Temporal Allele Frequency Change and Estimation of Effective Size in Populations With Overlapping Generations

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ABSTRACT

In this paper we study the process of allele frequency change in finite populations with overlapping generations with the purpose of evaluating the possibility of estimating the effective size from observations of temporal frequency shifts of selectively neutral alleles. Focusing on allele frequency changes between successive cohorts (individuals born in particular years), we show that such changes are not determined by the effective population size alone, as they are when generations are discrete. Rather, in populations with overlapping generations, the amount of temporal allele frequency change is dependent on the age-specific survival and birth rates. Taking this phenomenon into account, we present an estimator for effective size that can be applied to populations with overlapping generations.

THE effective population size is a fundamental concept in theoretical and applied population genetics. In spite of its importance, however, the effective size has been notoriously difficult to estimate in natural populations. Recent interest has been devoted to the possibility of estimating effective size from temporal changes of allele frequencies, the so-called temporal method (KRIMBAS and TSAKAS 1971; BEGON *et al.* 1980; NEI and TAJIMA 1981; POLLAK 1983; TAJIMA and NEI 1984; WAPLES 1989). This method is based on the following logic: if genetic drift is the only cause of allele frequency change over time, then effective population size can be estimated from empirical observations of temporal frequency shifts of selectively neutral alleles.

The theoretical development of the temporal method has primarily been confined to populations with discrete generations, although some authors have suggested that the basic theory should also be valid for populations with overlapping generations (NEI and TAJIMA 1981; POLLAK 1983). On the other hand, recent studies have indicated that temporal fluctuations of allele frequencies follow more complicated patterns when generations overlap than in the case of discrete generations (WAPLES 1990a,b; WAPLES and TEEL 1990). Those analyses, however, which were largely based on computer simulations, were directed toward the fairly unusual (semelparous) life history characteristics of Pacific salmon populations. Therefore, the generality and relevance of those results for other organisms are unclear.

In this paper, we examine the validity of applying discrete-generation theory for estimation of effective size of populations with overlapping generations. Our analysis concentrates on temporal allele frequency changes over relatively short periods of time relative to the lifespan of the organism, and we focus particularly

on shifts between consecutive cohorts. The emphasis on short time intervals is because we are interested in exploring the possibilities of obtaining an estimate of effective size without having to sample the population at intervals several generations apart, a practical consideration particularly important for long-lived organisms. Further, as indicated by WAPLES' analyses, in situations with overlapping generations the methods developed for discrete generations are most likely to lead to erroneous results when the time span between observations is short.

The presentation is structured as follows. First, we define the concept of effective population size and describe a simple model for populations with overlapping generations. Using this model we present the results of some computer simulations that illustrate the basic difference between populations with discrete and overlapping generations with respect to temporal shifts of allele frequencies. Second, we derive analytical expressions for quantifying genetic drift in terms of variances and covariances of allele frequencies. Next, we address the problem of relating observed allele frequency changes to the true effective population size considering samples of individuals. The results are incorporated into a general estimation procedure for effective size that is applicable to populations with discrete as well as with overlapping generations. Finally, we give a numerical example and discuss potential biases and limitations of the present approach.

THE MODEL

Effective population size: For definition of effective population size the pertinent reference is the simple Wright-Fisher model for finite populations (EWENS

1979). In this model the $2N$ genes in a particular generation are obtained by random binomial sampling from those present in the previous generation (EWENS 1979, p. 16). This sampling process is assumed to be the only cause of genetic change, implying that there is no mutation, migration or selection of genes. This model is often conceptualized through considering an infinite number of replicate populations of the same size (N) and initial frequency q of a particular allele (A_1). In a later generation (t) allele frequencies have drifted apart, and the variance of the frequency of A_1 among the replicate populations is defined as the drift variance, denoted $\sigma^2(t)$. After an infinite number of generations ($t = \infty$), a proportion q of populations are fixed for A_1 and the others ($1 - q$) are fixed for the alternate allele (A_2), so that $\sigma^2(t)$ has reached its limiting value of $q(1 - q)$. As long as the two alleles are still segregating, the drift variance increases with an amount that is constant relative to the amount remaining to be fixed (HILL 1979). Mathematically, this can be represented as the rate (cf. CHOY and WEIR 1978)

$$\frac{\sigma^2(t+1) - \sigma^2(t)}{q(1 - q) - \sigma^2(t)} = \frac{1}{2N}. \quad (1)$$

In accordance with the simple Wright-Fisher model, we define the variance effective population size by replacing N in Equation 1 with an effective number that reflects the true rate of increase of $\sigma^2(t)$ in the population under consideration, and we use this definition for populations with discrete as well as with overlapping generations. When time (t) is measured in generations, Equation 1 defines the effective size per generation (N_e). For populations with overlapping generations, however, time may be more conveniently measured in years (or some other time unit), in which case l defines the annual effective size (N_a), which is related to N_e through (HILL 1979)

$$N_e \approx \frac{N_a}{G}, \quad (2)$$

where G is the generation length (the average age of parents). Throughout this paper we assume a breeding interval of 1 year and measure time (t) in years. In this case N_a equals N_e when generations are discrete (implying $G = 1$). When generations overlap ($G > 1$) N_a is larger than N_e and corresponds to the size of a simple Wright-Fisher population with discrete generations and the same amount of drift per year (HILL 1979).

It should be noted that l does not necessarily yield a value for N_a (or N_e) that is constant over time. In particular, when generations overlap the rate of change of $\sigma^2(t)$ tends to fluctuate initially (*i.e.*, when t is small) and will only asymptotically approach a constant value

FIGURE 1.—Schematic description of the population model. Circles represent age groups consisting of N_i individuals, continuous arrows indicating survival, and dotted arrows reproduction from each particular age group. Columns of age groups constitute the total population in various years, rows the age classes (i) and diagonals (from upper left to lower right) represent cohorts, *i.e.*, individuals born in the same year.

(FELSENSTEIN 1971; CHOY and WEIR 1978; see below). This reflects a true population characteristic, however, and can easily be accommodated by admitting that the effective size is not constant through this asymptotic phase [see CHESSER *et al.* (1993) for a similar phenomenon arising in subdivided populations]. In any case, the time needed to reach a constant N_e is short for most populations, typically no more than a few generations.

The population model: To analyze the process of genetic drift in populations with overlapping generations, we define a model that incorporates the pertinent attributes of such populations. We do this by maintaining the characteristics of the simple Wright-Fisher model so that we can focus the analysis on the effect of overlapping generations, unaffected by other factors that are discussed later.

Specifically, we consider a population of monoecious diploids with constant size and age structure (Figure 1). At any time t there are k age classes with N_i individuals in the i th class ($1 \leq i \leq k$), the total population consisting of $N = \sum N_i$ individuals. We take time to be measured in years and count individuals and genes just before reproduction so that individuals are counted for the first time when they are 1 year old. The individuals born in a particular year constitute a cohort. We call individuals of age 1 “newborns” and those that are older “adults,” only to indicate that the former have entered the population after the previous count.

Reproduction is through random union of (selectively neutral) genes and occurs once every year. On average, an individual of age i produces $2b_i$ gametes that are incorporated into (diploid) offspring that survive to age 1. A total of N_1 newborn individuals enter the population each year, with $N_1 = \sum b_i N_i$. The fraction of newborns that survive to age i is l_i with $N_i = N_1 l_i$ and

$l_i = 1$ according to the definition of b_i . Because the population is of constant size, we have $\sum l_i b_i = 1$. Thus our model is stochastic with respect to genetic change but not with respect to population size and age structure. Noting that the product $l_i b_i$ is the probability that a gene in an individual was inherited from a parent of age i (FELSENSTEIN 1971), we call this product p_i . Reproduction is independent of mortality in the sense that whether or not an individual has reproduced affects neither its survival nor its future reproduction. The mean generation length is $G = \sum p_i i$ years, *i.e.*, the mean age of parents. This model is similar to those considered by FELSENSTEIN (1971), JOHNSON (1977), CHOY and WEIR (1978), EMIGH (1979) and HILL (1979).

