Demographic Genetics of Brown Trout (*Salmo trutta*) and Estimation of Effective Population Size From Temporal Change of Allele Frequencies

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ABSTRACT

We studied temporal allele frequency shifts over 15 years and estimated the genetically effective size of four natural populations of brown trout (*Salmo trutta* L.) on the basis of the variation at 14 polymorphic allozyme loci. The allele frequency differences between consecutive cohorts were significant in all four populations. There were no indications of natural selection, and we conclude that random genetic drift is the most likely cause of temporal allele frequency shifts at the loci examined. Effective population sizes were estimated from observed allele frequency shifts among cohorts, taking into consideration the demographic characteristics of each population. The estimated effective sizes of the four populations range from 52 to 480 individuals, and we conclude that the effective size of natural brown trout populations may differ considerably among lakes that are similar in size and other apparent characteristics. In spite of their different effective sizes all four populations have similar levels of genetic variation (average heterozygosity) indicating that excessive loss of genetic variability has been retarded, most likely because of gene flow among neighboring populations.

THE effective population size (N_e) is an essential concept when linking the theory of population genetics to the real world of natural populations (WRIGHT 1969, p. 211). This central position stems from the wide range of demographic factors incorporated by N_e, thereby allowing a unified theoretical treatment of random genetic changes of populations. It has been notoriously difficult to obtain reliable estimates of N_e in natural populations, however, because the direct assessment of N_e requires measurements of the entire array of demographic parameters on which it depends. Such measurements are difficult to carry out in natural populations. Moreover, the precise relationship between certain demographic factors, such as various forms of nonrandom mating, and N_e remain uncertain and subject to continued theoretical refinements (CROW and KIMURA 1970; CROW and DENNISTON 1988; RYMAN and LAIKRE 1991; CABALLERO and HILL 1992; ORIVE 1993; CABALLERO 1994; SUGG and CHESSER 1994; RYMAN *et al.* 1995; WANG 1995). Our knowledge of the effective size of natural populations is therefore very limited.

Indirect methods have been employed to circumvent the problem of directly evaluating N_e in natural populations (WRIGHT *et al.* 1942; KRIMBAS and TSAKAS 1971; HILL 1981; WAPLES 1991; BARTLEY *et al.* 1992). The most promising approach is based on measurements of the amount of random genetic drift between generations that is directly proportional to the effective population size (NEI and TAJIMA 1981; POLLAK 1983; TAJIMA and NEI 1984; WAPLES 1989). This so-called temporal method has been used to estimate N_e in natural populations from observed shifts of allozyme frequencies in various species characterized by either quite simple (*i.e.*, discrete generations: KRIMBAS and TSAKAS 1971; BEGON *et al.* 1980; HEDGECOCK *et al.* 1992; see also FRANKHAM 1995 and references therein) or rather peculiar (*i.e.*, semelparity: WAPLES 1990; WAPLES and TEEL 1990) life history patterns.

Although the above applications of the temporal method have been successful, it has been unclear how to apply it to the large group of organisms characterized by overlapping generations and that have the potential for repeated breeding during a lifetime (iteroparity). For this group of organisms we have recently evaluated the dynamics of random allele frequency change within a population and derived a method for estimating N_e from observed temporal shifts of allele frequencies (JORDE and RYMAN 1995). Here, we apply this method to allozyme data from brown trout (*Salmo trutta*) and estimate the effective size of four natural populations in Sweden.

MATERIALS AND METHODS

The estimation procedure (JORDE and RYMAN 1995) is based on observations of allele frequency differences among consecutive cohorts; this procedure requires genotypic data from individuals that have been classified with respect to age as well as information on the age-specific survival and reproduction rates for the population to which they belong. The results of the present study are based on a rather extensive set of data, and we have primarily focused on matters that relate directly to the estimation of effective size.

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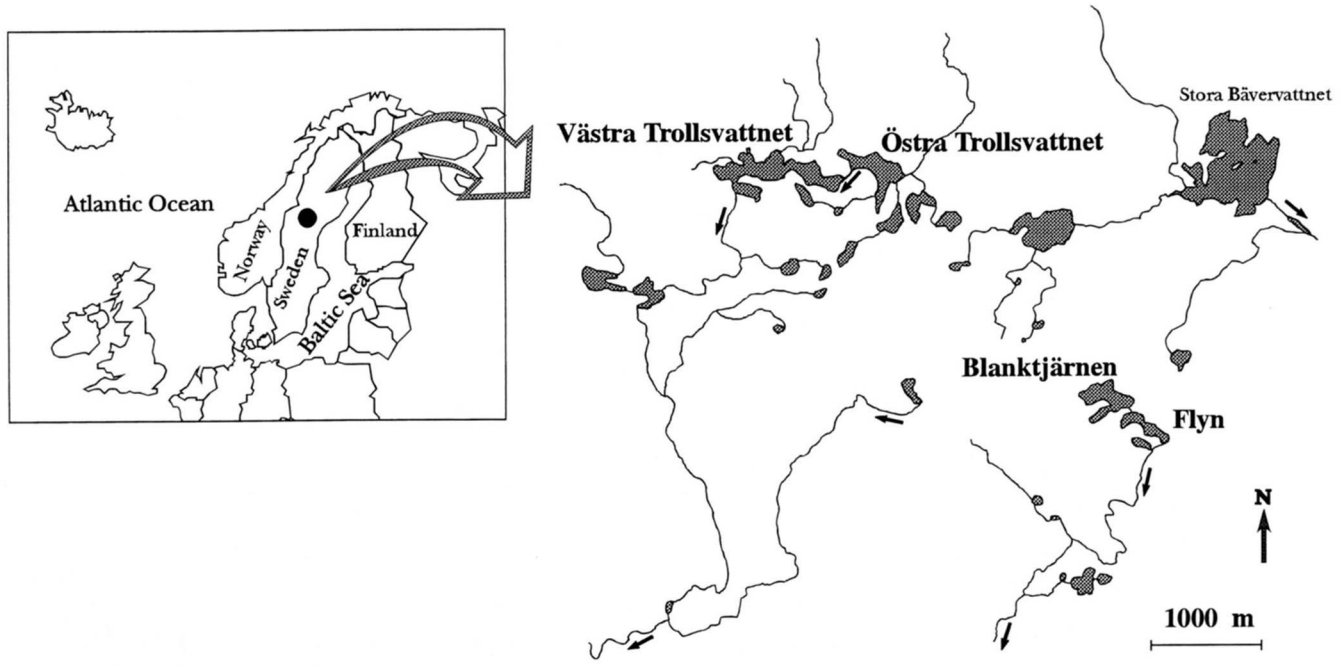

FIGURE 1.—Location of the study area in central Sweden (●) and schematic representation of the sampled lakes. Small arrows indicate the directions of waterflow.

Material: Within the scope of an on-going longitudinal genetic study of natural and introduced fish populations, we analyzed a total of 5899 fish collected in the period of 1979–1993 from four lakes in the province of Jämtland, Sweden, at the approximate elevation of 700 m (FIGURE 1; RYMAN *et al.* 1986). The lakes are rather small, measuring from ~300 to 1500 m across, and they typify the thousands of lakes inhabited by brown trout throughout the mountain range of Scandinavia. As indicated in Figure 1, the lakes represent two closely located pairs of lakes, Lakes Blanktjärnen and Flyn and Lakes Östra and Västra Trollsvattnet, respectively; within both pairs the lakes are interconnected by a small creek. The two pairs of lakes eventually drain into a common larger lake several kilometers downstream (not shown in Figure 1) with ~15 km waterway (creeks, streams, and small lakes that all contain brown trout) separating the two pairs of lakes. No information exists regarding the extent to which rapids, small waterfalls, and other potential barriers throughout this waterway may preclude upstream migration.

Each lake has been sampled annually from 1979 (Lake Östra Trollsvattnet) or 1980 (the other three) and thereafter, and in this report we include samples collected through 1993. Each year (July–September) ~100 brown trout were sampled from each lake using gillnets of various mesh sizes. For each fish we measured length and weight, determined sex and maturity (development of gonads), and collected otoliths for age determination and tissue samples (white skeletal muscle, liver, and eye) for protein electrophoresis. All samples were kept as cold as possible during the field work (on dry ice in later years) and were subsequently stored in an ultra freezer (−70°) until the electrophoretic analysis.

Electrophoresis: Screening of allozymes was done by conventional starch gel electrophoresis of known polymorphic loci (ALLENDORF *et al.* 1977; GUYOMARD and KRIEG 1983; TAGGART and FERGUSON 1984; JORDE *et al.* 1991; JORDE 1994). We follow the nomenclature proposed by SHAKLEE *et al.* (1990) for designation of loci and alleles. This nomenclature differs from the one that has been used in earlier publications from our group, and to allow comparisons with the information contained in those papers, we also provide the previous designations when different.

