Temporal Changes in Allele Frequencies and a Population's History of Severe Bottlenecks

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Abstract: *Monitoring temporal changes in genetic variation has been suggested as a means of determining if a population has experienced a demographic bottleneck. Simulations have shown that the variance in allele frequencies over time (*F*) can provide reasonable estimates of effective population size (*N_e*). This relationship between* F *and* N_e *suggests that changes in allele frequencies may provide a way to determine the severity of recent demographic bottlenecks experienced by a population. We examined allozyme variation in experimental populations of the eastern mosquitofish (*Gambusia holbrooki*) to evaluate the relationship between the severity of demographic bottlenecks and temporal variation in allele frequencies. Estimates of* F *from both the fish populations and computer simulations were compared to expected rates of drift. We found that different methods for estimating* F *had little effect on the analysis. The variance in estimates of* F *was large among both experimental and simulated populations experiencing similar demographic bottlenecks. Temporal changes in allele frequencies suggested that the experimental populations had experienced bottlenecks, but there was no relationship between observed and expected values of* F*. Furthermore, genetic drift was likely to be underestimated in populations experiencing the most severe bottlenecks. The weak relationship between* F *and bottleneck severity is probably due to both sampling error associated with the number of polymorphic loci examined and the loss of alleles during the bottlenecks. For populations that may have experienced severe bottlenecks, caution should be used in making evolutionary interpretations or management recommendations based on temporal changes in allele frequencies.*

Cambios temporales en frecuencias de alelos y la historia de una población con serveros cuellos de botella

Resumen: *El monitoreo de cambios temporales en variación genética ha sido sugerido como un medio para determinar si una población ha experimentado un cuello de botella demográfico. Simulaciones han mostrado que la varianza en frecuencias de alelos a través del tiempo (*F*) puede proveer estimaciones razonables del tamaño poblacional (*N_e*). Esta relación entre* F *y* N_e *sugiere que cambios en las frecuencias de los alelos pueden proveer una vía para determinar la severidad de cuellos de botella demográficos experimentados recientemente por una población. Para evaluar la relación entre la severidad de cuellos de botella demográficos y variaciones temporales en la frecuencia de alelos, analizamos la variación de alozimas en poblaciones experimentales de* Gambusia holbrooki*. Estimaciones de* F *obtenidas de la población de peces y por simulaciones de computadora fueron comparadas para tasas de derivación esperadas. Encontramos que los diferentes métodos empleados para la estimación de* F *tuvieron poco efecto en el análisis. La varianza en estimaciones de* F *para poblaciones sujetas a cuellos de botella demográficos similares fue grande entre las poblaciones experimentales y las simuladas. Cambios temporales en las frecuencias de alelos sugieren que las poblaciones experimentales han experimentado cuellos de botella, pero no existió relación entre los valores de* F *observados y los esperados. Más aún, la deriva génica fue aparentemente subestimada para las poblaciones que experimentaban los cuellos de botella más serveros. La débil relación entre* F *y la severidad del cuello de botella probablemente se deba a una error de muestreo asociado con el número de loci polimórficos*

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examinados y el número de alelos durante el cuello de botella. Se deberá ser cauteloso al hacer interpretaciones evolutivas o recomendaciones de manejo basadas en cambios temporales en las frecuencias de alelos para poblaciones que podrían haber experimentado cuellos de botella severos.

Introduction

The size of a population can have a large influence on its genetic variation and therefore on its evolution. Populations that experience large reductions in effective size, referred to as demographic bottlenecks, are expected to lose genetic diversity. Loss of this variation can affect both immediate and long-term population viability (Wayne et al. 1986; Leberg 1991; Borlase et al. 1993). Several conservation and resource biologists have stressed the importance of monitoring the effects of demography on genetic diversity (Smith et al. 1976; Meffe 1986; Wayne et al. 1986; Allendorf et al. 1987).

