

# The effect of population size on effective population size: an empirical study in the red flour beetle *Tribolium castaneum*

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## Summary

Despite the increasing number of studies on the magnitude of  $N_e/N$  ratios, much remains unknown about the effects of demographic and environmental variables on  $N_e/N$ . We determined  $N_e/N$  for seven population size treatments, ranging from  $N = 2$  to  $N = 960$ , in the red flour beetle *Tribolium castaneum*.  $N_e/N$  decreased with increasing  $N$ , as evidenced by a significant negative relationship between  $\log N$  and  $N_e/N$ . Our results are consistent with other published data on the relationship between  $N_e/N$  and  $N$ . Effective population sizes in large populations may be much smaller than previously recognized. These results have important implications for conservation and evolutionary biology.

## 1. Introduction

Theory of gene frequency change in small populations was developed for the idealized population in which all breeding individuals have an equal expectation of progeny (e.g. Falconer, 1989). Wright (1931) introduced the concept of effective population size ( $N_e$ ) to provide a means by which gene frequency change due to drift and inbreeding could be incorporated into population genetic theory such that theoretical expectations could be applied to natural populations. Natural populations are not likely to meet the assumptions of the idealized population. Many factors influence the relative contribution of genes to the gamete pool for sampling for the next generation. These include uneven sex ratio, variance in family size and fluctuations in the population size (e.g. Falconer, 1989; Frankham, 1995). Very few studies have considered how the magnitude of actual population size ( $N$ ) may affect  $N_e/N$ . This is not surprising given that the concept of  $N_e$  was developed to estimate gene frequency change in *small* populations (Wright, 1931).

This is a particularly relevant topic for conservation biologists interested in the relationship between  $N_e$  and the minimum viable population size. One of the primary objectives of conservation genetic planning is the maximization of evolutionary potential in the

form of genetic variation (e.g. Soulé, 1980; Lande & Barrowclough, 1987). Effective population size is considered an important predictor of a population's future adaptability, and management plans are often based, at least in part, on a minimum  $N_e$  in an effort to prevent extinction due to loss of evolutionary potential. Franklin (1980), and later Lande & Barrowclough (1987), proposed that an effective population size of 500 be considered the standard minimum. More recently, Lande (1995) suggested that an effective size of 5000 is a more accurate estimate. These numbers are based, however, on simple theoretical expectations. Many population and taxon-dependent parameters affect these expectations, making it difficult to generalize accurately among different populations and taxa. The concept of a minimum effective population size based on level of genetic variation is nonetheless important in conservation policy (e.g. Soulé, 1987; Mace & Lande, 1991; Tear *et al.*, 1995) and necessitates an empirical understanding of the relationship between actual and effective population size.

Nunney (1993) suggests that for most populations  $N_e/N$  ( $N$  being defined as number of adults) is expected to be greater than 0.5, and only in extreme circumstances is it going to be as low as 0.1.  $N_e/N$  ratios in studies involving small populations ( $N < 500$ ) range from approximately 0.5 to 0.9, in accordance with Nunney's prediction (e.g. Kerr & Wright, 1954*a, b*; Wright & Kerr, 1954; Buri, 1956; reviewed by Falconer, 1989; see also Crow & Morton, 1955;

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and Nunney & Elam, 1994). Frankham (1995), however, argues that estimates of the order of 0.1 are increasingly more common in the published literature. This is especially true for studies that report on  $N_e/N$  for large populations ( $N > 500$ ) (Nei & Graur, 1984; Avise, 1992; Briscoe *et al.*, 1992; see Fig. 3). For example, Nei & Graur (1984) summarize data from 64 studies on a wide variety of taxa ranging from primates to lizards to bacteria, with population sizes ranging from approximately  $10^4$  to  $10^{20}$ , and report a mean  $N_e/N$  ratio of 0.10 (SD 0.22). Avise (1992) examines  $N_e/N$  ratios for 16 marine populations ranging in size from approximately  $10^3$  to  $10^8$  and reports a mean  $N_e/N$  of 0.07 (SD 0.21). Briscoe *et al.* (1992) review the data for studies of  $N_e/N$  in large mass-mated populations of *Drosophila*,  $N$  ranging from approximately 500 to 5000, and report a mean  $N_e/N$  ratio of 0.08, SD 0.09 (excluding two outlier estimates greater than 1.0).