The following 14 loci were screened and found to be variable in the populations examined during the course of the present study (previous locus name in brackets, alleles in parenthesis): *sAAT-4* [*AAT-6*] (**100, *50, *25*); *CK-2* [*CPK-1*] (**115, *100*); *DIA* (**100, *95*); *bGALA-2* (**100, *95*); *bGLUA* [*BGA*] (**150, *100*); *G3PDH-2* [*AGP-2*] (**100, *50*); *IDDH-1* [*SDH-1*] (**100, *-50*); *sIDHP-1* [*IDH-2*] (**160, *100*); *LDH-5* (**105, *100*); *aMAN* (**100, *70*); *sMDH-2* [*MDH-2*] (**125, *100*); *ME* [*MEL*] (**120, *100*); *MPI* [*PMI*] (**105, *100*); and *PEPLT* (**100, *70*). Some of these polymorphisms were detected during the present study, and not all of them were scored before 1987.

Most of the loci appear to segregate independently in the brown trout and other salmonid species (*cf.* JOHNSON *et al.* 1987). The only exception seems to refer to the pair of loci that JOHNSON *et al.* (1987) designated *Dia* and *Ck-1*, which may correspond to *DIA* and *CK-2* in the notation used here. We have found no information on possible linkage relationships for the loci *bGALA-2, bGLUA, aMAN, ME,* and *PEPLT.*

As indicated above, all polymorphic loci except for *sAAT-4* segregated for two alleles only. The generally most common allele in Scandinavian populations is the one designated **100* (*cf.* RYMAN *et al.* 1979; RYMAN and STÅHL 1981; RYMAN 1983). In the present presentation we will focus on this **100* allele and refer to it as the "common" allele at each locus. As only two copies of the *sAAT-4*25* allele were observed in the course of the present study, they have been lumped with the **50* allele in the subsequent analyses.

In addition to the above 14 loci, previous electrophoretic screenings have revealed ~60 putative loci that appear monomorphic, or nearly so, in all four populations. These monomorphic loci were included when estimating average heterozygosity (NEI 1973).

Allele frequency statistics: Estimation of allele frequencies was performed through counting of alleles. At each of *IDDH-1* and *LDH-5* four fish could not be unambiguously classified due to difficulties in distinguishing the heterozygote from

one of the homozygote genotypes. In those cases the allele frequencies were estimated by the maximum-likelihood procedure described by JORDE and RYMAN (1990).

The statistical significance of allele frequency heterogeneities were assessed using G-tests with Williams' correction for small expected values (SOKAL and ROHLF 1981). Total gene diversity (expected heterozygosity: H_T), as calculated from observed allele frequencies, was analyzed by means of hierarchical gene diversity analyses accounting for both temporal and spatial variation following the methods of NEI (1973) and CHAKRABORTY *et al.* (1982).

Estimating effective size: The estimation of effective population size from observed allele frequency shifts among consecutive cohorts followed the method of JORDE and RYMAN (1995). In brief, the estimation included the following steps.

The magnitude of allele frequency shifts (F) between cohorts was computed from observed (sample) allele frequencies according to POLLAK's (1983) measure

$$F = \frac{2}{a-1}\sum_{i=1}^{a}\frac{(x_{i,t}-x_{i,t+1})^2}{x_{i,t}+x_{i,t+1}}, \tag{1}$$

where $x_{i,t}$ is the observed frequency of the ith allele in the sample from cohort t, and the summation is over all a alleles at this locus (here, $a = 2$ for each locus). This standardized variance of allele frequency shift (Equation 1) can be corrected for the expected contribution from sampling to give a new measure

$$F' = F - \frac{1}{2n_t} - \frac{1}{2n_{t+1}} + \frac{1}{N_1}, \tag{2}$$

where n_t is the number of individuals screened for this locus in the sample from cohort t and N_1 is the number of individuals in the cohort when they were newborn. As we do not know N_1 for the brown trout populations, we assumed that this number is large (a reasonable assumption for species with high fecundity) and used the approximate expression

$$F' \approx F - \frac{1}{2n_t} - \frac{1}{2n_{t+1}}. \tag{3}$$

These equations (2 and 3), being corrected for finite sampling, should represent unbiased measurements of temporal allele frequency shifts between cohorts. It is assumed that samplings from the two cohorts are independent, implying that removal of individuals from one cohort does not affect the other one. This assumption would not be met if the individuals sampled from the first cohort (t) are potential parents to those in the next one ($t + 1$) but are removed before reproduction and not returned to the population (corresponding to sampling scheme II of NEI and TAJIMA 1981). Clearly, for a cohort to contain parents of the next one, reproduction must start in the first year of life, and this is not the case for the brown trout (see below). It therefore seems safe to conclude that samples from the two cohorts are indeed independent, although individuals are sampled destructively and not returned to the population.

We obtained an overall measure of temporal allele frequency change ($\bar{F}'$) for each population through averaging the values of F' (Equation 3) obtained from all the combinations of loci and pairs of consecutive cohorts available for a particular population. This overall measure was used to estimate the effective population size as

$$\hat{N}_e = \frac{C}{2G\bar{F}'}, \tag{4}$$

where G is the mean generation length and C is a correction factor for overlapping generations (JORDE and RYMAN 1995).

To estimate the correction factor C, we need to take into account the fraction of individuals that survive to a given age i (the age-specific survival rate: l_i) and the average contribution to offspring by parents of that age (p_i). These demographic population parameters are estimated (see below) and summarized in the factor C that is particular to each population

$$C = \frac{f_{1,1}(t) + f_{1,1}(t+1) - 2f_{1,2}(t+1)}{f_{1,1}(t+1) - f_{1,1}(t)}, \tag{5}$$

where the $f_{i,j}$s are variables that trace the process of genetic drift in a population of the present age-structure. The $f_{i,j}$s were all set to zero at some arbitrary initial time, $t = 0$, and their subsequent values were found through iterating the following set of equations.

$$f_{1,1}(t+1) = 1 + \sum_{i=1}^{k}\sum_{j=1}^{k} p_i p_j f_{i,j}(t), \tag{6}$$

$$f_{i,i}(t+1) = \frac{1}{l_i} - \frac{1}{l_{i-1}} + f_{i-1,i-1}(t), \quad 1 < i \le k, \tag{7}$$

$$f_{1,j}(t+1) = \sum_{i=1}^{k} p_i f_{i,j-1}(t), \quad 1 < j \le k, \tag{8}$$

$$f_{i,j}(t+1) = f_{i-1,j-1}(t), \quad 1 < i < j \le k, \tag{9}$$

where the summations are over all k age classes in the population. After a few iterations (~50) of Equations 6–9 all $f_{i,j}$ become independent of their initial values and a constant value for C (Equation 5) is then obtained. This value was used in the estimator for effective size (Equation 4), along with the observed shifts of allele frequencies, (Equation 3; averaged over all loci and pairs of consecutive cohorts), and the generation length G (FELSENSTEIN 1971)

$$G = \sum_{i=1}^{k} p_i i. \tag{10}$$

We use two different approaches for assessing the precision of an estimated effective size both assuming that the largest uncertainty in $\hat{N}_e$ arises from sampling errors when determining the amount of allele frequency change (F), rather than in the factors C and G. First, under the assumption of F following a chi-square distribution with one degree of freedom (*i.e.*, the number of independent alleles used in computing each F value), we followed the suggestion of WAPLES (1989: Equation 16) and calculated 95% confidence limits for $\bar{F}'$. Those limits were then used in Equation 4 to estimate the corresponding limits for $\hat{N}_e$. Second, under the assumption of $\bar{F}'$ following approximately a normal distribution we set confidence limits for $\bar{F}'$ on the basis of its standard error calculated from all the individual F values, *i.e.*, from each combination of locus and pair of consecutive cohorts. These limits for $\bar{F}'$ were, again, converted into confidence limits for $\hat{N}_e$.

Demographic analyses: The age of the fish was assessed from otoliths by counting the number of slow-growing winter zones. Both otoliths from all fish were aged twice to reduce scoring errors. Occasionally, a third reading was done to resolve conflicts between the first two readings. Age is scored as the number of completed winter seasons, so that a fish that hatched (in the spring) the same year that it was sampled would be in its first year of life, frequently denoted as 0+ (zero, the plus indicating a fraction of a year). For the purpose of the present analysis, however, we will refer to individuals of this age (0+) as belonging to age class 1 (one), and so on for older fish (*cf.* FELSENSTEIN 1971; MURRAY and GÅRDING 1984).