Genetic drift when generations overlap: a simulation: As indicated above, the basic question when extending the temporal method to populations with overlapping generations may be put as follows: Is the direct relationship between effective size and allele frequency change that holds for discrete generations also true when generations overlap? To address this question, we performed a number of computer simulations based on the present population model.

All simulations, including those described subsequently, were performed by random drawings of the required (constant) number of genes for reproduction and death according to the set of parameters N_1 , b_i and l_i particular for each population. The drawings were done with and without replacement for reproduction and mortality, respectively. Simulated populations were initialized with the same allele frequency, q , in all age classes. Simulations were run for a sufficient number of years (≥ 50) to assure that the effective size (1) had stabilized before starting the analysis of allele frequency change. Sampling of individuals from the population was simulated by random drawing of the required number of genes from each age class, and sample allele frequencies were determined by counting alleles. Sampled genes were returned to the population before the next reproduction cycle. Each run was taken to represent one gene locus, and the entire procedure was repeated for each additional locus.

Simulation results show that when generations overlap there is not necessarily a direct relationship between effective size and temporal allele frequency fluctuations. In contrast to the discrete generation situation, populations with the same effective sizes and generation lengths may display dramatically different temporal shifts of allele frequencies. An example is illustrated in Figure 2, depicting simulation results for two populations of similar effective size, $N_e \approx 200$, that differ only in their age-specific birth rates. [Here, N_e has been computed according to the approximate formula of FELSENSTEIN (1971); applying our definition (1), as explained below, N_e becomes 199.9 and 206.2 for popu-

FIGURE 2.—Temporal allele frequency changes in two simulated populations with overlapping generations that are characterized by similar effective sizes and generation lengths. A total of $N_1 = 100$ newborns enter the populations each year and survive until age 4, when they all die, so that $l_i = 1$ ($i = 1 \dots 3$) for both populations. The two populations differ in their age-specific birth rates with $b_1 = b_2 = b_3 = 1/3$ for population I and $b_1 = 0.01$, $b_2 = 0.98$ and $b_3 = 0.01$ for population II. The mean generation length G is 2 years and the effective size per generation, $N_e \approx 200$, for both populations. (Exact values for N_e are 199.9 and 206.2 for population I and II, respectively; see text for details.) Graphs are given for the allele frequencies of separate age classes and for the total population, starting in year $t = 300$, and typify the results of one single run (or locus).

lations I and II, respectively.] Clearly, the two populations have very different amount of temporal allele frequency change, within age classes as well as for the total population. Further, the apparently nonrandom pattern of annual shifts, particularly obvious for population II, strongly suggests that those shifts are not exclusive reflections of random genetic drift. These observations imply that the relationship between short-term allele frequency change and effective size is not a simple one in populations with overlapping generations. Thus, before applying the temporal method to such populations this relationship must be examined in greater detail.

EXPECTED AMOUNT OF GENETIC DRIFT

In the present population model each individual can be classified as belonging to a particular cohort, the different cohorts comprising individuals born in different years and by different sets of parents. Reproduction is independent between years, and temporal changes in allele frequency must be considered separately for each age class. As before, we assume that there are two selectively neutral alleles, A_1 and A_2 , and denote the initial ($t = 0$) frequency of A_1 by q . Assuming also that this frequency is the same for all age classes, the expected allele frequency remains unchanged by genetic drift alone (CROW and KIMURA 1970, p. 333). The magnitude of allele frequency change of the total population in year t relative to the initial frequency is quantified by the drift variance [$\sigma^2(t)$] as defined in

Equation 1, which is initially zero. Similar to the drift variance of the total population, we also define analogous drift variances $[\sigma_i^2(t)]$ for each age class i . Greek letters are used to indicate that these are population characteristics as opposed to sample variances, which is discussed later.

The frequency of A_1 in the total population is the average of the frequency in each age class weighted by their number of individuals (N_i) relative to the total (N). Calculating the average of drift variances, being squared measures of allele frequency, requires a more complex procedure (CROW and KIMURA 1970, pp. 484–485; ELANDT-JOHNSON 1971, p. 112). Thus, for the total population in year t ,

$$\sigma^2(t) = \sum_{i=1}^k \left(\frac{N_i}{N} \right)^2 \sigma_i^2(t) + 2 \sum_{i=1}^k \sum_{j>i}^k \frac{N_i N_j}{N^2} \sigma_{ij}(t). \quad (3)$$

Here, the quantity $\sigma_{ij}(t)$ designates the covariance between the allele frequencies of age classes i and j in year t . This covariance appears when individuals in the two age classes are related by descent, as they usually are (below).

If we note that $\sigma_i^2 \equiv \sigma_{i,i}$, Equation 3 can be written in the more condensed but equivalent form:

$$\sigma^2(t) = \sum_{i=1}^k \sum_{j=1}^k \frac{N_i N_j}{N^2} \sigma_{ij}(t),$$

where $\sigma_{ij} = \sigma_{ji}$.

To evaluate (3), we derive the transition equations for the variance and covariance of allele frequencies within and between age classes. We note that the drift variances for newborns and adults are affected by two different processes, *viz.* sampling of genes at reproduction and sampling of surviving individuals in later years.

Reproduction: Reproduction can be conceptualized as if each breeder produces an infinite number of gametes that enter the pool from which newborns are to be drawn. The proportional contribution to this infinite gamete pool from each parental age class i is p_i , so that if we denote the frequency of allele A_1 in age class i by x_i , the frequency of the allele in the gamete pool is $x = \sum p_i x_i$. In accordance with the simple Wright–Fisher model, we now assume that the $2N_i$ genes that are incorporated into newborn individuals are drawn at random from the gamete pool. There is thus a probability p_i that a gene comes from a parent of age i , so that the actual proportion of genes from this age class that enters the offspring is a random (multinomial distributed) variable with a mean of p_i . At any rate, the drawing of offspring genes becomes binomial with a mean of x' and variance of $x(1-x)/2N_i$, where x' refers to the frequency of A_1 among the newborn individuals (*i.e.*, age class 1). Considering breeding in year t , the drift variance in age class 1 in year $t+1$ may thus be

found as $\sigma_1^2(t+1) = E[(x' - q)^2] = E_1 E_2[(x' - x)^2] + 2E_1[(x - q)E_2(x' - x)] + E_1[(x - q)^2]$, where E_2 and E_1 designate expected value operators for the current reproduction and for all previous years, respectively. Here, $E_1(x - q)$ and $E_2(x' - x)$ are both zero, whereas $E_1 E_2[(x' - x)^2] = E_1[x(1-x)]/2N_i = \{q(1-q) - E_1[(x - q)^2]\}/2N_i$, where $E_1[(x - q)^2]$ represents the variance of x (*i.e.*, the frequency of A_1 in the gamete pool). Recalling that $x = \sum p_i x_i$ and that $\sum p_i = 1$, we find $E_1[(x - q)^2] = E_1[(\sum p_i x_i - \sum p_i q)^2] = E_1[\sum p_i (x_i - q)]^2 = \sum \sum p_i p_j E_1[(x_i - q)(x_j - q)]$. This last expectation, however, represents the variances ($i = j$) and covariances ($i \neq j$) among parental age classes i and j , which we have already denoted by $\sigma_{ij}(t)$. Putting this together, we find the drift variance for the newborns in year $t+1$ as

$$\sigma_1^2(t+1) = \frac{q(1-q)}{2N_i} + \left(1 - \frac{1}{2N_i}\right) \sum_{i=1}^k \sum_{j=1}^k p_i p_j \sigma_{ij}(t). \quad (4)$$