Importantly, in all these studies involving large  $N$ , except for one estimate in Briscoe *et al.* (1992),  $N$  signifies approximate census size and not the number of breeding individuals, as in the studies involving small  $N$ . Because of this difference, it is difficult to compare  $N_e/N$  ratios accurately between the studies involving small and large populations. Also, uncontrolled demographic variables, especially fluctuations in population size, are likely to be important factors in the studies involving large populations, in contrast to the studies involving small populations in which these variables are kept constant. Here, we examine in a single experiment the changing relationship between  $N$  and  $N_e/N$ , where  $N$  is defined as the number of breeding individuals. Variables commonly known to affect  $N_e/N$  ratios, including fluctuations in population size, unequal sex ratio, overlapping generations and density, were carefully controlled.

## 2. Materials and methods

We measured the effective population size in populations initiated with 2, 4, 8, 16, 48, 480 and 960 individuals. There were 9 replicates for the  $N = 960$  treatment, 12 replicates for the  $N = 480$  treatment, and 25 replicates for all other treatments. The beetles in this experiment were descended from two *Tribolium castaneum* laboratory strains: *cSM +/+* and *cSM b/b*. In each replicate, the test individual was a *cSM +/+* female, and all the other beetles were *cSM b/b*. *cSM b/b* beetles are homozygous for an autosomal semi-dominant black body colour marker; *cSM +/+* beetles are red. Offspring of a cross between *cSM +/+* and *cSM b/b* are brown in colour and phenotypically distinguishable from black offspring of within-strain matings. Sex ratios were held constant. For example, in the treatment consisting of 480 founders, 1 red female beetle was placed in a jar with 239 black females and 240 black males. For the  $N =$

2 treatment, only 1 red female and 1 black male beetle were placed into a vial. The beetles were allowed to mate and oviposit for 11 days and then removed. The adult progeny were counted after 6 weeks and the numbers of brown and black offspring were recorded. The mean number of brown beetles within a treatment defines  $\mu_k$  ( $\mu_k = (\bar{X})(\bar{p})$ , where  $\bar{X}$  is the mean number of total offspring for each population size treatment and  $\bar{p}$  is the mean proportion of  $+/b$  offspring for each treatment; Wade, 1980, 1984); the variance in the number of brown beetles within a treatment defines  $V_k$  ( $V_k = (\sigma^2)(\bar{X}^2)$ , where  $\sigma^2$  is the variance in  $\bar{p}$ ; Wade, 1980, 1984). These statistics were used to calculate  $N_e$  (Crow & Morton, 1955; see below).

We tested only females in an effort to minimize factors known to increase variance in reproductive success. Females generally yield larger  $N_e/N$  ratios than do males because they generally have a lower variance in offspring number (e.g. Wade, 1980). We carefully controlled all variables commonly known to affect  $N_e/N$ , including unequal sex ratio (Wright, 1931), overlapping generations (Kimura & Crow, 1963), density (Nozawa, 1970; Wade, 1980) and fluctuating population size (Wright, 1931). Sex ratio was held constant as described above. Our estimate of  $N_e$  is based on data from a single generation; thus the effect of overlapping generations does not exist. We maintain constant density by varying the container size: the surface area to volume ratio for each treatment ranged from 0.45 to 0.65  $\text{mm}^{-1}$ , and the surface area per beetle ranged from 0.22 to 0.33  $\text{mm}^2$ , except for the  $N = 2$  treatment in which the ratio was 1.09  $\text{mm}^2$ . We corrected for the increase in population size from the parental to offspring generation using the methods of Crow & Morton (1955). Our adjusted variance in offspring number ( $V_a$ ) is the value that  $V_k$  would have if  $\mu_k$  were constant, i.e.  $\mu_k = 2$ . Specifically,  $V_a = s(1-s)\mu_k + s^2V_k$ , where  $s$  is a measure of probability of survival to reproductive maturity (Crow & Morton, 1955). In our experiment the  $+/b$  beetles were more fit than the  $b/b$  beetles as evidenced by a larger proportion of  $+/b$  offspring than expected by chance ( $\chi^2 = 1262$ , d.f. = 117,  $P < 0.001$ ), and therefore  $2/\mu_k$  provides the most accurate estimate of  $s$ . We calculated  $N_e$  according to the formula:  $N_e = 2N/(1 + V_a/2)$  (Crow & Kimura, 1970).

