ESTIMATING EFFECTIVE POPULATION SIZE: A REPLY TO NUNNEY

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In a recent article (Husband and Barrett 1992a), we reported a study of effective population size in ten populations of the annual aquatic plant *Eichhornia paniculata* (Pontederiaceae). We found the mean N_e to be 15.8, approximately 10.6% of the census number (N), and that this average, which was based on temporal changes in allozyme markers, was lower than any estimates calculated from demographic methods. Nunney (1995) has questioned our low estimate of N_e/N ($=0.11$) on the basis that N_e/N in species with nonoverlapping generations should approach a value above 0.5, according to his recent theoretical work (Nunney 1993). Nunney (1995) suggested that our low value of N_e/N and the discrepancies between genetic and demographic estimates are the result of errors in our application of demographic and genetic methods to *E. paniculata* and consistent downward biases associated with the genetic approach (although his recalculated estimate is still low, $N_e/N = 0.24$). Although we acknowledge that there will necessarily be some inaccuracies in our estimates of N_e, we wish to challenge Nunney's criticisms on three fronts. First, we believe that our approach was justified by our objectives, and that the alternate approaches suggested by Nunney were all considered within the original paper. Second, we feel that Nunney has not represented the upward and downward biases in available methods impartially. Finally, we believe that the theoretical expectations on which his judgments were originally founded are unrealistic for most species.

Our first priority in the original study (Husband and Barrett 1992a) was to evaluate the importance of drift to variation in the frequency of mating types in populations of *E. paniculata*. Therefore, it was critical that the estimates of N_e and N were not only accurate but also representative of populations throughout the geographic range. As a result, our approach was sometimes at odds with the objective of comparing ecological and genetic estimates of a single population. Nevertheless, Nunney criticized the manner in which we estimated census size, variance in reproductive success and variance in allele frequencies on this basis. In the original paper, we recognized that our approach may not be appropriate for all purposes and discussed at length how alternate approaches might influence the discrepancies between genetic and demographic estimates (Husband and Barrett 1992a, pp. 1881, 1886–1887); therefore, we will not discuss them in detail here. Briefly, with respect to census size, we agree that calculating N based solely on the second estimate of size rather than the mean of two census periods is most suitable for pairing ecological estimates to their respective genetic estimates of variance effective size for each popu-

lation. However, we felt that in a species where population size fluctuates so widely each year (see Husband and Barrett 1992b, table 1) estimates of N based on a single year would have no generality. To achieve this generality, we decided to include all N estimates available for the best measure of census size in these populations. We also acknowledge that substituting an average estimate of variance in flower number for those populations lacking estimates is less desirable when comparing ecological and genetic estimates for each population. However, for a species that is sparse yet widely distributed and whose flowering phenology is unpredictable at best, this represents the best data available, and we argue that the values may actually provide the most representative estimate for populations throughout the Brazilian range, which was in keeping with our priority for generality. All this seems relatively trivial, however, when one considers that the estimates, as we point out, are based on variation in flower number, rather than an actual measure of variance in reproductive success. Surely this will have more impact on the accuracy of ecological estimates of N_e.

We are less convinced of Nunney's criticisms that our application of the temporal method leads to underestimates of N_e. Waple's (1989) genetic approach provides two different sampling methods, plan I and II, for estimating changes in allele frequencies. Our estimate was based on plan II, in which individuals are sampled without replacement before reproduction. In *E. paniculata,* we sampled progeny equally from a random subset of maternal plants (or a complete sample in small populations). The number of progeny representing each maternal plant did not vary in proportion to the maternal parents reproductive success, although family size did vary primarily because of differences in seed production among maternal plants. Our decision to sample approximately equal progeny numbers would give a conservative estimate of allele-frequency variance since it reduces the variance in gamete contribution among plants.Therefore, our sampling approach would bias the estimate of N_e upward, and not downward, as suggested by Nunney. Because of this conservatism, we maintain that estimates of N_e based on Plan II are adequate; but to be sure, we also estimated N_e using Waples' Plan I using parental genotypes. This method does not suffer from the same criticism, and yet the mean N_e was even lower (11.8) than the estimate based on Plan II (although the confidence intervals tended to be larger), a point neglected in Nunney's review.