Age-specific survival rates (l_i) as expressed in an ordinary

life-table, *i.e.*, the probability of a newborn fish to survive to age i, were estimated from the observed age distributions in the total catch (*i.e.*, over all years) under the assumption that the probability of surviving from one year to another (S) was the same for all ages. This assumption was necessary because fishing did not capture the youngest age classes; clearly, they were underrepresented in our material (see below). We estimated S using the so-called CHAPMAN-ROBSON method (ROBSON and CHAPMAN 1961; YOUNGS and ROBSON 1978) that takes into account that young age classes may be underrepresented in the catch due to selective fishing for large individuals. On the basis of S, we then calculated the age-specific survival rates, $l_i = S^{i-1}$, for each age class i.

The relative reproductive success (p_i) at different ages was estimated in three steps. First, we estimated the proportion of breeders (sexually mature fish) in the various age classes, classification of sexual maturity being determined from gonadal development. Second, we used the average body weight as an indicator of relative gamete contribution and multiplied the proportion of breeders in each age class with the mean weight of the fish of that particular age and sex. The results were used as estimates of the mean number of offspring per male (b_i^m) and female (b_i^f) of age i after correcting the values so as to result in a constant population size (*i.e.*, net reproductive rate, $R_0 = \Sigma\, l_i b_i = 1$, for each sex). Third, using the age-specific estimates of b_i and l_i, we estimated p_i by giving the sexes equal weight: $p_i = l_i(b_i^m + b_i^f)/2$. The l_i and p_i values thus obtained were then used to compute the mean generation length (G; Equation 10) and the correction factor (C; Equations 5–9) as described above.

Selective neutrality: The possible existence of deviations from selective neutrality of alleles was tested by comparing the ratio of the observed variance of temporal allele frequency shifts (s^2) among loci to the variance expected when all loci are affected only by genetic drift (LEWONTIN and KRAKAUER 1973). In these tests we used the standardized variance of allele frequency shifts among cohorts (F; Equation 1) and computed the test statistic

$$X^2 = \frac{s_F^2}{2\bar{F}^2/\mathrm{df}}. \qquad (11)$$

Under the null hypothesis of selective neutrality this statistic is distributed approximately as $\chi^2_{\mathrm{d.f.}}/\mathrm{d.f.}$, where d.f. is the degrees of freedom, *i.e.*, the number of independent alleles used in computing each F value (here, d.f. = 1, always).

RESULTS

All the 14 loci were found to segregate for the same two alleles within each of the four populations. For each locus and population the relative frequency of the common (**100*) allele among all fish is given in Table 1 along with the variation (standard deviation, s) among cohorts and among year of sampling. Further, the overall variation of allele frequencies in space and time is described by means of hierarchical gene diversity analyses in Table 2. As indicated above, every allele frequency characterizing a particular combination of population, locus, and year of sampling is based on ~100 fish. In contrast, when classifying the fish with respect to cohort (year of birth) some cohorts were found to be represented by quite few individuals in some populations. To reduce the effects of sampling error we have only included cohorts containing a minimum of 50 individuals within a particular population in the subsequent analysis of cohort data (including those of Table 1). On the basis of Tables 1 and 2 we first comment on some general observations relating to the spatial and temporal distribution of genetic variability.

Distribution of genetic variation in time and space: Clearly, allele frequencies vary significantly over time (Tables 1 and 2). Thus, the present set of data makes an analysis of the causes of such changes meaningful. We note, however, that the variation due to allele frequency differences between sampling years within lakes only accounts for a minor portion (0.6%) of the total gene diversity (Table 2). As expected, our present estimate of the relative importance of temporal variation within natural populations of brown trout is somewhat larger than that obtained in a previous study (0.03%) that was based on a shorter time span (RYMAN 1983).

In addition to the temporal allele frequency heterogeneities within populations, there is significant divergence among populations at all loci (Table 2). The degree of differentiation, however, averaging 4.5 and 0.04% between and within pairs of lakes, respectively, is less conspicuous than what is generally observed for natural brown trout populations of this region (*e.g.*, RYMAN 1983) as well as in others (KRIEG and GUYOMARD 1986; FERGUSON 1989; GARCIA-MARIN *et al.* 1991).

Spatial heterogeneity: The comparatively low degree of divergence among populations (4.5%; Table 2) observed in the present study is due to relatively small allele frequency differences both between and within pairs of lakes. In particular, there are no indications that the two interconnected Lakes Blanktjärnen and Flyn represent genetically distinct populations.

The notion that Lakes Blanktjärnen and Flyn may actually represent one single randomly breeding population is supported by three independent observations. First, there is no statistically detectable overall divergence between these two interconnected lakes; for this pair of lakes there is only one significant allele frequency difference between them, and summing over all loci yields a nonsignificant result ($G = 14.9$, d.f. = 14, with $P > 0.05$). Second, there are no significant heterozygote deficiencies relative to the Hardy-Weinberg genotypic proportions at any locus, and this is true for the combined material as well as for each of the lakes of this pair considered separately. The only statistical significance observed in Lakes Blanktjärnen and Flyn refers to *PEPLT* that displayed both the weakly significant allele frequency difference mentioned above ($G = 5.5$, d.f. = 1 with $P < 0.05$) and a minor excess of heterozygotes ($\chi^2 = 5.7$, d.f. = 1 with $P < 0.05$) in Lake Blanktjärnen. An excess of heterozygotes is not indicative of population subdivision, however, and the significances observed at this locus most likely represent statistical type I errors. Third, the temporal shifts of allele frequencies in Lakes Blanktjärnen and Flyn are markedly correlated (Table 3). A significant positive correlation is observed at eight

TABLE 1

Temporal variation at 14 isozyme loci in four natural populations of brown trout

Population	Locus														All loci	
	sAAT-4	*CK-2*	*DIA*	*bGALA-2*	*bGLUA*	*G3PDH-2*	*IDDH-1*	*sIDHP-1*	*LDH-5*	*aMAN*	*sMDH-2*	*ME*	*MPI*	*PEPLT*	Sum of *G*	d.f.
Lake Blanktjärnen (n = 1415)																
$f(*100)$	0.55	0.98	0.70	0.90	0.79	0.67	0.73	0.56	0.74	0.94	0.96	0.89	0.26	0.65		
No. of years	11	12	10	7	7	14	12	9	14	7	14	7	7	7		
s_Y	0.026	0.011	0.037	0.025	0.018	0.053**	0.038	0.035	0.043*	0.018	0.019*	0.020	0.042	0.043	187.0***	124
No. of cohorts	11	11	11	7	7	14	11	10	14	7	14	7	7	7		
s_C	0.039	0.019*	0.070***	0.035	0.040	0.068***	0.040	0.040	0.051**	0.028	0.021**	0.067***	0.040	0.051	281.9***	124
Lake Flyn (n = 1456)																
$f(*100)$	0.53	0.98	0.72	0.91	0.80	0.68	0.74	0.55	0.75	0.94	0.96	0.90	0.25	0.61		
No. of years	11	12	10	7	7	14	12	9	14	7	14	7	7	7		
s_Y	0.041	0.011	0.040	0.022	0.015	0.041	0.051**	0.038	0.034	0.023	0.020**	0.049***	0.052**	0.032	232.0***	124
No. of cohorts	11	12	11	7	7	15	12	10	15	7	15	7	7	7		
s_C	0.059**	0.017*	0.075***	0.028	0.061***	0.052**	0.070***	0.050	0.052**	0.042***	0.033***	0.091***	0.054**	0.029	413.9***	129
Lake Östra Trollsvattnet (n = 1551)																
$f(*100)$	0.69	0.91	0.92	0.97	0.82	0.77	0.84	0.76	0.69	0.72	0.90	0.98	0.75	0.54		
No. of years	11	11	10	7	7	15	12	9	15	7	15	7	7	7		
s_Y	0.037	0.022	0.022	0.011	0.040*	0.030	0.029	0.017	0.036	0.020	0.025	0.013**	0.027	0.041	151.4	126
No. of cohorts	10	10	8	6	6	14	11	8	14	6	14	6	6	6		
s_C	0.034	0.027	0.028	0.014	0.044	0.038	0.022	0.043	0.036	0.036	0.026	0.013	0.026	0.036	139.2*	111
Lake Västra Trollsvattnet (n = 1477)																
$f(*100)$	0.72	0.92	0.94	0.97	0.81	0.74	0.84	0.71	0.71	0.71	0.90	0.98	0.78	0.54		
No. of years	11	12	9	7	7	14	12	9	14	7	14	7	7	7		
s_Y	0.040	0.020	0.021	0.015	0.030	0.050***	0.030	0.043	0.028	0.029	0.026	0.017	0.032	0.036	194.5***	123
No. of cohorts	9	11	9	6	6	13	11	8	13	6	13	6	6	6		
s_C	0.051**	0.022	0.020	0.015	0.020	0.053**	0.033	0.044*	0.039	0.035	0.030*	0.007	0.028	0.027	174.6***	109

n is the total number of fish analyzed. At each locus $f(*100)$ is the average frequency of the common ($*100$) allele; s_Y and s_C are the standard deviations of allele frequencies over years of sampling and cohorts (year of birth), respectively (number of years = the number of years the locus was screened). The statistical significance of temporal allele frequency shifts as determined by heterogeneity G tests is indicated by asterisks, and the sum of G (with d.f.) over all loci is given to the right. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2