Finally, we compared our results with published results on  $N_e/N$  ratios from other studies that examine  $N_e/N$  across a range of  $N$ , where  $N$  is defined as the number of breeding individuals (Nozawa, 1970; Wade, 1980; Husband & Barrett, 1995).

## 3. Results

### (i) This experiment

Estimated values of the mean number of offspring ( $\mu_k$ ),  $V_k$  (unadjusted variance in offspring number),  $\bar{p}$  and  $\bar{X}$  from which  $\mu_k$  and  $V_k$  are calculated, and  $N_e/N$

Table 1. Summary of  $N_e/N$  ratios and parameters calculated to estimate  $N_e/N$

$N$	$\bar{p}$ (SD)	$\bar{X}$ (SD)	$\mu_k$	$V_k$	$N_e/N$
2	1 (0)	46.88 (19.8)	46.88	0	1.02
4	0.52 (0.14)	88.68 (16.2)	45.85	146.49	0.95
8	0.26 (0.10)	157.20 (43.0)	42.52	217.94	0.91
16	0.13 (0.04)	332.14 (29.1)	44.49	222.65	0.92
48	0.06 (0.02)	923.72 (87.2)	53.92	276.23	0.93
480	0.005 (0.003)	9635.83 (657.3)	49.29	721.95	0.78
960	0.004 (0.002)	16792.44 (1096.4)	60.74	929.65	0.81

$\bar{p}$ , mean proportion of  $+/b$  offspring;  $\bar{X}$ , mean number total offspring;  $\mu_k$ , mean number of  $+/b$  offspring;  $V_k$ , variance in number of  $+/b$  offspring. See text for formulae for  $\mu_k$  and  $V_k$ .  $N_e/N = 2/[1 + (s(1-s)\mu_k + s^2V_k)/2]$ , where  $s = 2/\mu_k$ .

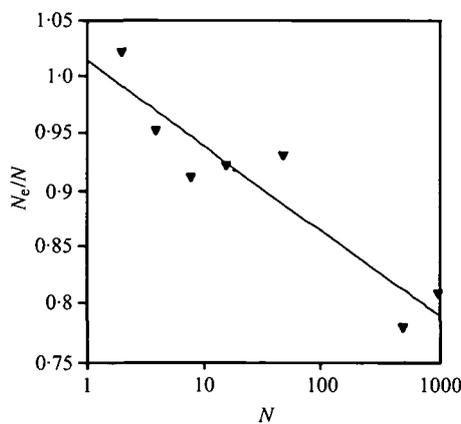


Fig. 1. The relationship between  $N$  and  $N_e/N$  ( $P = 0.0024$ ,  $r^2 = 0.86$ ).

are presented in Table 1. The relationship between  $N$  and  $N_e/N$  is best described by a logarithmic model:  $N_e/N = 1.013 - 0.0751 \log N$ ,  $r^2 = 0.86$ ,  $P = 0.0024$  (Fig. 1; Table 1).  $N_e/N$  decreases as  $N$  increases, and as population size increases the rate of change in  $N_e/N$  decreases.

Because  $N_e/N = 2/(1 + V_a/2)$ , a decrease in  $N_e/N$  is proportional to an increase in  $V_a$ . To evaluate why  $V_a$  increases as  $N$  increases, we tested the effect of  $N$  on the components of  $V_a$ , specifically  $V_k$  and  $\mu_k$ . There is a marginally significant increase in  $\mu_k$  with increasing  $\log N$  ( $r^2 = 0.56$ ,  $P = 0.05$ ) and a significant increase in  $V_k$  with increasing  $\log N$  ( $r^2 = 0.94$ ,  $P = 0.0003$ ).

(ii) The relationship between  $N$  and  $N_e/N$  in other published studies

We are aware of only one other study that includes  $N_e/N$  ratios for both small ( $N < 500$ ) and large ( $N >$