Nunney aptly points out that sampling errors made when estimating allele frequencies will necessarily exaggerate frequencies and hence lead to downward biases in the genetic estimate of N_e. Our concern, however, is that, first, this is accounted for by the estimator of N_e (Waples 1989) and, second, to imply that all genetic estimates will therefore be underestimates, or that this is the primary explanation for discrepancies between genetic and demographically based estimates is misleading. Rather, we believe that systematic upward biases in the genetic approach and shortcomings of the ecological approach should also receive attention. Are genetic estimates of N_e consistently biased downward as Nunney suggests? We question Nunney's assertion that inferring maternal genotypes from progeny arrays will result in a downward bias. This statement is not adequately explained

and, to our knowledge, there simply is no evidence that methods of inferring parentage are biased in any consistent direction (K. Ritland, pers. comm. 1994). In addition, Nunney does not consider a number of other potential biases, such as the effects of dispersal, balancing selection and sampling of progeny from maternal plants, which could in fact produce an upward bias or a downward bias in the genetic estimates depending on the circumstances. In final defense of the genetic approach, we emphasize that at present, this method is the only one that allows estimates of confidence limits, and that under certain circumstances (populations with small N_e), they need not be as large as Nunney implies.

Discrepancies between ecological and genetic estimates should not be attributed soley to marker-based approaches. Nunney focuses primarily on downward biases in the genetic approach without considering the properties of ecological methods. Clearly, the two approaches are fundamentally different: one prospective and the other retrospective. Genetic measures represent a realized measure of drift, since they are based on the actual fluctuations in allele frequencies. Ecological measures, however, are values predicted from demographic components of drift, and therefore, the two methods will be equivalent only if all demographic components of drift can be measured accurately in natural populations. This is unlikely ever to be achieved. Therefore, it is likely that ecological approaches are inaccurate because of their piecemeal quality as well as a result of sampling biases. For example, annual fluctuations in population size in *E. paniculata* are as important as variance in reproductive success to N_e. In addition mating systems, which range from t = 0.002 to 0.96 among populations in *E. paniculata* (Barrett and Husband 1990), can account for as much as a 50% change in N_e. In addition, there are other ecological characteristics rarely considered in ecological estimates that would decrease estimates of N_e further had they been considered. For example, current estimates of N_e based on variance in progeny number assume that reproductive asymmetries occur randomly among individuals. However, Nei and Murata (1966) showed that N_e is reduced further when fertility has some heritable basis, which is most likely the case. Neglecting any of these attributes will almost always lead to an overestimate of N_e. Nunney's earlier criticisms about our estimates of N and variance in reproductive success exemplify the very problem with demographically based estimates; that is, the difficulty in applying such methods to natural populations. As a result, we are often left with ecological estimates based on the best data available, and worse, with little information regarding the errors or biases involved.

Nunney's (1995) concerns seem largely based on the fact that his own theoretical studies indicate average N_e/N ratios in species with overlapping generations should approach 0.5 and should be higher with nonoverlapping generations. Our mean estimate of N_e is hardly a sufficient test of this theory, although other studies available also report N_e/N values of 0.1 or less (McKay 1980; Nei and Tajima 1981; Orive 1993), two of which are based on ecological approaches. Also, a recent review of published N_e/N estimates indicates that mean values based on both genetic and demographic measures are well below 0.5 (Frankham 1995). We find two aspects of Nunney's application of his theory troubling.

First, Nunney's focus on mean N_e/N masks the fact that we examined ten different populations and N_e/N ratios ranged from 0.002 to 0.17 (0.04–0.59 using his adjustment). The average value is adequate only to the extent that the ten populations were representative. Moreover, it is not clear what the meaning of an arithmetic average of N_e/N is for this species. We would argue that if an average is useful at all, a geometric mean would best represent the central tendency in a species with many small and few large populations. The geometric mean of N_e is 10.5, even lower than the original 15.8, and the geometric mean of N_e/N is reduced to 0.07 (0.17 using Nunney's adjustment). The second aspect is that, although we are not questioning his theoretical results, applying Nunney's theoretical conclusion seems overly simplistic because it is based on the assumption that N_e is essentially the product of one demographic feature in populations: variance in reproductive success. Although studies such as Wood's (1987) indicate that effective size is most sensitive to changes in parameters related to reproductive heterogeneity, his results corroborate ours in finding that parameters related to demographic recruitment were more likely to vary among populations and "are probably more important as causes of variation in effective size." This study and ours suggest that a substantially different conclusion may result when other complexities in life history are considered in the estimate of N_e.

Despite our specific disagreements, we believe Nunney has raised an important problem. The methods for estimating effective population size are many and, when applied in the field, are each subject to their own biases. However, virtually no comparisons of the statistical biases of these methods and the sensitivity of N_e estimates to these different biases have been conducted. Equally important yet lacking is better information on the magnitude of natural variation for these characters among populations and species, which cannot be predicted from the few studies that currently exist. Without this information it will remain difficult to determine the true magnitude of effective size in natural populations and the relative accuracy of genetic and ecological approaches.

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