Crow & Morton, 1955). Here, s is equal to the initial number of adults divided by the average number of total offspring, X .

For an example of the calculation, consider the three replicate populations established with 20 adults: 14 +/+ males, 5 +/+ females, and 1b/b female. The numbers of offspring produced for each replicate were (64 +/+, 13 +/b), (54 +/+, 9 +/b), and (56 +/+, 8 +/b). The mean and s.e. of the number of total offspring are 68.0 ± 4.51 as given in Table 1, line 12, last two columns. The mean proportion of +/b offspring is 0.146 and the $S_p^2 = 0.000484$. The quantity μ_k is the product of mean total offspring and mean proportion of +/b offspring or $(68.0)(0.146) = 9.9$ and V_k is given by the product $(68.0)^2(0.000484) = 2.238$. The average survivorship, $s = 0.2941$, is given by the ratio of the number of randomly chosen founding parents (20) to the average number of total offspring (68.0). The average number of offspring per female necessary to maintain a constant number of 20 breeding adults is $\mu_a = (20)/(6) = 3.33$ offspring per female and V_a is given by substituting the above parameter values of s , μ_k , and V_k in (1).

The variance-effective population number for a given sex ratio of breeding adults in a population of constant size with random sex ratio of offspring is given by

$$N_e = 4N/[mV_a + (1-m)/m + (1-m)V_a + m/(1-m)] \tag{2}$$

(Latter, 1959; Moran & Watterson, 1959; Pollak, 1977; Nagylaki, pers. comm.). The values of the parameters in equations (1) and (2) and the effective population numbers calculated with them are given in Table 3. It is clear from Table 3 that, except for the most extreme sex ratios, N_e is generally within 10–15% of N .

If the sex ratio of breeding adults varies at random from generation to generation in a population of constant size as is the case in the work of Wade (1977a, 1982) and Wade & McCauley (1980), then the harmonic mean of the individual effective numbers is the best representative of the effective population number over the whole period of time (Wright, 1938; Crow & Kimura, 1970, p. 360). The harmonic mean of the observed effective population numbers given in

Table 3 was calculated at both densities using the binomial distribution with an expected proportion of males equal to 0.5 in all cases as has been empirically determined to be the case for all initial sex ratios (Wade, 1977*b*). In calculating the harmonic mean of the effective numbers the extreme cases of all males or all females were excluded. In addition, the experimental design did not measure effective population numbers at all possible sex ratios. The effective numbers as

Table 3. *Estimates of the mean numbers of offspring (u_k), the unadjusted (V_k/u_k) and adjusted (V_a/u_a) ratios of mean to variance, and the ratio of effective to apparent population number (N_e/N) for each sex ratio treatment at both densities*

Proportion of males	Sex of b/b individual	Initial density of adults							
		10 adults				20 adults			
		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
0.20	Male	55.0	3.66	1.67	0.56	25.0	0.02	0.46	0.73
	Female	13.0	0.11	1.22					
0.30	Male	30.4	0.05	1.07	0.92	15.4	0.31	0.95	0.83
	Female	5.7	0.02	0.51					
0.40	Male	19.0	0.27	0.99	0.89	13.3	3.37	1.79	0.92
	Female	16.8	2.64	1.37					
0.50	Male	9.6	1.52	0.79	0.99	15.9	0.24	1.48	0.90
	Female	14.7	2.53	1.24					
0.60	Male	9.2	1.01	0.82	1.07	5.9	0.55	0.91	1.01
	Female	10.4	2.34	0.77					
0.70	Male	12.6	0.21	1.11	0.81	3.8	0.38	0.71	0.97
	Female	22.8	2.24	1.08					
0.80	Male	3.1	3.10	0.68	0.69	4.2	0.44	0.98	0.67
	Female	24.8	0.57	0.90					

determined from the ideal case, $N_e = 4m(1 - m)N$, or the average of the flanking observed expected numbers were used in the calculation of the harmonic mean.

The ratio of N_e to N at the lower density of 10 initial adults was 0.876 and the ratio at the higher density was 0.919.

4. DISCUSSION

The N_e values estimated for the cSM strain of *T. castaneum* at different sex ratios (Table 3) lie very close to the N_e values expected for the 'ideal' case given by Wright's formula, $N_e = 4m(1 - m)N$. This is owing to the observation that the ratio of mean-offspring number to the variance in offspring number is approximately unity for each sex at all sex ratios and densities. This finding is different from that reported by Crow & Morton (1955) for *Drosophila*, *Lymanaea*, and man where the variance in offspring numbers typically exceeded the mean and reduced N_e to approximately 0.75 N .

The results reported here also differ from those reported by Wade (1980) for a

different laboratory strain of *T. castaneum*, the Purdue University Foundation Stock using the black body-colour mutant, 'Chicago-black'. The Chicago-black mutation had been permitted to freely mate and hybridize with the Purdue stock for approximately 45 generations prior to the initiation of the earlier study (Wade, 1980, p. 2). Nevertheless, almost 35 % of the individuals homozygous for the body colour mutation, *b/b*, produced significantly more or less *+/b* offspring than expected (see Wade, 1980, table 4). Homozygous *b/b* individuals from the cSM stock, however, much more rarely exhibited significant deviations from the expected numbers of-offspring (8 of 84). There thus appears to be a marked difference between the two studies in the amount of selection operating on the genetic marker. The methods of Crow & Morton (1955) eliminate all but the second order effects of selection and these effects are expected to diminish with the strength of selection on the genetic marker used to identify offspring.

The harmonic mean effective population size at both densities is approximately 90 % of the apparent population size (Table 3). For this reason, in the studies of population structure by Wade (1977*a*, 1982) and Wade & McCauley (1980), where the sex ratio of the breeding adults was left to chance for technical reasons, the apparent population number, *N*, is representative of the effective population number, *N_e*. Although the effective number varies with the sex ratio, the extreme sex ratios have a sufficiently low probability of occurrence that the harmonic mean is not greatly affected.

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