Conservation Genetics of Pacific Salmon I. Temporal Changes in Allele Frequency

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Abstract: *Recent reductions in the abundance of all Pacific salmon species (*Oncorhynchus *spp.), coupled with large increases in artificial production, demand that careful attention be paid to genetic changes occurring in both wild and cultured populations. Analysis of electrophoretic data for chinook salmon (*O. tshawytscha*) from the Pacific coast of Oregon revealed substantial allele frequency changes over 2–4 years in hatchery, but not wild, populations. Unfortunately, our understanding of the causes of this result is hampered by a lack of theoretical models designed for organisms with life history features like those of Pacific salmon. We used computer simulations to provide a context for understanding genetic changes observed in the hatchery populations.*

Simulation results indicated that annual fluctuations in population allele frequencies due to genetic drift can typically be expected to be several percent, with the absolute magnitude determined primarily by the effective number of spawners each year rather than the age structure. Changes over 10- to 25-year periods were only slightly greater than short-term changes (1–5 years). The magnitude of allele frequency change over time was different for juvenile and adult samples. The probability of a significant test statistic comparing allele frequencies in temporally spaced samples increased with the ratio of sample size to effective number of breeders per year. This is an important consideration for conservation biologists, who typically are concerned with populations of small effective size.

Simulation results indicate that it is necessary to postulate

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Resumen: *Se han observado reducciones recientes en la abundancia de la especie de salmón del Pacifico (*Onoorhynchus *spp.). Estos cambios están relacionados con el incremento de la producción artificial, lo cual requiere que se preste atención especial a los cambios genéticos que están ocurriendo en las poblaciones silvestres y cultivadas de esta especie. Un análisis de los datos electroforéticos para el salmón chinook (*O. tshawytsha*) de la costa del Pacífico de Oregón reveló cambios substanciales en la frecuencia de alelos durante un período de 2–4 años en los criaderos, pero no en las poblaciones silvestres. Desafortunadamente, nuestro entendimiento de las causas de este resultado es limitado dado a la falta de modelos teóricos diseñados para organismos con ciclos de vida semejantes a las del salmón del pacifico. Nosotros utilizamos la simulación por computadoras para proporcionar un contexto para entender los cambios genéticos observados en las poblaciones del criadero.*

Los resultados de la simulación indicaron que las fluctuaciones anuales en la frecuencia de alelos de la población dado a la deriva genética, se espere que sean de varios porcentages. La magnitud absoluta es determinada principalmente por el número efectivo de hembras reproductivas cada año en vez de la estructura de edades. Cambios genéticos durante un período de 10 a 25 años fueron ligeramente mayores que cambios a corto plazo (1–5 años). La magnitud en los cambios en la frecuencia de alelos a través del tiempo fue diferente para las muestras de juveniles y de adultos. La probabilidad de un significativo test estadístico comparando frecuencias de alelos en muestras espaciadas en el tiempo aumentó con la relación de tamaño de la muestra a número efectivo de reproductores por año. Esta es una consideración importante para biólogos de conserva-

unrealistically large selection coefficients to explain the genetic changes in the hatchery populations by natural selection. The changes observed are consistent with a pure drift model provided that the effective number of breeders was as small as about 25–50 per year. Analysis of brood stock information for the hatcheries indicates that the effective population number may indeed have been this low, although the number of returning adults was often much larger. This conclusion underlines the importance of monitoring the genetic consequences of the large-scale artificial propagation programs involving Pacific salmon.

ción, a quienes típicamente les concierne el tamaño efectivo de poblaciones pequeñas.

Los resultados de la simulación indican que es necesario postular coeficientes de selección irrealisticamente grandes para explicar los cambios genéticos en la población reproductiva por selección natural. Los cambios observados son consistentes con un modelo puro de deriva provisto que el efectivo número de reproductores fue tan pequeño como 25 – 50 por año. El análisis de la información del linaje reproductive para los criaderos indica que el efectivo número poblacional en realidad pudo haber sido tan bajo, a pesar de que el número de adultos devueltos fue con frecuencia mucho más grande. Esta conclusión subraya la importancia de evaluar periodicamente las consecuencias genéticas de los programas de propagación artificial a larga escala que involucran al salmón del Pacífico.

Introduction

Anadromous Pacific salmon (*Oncorhynchus* spp.) spend one or more years at sea before returning to fresh water to spawn. The tenacity these fish demonstrate during the rigorous spawning migration — massive physiological changes associated with entry into fresh water, imposing natural obstacles such as rapids and waterfalls, predation by bears and other carnivores — has long been a source of wonderment to human observers. Unfortunately, Pacific salmon have not been as successful in overcoming obstacles created by man. Destruction of spawning habitat, blockage of migratory routes by dams, and overfishing have all made major inroads into spawning runs in most river systems. Current abundances of the five species in northwestern North America are substantially below historical levels (Fredin 1980; Fraidenburg & Lincoln 1985), and some populations have disappeared entirely or are on the verge of extinction (e.g., Williams 1989). To mitigate losses to wild stocks, considerable efforts have been made to boost artificial production in hatcheries. These efforts are carried out on a colossal scale: in 1987 in Washington state alone, over 330,000,000 Pacific salmon were released from over 130 facilities (Abrahamson 1988).

These two factors, depletion of wild stocks and increased hatchery production, can greatly accelerate the process of genetic change in Pacific salmon. Accelerated change may compromise the long-term fitness of the species by reducing overall levels of variability and eliminating adaptive gene complexes evolved over thousands of generations. Surviving wild populations are subject to selection in altered environments, immigration (from transplantation programs or strays from hatcheries), and genetic drift (through reduced population size). Rapid genetic change is an even greater concern in cultured populations: the opportunities for artificial selection are obvious, transfer of eggs or fish between hatcheries may introduce new genes or create novel genetic combinations through hybridization, and brood stock practices may cause the effective number of breeders to be much less than the number of returning adults (e.g., Simon et al. 1986).