Common measurements used for monitoring the genetic diversity of populations include heterozygosity, percent polymorphic loci, and numbers of alleles per locus. Experimental studies suggest that although these measures of genetic diversity tend to decrease as a result of population bottlenecks (McCommas & Bryant 1990; Leberg 1992), large sampling variance means they will be only weak predictors of population history (Leberg 1992). Thus, more-sensitive approaches are needed to better monitor the influence of bottlenecks on genetic variation.

Monitoring temporal changes in allele frequencies has also been suggested as a means of assessing changes in the genetic composition and demographic history of populations (Allendorf & Ryman 1987; Waples & Teel 1990; Hedgecock et al. 1992). The amount of genetic drift experienced by a population is inversely related to its effective size (Wright 1931; Wright 1938). Because of this relationship, it may be possible to estimate N_e, the effective population size, from F, the variance in allele frequencies over time (Krimbas & Tsakas 1971; Nei & Tajima 1981; Pollak 1983; Waples 1989). Although F and N_e have been estimated in natural and captive populations (Montchamp-Moreau & Katz 1987; Waples 1990; Hedgecock et al. 1992), relationships between population size and genetic drift are best understood from theoretical models and computer simulations (Pamilo & Varvio-Aho 1980; Nei & Tajima 1981; Pollak 1983; Waples 1989; Jorde & Ryman 1995).

Conservation biologists are often interested in genetic drift in populations that have experienced severe demographic bottlenecks; but most previous simulations of the relationship between F and N_e have examined populations where $N_e \geq 50$. Our objective was to determine whether temporal changes in allele frequencies reflect a population's history of severe demographic bottlenecks. We compared estimates of F obtained from experimental and simulated populations to theoretical expectations. A strong positive correlation between observed and expected changes in allele frequencies would suggest that monitoring of changes in allele frequencies would help conservation biologists determine if a population had experienced a severe demographic bottleneck. Failure to detect such a relationship would indicate that caution should be used when inferring a population's demographic history from observed rates of genetic drift.

Methods

Experimental Populations

We examined changes in allele frequencies in populations of the eastern mosquitofish (*Gambusia holbrooki*) that were established to study the effects of bottlenecks on population viability and heterozygosity (Leberg 1991; Leberg 1992; Leberg 1993). Three stock populations were established with offspring of pregnant females captured from the Savannah River in South Carolina. Offspring from these experimental stocks were selected at random to establish experimental populations. These populations were housed in circular pools (2.4 m wide by 30 cm deep) designed to replicate small ponds (Leberg 1992). Generation time was sufficiently short (60 days) for three generations of fish to be produced during one experimental period (Leberg 1992). Five treatments, representing bottlenecks of different severity, were established in the mesocosms (Fig. 1). These treatments are described more fully in Leberg (1992) and Richards (1994). Fecundity was high, so populations grew rapidly. After only 150 days, populations founded with only six fish usually contained 150–250 individuals (Leberg 1993).

An electrophoretic survey using 43 isozyme loci revealed six or seven polymorphic loci, depending on the genetic stock, that were consistently resolvable (Leberg 1992). The allele frequencies of these loci were determined from 100 fish sampled randomly from each of the three stock populations (Fig. 1). The allele frequencies of each of the experimental populations were determined for a sample of 45 individuals collected 2–3 generations after the last bottleneck event. Allele frequencies from the stock and mesocosm populations are presented in Leberg (1992).