Mortality: The allele frequency within a cohort changes through time because some individuals die and genes are thereby randomly removed from the cohort. This process, which is repeated each year, can be regarded as hypergeometric sampling of $2N_i$ out of the previous $2N_{i-1}$ genes (*cf.* NEI 1987, p. 353). Let x' now denote the frequency of A_1 in the age class that is i years old in year $t+1$, and x the frequency in the same cohort the preceding year (t) when the cohort members were of age $i-1$. Taking expectations as before, we find that $\sigma_i^2(t+1) = E[(x' - q)^2] = E_1 E_2\{[(x' - x) + (x - q)]^2\} = E_1 E_2[(x' - x)^2] + E_1[(x - q)^2]$. Here, $E_1[(x - q)^2]$ is the drift variance $[\sigma_{i-1}^2(t)]$ for the cohort in year t . Because x' is generated by hypergeometric sampling from a group with allele frequency x , it follows that

$$\begin{aligned} E_1 E_2[(x' - x)^2] &= E_1 \left[\frac{x(1-x)}{2N_i} \frac{2N_{i-1} - 2N_i}{2N_{i-1} - 1} \right] \\ &= \frac{1}{2N_i} \frac{2N_{i-1} - 2N_i}{2N_{i-1} - 1} \{q - q^2 - E_1[(x - q)^2]\}. \end{aligned}$$

Thus, for adult age classes ($i > 1$) the drift variance in year $t+1$ is given by

$$\begin{aligned} \sigma_i^2(t+1) &= \frac{q(1-q)}{2N_i} \frac{2N_{i-1} - 2N_i}{2N_{i-1} - 1} \\ &\quad + \frac{2N_{i-1}(2N_i - 1)}{2N_i(2N_{i-1} - 1)} \sigma_{i-1}^2(t). \quad (5) \end{aligned}$$

Covariances between age classes: It now remains to derive the various covariance terms of Equation 3. We start by considering the covariance of allele frequency change between newborns ($i = 1$) and an adult age

class ($j > 1$). Let x_i and x'_i designate the allele frequency of an age class i in year t and in year $t + 1$, respectively. The covariance of allele frequency change is then given by (e.g., CROW and KIMURA 1970, p. 484): $\sigma_{1,j}(t + 1) = E[(x'_1 - q)(x'_j - q)]$.

As discussed above, there are two independent sampling processes involved between year t and $t + 1$, i.e., reproduction that results in age class one and mortality in other age classes. Let E_1 and E_2 designate the expected value operators for these two events, respectively, and E that for all previous events. The expected allele frequency in the newborns, $E_1(x'_1)$, is the mean of allele frequencies over all parental classes in year t weighted by their average contribution to newborns in $t + 1$, or $\sum p_i x_i$. For any adult age class, the expected allele frequency $E_2(x'_j)$ is the same as that in the previous year, or x_{j-1} . In terms of the notation above, the covariance can now be written as $\sigma_{1,j}(t + 1) = E\{E_1 E_2[(x'_1 - q)(x'_j - q)]\} = E\{[E_1(x'_1) - q][E_2(x'_j) - q]\} = E[(\sum p_i x_i - q)(x_{j-1} - q)] = \sum p_i E[(x_i - q)(x_{j-1} - q)]$. But this last expectation is simply the covariance between age class i and $j - 1$ in year t . Thus, for $j > 1$,

$$\sigma_{1,j}(t + 1) = \sum_{i=1}^k p_i \sigma_{i,j-1}(t). \quad (6)$$

Allele frequency change within a particular cohort is due to mortality alone, which acts independently among cohorts. The covariance between any two cohorts therefore remains unchanged, and for $j > i > 1$

$$\sigma_{i,j}(t + 1) = \sigma_{i-1,j-1}(t). \quad (7)$$

By numerical iteration of Equations 3 through 7, the effective population size can be computed [by substituting q , $\sigma^2(t)$ and $\sigma^2(t + 1)$ into (1)] and the process of genetic drift can be studied in detail for the present model. Experimenting with different sets of parameters l_i and b_i (subject to the constraint $\sum l_i b_i = 1$), some general features of populations with overlapping generations emerge.

First, for some parameter sets (cf. EMIGH 1979), certain of the covariances remain zero, meaning that the population actually splits into two or more reproductively isolated units (demes) that evolve independently. This happens, for instance, when all individuals breed at the same age and die immediately thereafter, like in the pink salmon (*Oncorhynchus gorbuscha*). In such cases, (1) never reaches a constant value and no single effective size can be defined that adequately describes the amount of genetic drift in the total "population" as a unit (because it does not represent a single biological population). We therefore exclude such situations from further consideration in this study.

Second, and as mentioned previously, the drift variance of the total population (Equation 3) usually be-

haves erratically initially (i.e., when t is small) and then only asymptotically approaches a constant rate of increase. This delay is short for most populations, however, requiring only a few generations if there is a fair amount of mixing of genes from several age classes in the newborns (such as for population I in Figure 2). The same general pattern is also observed for the drift variances (and covariances) of each age class, $\sigma_i^2(t)$, which all approach the same constant rate:

$$r = \frac{\sigma_i^2(t + 1) - \sigma_i^2(t)}{q(1 - q) - \sigma_i^2(t)} \approx \frac{1}{2N_a}. \quad (8)$$

This is expected (cf. FELSENSTEIN 1971) because the drift variances of the separate age classes must all increase at the same rate as the drift variance of the total population, the latter being just the (weighted) average over all age classes (Equation 3).

Standardized measures of genetic drift: The equations developed above describe the exact drift variances and covariances at any time t given the initial frequency of allele $A_1(q)$, the number of newborns each year (N_1) and the age-specific survival and birth rates (l_i and b_i) of the population. For the purpose of finding more general patterns among the drift variances and covariances, it is of interest to see if it is possible to obtain measures that depend on fewer parameters. Inspection of Equations 4 through 7 shows that this can be achieved if we make the simplifying assumption that the number of individuals in each age class (N_i) is much larger than unity. The factor $(1 - 1/2N_1)$ in Equation 4 is then approximately equal to unity and the term $(2N_i - 1)$ in Equation 5 can be approximated by $2N_i$, which in turn equals $2N_1 l_i$. Introducing these approximations in Equations 4–7 and defining "standardized" measures of drift variance f_{ij} such that

$$\sigma_{i,j}(t) = f_{ij}(t) \frac{q(1 - q)}{2N_1}, \quad (9)$$

we see that the quantity $q(1 - q)/2N_1$ can be taken as a common factor in those expressions. Dividing Equations 4 through 7 with $q(1 - q)/2N_1$, we obtain the following measures of genetic drift. For newborns, Equation 4 can be written on the form

$$f_{11}(t + 1) \approx 1 + \sum_{i=1}^k \sum_{j=1}^k p_i p_j f_{ij}(t), \quad (10)$$

approximately. For adults ($1 < i \leq k$), Equation 5 transforms approximately into

$$f_{ii}(t + 1) \approx \frac{1}{l_i} - \frac{1}{l_{i-1}} + f_{i-1,i-1}(t). \quad (11)$$

Similarly, standardizing the covariances 6 between newborns and adult age classes ($1 < j \leq k$) yields

$$f_{1j}(t + 1) = \sum_{i=1}^k p_i f_{i,j-1}(t), \quad (12)$$

and standardizing those between adult age classes ($1 < i < j \leq k$) results (from Equation 7) in

$$f_{ij}(t+1) = f_{i-1,j-1}(t). \quad (13)$$

These new measures (Equations 10 through 13), which initially are zero (at $t = 0$), are obviously independent of both allele frequency and population size as long as the latter is not too small. Clearly, although the absolute values of the drift variances and covariances depend on population size and allele frequency, there exists a relationship among all the variances and covariances that is determined exclusively by the age-specific survival and birth rates. This important observation will be used below when developing an estimator for effective population size. Equations analogous to those presented here are also given by Felsenstein (1971), CHOY and WEIR (1978) and EMIGH (1979), obtained through different methods.