Our interest in the problem of genetic changes in Pacific salmon populations grew out of the analysis of electrophoretic data for a series of populations of chinook salmon (*O. tshawytscha*) from the Pacific coast of Oregon. Nine wild and nine hatchery populations were sampled in two different years, allowing comparisons of allele frequency at an average of about 10 polymorphic loci per population (Fig. 1). In the wild populations, the percentage of single-locus contingency chi square tests showing statistically significant allele frequency change (8%) was only slightly higher than the nominal α level for the test (5%). In eight of the nine hatcheries, however, the genetic changes were much more substantial (significant results in 22%–63% of single-locus tests; overall hatchery average = 36%).

This striking result emphasizes the need for monitoring the genetic consequences of large-scale artificial propagation programs. Identifying potential problems, however, is only the beginning; efforts to reduce rates of genetic change in cultured populations are unlikely to succeed unless the causes of the change can be identified. Distinguishing between the most likely causes of short-term genetic change (selection, migration, genetic drift) is difficult in any event, and the problem is even more complex in Pacific salmon because of the lack of an adequate model for genetic change. Most of the seminal work of Wright, Fisher, and Haldane was based on a discrete generation model, but among the Pacific salmon species this assumption is reasonable only for

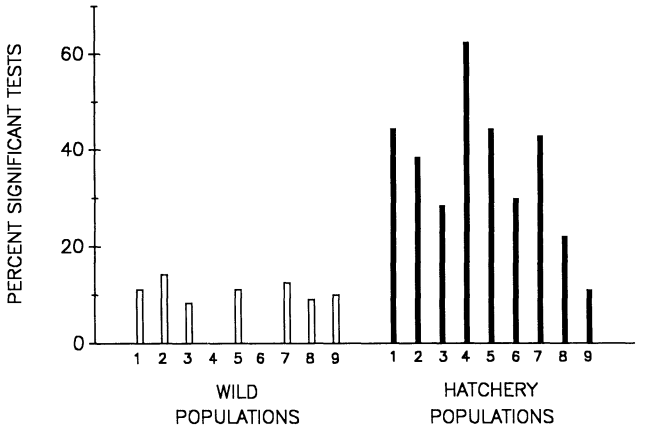

Figure 1. Contrasting patterns of genetic change over 2–4 years in nine wild and nine hatchery populations of chinook salmon from Oregon. For each population, allele frequencies in two temporally spaced samples (of approximately 100 juveniles each) were compared at an average of 10 polymorphic loci; the percentage of single-locus contingency chi square tests showing statistically significant allele frequency change is indicated by the height of the columns. All of the hatchery populations except #9 showed much higher rates of genetic change than did any of the wild populations.

the pink salmon, *O. gorbuscha,* which has a rigid 2-year life cycle in the Pacific Northwest (Aspinwall 1974). More recently (e.g., Hill 1979), it has been shown that many of the results obtained under more restrictive assumptions also hold for organisms in which generations overlap. Pacific salmon, however, do not have generations that overlap in the same sense as, say, most mammals or birds (Fig. 2). In most overlapping generation models, individuals breeding in one time period may survive to reproduce again; in such a population, composition of the breeding population changes gradually over time through death or senility and the maturity of juveniles. In contrast, *Oncorhynchus* spp. die after spawning, so mating occurs only with others returning in the same year. There is, however, some variation in age at spawning in all species except the pink salmon. A particular year's spawning population of chinook salmon, for example, might include fish aged 2, 3, 4, and 5 (or more) years. Thus, although there is 100 percent turnover in the spawning population each year (in contrast to a gradual turnover in other organisms with overlapping generations), a Pacific salmon spawning population typically includes genetic information derived from two or more previous brood years. The result is a pattern of overlapping year classes distinct from that of any existing population genetics model.

These life history features of Pacific salmon make the problem of temporal genetic change difficult to ap-

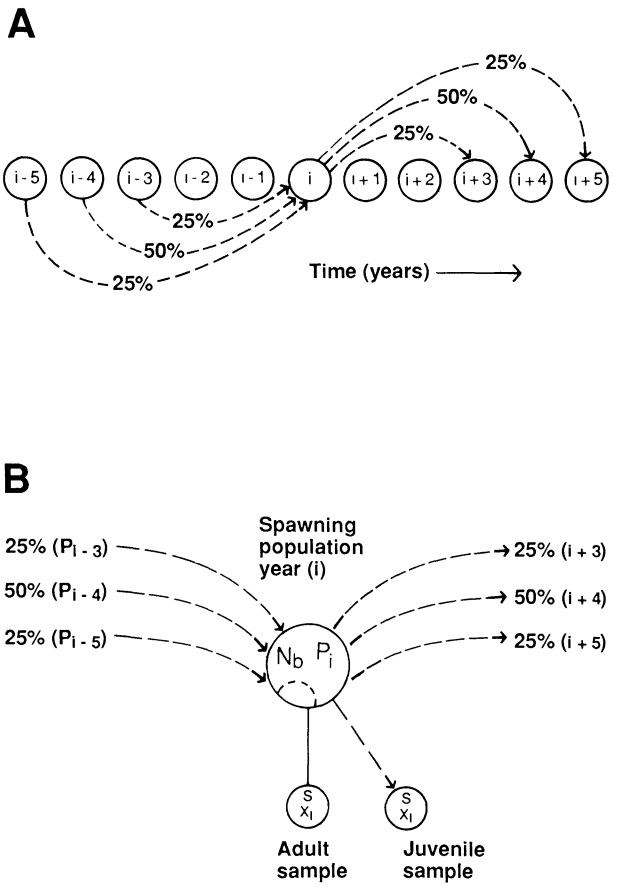

*Figure 2. **A.** Pattern of overlapping generations and migration between year classes used to model genetic change in Pacific salmon populations. Each circle represents a spawning population from a different brood year in the same stream. In the basic model, probability of spawning at age 4 was 0.5, so on average 50 percent of the spawners in year* i *were derived from offspring produced in brood year* i − 4, *with the remainder divided between years* i − 3 *and* i − 5. ***B.** Sampling plan. For each brood year* i, *samples of S individuals were drawn for genetic analysis. P_i is the population allele frequency for the N_b spawners in year class* i; X_i *is the allele frequency in the sample. Adults were sampled without replacement from the N_b spawners after reproduction (solid line); juveniles were sampled binomially from the pool of gametes produced by the spawning population (broken line).*