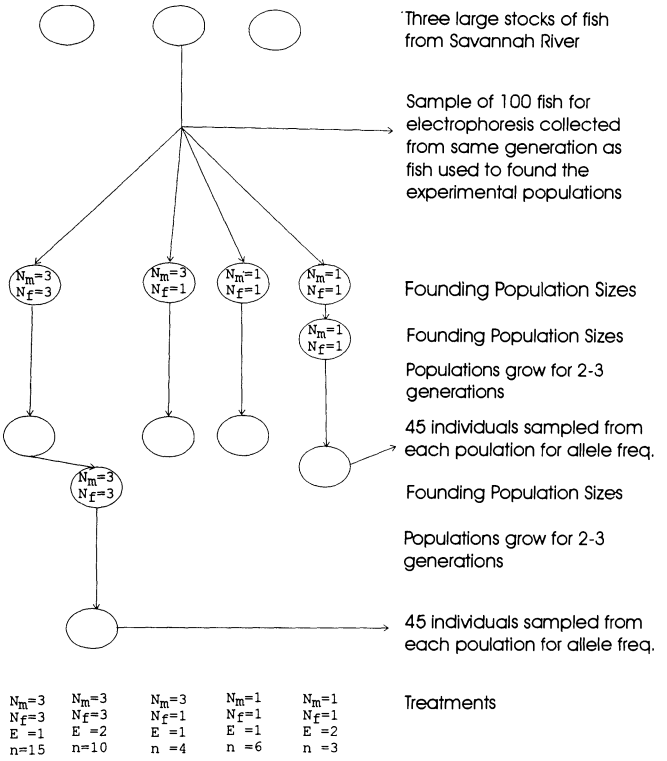

Figure 1. History of populations of mosquitofish used to study the effects of bottlenecks on F. *Treatments are identified by numbers of male and female founders* N_m *and* N_f*, respectively) and the number of bottleneck events the population experienced* (E)*. The total number of replicates* (n) *is also provided. Each of the stocks was used in some of the experimental treatments, although bottleneck treatments are shown for only one. A more detailed description of the source stocks and treatments is found in Leberg (1992) and Richards (1994).*

We used two methods to estimate F. Nei and Tajima (1981) estimate F as

$$\hat{F}_c = \frac{1}{K} \sum_{i=1}^{K} \frac{(x_i - y_i)^2}{(x_i + y_i)/2 - x_i y_i},$$

where K is the number of segregating alleles at a locus, and x_i and y_i are the allele frequencies of the *ith* allele in generation 0 and t, respectively. An alternative estimator of F, proposed by Pollak (1983), is

$$\hat{F}_k = \frac{1}{K-1} \sum_{i=1}^{K} \frac{(x_i - y_i)^2}{(x_i + y_i)/2}.$$

For each population, F_c and F_k were obtained for each locus. Mean estimates of F_c and F_k across these loci were calculated (Waples 1989); these multiple-locus estimates of F were used in all statistical analyses. All loci that were polymorphic in the stock populations were used to estimate F, regardless of whether or not they became fixed during the bottleneck event.

Determination of the theoretical expectations of F for the experimental treatments is problematic for three reasons. First, populations were allowed to grow after they were established, so N_e was not constant across generations. Second, populations of mosquitofish also had overlapping generations, complicating the relationship between F and population size (Waples 1989; Jorde & Ryman 1995). We know only the minimum generation times for these populations, not the actual number of generations that occurred between the allozyme samples. Finally, it was impossible to estimate accurately the effective size of the populations in any generation because both sex ratios and reproductive variance among individuals were largely unknown.

Because of the complications in determining the amount of genetic drift occurring after a bottleneck, we examined qualitative relationships between observed values of F with expected values resulting from the experimental bottlenecks. Because allele frequencies prior to the bottlenecks were determined by sampling without replacement (Plan II, Waples 1989), the expected change in allele frequencies, F, is $t/(2N_e + 1/2S_0\ 1/2S_t)$, where N_e is the effective population size, t is the number of generations between samples, and S_0 and S_t were the sample sizes of individuals used to estimate the change in allele frequencies at 0 and t generations (Waples 1989). To estimate the amount to genetic drift expected due to the experimental bottlenecks, we replaced t with E, the number of experimental bottlenecks, and S_0 and S_t became the sample sizes used to estimate allele frequencies at the beginning and end of the experiment, respectively. Because S_0 and S_t were the same for each experimental population (100 and 45 individuals, respectively), the quantity $1/2S_0 + 1/2S_t$ was a constant (0.016) that had no effect on the statistical analysis. For the treatment in which three males and one female founded each population, N_e of the founders was estimated as $4N_m \times N_f/(N_m + N_f)$, where N_m and N_f are the numbers of males and females, respectively. When sex ratios of founders were equal, we assumed that the effective size of the founding population equalled the number of founders. We also assumed that most of the temporal changes in allele frequencies in the populations were due to the experimental bottlenecks.