Hierarchical gene diversity analysis of spatial and temporal allele frequency variation at 14 isozyme loci in four populations of brown trout

Locus	No. of years	No. of fish	Total gene diversity (H_T)	Relative gene diversity component (%)			
				Within samples	Between years within lakes	Between lakes within pairs	Between pairs of lakes
sAAT-4	11	4488	0.470	96.4	0.51	0.10**	3.00***
CK-2	11–12	4865	0.093	97.1	0.54*	0.05	2.27***
DIA	9–10	3932	0.297	90.8	0.59*	0.06*	8.58***
bGALA-2	7	2847	0.120	97.6	0.52	0.02	1.88***
bGLUA	7	2835	0.312	99.5	0.42	0.01	0.08*
G3PDH-2	14–15	5899	0.407	98.2	0.87***	0.03	0.86***
IDDH-1	12	4942	0.338	97.6	0.81**	0.01	1.57***
sIDHP-1	9	3645	0.459	95.7	0.47	0.14**	3.67***
LDH-5	14–15	5896	0.401	99.2	0.59*	0.02	0.20***
aMAN	7	2839	0.288	90.5	0.31	0.01	9.20***
sMDH2	14–15	5897	0.128	97.9	0.77***	0.01	1.37***
ME	7	2851	0.120	96.1	1.15***	0.00	2.75***
MPI	7	2847	0.500	72.8	0.53*	0.06	26.62***
PEPLT	7	2851	0.486	98.6	0.51	0.11	0.76***
Mean			0.316	94.86	0.61***	0.04**	4.49***
SE			0.040	1.85	0.06	0.01	1.86

Significance levels of the gene diversity components were obtained from contingency G tests of allele frequency heterogeneity at each hierarchical level. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

out of 14 loci, and these eight loci are those that display the most conspicuous temporal frequency changes within both lakes (*cf.* Table 1).

The two lakes of the other pair, Lakes Östra and Västra Trollsvattnet, also exhibit allele frequencies that are rather similar to each other, but the overall allele frequency difference is statistically significant (summing over all loci yields $G = 40.3$, d.f. = 14 with $P < 0.001$). The impression that these two lakes represent more than one population is further supported by a marked deficiency of heterozygotes in the pooled sample (summing over all codominant loci yields $\chi^2 = 137.1$, d.f. = 13 with $P \ll 0.001$). The number of populations and their spatial distribution in these two lakes is, however, somewhat unclear. There is evidence for population admixture within both lakes as revealed by a significant heterozygote deficiency at eight out of the 13 codominant loci tested in Lake Västra Trollsvattnet

TABLE 3

Correlation coefficients between allele frequency changes among consecutive cohorts of interconnected lakes

Locus	Lake Blanktjärnen *vs.* Lake Flyn			Lake Östra Trollsvattnet *vs.* Lake Västra Trollsvattnet		
	r	No. of cohorts	P	r	No. of cohorts	P
sAAT-4	0.4718	10	NS	0.4486	9	NS
CK-2	0.6687	11	<0.05	0.7279	9	<0.05
DIA	0.7332	10	<0.05	0.2560	7	NS
bGALA-2	0.7992	6	NS	−0.2888	5	NS
bGLUA	0.3374	6	NS	0.6464	5	NS
G3PDH-2	0.5676	14	<0.05	0.3253	13	NS
IDDH-1	0.8021	11	<0.01	0.0680	10	NS
sIDHP-1	−0.1233	9	NS	0.4854	7	NS
LDH-5	0.7560	14	<0.01	0.0440	13	NS
aMAN	0.7839	6	NS	0.2717	5	NS
sMDH-2	0.6366	14	<0.05	0.1655	13	NS
ME	0.9100	6	<0.05	−0.3127	5	NS
MPI	0.8853	6	<0.05	−0.0483	5	NS
PEPLT	0.6225	6	NS	0.8701	5	NS

r, correlation coefficient; P is the significance level; NS, not significant at the 5% level.

(Wright's fixation index F_{IS} = 0.065 when averaged over 13 loci; χ^2 = 110.5, d.f. = 13 with $P \ll 0.001$) and at five out of 13 loci in Lake Östra Trollsvattnet (F_{IS} = 0.036; χ^2 = 48.8, d.f. = 13 with $P < 0.001$).

Although the above observations strongly indicate the existence of more than one population within Lakes Östra and Västra Trollsvattnet, the present data do not allow us to determine the number of genetically distinct populations that may occur in the two lakes. There are two alternative explanations for our observations as we do not know to what extent fish migrate between the lakes. First, each lake may harbor its own genetically distinct population, but individuals from the two populations migrate between lakes and are mixed in the samples. Second, fish from the two lakes do not mix, but there is more than one population within both lakes. The correlations of temporal allele frequency shifts between the two lakes (Table 3) do not provide conclusive information with respect to these alternatives. Only one correlation is statistically significant, but the excess of positive correlation coefficients (11 out 14; χ^2 = 4.6, d.f. = 1 with $P < 0.05$) may favor the first alternative; under the assumption of selective neutrality (see below) temporal allele frequency shifts are independent among reproductively isolated populations, whereas a positive correlation is expected in samples drawn from a mixture of the same two populations. The implications, with respect to the estimation of effective size, of sampling a mixture of populations within each of these two lakes is discussed below.

Temporal variation: Clearly, temporal allele frequency shifts are generally larger between cohorts than between sample years (Table 1). This observation conforms with theoretical expectation when genetic drift is the primary cause of short term fluctuations (JORDE and RYMAN 1995). As indicated in Figure 2, however, there are considerable differences between lakes with respect to the magnitude of those shifts.

The largest temporal differences are found in Lake Flyn where highly significant allele frequency shifts among cohorts are observed at 11 out of 14 loci (Table 1), and the variation is also appreciable in Lakes Blanktjärnen (significant at six loci) and Västra Trollsvattnet (four loci). In contrast, brown trout from Lake Östra Trollsvattnet display only little variation among cohorts; no single locus displays significance at the 5% level, and there is just about a significant temporal change when summing over all the 14 loci (G = 139.2, d.f. = 111 with $P < 0.05$). Further, this weak significance is largely due to the allele frequencies observed at two loci from the first cohort (1974); if this cohort is excluded there is no overall significance for this lake (G = 129.8, d.f. = 108 with P = 0.08). Thus, in contrast to the other three lakes there is no strong evidence for true temporal allele frequency changes in Lake Östra Trollsvattnet.

Selective neutrality of allozymes: There are no indications of natural selection causing the observed temporal allele frequency shifts. The results of the LEWONTIN-KRAKAUER tests for selective neutrality are shown in Table 4. Clearly, none of the X^2 values exceeds the nominal value of 3.84 at the 5% rejection level; if anything there is a tendency for the variance among loci to be somewhat low as judged by this test, with average X^2 ranging from 0.55 (Lake Västra Trollsvattnet) to 0.87 (Lake Blanktjärnen) as compared to the expected value of 1.0 (Table 4, bottom line).

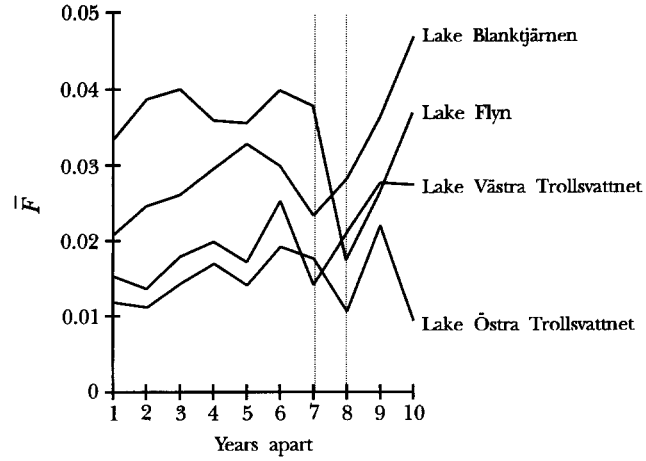

FIGURE 2.—Amount of temporal allele frequency change as measured by F (Equation 1) averaged over loci for cohorts born at various numbers of years apart. The vertical dotted lines represent the range of generation intervals (G) estimated for the present four populations on the basis of demographic data (*i.e.*, 7.15–7.97 years; Table 6).