proach analytically, but the system is amenable to study by simulation. Using the model described below, we address three major questions: (1) What magnitude of change in population allele frequency between years is expected for Pacific salmon in the absence of any directional forces? (2) How should the results of statistical tests of allele frequency change over time be inter-

preted? (3) Based on results for (1) and (2), can we identify the probable causes of the genetic changes observed in the Oregon hatchery populations? Answers to questions (1) and (2) are important because they provide a context for understanding changes in allele frequency observed in salmon populations. As we shall see, the answer to the third question provides important insight for those interested in conserving the genetic resources of cultured populations.

Materials and Methods

Electrophoresis

Samples of approximately 100 juveniles each were taken in 1981 (1980 brood year) from nine wild and nine hatchery populations along the Pacific coast of Oregon. Comparable samples were taken from the wild populations in 1983 (1982 brood) and from the hatcheries in 1985 (1984 brood). The hatchery and wild populations were not paired in a statistical sense; rather, they are part of a geographically more extensive program to sample the major chinook-producing populations in North America (cf. Utter et al. 1989). Sample preparation, starch gel electrophoresis, and interpretation of banding patterns followed Aebersold et al. (1987) and Utter et al. (1987). Loci found to be polymorphic in the hatchery samples are indicated in Table 1. For each locus in each population, a contingency chi square test was used to test the hypothesis that population allele frequencies did not change between the times of sampling. Isoloci (pairs of duplicated loci sharing alleles with identical electrophoretic mobility; see Allendorf and Thorgaard 1984; Waples 1988) were not used in this analysis, nor were they modeled in the simulations. Analysis of temporal changes at duplicated loci is somewhat more complicated (Waples, manuscript II, in press).

Computer Simulations

We adopted a model similar to that used by several authors (Nei & Tajima 1981; Pollak 1983; Waples 1989*a*) studying temporal variation in organisms with discrete generations, with certain modifications dictated by the unique life history features of Pacific salmon. Consider a population in which the effective number of breeders returning to spawn each year is N_b (N_b is analogous to effective populations size, N_e, except that it refers to the number per year rather than per generation). Change was monitored at a single gene locus with two alleles (A,a). In a given year's spawning population (e.g., brood year i), the breeders include individuals of different ages (ages k = 3,4,5) in the expected proportions f_k ($\Sigma f_k = 1$). The $2N_b$ genes representing the breeding population in year i were selected in a series of binomial draws from previous brood years (the selection being from brood year $i - k$ with probability f_k), and the probability of drawing an A allele was P_{i-k} = the frequency of allele A in brood year $i - k$. Offspring produced from the spawn also return to breed at various ages, thus continuing the pattern of overlapping year classes.

Samples for genetic analysis in a particular brood year

Table 1. Significance level of single-locus χ^2 tests of temporal stability of allele frequencies in chinook salmon from nine Oregon hatcheries. Sample sizes in 1980 and 1984 brood years, respectively, are given in parentheses below hatchery names. Samples from two different stocks (spring and fall run) were taken at two hatcheries. For loci showing significant temporal changes (n.s. = not significant), the direction of frequency change of the common allele is indicated in parentheses. A dash indicates no test was performed (locus monomorphic in both samples or no data from one sample).

Locus	*Cedar Creek (99,100)*	*Cole Rivers (spring) (11,350)*	*Cole Rivers (fall) (50,100)*	*Elk River (100,100)*	*Fall Creek (100,100)*	*Rock Creek (100,100)*	*Salmon River (99,100)*	*Trask River (fall) (100,100)*	*Trask River (spring) (100,100)*
Aat-3	n.s.	n.s.	n.s.	—	—	n.s.	n.s.	—	n.s.
Ada-1	—	—	—	0.01(+)	n.s.	—	n.s.	n.s.	—
Adh	—	n.s.	—	—	—	n.s.	—	—	n.s.
Ah-4	0.05(+)	n.s.	n.s.	n.s.	0.001(−)	0.01(+)	0.05(+)	n.s.	0.01(−)
Gl-1	n.s.	0.05(−)	n.s.	n.s.	n.s.	0.05(+)	0.01(+)	n.s.	n.s.
Gr	0.001(−)	—	0.05(+)	n.s.	—	n.s.	—	—	n.s.
Idh-2	—	—	0.01(+)	—	—	—	—	—	—
Ldh-4	—	—	0.01(+)	n.s.	—	—	—	—	—
Ldh-5	0.05(−)	n.s.	0.05(+)	—	—	—	—	—	n.s.
Lgg-1	n.s.	n.s.	n.s.	n.s.	0.05(−)	0.01(+)	0.001(+)	0.001(−)	0.05(+)
Ltp	—	—	n.s.	—	—	—	—	—	—
Mpi	n.s.	n.s.	n.s.	0.001(−)	n.s.	n.s.	0.05(+)	0.05(+)	0.05(−)
Pgdh	—	—	n.s.	—	—	—	—	—	—
Pgk-2	n.s.	n.s.	n.s.	n.s.	n.s.	0.01(−)	0.05(+)	n.s.	n.s.
Sod-1	0.001(−)	n.s.	0.05(−)	n.s.	n.s.	n.s.	n.s.	0.05(−)	n.s.
Combined χ^2 test over all loci:									
Significance level	0.001	n.s.	0.01	0.001	0.01	0.001	0.001	0.001	0.01

(e.g., year i) were chosen in two ways, corresponding to procedures commonly used in studies of Pacific salmon. Adults for genetic analysis are generally taken as post-spawn mortalities (in the wild) or as poststripping sacrifices (in hatcheries). These adults (and the genes they carry) represent a sample *without replacement* of the actual spawning population in brood year i. Juvenile samples are generally taken from the large pool of offspring resulting from the previous year's spawn. A juvenile sample can be considered to be a binomial sample (i.e., a sample with replacement) from gametes produced by the spawners of brood year i. Figure 2 illustrates these sampling schemes and the pattern of overlapping year classes typically found in chinook salmon.