Computer Simulations

A model designed to simulate the dynamics of fish populations in the mesocosms was used to assess effects of the experimental treatments on allele frequencies. The complete model is provided in Richards (1994) and is described by Leberg (1992). For each experimental treatment applied to a genetic stock of fish, 300 replicate populations were created with the simulation model (Leberg 1992). Because allele frequencies were based on 100 individuals from each genetic stock and 45

individuals for each experimental population, the same number of individuals was sampled from the simulated populations. As in the case of the experimental populations, allele frequencies were sampled prior to reproduction before bottleneck events and again after three generations of population growth. Unlike the experimental populations, the estimates of allele frequencies before the bottlenecks ($t = 0$) were based on sampling with replacement. Expectations of F under the plan II sampling scheme still apply (Waples 1989), however, because the population sizes of genetic stocks in the simulations were infinitely large. Other aspects of estimation of F were identical to those used for the experimental populations.

To evaluate the effects of sample size on the variance of $\hat{F}$ among replicates experiencing the same bottleneck, Var($\hat{F}$), we estimated F for both a sample of 45 individuals and the entire simulated population. In the latter case allele frequencies were measured without sampling error. The simulation model was also used to evaluate the effects of population growth after the experimental bottleneck on estimates of $\hat{F}$. Estimates of $\hat{F}$ from the simulations of fish populations, which grew rapidly, were compared to estimates from simulated populations in which there was no growth after the bottleneck events. In the latter case 45 offspring resulting from the random union of gametes from individuals founding the population were sampled to obtain allele frequencies. For these simulated populations, unlike the simulations of the experimental populations, t equaled E.

Statistical Analyses

The $\hat{F}_c$ and $\hat{F}_k$ are expected to have skewed distributions (Waples 1989). Therefore, nonparametric Spearman's rank correlation tests were used to examine correlations between the two estimates of F and between the observed and expected values of F. A one-sample sign-rank test was used to determine if differences between the expected and observed values of F were significantly different from zero for each bottleneck treatment. In the case of the experimental populations only a few replicates were available in some of the treatments. Therefore, in comparisons of observed and expected values of F using the sign-rank test, replicates of treatments $N_m = 3$, $N_f = 3$, $E = 2$, and $N_m = 3$, $N_f = 1$, $E = 1$ were combined, as were replicates of $N_m = 1$, $N_f = 1$, and $E = 1$ or 2.

Results

Experimental Populations

The correlation between $\hat{F}_c$ and $\hat{F}_k$ was very high ($R = 0.996$, $p = 0.0001$), and the choice of estimators made no difference in the outcome of comparisons discussed below. Hedgecock et al. (1992) suggest that estimates of drift could be based on F_c for loci with two alleles and on F_k for loci with three or more alleles. Differences between the two estimates of F were so small, however, that this exercise does not affect our results. For simplicity, we use $\hat{F}_c$ in all subsequent analyses.