We also tested for the existence of natural selection through comparing allele frequencies among age classes of fish of a common cohort. Here, we take advantage of the fact that each cohort occurs in the catches of multiple years (5 years on average). We performed heterogeneity G tests separately for each locus and cohort within each of the four populations and obtained results that can be summarized as follows. Out of a total of 526 separate tests we found 28 to be significant at the 5% level, including two that were significant also at the 1% level. Eleven out of the 28 significances occur in Lake Västra Trollsvattnet and five, six, and six in Lakes Blanktjärnen, Flyn, and Östra Trollsvattnet, respectively (the total number of tests in those lakes were 123, 136, 142, and 125, respectively). All but three loci (*DIA, aMAN,* and *PEPLT*) out of the 14 ones analyzed were represented at least once among the 28 significances, neither of them seemed particularly subject to change, and we could see no consistent trend or direction of allele frequency shifts at the 11 loci yielding one or more significances. Thus, except for a small excess of significances in Lake Västra Trollsvattnet, no pattern could be detected suggesting the operation of selective forces. In view of the lack of a discernable pattern and of the fact that the proportion of significant tests (28/526 = 0.053) is very close to what is expected from

TABLE 4

Amount of allele frequency change (F; Equation 1) between consecutive cohorts and test for heterogeneity of F among loci

	Lake Blanktjärnen				Lake Flyn				Lake Östra Trollsvattnet				Lake Västra Trollsvattnet			
Cohorts	No. of loci	F	s_F^2	X^2	No. of loci	F	s_F^2	X^2	No. of loci	F	s_F^2	X^2	No. of loci	F	s_F^2	X^2
1974–1975	—	—	—	—	—	—	—	—	3	0.055395	0.002086	0.34	3	0.015027	0.000043	0.10
1975–1976	3	0.029902	0.002140	1.20	3	0.021767	0.000492	0.52	3	0.016430	0.000056	0.10	3	0.002920	0.000016	0.93
1976–1977	3	0.038588	0.001060	0.36	3	0.001998	0.000005	0.63	3	0.011243	0.000161	0.64	5	0.022072	0.000120	0.12
1977–1978	3	0.022514	0.000379	0.37	3	0.031482	0.000686	0.35	4	0.004909	0.000067	1.39	5	0.003712	0.000007	0.27
1978–1979	7	0.000671	0.000000	0.49	5	0.006715	0.000088	0.97	6	0.012003	0.000208	0.72	7	0.003162	0.000020	0.99
1979–1980	8	0.004546	0.000024	0.58	7	0.007227	0.000093	0.89	6	0.007740	0.000030	0.25	8	0.014110	0.000206	0.52
1980–1981	8	0.009465	0.000165	0.92	8	0.020962	0.001129	1.29	8	0.017294	0.000209	0.35	8	0.032367	0.000589	0.28
1981–1982	8	0.041821	0.003578	1.02	8	0.076692	0.003777	0.32	8	0.013335	0.000239	0.67	14	0.018491	0.000432	0.63
1982–1983	14	0.010895	0.000411	1.73	8	0.017141	0.000268	0.46	14	0.006572	0.000083	0.97	14	0.013113	0.000241	0.70
1983–1984	14	0.017815	0.000436	0.69	14	0.015159	0.000756	1.65	14	0.009492	0.000163	0.90	14	0.011971	0.000157	0.55
1984–1985	14	0.012002	0.000159	0.55	14	0.028625	0.000627	0.38	14	0.004340	0.000036	0.96	14	0.009725	0.000147	0.78
1985–1986	14	0.017117	0.000459	0.78	14	0.021066	0.000550	0.62	14	0.009070	0.000183	1.11	14	0.023969	0.000837	0.73
1986–1987	14	0.033705	0.001752	0.77	14	0.061756	0.008516	1.12	14	0.020183	0.000378	0.46	—	—	—	—
1987–1988	14	0.040527	0.006250	1.90	14	0.067292	0.019207	2.12	—	—	—	—	—	—	—	—
1988–1989	—	—	—	—	14	0.029473	0.000699	0.40	—	—	—	—	—	—	—	—
Average X^2				0.87				0.84				0.68				0.55

s_F^2 is the observed variance of F among loci, calculated separately for each pair of consecutive cohorts. X^2 is the LEWONTIN-KRAKAUER test statistic (Equation 11) that is approximately χ^2-distributed with one d.f. (the number of independent alleles used in computing each F value). None of these tests are significant at the 5% level.

chance alone, we conclude that there is no evidence for the operation of natural selection on any of the 14 loci observed.

Using a somewhat different approach when testing for selection, we also looked for tendencies for allele frequencies in different cohorts and populations to change in the same direction from one year to another, *i.e.*, in the interval from year t to $t + 1$. In these tests we only included population-cohort-interval combinations where the allele frequencies at both years (t and $t + 1$) were based on 10 or more fish. For example, for locus *G3PDH-2* and interval 1987–1988 the number of cohorts meeting the sample size criterion of 10 fish were four, three, four, and three in Lakes Blanktjärnen, Flyn, Västra Trollsvattnet and Östra Trollsvattnet, respectively. Eight of these 14 allele frequency changes were in the negative direction, but this frequency of shifts in one direction (8/14 = 0.57) is not significantly different from 0.5 as expected under a null hypothesis of independence (two-sided exact binomial $P = 0.79$). This two-sided binomial test cannot result in statistical significance, *i.e.*, the power is zero, when less than six cohorts are compared, and a total of 120 combinations of locus and time interval had six or more cohorts with sufficient sample sizes. Of these 120 combinations only four (3.3%) yielded a statistically significant excess of positive or negative shifts, and those significances were all weak ones (in the interval 0.01–0.05) and represented different loci. The four significances were all from interval-locus combinations comprising nine or more cohorts, *i.e.*, combinations with a statistical power for detection of a trend greater than those with fewer (six to eight) cohorts. Confining the analysis to combinations with a minimum of nine cohorts, however, only results in a minor increase of the frequency of significant tests (4/95 = 4.3% nominal significances at the 5% level). Thus, we could find no evidence for allele frequency shifts from one year to another to be correlated among cohorts.

Life history characteristics: The demographic characteristics were fairly similar among populations (Table 5). The proportion of males and females was close to 50% at all ages and localities, except for an excess of males in the younger age classes (Figure 3). There were, however, marked differences between the sexes with respect to the age for sexual maturity and to the proportion of breeders at various ages. Sexually mature males first occur at the fifth year of life (*i.e.*, at age 4+) with 10–30% of the males of this age being breeders. At older ages 30–50% of the males in any particular year were reproducing. Females become sexually mature one or two years later than males; <10% of the females of age 5+ were about to breed, whereas at older ages the proportion of breeders was 50–80%.

The age-specific estimates of the life table parameters l_i and b_i are shown in Table 5 along with the corresponding p_i values that are necessary for assessment of the C factor. Clearly, the probability of surviving from one year to another (S) is fairly low (~0.5). The high annual mortality rates require quite large reproduction rates among those individuals that survive to sexual maturity, as is reflected in the b_i values. These values may seem conspicuously large, but the estimates refer to the numbers of offspring at age zero, *i.e.*, when they are just

TABLE 5

Demographic statistics for four natural populations of brown trout

Age	Age class i	Lake Blanktjärnen				Lake Flyn				Lake Östra Trollsvattnet				Lake Västra Trollsvattnet			
		l_i	b_i^m	b_i^f	p_i	l_i	b_i^m	b_i^f	p_i	l_i	b_i^m	b_i^f	p_i	l_i	b_i^m	b_i^f	p_i
0+	1	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
1+	2	0.4800	0	0	0	0.4000	0	0	0	0.5300	0	0	0	0.5300	0	0	0
2+	3	0.2304	0	0	0	0.1600	0	0	0	0.2809	0	0	0	0.2809	0	0	0
3+	4	0.1106	0	0	0	0.0640	0	0	0	0.1489	0	0	0.	0.1489	0	0	0
4+	5	0.0531	1.9	0	0.0503	0.0256	10.0	0	0.1285	0.0789	1.3	0	0.0497	0.0789	2.9	0	0.1124
5+	6	0.0255	11.7	1.9	0.1733	0.0102	31.0	11.6	0.2173	0.0418	6.1	0.6	0.1404	0.0418	6.5	0	0.1363
6+	7	0.0122	17.8	26.8	0.2720	0.0041	51.4	89.3	0.2884	0.0222	13.0	11.9	0.2766	0.0222	8.9	10.2	0.2120
7+	8	0.0059	26.7	43.7	0.2076	0.0016	75.9	171.4	0.1978	0.0117	10.9	25.6	0.2132	0.0117	10.5	24.7	0.2060
8+	9	0.0028	34.4	65.5	0.1398	0.0007	81.0	194.5	0.0964	0.0062	15.9	29.3	0.1402	0.0062	11.7	34.2	0.1424
9+	10	0.0014	40.7	73.7	0.0800	0.0003	94.1	263.9	0.0537	0.0033	18.6	31.8	0.0832	0.0033	15.4	38.2	0.0884
10+	11	0.0006	67.6	72.7	0.0420	0.0001	94.1	263.9	0.0179	0.0017	20.0	37.0	0.0484	0.0017	17.0	43.3	0.0513
11+	12	0.0003	67.6	72.7	0.0210	—	—	—	—	0.0009	20.0	37.0	0.0256	0.0009	17.0	43.3	0.0271
12+	13	0.0001	67.6	72.7	0.0070	—	—	—	—	0.0005	20.0	37.0	0.0142	0.0005	17.0	43.3	0.0151
13+	14	0.0001	67.6	72.7	0.0070	—	—	—	—	0.0003	20.0	37.0	0.0085	0.0003	17.0	43.3	0.0090