Monte Carlo methods were used to simulate the pattern of genetic change in Pacific salmon. To initialize the simulations, allele frequencies in the N_b breeders in years i = 1–5 were set to 0.5. Beginning in year 6, population allele frequencies (P_i) were determined by sampling from brood years up to 5 years before, as described above. Sample allele frequencies (X_i) were also computed for adults and juvenile progeny taken each year. This process was repeated until year 50 to allow the system to reach a dynamic equilibrium. For each year from i = 51 to 100, the difference in allele frequencies ($|X_i - X_{i-k}|$) was computed for samples taken in the current year and k = 1, 2, 3, 4, 5, 10, and 25 years previously. Differences in population allele frequencies ($|P_i - P_{i-k}|$) were computed in a similar manner. After year 100, allele frequencies were reinitialized to avoid gene extinction, and the process was repeated. Two hundred replicates were performed for each set of initial parameters, with data taken over the last 50 years in each replicate. Thus, 50 × 200 = 10,000 data points ($|X_i - X_{i-k}|$ or $|P_i - P_{i-k}|$) were available for each value of k years separating two samples. Means and variances of these differences in allele frequency were computed over the 10,000 data points. A 2 × 2 contingency chi square test of equality of allele frequencies was performed for each comparison of juvenile or adult samples, and the percentage of statistically significant test results (α = 0.05) over the 10,000 trials was recorded.

Considerable variation in age at spawning occurs both within and between species of Pacific salmon. In the basic model (Fig. 2), probability of spawning at age 4 (f_4) was 0.50, and probability of spawning at ages 3 and 5 was 0.25. This age structure is similar to that found in chinook salmon populations from many areas of the Pacific Northwest, including coastal areas of Oregon (Nicholas & Hankin 1988). In the simulations, an average of 50% of the genes were "immigrants" from brood years other than the one 4 years before, resulting in a "migration" rate (m) or rate of gene flow among year classes of 0.5. We also performed simulations in which m ranged from 0.1 (90% from year i − 4, 5% each from years i − 3 and i − 5) to 0.7 (30% from year i − 4, 35% each from years i − 3, and i − 5) to encompass some of the intra- and interspecific variation in age structure found in Pacific salmon.

Results

Magnitude of Allele Frequency Change

Figure 3A shows the mean difference in allele frequency $|P_i - P_{i-k}|$ in spawning populations k years apart. Even with 50% of the breeders "migrating" between year classes, P_i typically varied several percent from year to year. In the simulations shown in Figure 3A, initial allele frequency was set to 0.5. As expected, the magnitude of allele frequency change was somewhat smaller in simulations with initial frequency closer to 0 or 1 (unpublished data). Relatively small frequency differences (about 1–2%) were found if the number of breeders each year was very large (N_b = 500 to 1000), whereas

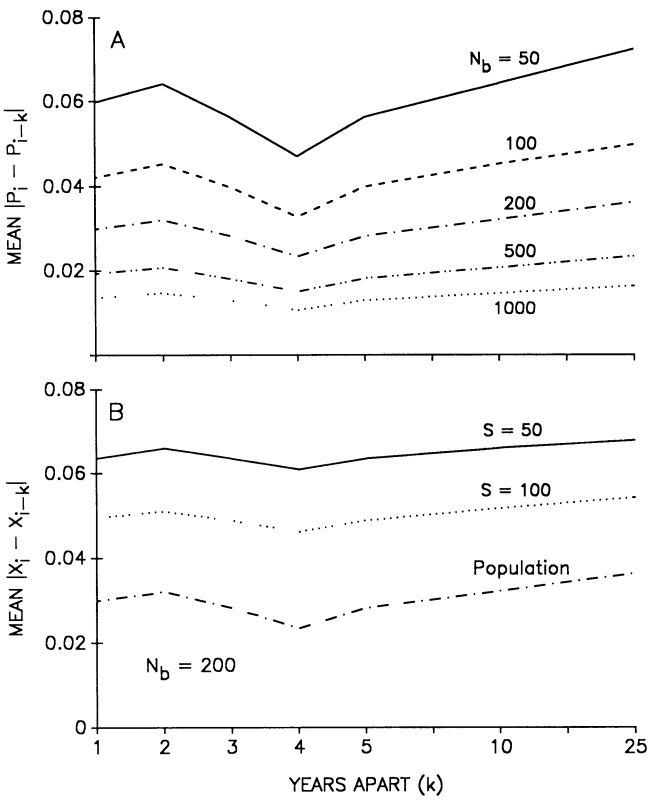

*Figure 3. The magnitude of temporal changes in allele frequency observed in the Pacific salmon model. **A.** Mean change in frequency (P_i) in spawning populations over a period of* k *years as a function of the effective number of spawners per year (N_b). **B.** Mean change in sample allele frequency (X_i) for juvenile samples of S = 50 and S = 100 taken* k *years apart from a population with N_b = 200 breeders per year. Results are from simulations with P = 0.5 and age structure as shown in Figure 2A.*

simulations using fewer spawners (N_b = 50 to 100) resulted in larger changes between years (about 4–6%). The inverse relationship with N_b was expected because the changes in population allele frequency are a consequence of drawing a finite number of breeding individuals each year. Because of the complete turnover each year in the spawning population, annual fluctuations of allele frequency were larger in the Pacific salmon model than are expected using standard models assuming overlapping or discrete generations (Waples, manuscript I, in press). Estimating N_b in spawning populations is difficult; however, given the wide range of run sizes found in most species of Pacific salmon, this number probably differs by several orders of magnitude among populations.