DRIFT VARIANCE IN RELATION TO ALLELE FREQUENCY SHIFTS

Although the drift variances serve as useful theoretical devices, they are not accessible for observation in a natural population (BEGON *et al.* 1980). The analysis of real populations must be based on observable quantities such as allele frequency and its change through time. In this section, we explore the relationship between temporal allele frequency change and effective population size, concentrating on short-term (annual) changes.

Sampling: Previous work on estimation of effective size from observed changes of allele frequencies have identified two different sampling schemes (NEI and TAJIMA 1981). In scheme 1, the effective size is assumed to be equal to the actual number (N) of individuals in the population, and individuals are sampled for analysis after reproduction or are replaced to the population before reproduction occurs. In scheme 2, the actual number of individuals is assumed to be considerably larger than the effective population size, and individuals are sampled after reproduction.

For the present model with overlapping generations, it is not clear what is meant by "before" and "after" reproduction because individuals may breed several times in a lifetime. The critical distinction between the two sampling schemes, however, lies in whether the act of sampling affects the population under study and thereby also affects samples drawn at later times (NEI and TAJIMA 1981). In this paper we assume that individuals collected for genetic analysis are drawn without replacement (so that no individual occurs more than once in a particular sample) but are subsequently returned to the population (so they may reproduce and possibly occur again in later samples). Thus, sampling follows scheme 1 and, as shown by WAPLES (1989), the

restriction that N_e is the same as the actual population size (N) is unnecessary. Samples of individuals drawn from the population at different times can then be regarded as independent hypergeometric samples of genes from the population.

Temporal change of allele frequency: As discussed previously, an age-structured population does not constitute a homogeneous breeding unit and temporal changes of allele frequencies must be considered separately for each age class. In the following we assume that two samples are drawn from the population in year t and $t+1$ according to sampling scheme 1. Grouping individuals according to age, we compare the observed allele frequencies in the 2 years for individuals belonging to the same age class, i . The comparison is thus between samples from two consecutive cohorts born 1 year apart (*cf.* Figure 1). We let x designate the observed allele frequency among those n_t individuals that were of age i when drawn in year t , and y that among the n_{t+1} individuals that were of the same age when sampled the following year ($t+1$). These sample allele frequencies will generally differ from the true, but unknown, frequencies q_x and q_y .

To estimate the amount of genetic drift that has occurred in the population over the sample interval, previous authors have used the observed amount of allele frequency difference in the two samples, standardized in a fashion similar to WRIGHT's F_{ST} (KRIMBAS and TSAKAS 1971; BEGON *et al.* 1980; NEI and TAJIMA 1981; POLLAK 1983; WAPLES 1989). In particular, NEI and TAJIMA (1981) recommended the measure

$$F_c = \frac{1}{a} \sum_{i=1}^a \frac{(x_i - y_i)^2}{(x_i + y_i)/2 - x_i y_i}, \quad (14)$$

for this purpose. Here, a is the number of alleles at the locus under study and the summation is over all alleles. POLLAK (1983), on the other hand, recommended the closely related measure

$$F_k = \frac{1}{a-1} \sum_{i=1}^a \frac{(x_i - y_i)^2}{(x_i + y_i)/2}, \quad (15)$$

in place of F_c , arguing that the sampling properties are better. Our main purpose is not to discuss the relative merits of these two measures (see WAPLES 1989) but rather to investigate the relationship between temporal allele frequency change and the effective size of populations with overlapping generations. The following theoretical analysis focuses on F_c (Equation 14), and computer simulations are used for comparing the results of (14) and (15).

To investigate the relationship between observed allele frequency change and effective population size, we need to find the expectation of F_c with respect to a common reference allele frequency. In the discrete generation case, the true allele frequency in the last

sample year (here q_y) depends only on the amount of drift over the sample interval and the allele frequency, q_x , in the first year, so that q_x can serve as a point of reference (NEI and TAJIMA 1981). When generations overlap, however, the dependency of q_y on previous allele frequencies is more complicated (*cf.* Figure 1). It is therefore necessary to take the expectation of F_c with respect to the initial ($t = 0$) allele frequency, q , which is the only common point of reference.

The expectation of F_c can be written approximately (WAPLES 1989) as

$$E(F_c) \approx \frac{E[(x - y)^2]}{E[(x + y)/2 - xy]} \\ = \frac{\text{Var}(x) + \text{Var}(y) - 2 \text{Cov}(x, y)}{q(1 - q) - \text{Cov}(x, y)}, \quad (16)$$

where $\text{Var}(x)$ is the variance of x and $\text{Cov}(x, y)$ is the covariance between x and y . Imagining, as before, an infinite number of replicates of the population, $\text{Var}(x)$ can be conceptualized as the variance of allele frequencies found in samples from age class i in year t . Thus, $\text{Var}(x)$ embodies both the true allele frequency change until year t , as well as a variance due to sampling in year t .

The two components of $\text{Var}(x)$ can be identified as follows. Let E designate the expected value operator for the change of allele frequency of age class i from year 0 to t , and E_1 that for sampling in year t . Then, $\text{Var}(x) = EE_1[(x - q)^2] = EE_1[(x - q_x)^2] + E[(q_x - q)^2]$. Because x is obtained by hypergeometric sampling of $2n_t$ genes from an age class with $2N_t$ genes and allele frequency q_x , it follows that

$$EE_1[(x - q_x)^2] = E \left[\frac{q_x(1 - q_x)}{2n_t} \frac{2N_t - 2n_t}{2N_t - 1} \right] \\ = \frac{2N_t - 2n_t}{2n_t(2N_t - 1)} \{q(1 - q) - E[(q_x - q)^2]\},$$

where $E[(q_x - q)^2]$ is the drift variance for age class i in year t , or $\sigma_i^2(t)$, as found previously. Thus,

$$\text{Var}(x) = \frac{q(1 - q)(2N_t - 2n_t)}{2n_t(2N_t - 1)} \\ + \left[1 - \frac{(2N_t - 2n_t)}{2n_t(2N_t - 1)} \right] \sigma_i^2(t).$$

Note that $\text{Var}(x)$ equals $\sigma_i^2(t)$ if the entire age class is sampled (*i.e.*, $n_t = N_t$); all the remaining factors represent the effect of sampling only a fraction of the age class. Interestingly, if we sample a given number of individuals (n_t) from a cohort, $\text{Var}(x)$ will be the same regardless of the age at which they are sampled. For newborns ($i = 1$) the age class consists of N_1 individuals and the drift variance, $\sigma_1^2(t)$, is given by (4). At older

ages, the drift variance increases due to mortality (Equation 5), whereas the sampling term decreases for the same reason (a larger proportion of the individuals is sampled). It can be shown that this simultaneous increase and decrease of the components of $\text{Var}(x)$ cancel out exactly. Thus, the variance of the observed allele frequency in a sample of n_t individuals from a cohort whose members were newborns in the year $t - i + 1$ is given by

$$\text{Var}(x) = \frac{q(1 - q)(N_1 - n_t)}{n_t(2N_1 - 1)} \\ + \left[1 - \frac{(N_1 - n_t)}{n_t(2N_1 - 1)} \right] \sigma_1^2(t - i + 1), \quad (17)$$

regardless of when or at which age the cohort was sampled. The expression for $\text{Var}(y)$ is analogous.