The age-specific survival rates, l_i, were estimated from the number of fish in each age class using the CHAPMAN-ROBSON method. No fish older than 13 years (corresponding to age class 14) were found in any population. The birth rates, b_i^m and b_i^f, were estimated from the observed proportions of male (m) and female (f) breeders in various age classes (i), multiplied by the average weight of fish of that age and sex, and adjusted so as to result in a constant population size (*i.e.*, $\Sigma l_i b_i = 1$). $p_i = l_i(b_i^m + b_i^f)/2$ is the estimated probability that a gene in an individual was inherited from a parent of age i.

hatched, and the numbers calculated are consistent with previous observations on brown trout in Scandinavia (JONSSON 1977).

The p_i values (Table 5) indicate that almost 80% of the offspring are produced by parents that are between 6 and 9 years old, and the mean generation length, G (Equation 10), of the four populations varies between 7.15 and 7.97 years (Table 6). Using the l_i and p_i values from Table 5 in Equations 6–9, we obtained constant values for C (Equation 5) after some 50 iterations. The resulting values of C (Table 6) range from $C = 11.29$ (Lake Västra Trollsvattnet) to $C = 15.90$ (Lake Flyn).

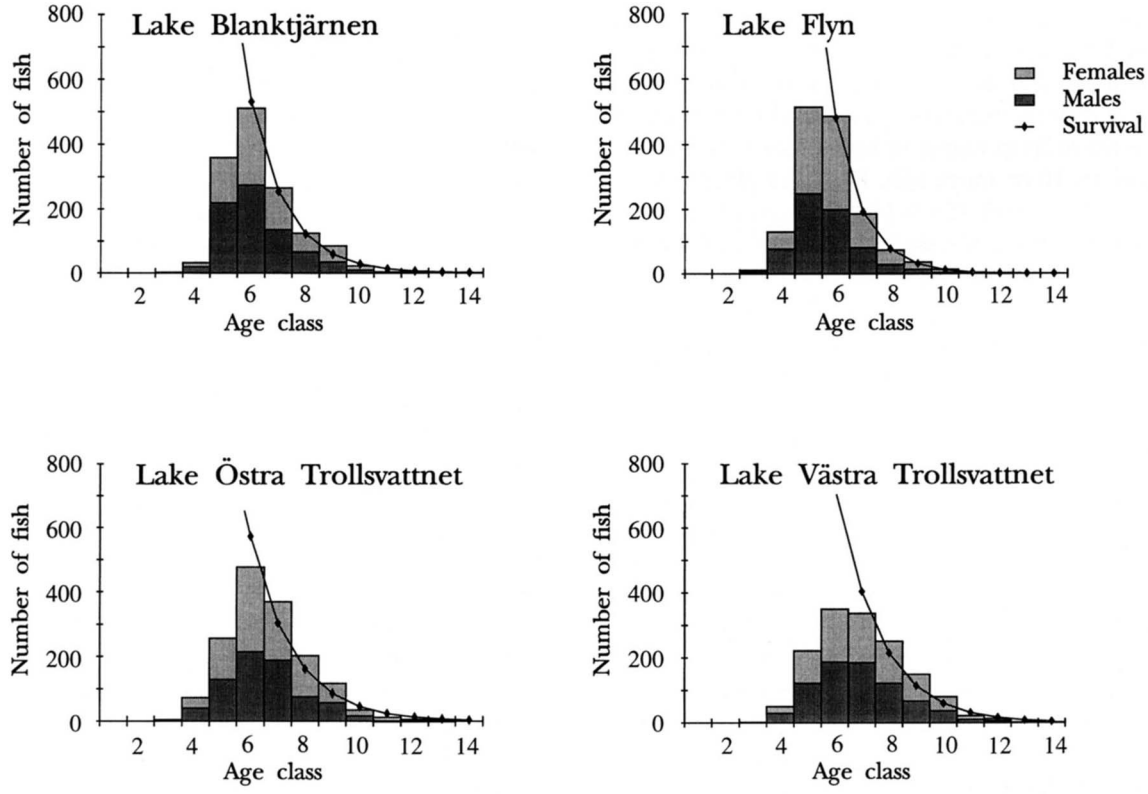

FIGURE 3.—Observed number of fish in various age classes in the total catches (total for the entire period of 1979–1993). The curves represent the estimated age-specific survival rates (l_i) and are scaled to fit the number of fish of higher age classes.

TABLE 6

Observed allele frequency change and estimates of effective size in four natural populations of brown trout

	Lake Blanktjärnen	Lake Flyn	Lake Östra Trollsvattnet	Lake Västra Trollsvattnet
Demographic data				
C	13.17	15.90	12.67	11.29
G	7.82	7.15	7.97	7.89
Temporal allele frequency change				
No. of F values	124	129	111	109
$\bar{F}$	0.020752	0.033298	0.011960	0.015215
$\bar{F}'$	0.008708	0.021470	0.001657	0.005096
SE($\bar{F}'$)	0.003357	0.005466	0.001466	0.001777
Estimated effective size				
$\hat{N}_e$	97	52	480	140
CI (method I)	56–195	35–76	142–∞	70–418
CI (method II)	55–396	35–103	175–∞	83–443
Average heterozygosity				
$\hat{H}$	0.059	0.061	0.052	0.056

C and G are the correction factor for overlapping generations and the mean generation length, respectively. $\bar{F}$ (Equation 1) is the observed amount of temporal allele frequency change averaged over all measurements (combination of loci and consecutive cohorts), and $\bar{F}'$ (Equation 3) is the corresponding quantity corrected for the expected contribution from sampling [standard error = SE($\bar{F}'$)]. The resulting estimates of effective population size, $\hat{N}_e$ (Equation 4) and their 95% confidence intervals (CI) are given below. The CIs were computed in two different ways: method I follows WAPLES (1989), and method II utilizes SE($\bar{F}'$) (see text for details). $\hat{H}$ is the observed heterozygosity averaged over 74 loci (including 60 monomorphic ones).

Clearly, the calculated values for C and G do not differ greatly between populations despite some considerable differences in the estimated l_i and p_i values between the populations (*cf.* Table 5). This indicates that the estimation method is fairly robust to uncertainties in the estimated demographic parameters.

Estimation of effective population size: As indicated above, we estimated effective population size from the standardized variance of allele frequency shifts between consecutive cohorts (F'; Equation 3). Within each population the single locus F' values were averaged over all combinations of locus and consecutive cohorts, and the resulting average ($\bar{F}'$; Table 6) was used in Equation 4 along with the corresponding values of C and G (Table 6).

The estimated effective sizes range from 52 for Lake Flyn to 480 for Lake Östra Trollsvattnet. Although the point estimates are subject to considerable sampling error, the confidence intervals indicate that the populations differ with respect to their effective sizes. For example, the confidence intervals do not overlap for the estimates from Lakes Flyn and Östra Trollsvattnet, and the point estimate for the latter lake is beyond the upper confidence limits of all the other estimates (Table 6). We also note that these conclusions hold true regardless of what method is used for placing confidence limits to the point estimates; both methods yield limits that are reasonably similar in spite of the difference in logic and approach. Employing a randomization test (SOKAL and ROHLF 1981) on the observed F' values yields highly significant differences in the average among populations ($P = 0.0003$, based on 100,000 random partitions of the total of 473 observed values into four samples). This significance indicates the existence of truly different amounts of temporal allele frequency shifts, implying different effective sizes.

Although the estimates of effective size differ by a factor of 10, the average heterozygosities (H) are all fairly similar among populations (Table 6). Based on a total of 74 loci (including 60 monomorphic ones) H ranges from 0.052 in Lake Östra Trollsvattnet to 0.061 in Lake Flyn. Below we discuss the probable cause of this inconsistency with the relationships expected theoretically.