Another important result illustrated in Figure 3A is that the change in population allele frequencies over 10 or 25 years was not much larger than the change over much shorter periods (1–5 years). This result is due to the pattern of overlapping year classes found in Pacific salmon. Each spawning population incorporates genetic information from several brood years, thus dampening the effects of random drift for the system as a whole. Whereas micro variation (differences between consecutive brood years) is a function of the number of breeders each year, macro variation (over generations, or 3- to 5-year periods) is a function of the much larger number of spawning individuals that comprise an entire generation (about 4 years in the present model).

Normally, data are not available for the entire spawning population, and one must estimate allele frequencies from a sample. Two points can be made regarding temporal differences observed in samples drawn in the simulations (Fig. 3B). First, because sampling from the population introduces a source of random error in addition to genetic drift, these samples show greater frequency differences than are found in the entire breeding population. Second, the magnitude of this sampling error is inversely proportional to sample size, so larger absolute differences are found in smaller samples (e.g., larger differences for S = 50 than S = 100 in Figure 3B). The data in Figure 3B and subsequent figures are for juvenile samples. Allele frequency differences in adult samples were generally somewhat smaller, but results for adult sampling are more complicated because they also depend on the ratio of N_b to N, the number of actual spawners (see below).

Test for Equality of Allele Frequencies

The results depicted in Figure 3 indicate that tests of the equality of allele frequencies in temporally spaced samples must be interpreted with caution. This is because standard tests (such as the homogeneity χ^2 test) assume as a null hypothesis that both samples are binomially drawn from the same probability distribution. In the case of temporal variation, the population allele frequencies change over time, so this assumption is invalid. Temporally spaced samples will on average differ more than independent binomial samples drawn from the same population, resulting in a higher proportion of "significant" test results than the nominal α level of the test would suggest, even if no directive forces are involved. The probability of a significant test comparing temporally spaced samples can be obtained analytically in the discrete generation model (Waples 1989*b*), but the complicated life history features of Pacific salmon required a simulation approach.

Results of these simulations (Fig. 4) clearly show that for a given spawning population size (N_b), more tests were significant when a larger sample was taken. The explanation for this result is straightforward: power of a

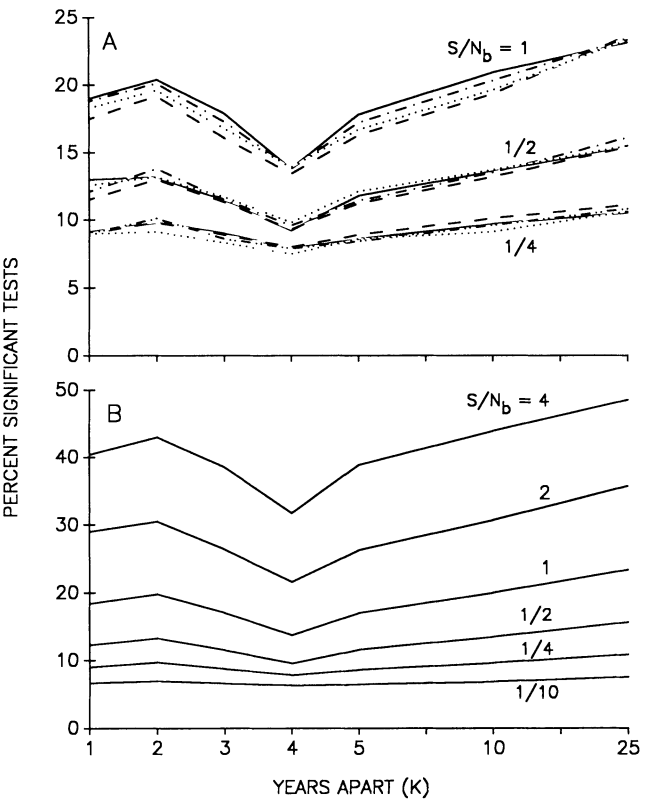

Figure 4. Percent significant χ^2 tests ($p < 0.05$) of homogeneity of allele frequencies in juvenile samples from the same population taken k *years apart. **A.** Results from simulations with N_b = 100 (solid line), 200 (dashed line), 500 (dashed-dotted line), and 1000 (dotted line) and sample sizes such that S/N_b = 0.25, 0.5, and 1. Probability of a significant test result depends not on absolute value of S or N_b, but rather their ratio. **B.** Means of all simulations in **A** for indicated ratios of sample size to effective number of breeders. In the simulations, initial allele frequency was 0.5, and age structure was as shown in Figure 2A.*

statistical test increases with sample size if the null hypothesis is false. In analyzing temporal variation, the null hypothesis for the standard chi square test (two samples taken from the same probability distribution) is always violated, and larger samples more accurately reflect the actual temporal differences that exist in the breeding populations. In fact, the probability of a significant test depends not on the actual values of S or N_b, but on the ratio S/N_b. Figure 4A depicts results of simulations with N_b = 100, 200, 500, and 1000 and juvenile samples with $S = 0.25N_b$, $S = 0.5N_b$, and $S = N_b$. It is clear that the ratio S/N_b explains almost all of the differences between simulations. Waples (1989*b*) reported a similar result in the discrete generation model. This means that for a sample of any size S, the probability of a significant test (assuming only drift and sampling error are involved) can be predicted as a function of N_b. Figure 4B, which can be used for this purpose, shows the mean percentage of significant tests for all simulations with values of S/N_b between 0.1 and 4.