To evaluate (16), it now remains to find the covariance, $\text{Cov}(x, y)$, between sample allele frequencies. Using designations as above, and introducing E_2 for the expected value operator for samples from age class i in year $t + 1$, we find $\text{Cov}(x, y) = E[E_1(x - q)E_2(y - q)] = E[(q_x - q)(q_y - q)] = \sigma_{i,i+1}(t + 1)$. That is, the covariance between sample allele frequencies equals the covariance of the true allele frequencies among the two cohorts. Using (7), this covariance can be expressed as

$$\text{Cov}(x, y) = \sigma_{1,2}(t - i + 2). \quad (18)$$

Substituting (17) and (18) into Equation 16 and rearranging, we find the expectation for the observed shift in allele frequencies between two consecutive cohorts as

$$E(F_c) = \frac{q(1 - q) - \sigma_1^2(t - i + 1)}{q(1 - q) - \sigma_{1,2}(t - i + 2)} \\ \times \left[\frac{N_1 - n_t}{n_t(2N_1 - 1)} \right] \\ + \frac{q(1 - q) - \sigma_1^2(t - i + 2)}{q(1 - q) - \sigma_{1,2}(t - i + 2)} \\ \times \left[\frac{N_1 - n_{t+1}}{n_{t+1}(2N_1 - 1)} \right] \\ + \frac{\sigma_1^2(t - i + 1) + \sigma_1^2(t - i + 2) - 2\sigma_{1,2}(t - i + 2)}{q(1 - q) - \sigma_{1,2}(t - i + 2)}. \quad (19)$$

Despite its apparent complexity, Equation 19 is easily interpreted. The last term is the standardized variance of the true shift of allele frequency between cohorts (*cf.* Equation 16), which is the quantity of interest. We refer to this quantity as $E(F'_c)$, the apostrophe indicating that it has been corrected for sampling and thus

TABLE 1

Comparisons of the amount of allele frequency change in two simulated populations (I and II) that differ only in their age-specific birth rates

Age class	Time interval between observations					
	1 year		1 generation		3 generations	
	$\bar{F}_c$	$1/2\bar{F}_c$	$\bar{F}_c$	$1/2\bar{F}_c$	$\bar{F}_c$	$3/2\bar{F}_c$
Population I						
1	0.007504	67	0.007324	68	0.011614	129
2	0.007448	67	0.007565	66	0.011773	127
3	0.007519	66	0.007491	67	0.011744	128
Total	0.000799	626	0.001876	267	0.006949	216
Population II						
1	0.110666	5	0.004911	102	0.013906	108
2	0.111244	4	0.004807	104	0.014615	103
3	0.111127	4	0.005139	97	0.014464	104
Total	0.013126	38	0.001567	319	0.006595	227

Both populations I and II have the same absolute size ($N = 300$), effective size ($N_e \approx 200$) and generation length ($G = 2$ years) (see Figure 2 for details). Allele frequencies were measured with intervals of 1 year, 2 years (one generation) and 6 years (three generations) starting in year $t = 300$. F_c was calculated from Eq. 14 and averaged ($\bar{F}_c$) over 5,000 replicate runs for each age class and for the total population. The inverse of $2\bar{F}_c$ represents the "effective size" that would be inferred from these values if the changes were thought to be entirely due to drift over the time interval (cf. Equation 26).

represents the true allele frequency shift. Note that $E(F_c)$ remains constant over years because all the variance and covariance components increase at the same rate when t is large so that (19) is not restricted to any particular year, and $t - i + 1$ can be replaced by t .

The first two terms of (19) represent the contribution to F_c from sampling a limited fraction of the two cohorts (n_t and n_{t+1} individuals, respectively). It is unfortunate that they both include the drift variances, because this contribution from sampling must eventually be subtracted from F_c . However, a simple approximation is possible if we assume that the fractions involving these variance components are both close to unity. This assumption is reasonable because the drift variances in newborn cohorts (σ_1^2) and the covariance between them ($\sigma_{1,2}$) cannot be very different because $\sigma_{1,2}$ is limited upward by σ_1^2 (cf. Equation 6) and downward by the fact that the two cohorts are related by descent. At the worst, exemplified by population II in Figure 2, we find $\sigma_1^2(t) = 0.08195$ and $\sigma_{1,2}(t+1) = 0.07097$ after $t = 300$ iterations of Equations 4–7, starting with $N_1 = 100$ and $q = 0.5$. With these values the fraction $[q(1 - q) - \sigma_1^2(t)] / [q(1 - q) - \sigma_{1,2}(t+1)]$ in (19) equals 0.94, which is not very different from unity, especially considering that this example represents an extreme situation where almost all reproduction is confined to one single age class. Also, in practical applications of the temporal method to real populations, one will use loci that are reasonably variable, *i.e.*, far from being fixed. For such loci the variance, σ_1^2 , as well as the covariance, $\sigma_{1,2}$, must both be considerably smaller than $q(1 - q)$ because the situation where σ_1^2

$= q(1 - q)$ corresponds to complete fixation. Therefore, Equation 19 can be approximated by

$$E(F_c) \approx \frac{1}{2n_t} + \frac{1}{2n_{t+1}} - \frac{1}{N_1} + \frac{\sigma_1^2(t) + \sigma_1^2(t+1) - 2\sigma_{1,2}(t+1)}{q(1 - q) - \sigma_{1,2}(t+1)}. \quad (20)$$

This equation thus quantifies the amount of allele frequency difference expected between two samples of n_t and n_{t+1} individuals drawn from each of two consecutive cohorts. As discussed above, $E(F_c)$ will be the same regardless of what year and at what age the members of the two cohorts are sampled (provided that t is large). This characteristic of F_c greatly simplifies the analysis because 20 can be applied to a number of sampling situations. For example, for a given sample size, $E(F_c)$ is the same between 2-year-old individuals sampled in consecutive years as between 2- and 3-year-old individuals sampled in the same year, and so on.

RELATIONSHIP BETWEEN F_c AND EFFECTIVE SIZE

As pointed out previously, there exists a relationship between variances and covariances within and among age classes that depends on the age-specific birth and survival rates of the particular population. This dependency has two important implications for F_c (Equation 20) when dealing with populations with overlapping generations. First, F_c among cohorts must be larger than in a population of the same effective size with discrete generations. This is because the covariance cannot be larger than the

drift variances, and typically it is smaller; thus, the last term of (20) takes a larger value than in the corresponding discrete generation situation where this term equals $1/2 N_e$ (see below). Therefore, when generations overlap we expect a value of F_c that is larger than what can be explained only by genetic drift over 1 year.

The second important implication of the dependence of F_c on l_i and b_i is that F_c is not uniquely determined by the effective size when generations overlap. As a result, two populations of the same effective size may display different amounts of temporal allele frequency shift if their l_i and b_i values differ (as observed in Figure 2). This phenomenon is illustrated in Table 1, depicting results from more extensive computer simulations (as compared with those in Figure 2) of the two hypothetical populations I and II. Clearly, the magnitude of allele frequency shift (F_c) differs dramatically between the two populations, although they have (approximately) identical effective sizes and generation lengths. This means that the age-specific survival (l_i) and birth (b_i) rates of the particular population must be accounted for when estimating the effective size from observed short-term allele frequency changes.