DISCUSSION

The major findings of the present study may be summarized as follows. First, we have established the existence of true genetic changes over time in natural populations of brown trout, a species that is characterized by overlapping generations and iteroparity. Second, it has been possible to detect these changes from limited numbers of individuals sampled from the populations over a restricted time period. Third, most or all of the temporal allele frequency shifts within populations appear to represent random genetic drift. Fourth, it has been possible to obtain reasonably accurate estimates of effective population size from the temporal changes observed. Fifth, the populations studied were found to differ considerably in effective size although they occur in lakes that appear very similar with respect to both

biotic and abiotic characteristics. Finally, the differences in effective size are not coupled with apparent corresponding differences in the amount of genetic variability (average heterozygosity, H) of these populations. In the following we discuss the validity and implications of these conclusions.

Genetic drift: As mentioned above there is no evidence for the temporal allele frequency shifts being caused by natural selection. Both the LEWONTIN-KRAKAUER tests (Table 4) and the tests for temporal changes within cohorts are in agreement with this contention. The only possible indication of temporal shifts not being caused by random drift refers to Lake Västra Trollsvattnet where 11 out of 123 (8.9%) of the tests for allele frequency heterogeneity within cohorts were statistically significant. In this lake, however, we also observed strong evidence for the existence of multiple populations, and the significances observed may very well have been caused by sampling of different proportions of the contributing populations (*cf.* NEI and TAJIMA 1981).

A completely independent piece of information supporting the notion of genetic drift being the predominant cause of temporal change is illustrated in Figure 2. In this figure we plotted the standardized allele frequency shifts (F, averaged over loci) between cohorts born various number of years apart. As discussed previously (JORDE and RYMAN 1995), in a population where genetic drift is the only factor causing allele frequency changes, F is expected to take different values depending on the time that separates the birth of two cohorts. In particular, a low value of F is expected between cohorts that are born exactly one generation interval apart (see also WAPLES and TEEL 1990). Such a minimum is precisely what is observed in all lakes for cohorts that are born 7–8 years apart, *i.e.,* one generation interval apart as determined from the demographic analyses (Tables 5 and 6). This good agreement between our observations and the theoretical expectation for selectively neutral alleles adds further support to the presumption that the main cause of the observed allele frequency changes is a limited effective population size, *i.e.,* genetic drift.

Population admixture: The clear indications of the samples from each of Lakes Västra and Östra Trollsvattnet being drawn from more than one single population complicates the interpretation of the estimates of effective size from these two lakes. As discussed by NEI and TAJIMA (1981), the effect on the estimates of N_e of sampling from more than one genetically distinct population depends on the sampling strategy. If the samples are taken so that all populations are proportionally represented, the estimated effective size will be that of the total (combined) population. In other cases the estimate may be biased downward, and it may also be unclear to which population the estimate refers.

For Lake Östra Trollsvattnet, the possibility of a downward bias is of little practical concern because the estimated effective size is quite large (with an upper 95% confidence limit of infinity). Regardless of any such bias we must conclude that Lake Östra Trollsvattnet harbors at least one population of substantial effective size.

The situation is somewhat different for Lake Västra Trollsvattnet, however, both because the estimated N_e of 140 (Table 6) is not very large and because the indications of population subdivision are much stronger than for the other lake of this pair (Östra Trollsvattnet). For Lake Västra Trollsvattnet the above mentioned allele frequency heterogeneities within cohorts (8.9% of the tests yielding statistical significance) may directly indicate a variable representation of genetically distinct populations in the samples. Such a variable representation would lead to higher F values than expected by genetic drift alone and thus result in an estimated effective size that is too small. Thus, the estimated effective size for Lake Västra Trollsvattnet should probably be viewed with some caution.

For Lakes Blanktjärnen and Flyn there are no indications of population subdivision that might bias the estimates of effective size. Rather, we are left with the impression that the same population occupies both lakes. If this is true, the same parametric effective size is estimated from both lakes of this pair. Indeed, the two estimates are fairly similar (97 and 52; Table 6) though not identical. A randomization test on the observed F' values from the two lakes results in a just about significant ($P = 0.04$) difference between their average $\bar{F}'$s (Table 6), indicating different effective sizes. A tentative explanation to the difference between the estimates, under the assumption of one single population occupying both lakes, would be that the offspring from somewhat different segments of the pool of breeders are found in the two lakes.

Robustness to assumptions: When correcting the observed temporal allele frequency shifts (F) for the expected contribution from sampling, we assumed that the actual number of newborns (N_1) in these populations is large so that the term $1/N_1$ can be ignored (*cf.* Equations 2 and 3). This assumption seems reasonable in view of the high fecundity characterizing salmonid fishes. Further, if the survival among young fish is indeed as low as estimated for the older age classes (~50% per year), N_1 must at least be of the order of 1000 for the number of fish at catchable ages to approach 100 (the average number of individuals that were sampled from each cohort). If so, the use of Equation 3 appears fully appropriate.

Although the above arguments speak in favor of N_1 being large, it is still possible that it is not large enough to justify that the term $1/N_1$ is ignored. To check how sensitive our estimated effective sizes are to the assumption of large N_1, we compared those estimates to the ones obtained under the most extreme alternative to

our assumption of large numbers of newborns. Clearly, the number of newborns in a cohort cannot be smaller than the number of individuals actually sampled from that cohort, so we used that number for N_1 to see how the estimates of effective size were affected. If N_1 equals the number of individuals actually sampled from a cohort, Equation 2 reduces to $\bar{F}' = \bar{F}$, and because there is no mortality at young ages under this scenario, we set $l_i = 1$ for age classes $i = 1$ to 5 and calculated l_i for older age classes from the estimated annual survival (S) as before. Using these survival rates when calculating the correction factor C, we obtain $C = 31.6$, 32.7, 35,6, and 34.2 for Lakes Blanktjärnen, Flyn, and Östra and Västra Trollsvattnet, respectively, which results in estimates of effective sizes (Equation 4) of 97, 69, 187, and 142 for the four populations. With the exception for Lake Östra Trollsvattnet, these estimates are essentially identical to those obtained previously (*cf.* Table 6), indicating that the estimation procedure is robust to assumptions regarding the number of newborns. As for Lake Östra Trollsvattnet, we note that the observed $\bar{F}$ is considerably smaller for this lake than for the other ones, with barely significant temporal allele frequency shifts, and the estimated effective size in this lake is thus subject to larger uncertainty.

Maintenance of genetic variation: The present study has shown that the effective size of natural brown trout populations may be quite small and differ considerably among lakes. Because we do not have estimates of the number of fish (N) of the different lakes, we cannot compare directly the ratios N_e/N for the present populations. Indirect observations, however, indicate that a ranking of the lakes with respect to their absolute population sizes corresponds with that of the effective ones (*cf.* Table 6). First, each of the two Lakes Östra and Västra Trollsvattnet appears to harbor more fish than either of the two other lakes as judged from the relative amount of fishing effort needed for the annual sampling of fish (one *vs.* two or more nights of gillnetting for each lake of the two pairs, respectively). Second, there is a crude correspondence between the physical characteristics of the lakes and the effective population sizes estimated here. These characteristics include the surface areas and the number of creeks (*i.e.*, potential spawning sites) flowing in and out of the lakes (Figure 1). In particular, the smallest effective sizes are estimated for those lakes (Blanktjärnen and Flyn) that appear to have the smallest number of suitable breeding and/or nursery grounds, *i.e.*, inlet and outlet streams. Thus, although the evidence is circumstantial, there appears to be a reasonable congruence between the estimates of effective size and our general impression of the actual number of fish in these lakes and their potential for supporting self-sustaining populations.

There is an apparent inconsistency, however, between the estimated effective sizes of these populations on one hand and their relatively high and similar levels of genetic variation (average heterozygosity, H: Table 6) on the other. Clearly, there must be some factor(s) preventing excessive loss of genetic variation in these populations. The most apparent explanation to the apparent retention of heterozygosity is migration of fish from neighboring populations. A low level of migration (gene flow) is sufficient to prevent fixation of alleles due to genetic drift within populations, and under the assumption of gene flow, the average heterozygosities do not necessarily reflect the effective sizes of the local populations, *i.e.*, the quantities estimated here. Rather, the heterozygosities are reflections of the much larger effective neighborhood size (WRIGHT 1969).