All of the populations had estimates of F that were much larger than expected on the basis of sampling error (0.016; Fig. 2). This suggests that the allele frequencies were being strongly affected by small population sizes. There was, however, high within-treatment variance in $\hat{F}$ (Fig. 2) and considerable overlap in $\hat{F}$ among the different treatments. There was also no correlation between observed values of F and those expected to result from the experimental bottlenecks ($R = 0.162$, $p = 0.484$). The estimates of $\hat{F}$ were not significantly different from the expected value for the least severe bottleneck ($N_m = 3$, $N_f = 3$, $E = 1$, $p = 0.437$). But estimates

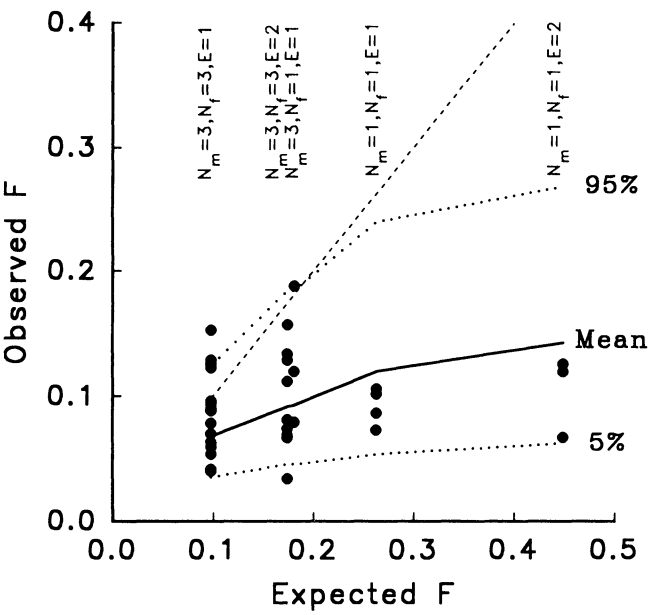

Figure 2. Comparison of estimates of F *from the populations of mosquitofish (data points) with expected estimates of* F *based on the number of individuals founding the population as well as the number of bottlenecks it experienced. The solid line is the mean of the estimates from the simulated populations, and the dotted lines represent their distribution. The percentage of estimates for the simulated populations occurring below a dotted line is given to the right of each line. The number of male and female founders (*N_m *and* N_f*) and the number of bottlenecks (*E*) are provided above the data points associated with each experimental treatment. The dashed line represents the values of* F *expected to result from the experimental bottlenecks.*

for the other combinations of treatments were significantly less than the expected values of F ($p < 0.02$).

Computer Simulations

As with the experimental populations, estimates of genetic drift were much less than would have been expected based on the severity of the bottlenecks experienced by the simulated populations. This bias became larger as the severity of the bottleneck increased (Fig. 2). Only for the least severe bottleneck treatment did the mean of F approach the expected value, and the estimates were significantly smaller than the expectations for all treatments ($p < 0.001$). Also, as in the case of the experimental populations, the within-treatment variance of $\hat{F}$ for the simulated populations was large (Fig. 2).

The distribution of $\hat{F}$ from the simulated and experimental populations were similar (Fig. 2). Estimates for the experimental populations all occurred within the range of F from the simulated populations. All but three of the estimates from the experimental populations fell within the range around the mean where 90% of the estimates from the simulations occurred (Fig. 2).

Results of the simulations to evaluate the effects of sample size on the Var($\hat{F}$) for the two most several bottleneck treatments (Table 1) are comparable to the remaining treatments that are not presented. Var($\hat{F}$) was reduced only slightly when estimates were based on the entire population (Table 1). Sampling error resulting from use of 45 fish to estimate allele frequencies explains little of the large variance in $\hat{F}$ among populations experiencing identical bottlenecks.

There is little evidence that population growth after the bottlenecks greatly increased the bias of $\hat{F}$ (Table 1). Because the bias increased with the severity of the bottlenecks, only comparisons of the two most severe bottleneck treatments are presented. Estimates of F did increase in simulated populations in which there was no population growth following the bottleneck (Table 1). These values of $\hat{F}$, however, were still substantially less than expected from $t/(2N_e + 1/2S_o + 1/2S_t)$. Variance in $\hat{F}$ among replicates increased slightly in populations in which there was no population growth following the experimental bottlenecks (Table 1).