To use F_c for estimation of effective size, we need to find an expression for the relationship between the true allele frequency shift among two cohorts born in year t and $t + 1$ [$E(F'_c)$] and the amount of drift over the same period (r ; Equation 8). We define a quantity C such that

$$C = \frac{E(F'_c)}{r}. \quad (21)$$

When t is large, C approaches a constant value because both F_c and r are then constant, as discussed previously. In addition, the value of C is determined exclusively by l_i and b_i , and it is therefore independent of population size and allele frequency. This can be shown through noting that C can be expressed as

$$C = \frac{\sigma_1^2(t) + \sigma_1^2(t+1) - 2\sigma_{1,2}(t+1)}{\sigma_1^2(t+1) - \sigma_1^2(t)} \times \left[\frac{q(1-q) - \sigma_1^2(t)}{q(1-q) - \sigma_{1,2}(t+1)} \right], \quad (22)$$

where the fraction within brackets is approximately equal to unity and cancels out, as noted in connection with (19). If we divide both the nominator and the denominator of the remainder of (22) with $q(1-q)/2N_1$ and substitute for (9) we obtain

$$C \approx \frac{f_{11}(t) + f_{11}(t+1) - 2f_{12}(t+1)}{f_{11}(t+1) - f_{11}(t)}. \quad (23)$$

The f_{ij} s in (23) depend only on the age-specific survival and birth rates (*cf.* Equations 10–13) so that C is independent of the effective population size and takes

FIGURE 3.—Relationships between the amount of temporal allele frequency change and effective size as summarized by the term C for two different populations (I and II; Figure 2). The graphs show the values of C (Equation 23) through time from $t = 0$ until constant values are reached, *i.e.*, until $C = 6.0$ for population I and $C = 102.0$ for population II. Note the different scales of the two graphs. (The graph for population II has been truncated at $C = 1400$ for $t < 10$.)

different values depending on the particular survival and birth rates. The value of C can be calculated by iterating Equations 10–13 for the population under study until (23) approaches a constant value. As an example, the behavior of C for the previously described hypothetical populations I and II is illustrated in Figure 3. Clearly, C fluctuates initially and eventually approaches a constant value that is quite different for the two populations, as is the time required to stabilize. We may note that in both cases C cycles around its harmonic mean during the approach to equilibrium.

It follows from (20) and (22) that

$$E(F_c) = \left(\frac{1}{2n_t} + \frac{1}{2n_{t+1}} - \frac{1}{N_1} \right) = rC = \frac{C}{2N_a} = \frac{C}{2N_e G}. \quad (24)$$

Substituting the observed F_c for $E(F_c)$ in 24 and solving for N_e , we obtain an estimator for effective size that is applicable to populations with overlapping generations:

$$\hat{N}_e = \frac{\hat{N}_a}{G} = \frac{C}{2G \left(F_c - \frac{1}{2n_t} - \frac{1}{2n_{t+1}} + \frac{1}{N_1} \right)}, \quad (25)$$

where F_c is the observed amount of allele frequency shift between two consecutive cohorts (Equation 14), n_t and n_{t+1} are the number of sampled individuals from each of the two cohorts, N_1 is the number of newborns each year, G is the generation length in years and C may be seen as a “correction” term that is determined

TABLE 2

Estimates of effective size from temporal sample allele frequency shifts within age classes in a simulated population

Age class i	Population parameters		Observed annual allele frequency changes				Estimated effective size	
	l_i	b_i	$\bar{F}_c$	$s_{F_c}^2$	$\bar{F}_k$	$s_{F_k}^2$	$\hat{N}_{e(F_c)}$	$\hat{N}_{e(F_k)}$
1	1.0	0.00000	0.032926	0.001916	0.033704	0.002088	509	471
2	1.0	0.00000	0.032790	0.002005	0.033590	0.002199	516	476
3	0.9	0.00000	0.032849	0.001902	0.033622	0.002080	513	474
4	0.9	0.13333	0.032987	0.002042	0.033800	0.002237	506	466
5	0.8	0.39375	0.032464	0.001991	0.033255	0.002185	535	492
6	0.7	0.39286	0.032781	0.002097	0.033607	0.002311	517	475
7	0.6	0.26667	0.032999	0.001978	0.033795	0.002161	505	467
8	0.5	0.13000	0.032642	0.002020	0.033445	0.002228	524	483
9	0.5	0.13000	0.033299	0.002111	0.034137	0.002325	490	452
10	0.5	0.00000	0.034167	0.002159	0.035032	0.002371	451	417
Average			0.032990		0.033799		506	466

Allele frequencies were generated by computer simulations for a population with $N_1 = 100$ and l_i and b_i as indicated in the table. The true effective size is $N_e = 456$ (per generation) with a generation length of $G = 5.93$ years. Samples of $n_t = n_{t+1} = 30$ individuals were drawn from each age class in year $t = 50$ and $t + 1 = 51$. F_c and F_k were computed from Equation 14 and 15, respectively, and averaged over 5000 replicate runs to give $\bar{F}_c$ and $\bar{F}_k$ and their respective sample variances s^2 . Effective population size ($\hat{N}_e$) was estimated using Equation 25 with the correction factor $C = 57.9$ (Equation 23).

by the particular values of l_i and b_i of the population under study. In practice, the quantity $1/N_1$ may be difficult to obtain for many organisms. The estimation is greatly simplified, however, if it can be assumed that N_1 is much larger than the sample sizes, in which case the term $1/N_1$ can be safely ignored. Such an assumption may be quite realistic for many natural populations with high juvenile mortality.

It should be noted that the estimator (25) is identical to those for discrete generations given by NEI and TAJIMA (1981; Equation 16 assuming $N = N_e$) and WAPLES (1989; Equation 12) except that it contains the generation interval G and the correction factor C . In the case of discrete generations, however, both G and C take the value of unity. This is because discrete generations implies that there is only one single age class with $p_i = 1$ and $N_1 = N$ so that $G = \sum p_i = 1$, and from (12) we see that $f_{i2}(t+1) = f_{i1}(t)$ in Equation 23 so that $C = 1$. Under those condition (25) reduces to

$$\hat{N}_e = \frac{1}{2 \left(F_c - \frac{1}{2n_t} - \frac{1}{2n_{t+1}} + \frac{1}{N} \right)}, \quad (26)$$

as it should.

Numerical example: To check the suggested estimator (25), we applied it to observed allele frequencies in a simulated population of known effective size (Table 2). Our discussion deals primarily with F_c for quantifying allele frequency shifts, but POLLAK's (1983) measure F_k was also calculated and used in 25 as a substitute for F_c when estimating the effective size (see below). The true annual effective size of the simulated popula-

tion was $N_e = 2702$ as computed from Equation 1 after iterating (3) – (7) until reaching a constant value (obtained after <50 iterations). With a generation interval of $G = \sum p_i = 5.93$ years, this annual effective size corresponds to an effective size per generation of $N_e = 456$ (Equation 2). These are thus the true parameter values to which the estimates are to be compared.

From each of the 10 age classes, we sampled 30 individuals (60 genes) in two consecutive years, 50 and 51, and determined allele frequencies (not shown) by counting of alleles. The initial allele frequency (in year $t = 0$) was $q = 0.5$ (two alleles) in all age classes, and sampling was as described previously. We calculated F_c and F_k (Equations 14 and 15) separately for each age class from the observed allele frequencies in the 2 years ($t = 50$ and 51). The results were averaged over 5,000 replicate runs (corresponding to 5000 loci) as presented in Table 2. As expected, $\bar{F}_c$ (as well as $\bar{F}_k$) are approximately equal over all age classes, so we can use the averages $\bar{F}_c = 0.032990$ and $\bar{F}_k = 0.033799$ (Table 2, bottom row).