The results of this study have several implications for the understanding of evolutionary change and for the management of brown trout and other species with similar life history characteristics. For example, the information that the effective size may be of the magnitude of 50–100 individuals tells us that natural selection must be fairly strong to override random changes of allele frequencies in many natural populations (*cf.* KIMURA 1983, p. 48). Thus, random genetic drift may explain the marked genetic differentiation commonly observed in this species and other explanations for this observation, such as initial founder effects, are unnecessary. We will not expand on these issues here, but return to them in forthcoming papers. In conclusion, we note that the smallest estimates of N_e obtained in this study are not very different from the numbers generally considered as the minimum acceptable size for short-term conservation of genetic variability when discussing management of endangered and threatened populations (*e.g.*, RYMAN and STÅHL 1980; SOULÉ 1980). This observation emphasizes the need for conserving population systems including both the constituent subpopulations and the potential for gene flow between them.

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LITERATURE CITED

ALLENDORF, F. W., N. MITCHELL, N. RYMAN and G. STÅHL, 1977 Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. Hereditas **86:** 179–190.

BARTLEY, D., M. BAGLEY, G. GALL and B. BENTLEY, 1992 Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. Conserv. Biol. **6:** 365–375.

BEGON, M., C. B. KRIMBAS and M. LOUKAS, 1980 The genetics of *Drosophila subobscura* populations. XV. The effective size of a natural population estimated by three independent methods. Heredity **43:** 335–350.

CABALLERO, A., 1994 Developments in the prediction of effective population size. Heredity **73:** 657–679.

CABALLERO, A., and W. G. HILL, 1992 Effective size of nonrandom mating populations. Genetics **130:** 909–916.

CHAKRABORTY, R., M. HAAG, N. RYMAN and G. STÅHL, 1982 Hierarchical gene diversity analysis and its application to brown trout population data. Hereditas **97:** 17–21.

CROW, J. F., and C. DENNISTON, 1988 Inbreeding and variance effective population numbers. Evolution **42:** 482–495.

CROW, J. F., and M. KIMURA, 1970 *An Introduction to Population Genetics Theory.* Burgess, Minneapolis.

FELSENSTEIN, J., 1971 Inbreeding and variance effective numbers in populations with overlapping generations. Genetics **68:** 581–597.

FERGUSON, A., 1989 Genetic differences among brown trout, *Salmo trutta,* stocks and their importance for the conservation and management of the species. Freshw. Biol. **21:** 35–46.

FRANKHAM, R., 1995 Effective population size/adult population size ratios in wildlife: a review. Gen. Res. **66:** 95–107.

GARCIA-MARIN, J. L., P. E. JORDE, N. RYMAN, F. UTTER and C. PLA, 1991 Management implications of genetic differentiation between native and hatchery populations of brown trout (*Salmo trutta*) in Spain. Aquaculture **95:** 235–249.

GUYOMARD, R., and F. KRIEG, 1983 Electrophoretic variation in six populations of brown trout (*Salmo trutta* L.). Can. J. Genet. Cytol. **25:** 403–413.

HEDGECOCK, D., V. CHOW and R. S. WAPLES, 1992 Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture **108:** 215–232.

HILL, W., 1981 Estimation of effective population size from data on linkage disequilibrium. Genet. Res., Camb. **38:** 209–216.

JOHNSON, K. R., J. E. WRIGHT and B. MAY, 1987 Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics **116:** 579–591.

JONSSON, B., 1977 Demographic strategy in a brown trout population in Western Norway. Zool. Scr. **6:** 255–263.

JORDE, P. E., 1994 Allozymes in Scandinavian brown trout (*Salmo trutta* L.). Report from the Division of Population Genetics, Stockholm University, Stockholm.

JORDE, P. E., and N. RYMAN, 1990 Allele frequency estimation at loci with incomplete co-dominant expression. Heredity **65:** 429–433.

JORDE, P. E., and N. RYMAN, 1995 Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics **139:** 1077–1090.

JORDE, P. E., A. GITT and N. RYMAN, 1991 New biochemical genetic markers in the brown trout (*Salmo trutta* L.). J. Fish Biol. **39:** 451–454.

KIMURA, M., 1983 *The Neutral Theory of Molecular Evolution.* Cambridge University Press, Cambridge.

KRIEG, F., and R. GUYOMARD, 1986 Population genetics of French brown trout (*Salmo trutta* L.): large geographical differentiation of wild populations and high similarity of domesticated stocks. Génét. Sél. Evol. **17:** 225–242.

KRIMBAS, C. B., and S. TSAKAS, 1971 The genetics of *Dacus oleae.* V. Changes of esterase polymorphism in a natural population following insecticide control—selection or drift? Evolution **25:** 454–460.

LEWONTIN, R. C., and J. KRAKAUER, 1973 Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics **74:** 175–195.

MURRAY, B. G., and L. GÅRDING, 1984 On the meaning of parameter *x* of Lotka's discrete equations. OIKOS **42:** 323–326.

NEI, M., 1973 Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70:** 3321–3323.

NEI, M., and F. TAJIMA, 1981 Genetic drift and estimation of effective population size. Genetics **98:** 625–640.

ORIVE, M. E., 1993 Effective population size in organisms with complex life-histories. Theor. Pop. Biol. **44:** 316–340.

POLLAK, E., 1983 A new method for estimating the effective population size from allele frequency changes. Genetics **104:** 531–548.

ROBSON, D. S., and D. G. CHAPMAN, 1961 Catch curves and mortality rates. Trans. Am. Fish. Soc. **91:** 181–189.

RYMAN, N., 1983 Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture **33:** 1–21.

RYMAN, N., and L. LAIKRE, 1991 Effects of supportive breeding on the genetically effective population size. Conserv. Biol. **5:** 325–329.

RYMAN, N., and G. STÅHL, 1980 Genetic changes in hatchery stocks of brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. **37:** 82–87.

RYMAN, N., and G. STÅHL, 1981 Genetic perspectives of the identification and conservation of Scandinavian stocks of fish. Can. J. Fish. Aquat. Sci. **38:** 1562–1575.

RYMAN, N., F. W. ALLENDORF and G. STÅHL, 1979 Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). Genetics **92:** 247–262.

RYMAN, N., R. ÖHMAN, G. STÅHL, A. NILSSON, U. LAGERCRANTZ *et al.,* 1986 Genetisk variation hos laxartad fisk - en snabbt försvinnande naturresurs: Information om genetisk forskning i Jämtland. (Genetic variation in salmonid fishes - a rapidly declining natural resource: Information on the scientific work in the Lakes Bävervattnen area in Jämtland, central Sweden. [In Swedish]). Resta Grafiska AB, Stockholm.

RYMAN, N., P. E. JORDE and L. LAIKRE, 1995 Supportive breeding and variance effective population size. Conserv. Biol. **9:** 1619–1628.

SHAKLEE, J. B., F. W. ALLENDORF, D. C. MORIZOT and G. S. WHITT, 1990 Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. **119:** 2–15.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry,* Ed. 2. Freeman, San Francisco.

SOULÉ, M. E., 1980 Thresholds for survival: maintaining fitness and evolutionary potential, pp. 151–169 in *Conservation Biology. An Evolutionary-Ecological Perspective,* edited by M. E. SOULÉ and B. A. WILCOX. Sinauer Associates, Sunderland, MA.

SUGG, D. W., and R. K. CHESSER, 1994 Effective population sizes with multiple paternity. Genetics **137:** 1147–1155.

TAGGART, J. B., and A. FERGUSON, 1984 Allozyme variation in the brown trout (*Salmo trutta* L.): single locus and joint segregation inheritance studies. Heredity **53:** 339–359.

TAJIMA, F., and M. NEI, 1984 Note on genetic drift and estimation of effective population size. Genetics **106:** 569–574.

WANG, J., 1995 Exact inbreeding coefficient and effective size of finite populations under partial sib mating. Genetics **140:** 357–363.

WAPLES, R. S., 1989 A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics **121:** 379–391.

WAPLES, R. S., 1990 Conservation genetics of Pacific salmon. III. Estimating effective population size. J. Heredity **81:** 277–289.

WAPLES, R. S., 1991 Genetic methods for estimating the effective size of cetacean populations. Rep. Int. Whal. Commn. (special issue) **13:** 279–300.

WAPLES, R. S., and D. J. TEEL, 1990 Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. Conserv. Biol. **4:** 144–156.

WRIGHT, S., 1969 *Evolution and the Genetics of Populations, Vol. 2, The Theory of Gene Frequencies.* University of Chicago Press, Chicago.

WRIGHT, S., T. DOBZHANSKY and W. HOVANITZ, 1942 Genetics of natural populations. VII. The allelism of lethals in the third chromosome of *Drosophila pseudoobscura.* Genetics **27:** 363–394.

YOUNGS, W. D., and D. S. ROBSON, 1978 Estimation of population number and mortality rates, pp. 137–164 in *Methods for Assessment of Fish Production in Fresh Water,* edited by T. BAGENAL. IBP Handbook No. 3, Ed. 3, Blackwell Scientific, Oxford.

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