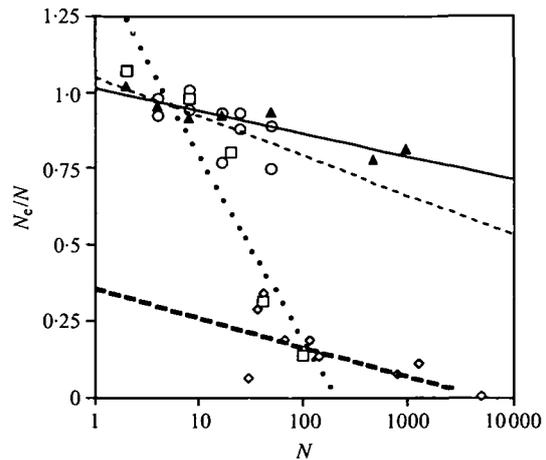


Fig. 2. A comparison of our data set (triangles, continuous line) with data sets from three other experiments in which  $N$  is defined as the number of breeding individuals;  $\blacktriangle$ , this paper ( $P = 0.0024$ ,  $r^2 = 0.8$ );  $\circ$  (---), Wade (1980),  $P = 0.06$ ,  $r^2 = 0.37$ ;  $\square$  (.....), Nozawa (1970),  $P = 0.02$ ,  $r^2 = 0.87$ ;  $\diamond$  (-.-.-), Husband & Barrett (1995),  $P = 0.03$ ,  $r^2 = 0.46$ . Although conditions that affect  $N_e/N$  vary among experiments, the negative regression of  $N_e/N$  on  $\log N$  is common among studies. Data from Husband & Barrett (1992) are their ' $N_{comb}$ ' estimates.

500) population sizes in a single experiment. Husband & Barrett (1992) compare genetic and ecological measures of  $N_e$  in 10 populations of the plant *Eichhornia paniculata* (Pontederiaceae). For their ecological measure of  $N_e$  (' $N_{comb}$ '),  $N_e/N$  decreases as  $\log N$  increases ( $P = 0.03$ ,  $r^2 = 0.46$ ) and ranges from 0.34 ( $N = 42.5$ ) to 0.008 ( $N = 5040$ ), with a mean of 0.16 (SD = 0.10), where  $N$  is defined as the number of breeding individuals. For their genetic measure of  $N_e$ , the negative linear regression of  $N_e/N$  on  $\log N$  is also significant ( $P = 0.0008$ ,  $r^2 = 0.77$ ), with  $N_e/N$  ranging from 0.002 to 0.17 with a mean of 0.11 (SD = 0.07). Husband & Barrett (1992) observe that their estimates of  $N_e$  are lower than those typically reported for animals, even for small populations (see Fig. 2), and they suggest that this may be due to population structure. Nunney (1995) recalculated what may be a more accurate estimate of  $N$  and  $N_e/N$  for both the ecological and genetic estimates of  $N_e$  (but see Husband & Barrett, 1995). With these new estimates, the negative trend in  $N_e/N$  is still significant for the genetic estimate of  $N_e$  ( $P = 0.01$ ,  $r^2 = 0.54$ ); the trend for the recalculated ecological estimates cannot be similarly analysed, however, because the data points are not independent. To our knowledge, there has been no study in which  $N_e/N$  ratios have been reported for a range of both small and large population sizes for which population structure is not a factor.

Nozawa (1970) and Wade (1980) both reported lower  $N_e/N$  in larger populations of *Drosophila melanogaster* and *T. castaneum*, respectively ( $2 \leq N \leq 100$  in Nozawa, 1970; and  $4 \leq N \leq 48$  in Wade, 1980; Fig. 2). Both studies were designed to test density

effects, however, and in our experiment we held density constant. Also, our study included larger populations.

#### 4. Discussion

Our results show that the ratio  $N_e/N$  ranges from 0.91 to 1.02 in small populations ( $N$  ranging from 2 to 48) and decreases to 0.78 to 0.81 in larger populations ( $N = 480$  and  $N = 960$ ). The trend in our data is small but consistent and statistically highly significant. Almost all the variation in the data is explained by the model ( $r^2 = 0.86$ ). The trend in our data is consistent with other empirical studies that provide results on the relationship between  $N_e/N$  and  $N$ , where  $N$  is defined as the number of breeding individuals (Fig. 2; Nozawa, 1970; Wade, 1980; Husband & Barrett, 1992).