We calculated the correction factor C by iterating Equations 10 through 13 using the demographic parameters l_i and b_i as given in Table 2 (recall that $p_i = l_i b_i$). After fluctuating during the first few generations, C stabilizes at a constant value of 57.9. This means that for this particular population F_c between subsequent cohorts is expected to be 57.9 times larger than in the corresponding discrete generation case (i.e., a simple Fisher–Wright population with a breeding interval of 1 year and a population size of 2,702). Now, substituting $C = 57.9$ into (25), along with the observed $\bar{F}_c = 0.032990$ and the expected contribution from sampling

(0.02333: the quantity within brackets in Equation 24), we obtain an estimate of the effective size per generation of $\hat{N}_e = 57.9 / [2 \cdot 5.93 \cdot (0.032990 - 0.023333)] = 506$, which corresponds to an annual effective size of $\hat{N}_a = 5.93 \cdot 506 = 2998$. These estimates are close to, but slightly larger than, the true values of $N_e = 456$ and $N_a = 2702$.

The reason for the upward bias is due to the approximation we made in generating Equation 20, and possibly also to the one in Equation 16. Conducting a series of computer simulations, WAPLES (1989) found that for populations with discrete generations F_c (and to a lesser extent F_k) consistently tends to somewhat overestimate the effective population size when computed from di-allelic loci. Indeed, using $\bar{F}_k = 0.033799$ (Table 2) in place of $\bar{F}_c$ in Equation 25 (*cf.* POLLAK 1983), we obtain the more accurate estimates of $\hat{N}_e = 466$ and $\hat{N}_a = 2766$. These latter estimates deviate from the true values by no more than 2%, so it will seem that F_k is a less biased measure of allele frequency change than F_c . Note, however, that the sampling variance of F_k is somewhat larger than that of F_c (Table 2), so it is not necessary that F_k is always to be preferred (TAJIMA and NEI 1984; WAPLES 1989). At any rate, the level of bias observed here must be regarded as negligible in view of the large uncertainty associated with actual estimates of effective size from a relatively small number of loci and individuals (NEI and TAJIMA 1981; POLLAK 1983; WAPLES 1989; below).

In addition to the potential bias, the precision of the point estimates is affected by sampling a restricted number of individuals and genes. The precision of $\hat{N}_e$ can be evaluated from the sampling distribution of $\bar{F}_c$ (or $\bar{F}_k$). As shown by NEI and TAJIMA (1981), the quantity $g\bar{F}_c/\bar{F}_c$ is expected to be approximately chi-square distributed with g degrees of freedom, where g is the total number of independent alleles used for calculating F_c . The fit to the appropriate chi-square distribution can be tested by noting that the quantity $gs^2/\bar{F}_c^2$, where s^2 is the variance among observed F_c values, should equal 2 for a perfect fit (NEI and TAJIMA 1981). Using data from Table 2 (with $g = 1$), we find that $gs^2/\bar{F}_c^2$ lies in the range of 1.76–1.95, and the corresponding range for $gs^2/\bar{F}_k^2$ is 1.84–2.05. These values are close to those found in simulations of discrete generation cases (NEI and TAJIMA 1981; WAPLES 1989), indicating that the chi-square approximation for F_c and F_k appears to hold also when generations overlap. The method for placing confidence limits to the estimated effective size on the basis of the distribution of F_c (WAPLES 1989) should therefore be applicable regardless of population model.

As pointed out previously, the allele frequency differences among cohorts can often be measured in several ways through grouping the data differently. For the purpose of the present example, we arbitrarily chose to

perform the analysis through comparisons within age classes between years. Alternatively, we could have (1) compared age classes within a sample-year or (2) compared cohorts through grouping all individuals belonging to the same cohort regardless of what year they were sampled. The estimator (25) is applicable to each approach and should result in similar estimates. The grouping to be preferred in a particular situation is somewhat unclear, however, and a detailed examination of this problem is beyond the scope of the present analysis. The ambiguity arises because different groupings of individuals may result in different sample sizes (n) and different number of comparisons to be pooled.

DISCUSSION

Our present analysis has shown that information on the amount of temporal allele frequency shifts alone is not sufficient for estimating effective size of populations with overlapping generations. Thus, for such populations application of the temporal method for estimation of effective size may lead to serious errors unless some particular characteristics of the allele frequency dynamics are taken into account.

The key difference from the discrete generation situation is that an age-structured population does not constitute a homogeneous breeding unit. Rather, the population is made up of cohorts that differ in their allele frequencies to an extent that depends on the covariance between them, *i.e.*, on the genetic relationship between cohorts. With several cohorts present the sample allele frequencies depend on the relative contribution to the sample from the various cohorts, and information regarding the effective size cannot be inferred from observed allele frequency differences without knowledge of that contribution. In practice, this appears to require either that individuals can be aged or that the population can be randomly sampled with respect to age. When age determination is possible, the most direct way of estimating effective size is to compare consecutive cohorts, which is the focus of the present study. The extension to cohorts born >1 year apart is straightforward as long as we can calculate the covariance of allele frequencies between them. For instance, with cohorts born 2 years apart, the appropriate correction term C is calculated from Equation 23 using $f_{11}(t+2)$ and $f_{13}(t+2)$ in place of $f_{11}(t+1)$ and $f_{12}(t+1)$.

A situation where it appears that effective size can be estimated more or less directly from temporal allele frequency shifts is when cohorts are sampled representatively from the entire population with an interval of more than a single generation. As indicated from the simulation results presented in Table 1, the inferred estimate of N_e based on the total population is very poor when allele frequencies are measured only 1 year apart.

Those inferred estimates, however, appear to approach the true value when measurements are made over longer time intervals (Table 1, right column). We have not examined this situation theoretically, but it appears intuitively that the relative contribution from genetic drift to F_i should increase with the number of generations occurring between sampling. Then, with a larger contribution from drift, the relative effects of the age structuring on the temporal shifts should be less important. The practical use of such an approach may be limited, however. When the generation interval is long, say many years, the estimation procedure would take an excessively long time to complete. Further, unbiased estimates of allele frequencies from the population as a whole requires representative samples of individuals from all age classes, and this may be difficult to obtain from natural populations. For example, in many species individuals of different ages have different behavior and habitat preferences; they may not even coexist in the same region, making identification of the appropriate biological population and representative sampling from it difficult or impossible.

Application to real populations: Application of the suggested estimation procedure requires at least a rough idea of the age-specific survival and birth rates (l_i and b_i) of the population under study. This information, which can be summarized in a correction factor C , is needed to relate observed allele frequency differences to the amount of drift expected to occur in the population over the time interval between the birth of the cohorts being compared. Clearly, the age-specific survival and birth rates may be difficult to estimate from natural populations with a high degree of precision. It should be noted, however, that the precision required in a particular situation must be evaluated on a case by case basis. The sensitivity of C to particular values of l_i and b_i varies among populations and may be evaluated from preliminary estimates of those parameters. It appears, for instance, that obtaining very precise estimates may not be overly critical for providing reasonably accurate values of C when reproduction is distributed over several age classes.

With separate sexes the process of genetic drift is the same as for a monoecious population as long as the sex ratio is even and the demographic parameters are the same for both sexes (*cf.* CROW and KIMURA 1970, p. 103). The theory developed in this paper is then directly applicable. If survival and/or reproduction is different for males and females, we need to clarify the precise meaning of l_i and p_i ($=l_i b_i$) in the computation of the factor C and the generation length G . Retracing our derivations, we note that p_i in Equations 4 or 10 and 6 or 12 is the probability that an individual inherited a gene from a parent of age i . There is a probability of one half that the gene came from a male (or a female) parent, so the relevant value for p_i in computing C and

G is the mean over the two sexes, or $p_i = (p_i^m + p_i^f)/2$, where the superscripts refer to the values for males and females. Finally, l_i in Equation 11 arises because of mortality within adult age classes. This mortality generates an allele frequency change within cohorts that is independent of the sex of the individuals that die, so that l_i is simply the proportion of individuals that survive to age i , regardless of their sex. With these modifications our estimation procedure is readily applicable to populations with separate sexes also.