Table 1. Comparison of mean $\hat{F}$ and its variance among replicates.*

	$N_m = 1, N_f = 1, E = 1$		$N_m = 1, N_f = 1, E = 2$	
Source of F and simulation	$\hat{F}$	*Var*($\hat{F}$)	$\hat{F}$	*Var*($\hat{F}$)
$t/(2N_e + 1/2S_o + 1/2S_t)$	0.266	——	0.516	——
Simulation of experimental populations	0.120	0.002	0.144	0.004
populations with no sampling error	0.120	0.002	0.142	0.004
populations with no growth following bottlenecks ($E = t$)	0.142	0.003	0.157	0.005

**From the simulations of the two most severe experimental bottlenecks to the expectation from $t/(2N_e + 1/2S_o + 1/2S_t)$, simulations in which allele frequencies were estimated without error, and simulations in which populations did not grow for 2–3 generations following the bottlenecks.*

Discussion

There was no correlation between expected values and $\hat{F}$ from the experimental populations. The lack of positive relationship was due partially to the high variance of $\hat{F}$ among populations within the same treatment. This variability makes it impossible to distinguish between individual populations established with large and small numbers of founders. Furthermore, $\hat{F}$ was usually lower than expected values. As in the experimental populations, $\hat{F}$ for the simulated populations was smaller than expected and was highly variable among populations with the same history of bottlenecks.

Explanations for Bias and High Variance of $\hat{F}$

The lack of concordance of $\hat{F}$ with theoretical expectations, as well as the large Var($\hat{F}$), probably result from sampling error associated with estimates of genetic drift based on only a few polymorphic loci. Based on simulations, Pamilo and Varvio-Aho (1980) believed it unlikely that enough polymorphic loci could be examined to obtain useful estimates of genetic drift and N_e. Nei and Tajima (1981) suggested that more than 20 polymorphic loci would be needed to obtain estimates that were highly correlated with expected values. We examined drift at 6–7 polymorphic loci. Although this number is small relative to the recommendations of Nei and Tajima (1981), it is similar to the number of loci examined in most studies (Mueller et al. 1985; Montchamp-Moreau & Katz 1987; Hedgecock et al. 1992) that employ the temporal changes in allele frequencies to estimate genetic drift (x = 6.8 loci, range 3–14). If these studies had examined loci with large numbers of alleles, fairly precise estimates of F might be possible; as in our study, however, the majority of the allozyme loci had less than four alleles per population.

Increasing the number of individuals used to estimate allele frequencies has been proposed as a solution to improve the precision of $\hat{F}$ (Waples 1989). This would have had virtually no effect in our study. In the case of the simulated populations, allele frequencies based on a sample of 45 individuals were very similar to those for the entire population and contributed little to Var($\hat{F}$).

Although sampling error due to small numbers of

polymorphic loci or independent alleles may have produced the high Var($\hat{F}$), it is not clear how this error would have produced the biased estimates of F. The most likely explanation for the bias in $\hat{F}$ for the experimental and simulated populations is the extinction of alleles during the experimental bottlenecks. The loss of alleles places an absolute limit on the temporal change in allele frequencies that can result from a bottleneck. The more alleles that become extinct, the greater the underestimate of $\hat{F}$. This may explain why the bias of $\hat{F}$ was greatest in the bottleneck treatments founded by a pair of siblings, because the average number of alleles that were lost (8.6 alleles out 15) was also highest in this treatment (Leberg 1992). Allele extinction would be much less uncommon when effective population size is greater than 50, so this bias would not be observed in studies that modeled genetic drift in larger populations (Waples 1989). Waples (1989) did observe, however, that changes in the frequencies of alleles that had initial frequencies near 0 and 1 produced the most biased estimates of N_e; it is in these cases that allele extinction is most likely.