There are several features of the experimental design that could have affected our results. These include the sex and genotype of the test individual, and the possibility of frequency-dependent selection. For example, because males generally show greater variation in reproductive success (Wade, 1980), one might expect to see a greater trend if we had tested males instead of females. In his study on the effect of density on  $N_e/N$ , Wade (1980) reports that the effect is indeed greater for males than females. However, Nozawa (1970) reports on similar density effects between males and females. In our experiment, the genotype of the test individual for each assay had a higher fitness, on average, than the genotypes of the other beetles in the populations (Table 1). The only

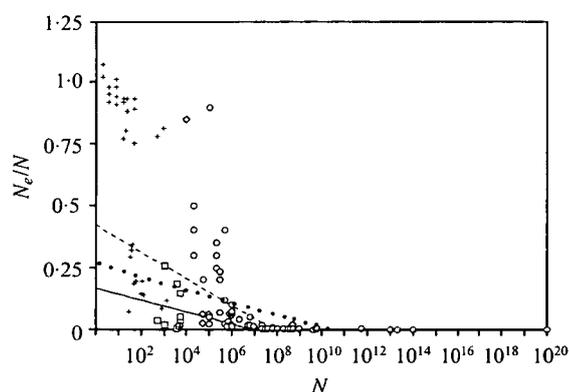


Fig. 3. A summary of data from studies involving large  $N$ , where  $N$  is defined as approximate census size. Importantly, an accurate comparison between studies in Fig. 2 (+) and those shown here is difficult because of differences in how  $N$  is defined and the likelihood of uncontrolled demographic variables in all the studies in Fig. 3. Conditions that affect  $N_e/N$  and methods of estimating  $N_e$  vary both among and within data sets in Nei & Grauer (1984),  $\circ$  (...); Avise (1992),  $\diamond$  (—) and Briscoe *et al.* (1992),  $\square$  (—). Only one of the regressions for these three data sets is statistically significant (for Nei & Grauer,  $P = 0.0003$ ,  $r^2 = 0.19$ , excluding one outlier; for Avise,  $P = 0.09$ ,  $r^2 = 0.19$ ; and for Briscoe *et al.*,  $P = 0.93$ ,  $r^2 < 0.01$ ).

study that we are aware of that examines genotypic effect on  $N_e/N$  in *Tribolium* is Wade (1980). Wade (1980) conducted two sets of assays, one using *cSM*  $+/+$  test individuals and the other using *cSM*  $b/b$  test individuals, and for both genotypes  $N_e/N$  decreases as  $\log N$  increases (see Fig. 2 for a graph of combined data set). Although the trend is steeper for the  $+/+$  beetles, it is statistically significant only for the  $b/b$  beetles, whether testing males, females or both sexes combined. This suggests that even if we had tested a less fit genotype such as  $b/b$ , the results would still be significant. With regard to the possibility of frequency-dependent selection, whether we had tested  $+/+$ ,  $b/b$  or any other genotype, the proportion of the test individuals' offspring in the total population decreases as  $N$  increases. Frequency-dependent viability selection has been shown to exist in *T. castaneum* (Schwartz *et al.*, 1996). However, one would expect rare genotype advantage to cause an increase, not a decrease, in  $N_e/N$  with increasing  $N$ . Thus rare genotype advantage cannot explain our results.

$N_e/N$  ratios in larger populations are generally smaller than  $N_e/N$  in small populations (Fig. 3), primarily because larger populations are more likely to experience fluctuations in population size (Nei & Graur, 1984; Frankham, 1995). Importantly, fluctuating population size and other variables commonly known to affect  $N_e/N$  were carefully controlled in our experiment, and yet we still observed a significant decrease in  $N_e/N$  with increasing  $N$ , suggesting that other factors not commonly associated with increasing population size may also affect  $N_e/N$ . Thus, although fluctuating population size may be the most important variable affecting  $N_e/N$  ratios in Fig. 3, as the authors of those papers explain, our results suggest that there may be additional factors associated with population size, such as increased competition, that may also lower  $N_e/N$  ratios.

In conservation planning the effect of population size on  $N_e/N$  needs to be considered when predicting  $N_e/N$  ratios. Otherwise, long-term predictions are likely to underestimate the actual population size necessary to maintain a minimum effective population size. For many taxa it is likely that the ratio  $N_e/N$  is very small for effective populations as large or larger than  $10^4$  (see Fig. 3), a recently suggested minimum  $N_e$  (Lande, 1995). The minimum viable population size based on level of genetic variation probably varies, however, and needs to be empirically studied in order to set realistic and appropriate conservation goals.

The trend in our data also signifies the importance of designing experiments on  $N_e/N$  such that any one parent's contribution to the total progeny is small (J. F. Crow, personal communication). In this way, experimental effects would not be confounded by uncontrolled factors associated with population size, and results could be more accurately extrapolated to larger, natural populations.

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