We have assumed throughout this paper that individuals can be reliably aged (from annual growth rings or by other means), but this condition is not necessarily a prerequisite for application of our suggested estimator. If direct age determination is difficult or unrealistic for the organism under consideration, an alternative may be to sample only a subset of the population for which the age can be inferred (*e.g.*, larvae, pupae, seeds or yearlings). Because the expected value of F_i is the same for all age classes, the effective population size can be estimated using allele frequencies from a single age class only.

Finally, our derivation of the expectation of F_i rests on the assumption that the individuals sampled for allele frequency estimation are drawn without replacement but are subsequently returned to the population. If the actual number of individuals in the population is large, however, no major bias will be introduced if sampling is destructive (*cf.* WAPLES 1989).

Robustness to assumptions: We have assumed throughout this paper that the genes investigated are selectively neutral, that there are no mutations and that the population is closed and does not receive genes from immigrants. These assumptions are in common with the discrete generation case, as discussed by NEI and TAJIMA (1981) and POLLAK (1983), and here we only address assumptions that are specific to populations with overlapping generations, *i.e.*, those associated with the demographic structure of the population.

Demographic variability: In our model the population maintains a constant size and age distribution over time. This means that the proportion of individuals l_i that survive to age i is treated as a fixed quantity, as is the number of newborns N_1 . On the other hand, the proportion of genes from age class i that is incorporated into the offspring is a multinomially distributed random variable with a mean of p_i and a variance of $p_i(1 - p_i)/2N_1$. Thus, for finite populations this proportion will vary according to chance and, in this respect, our model is stochastic. In real populations, however, p_i itself may vary over time, as may l_i and N_1 .

There are presently two different routes that may be taken to evaluate the effect of demographic variability on our estimation procedure, *i.e.*, on the correction factor C . First, minor variations in l_i and p_i do not generally have a large effect on C , and even less on the

quantity C/G that appears in Equation 25. This can be checked by computing C according to (23) for a seemingly reasonable range of values for l_i and p_i for the population considered. If C (or C/G) does not turn out to be very different over this range the estimator (25) can be safely used.

In the case of occasional demographic disturbances, C may be temporarily pushed away from its equilibrium value. This does not necessarily prevent estimation of effective size from allele frequency shifts (F_e) provided that the shifts are monitored over a number of consecutive years. As indicated in Figure 3, the relation between F_e and the equilibrium effective size (N_e) as described by the factor C may fluctuate dramatically, but the fluctuations center around the harmonic mean of the asymptotic value of C . Thus, if the population is sampled for sufficiently many years and if F_e is computed as a mean over all years, the estimated N_e should converge toward the harmonic mean effective size over the interval, *i.e.*, toward the quantity of interest. It therefore appears that the temporal method should also be applicable to populations that have experienced demographic perturbations, provided that the population is monitored over some time.

Dependence between reproduction and survival: We have assumed that reproduction and survival of individuals in a year $t + x$ is independent of whether or not they reproduced in an earlier year t . Clearly, there are many organisms for which the assumption of independence is violated in a greater or less degree. The effect of such deviations from independence on the estimated effective size is not immediately obvious, in part because of the multitude of possible forms the dependence may take but also because the true effective population size is affected by lack of independence.

To address this problem, we performed a number of computer simulations along the lines described previously, but with certain modifications to reflect situations where survival (and thus future reproduction) is not independent of previous reproduction. Specifically, we let only a portion of the individuals within a age class reproduce (the "breeders") and assigned a certain obligate mortality (m) to those breeders ($0 \leq m \leq 1$). In the case of $m = 0$, survival is independent of reproduction such that breeders and nonbreeders share the same probability of survival that is determined only by the l_i value of the particular age class (as in our original model). When $m = 1$ all breeders die immediately after reproduction, which corresponds to semelparity. The obligate mortality (m) assigned to the breeders is included in l_i such that we can compare the results from simulation that differ only with respect to m . We estimated the effective population size from sample allele frequencies, as described previously, and compared it with the true effective population size. This true effective size was computed from (1) using the

FIGURE 4.—Effect of lack of independence between reproduction and survival on the estimated ($\hat{N}_e$) and the true (N_e) effective population size. Two different sets of populations (A and B) with different l_i and b_i values were simulated for various levels of obligate breeder mortality m . The mortality rate m represents the fraction of breeders that die immediately after reproduction and ranges from zero (survival independent of reproduction) to unity (semelparity). Additional mortality as determined by the l_i values was independent of reproduction. A sample of 25 individuals (50 genes) was drawn in year 50 and 51 for allele frequency measurement and estimation of effective population size (Equation 25). Case A: $N_1 = 100$, $l_1 = 1$, $l_2 = 1$, $l_3 = 0.75$, $l_4 = 0.25$, $b_1 = 0$, $b_2 = 0.25$, $b_3 = 0.6667$ and $b_4 = 1$. The computed correction factor $C = 17.80$ (Equation 23, derived under the assumption of $m = 0$) and mean generation length $G = 3.00$ years. A proportion of b_i individuals from each age class i were randomly selected as breeders resulting in a total of $25 + 50 + 25 = 100$ breeders per year. Case B: $N_1 = 400$, $l_1 = 1$, $l_2 = 0.1$, $b_1 = 0.9$ and $b_2 = 1$, with $C = 1.22$ and $G = 1.10$ years. The proportion of breeders in age class 1 and 2 were 0.1 and 0.5, respectively (total number of breeders = 60).

simulated population drift variances $[\sigma^2(t)]$ and the initial allele frequency q . In these particular simulations, the true effective sizes are based on 50,000–100,000 runs and the estimates on 10,000 runs.

The results of such computer simulations for two sets of populations (A and B) with different l_i and b_i values are summarized in Figure 4. The l_i and b_i values were chosen to illustrate populations with low (A) and high (B) total mortality. In both cases the estimator coincides with true effective population size when $m = 0$, as it should. With respect to case A, the true effective size is seen to increase at higher values of m . This is apparently because more individuals are engaged in the production of the (fixed number of) newborns when fewer breeders can reproduce more than once in a lifetime, thereby reducing the variance of lifetime offspring number and resulting in a larger effective size (HILL 1979). In contrast, the estimated effective size becomes smaller as m approaches unity, thus deviating increasingly from the true parameter value. The reason why the estimate declines is that the obligate mortality tends to reduce the covariance of allele frequencies between successive cohorts, below that given by (6), such that F_e becomes larger yielding an estimate of the effective size that is biased downward.

The general behavior of the true effective size and the estimate is similar in case B, but here the magnitude of the difference is very small (Figure 4) and therefore of no practical concern when estimating effective size. The reason for the small effect is that in case B the mortality is so large that only a minor fraction of the breeders at age 1 will survive to reproduce again, even for $m = 0$. Thus, at this total mortality rate there is little practical difference between the situation where mortality is independent of reproduction ($m = 0$) and semelparity ($m = 1$).

There is an infinite number of possible deviations from our model with respect to the assumption of independence between reproduction in 1 year and future mortality and reproduction. Nevertheless, the simulation results in Figure 4 indicate that our estimator is quite robust to deviations from the basic assumptions. Only in a situation where most mortality is associated with breeding (e.g., case A with m close to unity), the bias may be of practical concern. Such situations may call for special attention (WAPLES 1990b), but life-history characteristics of this particular kind should in many cases be known or easily identified by the investigator. In a forthcoming paper we will use the present method for estimating the effective size of natural brown trout (*Salmo trutta*) populations from allozyme data.

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