Note that results in Figures 4, 5, and 7 are for χ^2 tests at a single locus with two alleles. In comparing temporally spaced samples, the probability of a significant test increases with both the number of alleles per locus and the number of loci used (if an overall test is used combining results for multiple loci). See Waples (1989*b*) for a discussion of these points for the case of discrete generations.

The Oregon Hatchery Data

The simulation results provide a context for evaluating possible causes for the relatively large allele frequency changes observed in the hatchery populations. One possibility — that the changes were larger in the hatchery samples because they were taken 4 years apart as opposed to 2 years for the wild samples — can be ruled out. In simulations with $m \leq 0.5$, the magnitude of genetic change (and the proportion of significant χ^2 tests) was lowest for samples taken exactly 4 years apart. This is a consequence of the assumption in the model that chinook salmon are more likely to return at age 4 than at any other age: samples taken 4 years apart can be expected to be the most similar if the majority of the spawners are 4 years old. If anything, therefore, we would expect the timing of the samples to produce slightly larger changes in the wild populations.

The simplest explanation for the temporal changes is that they are due to stochastic factors alone (genetic drift and sampling error). Examination of Figure 4B indicates that these factors can be expected to produce the proportion of significant tests observed in the Oregon hatcheries (36%) only if the ratio S/N_b was somewhat larger than 2. Because most of the hatchery samples were of 100 individuals (Table 1), it is necessary to postulate $N_b < 50$ to explain the observed changes by stochastic factors alone.

Before examining the possibility of restricted population size in more detail, it is necessary to consider several other factors that might be responsible for the changes:

1. Inaccurate or artifactual data for at least one year's data. This is possible, but it would not explain why only the hatchery samples were affected. Furthermore, the enzymes used in the analyses have been routinely surveyed for many years, and breeding experiments have verified the Mendelian basis of observed variation for most systems (James B. Shaklee, Washington Department of Fisheries, personal communication).

2. Nonrandom sampling of juveniles for genetic analysis. It is possible that allele frequencies in the hatchery populations as a whole were relatively stable, and that changes observed in the samples were due to one or both samples being nonrepresentative. This might occur, for example, if one of the samples were progeny of only a few adults (see Allendorf and Phelps 1981 for discussion of this point). Available data, however, indicate that eggs from different families were mixed shortly after spawning and long before our sampling (Edward Cummings, Oregon Department of Fish and Wildlife, personal communication). It thus seems reasonable to assume that the samples were approximately random with respect to the surviving progeny.

3. Natural or artificial selection. Although it is often assumed that the majority of electrophoretically detectable polymorphisms are selectively neutral, a number of studies suggest that natural selection is important in maintaining polymorphisms at some electrophoretically assayed gene loci in some organisms. Artificial selection was presumably absent in the wild populations, but a variety of hatchery practices might result in the disproportionate representation of certain genotypes in the spawners or offspring.

If strong selection involving these electrophoretically screened loci (or closely linked loci) was occurring in the hatcheries, it apparently was not uniform in direction in all hatcheries. Table 1 gives the direction of change in frequency of the common allele for all loci with significant χ^2 tests, and no clear pattern is evident. At most loci, the common allele increased in frequency in some hatcheries and decreased in others. Furthermore, significant test results were distributed over 11 different polymorphic loci. Nevertheless, selection cannot entirely be ruled out on this basis because it might act differently in different environments or genetic backgrounds.

To examine the more general possibility that selection was responsible for the observed changes, we considered a two-allele model with the fitness of the three genotypes (*AA, Aa, aa*) given by $w_1 = 1$, $w_2 = 1 - s$, $w_3 = 1 - 2s$, respectively, where s is the selection coefficient. If a population with allele frequency P is in Hardy-Weinberg equilibrium before selection, expected

genotypic frequencies after one generation of selection are $P^2w_1/\bar{w}$, $2P(1 - P)w_2/\bar{w}$, and $(1 - P)^2w_3/\bar{w}$, respectively, where $\bar{w}$ (mean fitness) $= P^2w_1 + 2P(1 - P)w_2 + (1 - P)^2w_3$. This model is based on the assumption that the alleles are codominantly expressed (as in most electrophoretic phenotypes), and the result is strong directional selection against the *"a"* allele. This type of selection will not, however, cause substantial departures from Hardy-Weinberg equilibrium because genotypic frequencies after selection will be close to those expected based on the new allele frequencies (cf. Lewontin & Cockerham 1959). This is important because no substantial Hardy-Weinberg departures were observed in samples from the Oregon hatcheries (unpublished data).

We performed a series of simulations as described previously, with the following exception: an episode of natural selection was introduced between the formation of the spawning population in year i and the samples of 100 juveniles taken from their progeny. For years i = 51 to 100, an array of samples (corresponding to the array of selection coefficients s = 0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.5) were binomially drawn from the spawning population. In each sample, 100 genotypes were chosen by random draw with the probabilities of each genotype as given above for the corresponding value of s. Allele frequencies (x_i) in these samples were compared with those in samples taken 4 years previously which were not subject to selection (s = 0). Sampling was performed in this way because we are interested in the effects of selection in a single generation, and selection was presumed to have occurred after the initial sample was drawn. Long-term selection should lead to similar results when considering comparisons over a single 4-year period, but such a model was not used to avoid the problem of gene extinction.

Results of these simulations are shown in Figure 5. Selection coefficients in the range 0.01–0.1 had little effect on the probability of a significant test. To explain the differences observed (36% significant tests) in terms of selection and drift, it is necessary to postulate selection coefficients of $s > 0.2$ over all the loci surveyed. Selection of this intensity causes more than a 40 percent reduction in fitness of the *aa* homozygote — a far greater loss of fitness than has been convincingly demonstrated for other electrophoretically detectable polymorphisms (see review by Eanes 1987). Other models of selection are possible, of course, but most do not result in such intense directional change in allele frequency. It seems unlikely, therefore, that natural or artificial selection was responsible for all, or even a substantial part, of the changes observed in the Oregon hatchery populations.