Other Assumptions and Considerations

We have noted that our experimental system did not meet all of the assumptions of the temporal method, so a discussion is warranted of how violation of those assumptions might have affected our results. For example, we assumed that little of the Var($\hat{F}$) was due to drift after the bottlenecks because populations grew rapidly, but some drift must have occurred during this recovery period. Although variation in the growth rate of mesocosm populations may have produced some of the variability in $\hat{F}$ among replicates, differential growth rates were not a major contributor to this large variance because the populations within each bottleneck treatment had similar growth rates (Leberg 1992; Leberg 1993). Furthermore, the within-treatment variance in $\hat{F}$ was also large in the simulated populations in which growth rate was constant across all populations. Finally, simulations indicate that drift during the generations following the bottlenecks did not increase Var($\hat{F}$).

Drift occurring after the experimental bottlenecks also could not explain why F is small relative to expectations. Our simulations suggest that only a small portion of the bias in $\hat{F}$ is attributable to the period of population growth following the bottlenecks. Also, because populations founded with one female tended to grow slower than those established with three females, the effects of drift after the bottlenecks would have been greatest in populations experiencing the most severe bottlenecks. It is in these treatments, however, that the observed rate of drift is much less than that expected based solely on population size during the bottleneck.

We also assumed that the effective size during the experimental bottlenecks was equal to the number of founders, with the exception of adjustments for unequal sex ratios. It seems likely that effective population size was sometimes smaller than the number of founders. There are two reasons, however, why differences between founder number and effective size could not account for the Var($\hat{F}$) or the underestimates of F in the most severe bottlenecks. First, N_e equaled N during the bottlenecks in the computer simulations, yet the within-treatment variance and the bias in $\hat{F}$ were similar for the simulated and experimental populations. Second, N_e had to equal founder number for the populations established with only a pair of fish, but it is in these populations that the deviation between observed and expected values of F is the greatest.

One major concern in estimating genetic drift should be errors in genotype determination, because such errors would result in erroneous estimates of allele frequencies before and after the experimental bottleneck. If errors in genotype determination were random, they would result in overestimates of the amount of genetic drift that had occurred. We avoided this problem by examining only loci for which genotype determination was not ambiguous. Furthermore, our observed rates of genetic drift were lower than expected values, opposite of the result usually expected from errors in genotype determination. Finally, results from the experimental populations were similar to those from the computer simulations in which genotypes were assigned without error.

Selection could possibly have produced genetic change within the experimental populations, but it was not included in the simulation model. The similarity of bottleneck effects on genetic diversity between the experimental and simulated populations in both our study and that of Leberg (1992) suggests that genetic drift—not selection—was the major evolutionary force influencing changes in allele frequencies.

Most applications of the temporal method assume discrete generations (Waples 1989). There appeared to be a consensus, however, that the relationship of $F = 1/2N_e$ holds reasonably well for populations that have overlapping generations as long as age structures are relatively stable (Nei & Tajima 1981; Waples 1989). Recently, Jorde and Ryman (1995) have argued that overlapping generations can contribute to temporal variance in allele frequencies. In their model, populations with overlapping generations would have higher values of F than expected on the basis of N_e alone. It is hard to see how this model would explain the low values of F observed in our study. We can also see no reason why a changing age structure would cause the large variability of $\hat{F}$ within treatments because the replicate experimental populations experienced similar demographic trends, and because the age structures in the simulated populations were identical. The changing demography of our

populations could have biased $\hat{F}$, but Pollak (1983) believed that such changes would lead to an overestimate of F, not the underestimates we observed. Finally, our simulations in which $t = E$ (discrete generations) produce estimates of F similar to those of the simulations of the fish populations, suggesting that changing age structures or overlapping generations had little effect on the bias or the within-treatment variance of $\hat{F}$.

Finally, although $\mathrm{Var}(\hat{F})$ is large, it probably underestimates the true variance. Because we sampled allele frequencies in the stock populations only once, sampling errors resulting from independent sampling to determine x_i for each experimental population were not included in our estimates of drift. This would underestimate the true within-treatment variance of F (R. S. Waples, personal communication).