4. Mixing of gene pools. Our simulation model incorporated gene flow only between year classes, but a certain amount of straying is known to occur between different streams in all species of Pacific salmon (Ricker 1972; Lister et al. 1981). In addition, it is not uncommon for hatcheries with surplus production in a particular year to transfer progeny (usually eyed eggs) to other hatcheries that have been less successful. Such mixtures of gene pools can considerably change allele frequencies in the recipient population, and there is some indirect evidence that this may have happened to some of the Oregon hatchery populations.

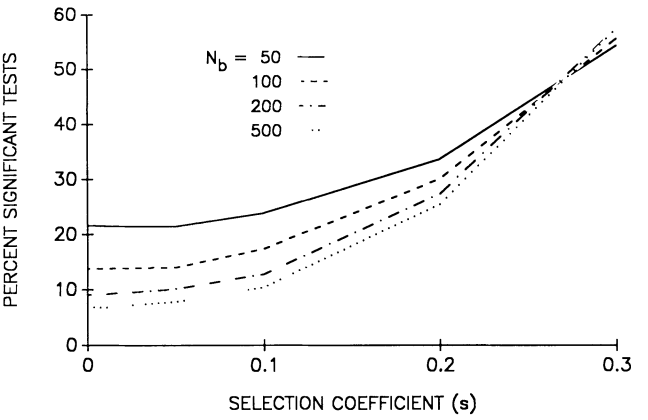

Figure 5. Percent significant χ^2 tests of homogeneity of allele frequencies as a function of selection coefficient (s) and effective number of breeders per year (N_b). Results are from simulations with two samples (100 juveniles each) taken 4 years apart, with one episode of selection before the second sample. Relative fitnesses of the three genotypes (AA, Aa, aa) were 1, 1 − s, and 1 − 2s, respectively. Initial allele frequency was 0.5, and age structure was as shown in Figure 2A.

Nei and Li (1973) pointed out that gametic disequilibrium (nonzero correlations between alleles at different gene loci) is expected in mixed populations containing two or more distinct gene pools. Waples and Smouse (1990) found statistically significant ($p < 0.05$) gametic disequilibrium in four of the nine Oregon hatchery samples from the 1980 brood and in five of the nine from 1984. In contrast, none of 18 samples from Oregon wild populations showed significant disequilibrium. The existence of substantial gametic disequilibrium is consistent with the hypothesis that fish from distinct gene pools made genetic contributions to the hatchery populations prior to 1985. However, the absence of a consistent heterozygote deficiency (Wahlund effect) in samples showing significant gametic disequilibria (Waples & Smouse 1990) argues against the hypothesis that the allele frequency differences in general were due to the transfer of eggs that produced the 1984 brood. Gametic disequilibrium without the Wahlund effect would be expected if an episode of random mating occurred after the admixture event (e.g., if parents of the 1984 brood were a mixture), but Waples and

Smouse (1990) showed that in that case the allele frequency differences between the contributing populations must have been quite substantial for the residual disequilibrium to be as large as observed. Furthermore, hatchery records indicate that in the period 1979–1984, Cedar Creek and Fall Creek were the only two hatcheries receiving imports that were released on site. Natural straying into the hatcheries might have contributed to the disequilibrium but, again, the immigrants would have to have been fairly numerous and genetically distinct. Mixtures of gene pools thus may have contributed to the temporal changes in allele frequency in at least some populations, but this phenomenon seems an unlikely cause of all the changes observed.

Interestingly, Waples and Smouse (1990) found that the multilocus test they used is also very sensitive to disequilibrium arising from genetic drift (all progeny produced by a finite number of parental genotypes). Simulation results demonstrated that it is necessary to postulate N_b of no more than about 50 per year to account for the observed levels of disequilibrium by drift—about the same population size necessary to account for the allele frequency changes by drift.

It is thus possible to explain both the allele frequency changes and the gametic disequilibrium by assuming N_b was no larger than about 50 per year in these hatcheries. In view of the fact that over 1000 adults a year return to some of these hatcheries (Nicholas & Hankin 1988), this number may seem unrealistically small. However, some hatcheries have many fewer returning adults, and even those hatcheries with a large run size may use only a small percentage of the total for spawning. In addition, at some hatcheries progeny (usually eggs) are exported to other hatcheries or are released off site. Exports often involve the entire production of some families, thus effectively eliminating them from the breeding pool responsible for the remaining progeny. High variance of reproductive success among families may further limit the effective number of breeders per year. Simon et al. (1986) showed that the above factors can cause a substantial reduction in effective population size in coho salmon.

The data necessary to compute N_b for the nine Oregon hatchery populations are not all available, but we obtained the following information for the period 1979–1984 (Edward Cummings, Oregon Department of Fish and Wildlife, personal communication; see Table 2). The number of females used for spawning ranged from a low of 2 in one year at Cedar Creek to over 1800 at Cole Rivers (spring run). In most cases, eggs from four to six females were mixed with milt from at least two males; hence the typical sex ratio of spawners was about 1:2 male:female. Exports (generally eggs) ranged from none (at several hatcheries) to over 90 percent of the total production in one year for Cole Rivers fall run. Using data for the number of females that produced the exported eggs, we have estimated the number of females (F^*) responsible for progeny remaining in the hatchery. For each population, F^* was computed each year, as well as the harmonic mean $\tilde{F}^*$ over the 6-year period.