Implications

Temporal variation in allele frequencies should reflect the effective number of breeding individuals in a population between sampling events. Because this relationship would be most efficiently measured in small populations, Waples (1989) felt that estimation of temporal changes in allele frequencies would have many applications in conservation biology. But if populations become small enough to make loss of alleles likely, the relationship between $\hat{F}$ and N_e apparently weakens. We find large variance in F populations experiencing the same history of bottlenecks, and we find underestimates of $\hat{F}$ in populations experiencing severe bottlenecks. These observations call into question the usefulness of temporal surveys of allele frequencies under the conditions we examined.

Studies of evolutionary processes such as natural selection and genetic drift may be adversely influenced by imprecise estimates of F. Temporal changes in allele frequencies have often been used to evaluate the relative influence of drift and selection. On the basis of whether $\hat{F}$ agreed or disagreed with expectations, drift or selection, respectively, are usually invoked as the major influence on the genetic variation in a population. It is possible that the results of such studies have been influenced by both allele extinction and the examination of small numbers of polymorphic loci.

Unfortunately, the number of polymorphic allozyme loci that can be consistently resolved from a population is limited. Based on Nevo et al. (1984), most studies (90%) examine 11 or fewer polymorphic loci per population. The average number of polymorphic loci used per study was six. A smaller survey of more-recent allozyme studies found that the average number of polymorphic loci examined per population was only four (Leberg 1996). In our study we could consistently resolve polymorphism at only 7 of the 43 loci examined. Perhaps analysis of highly polymorphic loci such as microsatellite loci (Bruford & Wayne 1993) will greatly expand our ability to examine polymorphic loci and independent alleles. Examination of a large number of alleles at a few microsatellite loci might be a more practical way of reducing $\mathrm{Var}(\hat{F})$ than the analysis of a much larger number of less polymorphic allozyme loci.

Allele extinction should also be of concern to conservation biologists using temporal surveys to gain insight into a population's history. If the population has gone through a severe bottleneck, as is often the case with endangered taxa, alleles can often be expected to become lost or fixed. If this occurs at several loci, it will indicate that the population has gone through a bottleneck. It would be unwise, however, to use $\hat{F}$ to determine just how small the effective size was during the bottleneck because allele extinction would result in large underestimates of the genetic drift—and thus the bottleneck—experienced by the population.

The usefulness of the temporal assays of allele frequencies for conservation and evolutionary biologists will depend on how precise an estimate of genetic drift is needed and how small the effective population size was between the sampling periods. For populations large enough to make allele extinction uncommon, reasonable estimates of genetic drift and demographic history may be possible from temporal shifts in allele frequencies (Waples 1989; Jorde & Ryman 1995), given that enough alleles or polymorphic loci can be examined. But when only a few polymorphic loci or alleles are available for examination, or when a population has experienced a bottleneck sufficient to cause loss of alleles, examination of temporal changes in allele frequencies may be misleading.

Our study of severe bottlenecks suggests that monitoring changes in allele frequencies would indicate that a population had experienced a reduction in size; this would probably be sufficient for many conservation applications. Furthermore, this would be an improvement over monitoring multiple-locus heterozygosity, which was relatively insensitive to the types of bottlenecks we examined (Leberg, 1992). But temporal variation in allele frequencies provided little information about the relative magnitude of the bottlenecks. In situations in which populations have experienced severe bottlenecks, the number of alleles lost is a much better predictor of bottleneck size (Leberg 1992). Although the relationship between bottleneck severity and the number of alleles lost was imprecise for our populations (Leberg 1992), the correlation ($r = 0.77$) was far stronger than the relationship between bottleneck severity and temporal variation in allele frequencies. This observation does not negate the importance of genetic monitoring in small populations. Rather, it suggests that the most sensitive measure of demographic effects on genetic variation will change with population size.

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