The effective number of breeders can be computed using a variation of Wright's (1938) formula: $N_b = 4MF/(M + F)$, which, assuming the ratio of spawning males (M) to females (F) was 1:2, reduces to $N_b = 4F/3$. Under this assumption, N_b in the period 1979–1984 was less than 100 for five populations (Cedar Creek, Cole Rivers (fall run, Fall Creek, Rock Creek, Salmon River), about 100–200 for Trask River (spring and fall run), over 200 for Elk River, and over 1100 for Cole Rivers (spring run) (Table 2). These numbers are, in most cases, somewhat higher than the average of $N_b = 50$ or less required to explain the allele frequency changes by genetic drift alone. However, N_b computed using only the sex-ratio adjustment assumes that each male (or each female) has an equal opportunity to produce progeny that survive to

Table 2. Breeding information for nine chinook salmon stocks from Oregon hatcheries during the period 1979–1984.

Hatchery	*Number of females spawned*	*Percent exported*[a]	$\tilde{F}^*$[b]	*Maximum* N_b[c]	*Percent significant tests*[d]
Cedar Creek	2–25	0	8	11	44.4
Cole Rivers (spring)	610–1861	0–36	863	1151	11.1
Cole Rivers (fall)	22–210	0–93	26	35	38.4
Elk River	149–378	0–23	209	279	22.2
Fall Creek	19–69	0–12	41	55	28.6
Rock Creek	43–153	0–59	72	96	44.4
Salmon River	53–79	0–23	60	80	62.5
Trask River (spring)	94–160	0–35	104	139	30.0
Trask River (fall)	164–324	25–61	129	172	42.9

Based on information provided by Oregon Department of Fish and Wildlife.
[a] *Estimated percentage of females whose entire production was exported off-site.*
[b] *Harmonic mean (years 1979–1984) number of females responsible for progeny remaining in hatchery after adjusting for exports.*
[c] *Effective number of breeders computed using $\tilde{F}^*$, assuming 1:2 ratio of spawning males and females and a binomial distribution of progeny number per parent.*
[d] *Single locus χ^2 tests comparing allele frequencies in samples from 1980 and 1984 brood years (see Tables 1 and 2).*

reproduce the next generation. This calculation does not consider possible reduction in N_b due to high variance in reproductive success among individuals of the same sex, and it therefore must be considered a maximum estimate. Apart from the probability that survival to spawning age of individuals from different families is nonrandom, the practice of pooling sperm and eggs from multiple individuals for fertilization has been shown to result in disproportionate contributions by some individuals at the expense of others (Simon et al. 1986; Withler 1988), further reducing the effective size. In the absence of data on variance in reproductive success, it is impossible to calculate N_b more accurately for these Oregon hatcheries. However, it is instructive that in a coho salmon hatchery to which thousands of adults returned each year, Simon et al. (1986) found that (1) as few as 4 percent of the returning adults were used for spawning in a given year; (2) the variance in family size was typically several times the mean; and (3) in some years the effective number of breeders may have been as low as 16–22 of each sex. Therefore, it is clearly possible that actual N_b may have been 50 or less, at least in the five hatcheries with maximum $N_b < 100$, and perhaps in Trask River as well.

Additional support for the hypothesis that drift was a major factor contributing to the changes observed in the hatcheries is the clear, inverse relationship between the number of females used for spawning and the magnitude of temporal change. This relationship was apparent even using the crude measure of the percentage of significant single-locus χ^2 tests (Fig. 6). For example, the three hatcheries with the highest percentages of significant tests (Salmon River, Cedar Creek, Rock Creek) used relatively few females for spawning, and the two hatcheries using the most females (Elk River, Cole Rivers spring run) had the lowest proportions of significant tests.

Variations on the Basic Model

The simulation results described above were obtained with $m = 0.5$ and initial allele frequency set to $P = 0.5$. Certain simulations were repeated with different m and P values to evaluate the sensitivity of the analysis to these parameters. Because the rate of change in allele frequency per generation is a function of $P(1 - P)$, it is clear that the absolute magnitude of the changes shown in Figure 3A is specific for $P = 0.5$. The probability of a significant test statistic, however, in theory is independent of allele frequency (Waples 1989*b*). In practice, this independence will break down if P is too close to 0 or 1 or if S is too small. In simulations using values of P larger than 0.5, little effect of allele frequency was observed for $P \leq 0.9$ and $S \geq 50$. For smaller sample sizes or very extreme allele frequencies, the probability of a significant test is slightly less than shown in the figures.

The effects of different age structures were evaluated by varying m from 0.1 to 0.7. On average, each spawning population comprised $(m/2)N_b$ breeders derived from years $i - 3$ and $i - 5$ and $(1 - m)N_b$ breeders from year $i - 4$. Figure 7 shows results of simulations with $N_b = 200$ and juvenile samples of $S = 50$ fish. For values of m that are realistic for most chinook salmon populations ($m \approx 0.3$ to 0.7), migration had little effect on the probability of a significant test. Apparently, 30 percent migration from other brood years is sufficient to smooth out allele frequencies over time about as much as they can be equalized, given that each spawning pop-

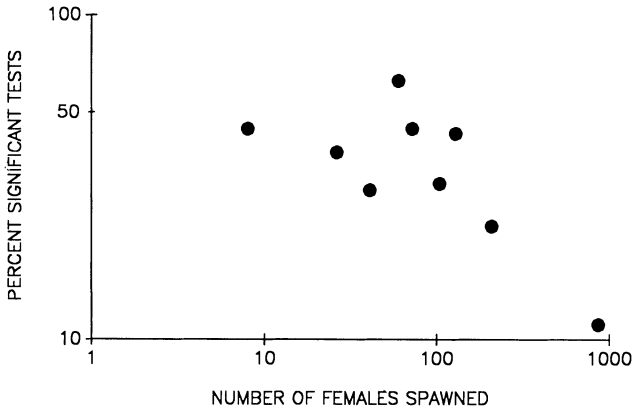

Figure 6. Relationship between temporal variability in allele frequencies in nine Oregon hatchery stocks of chinook salmon and the harmonic mean number of female spawners for the period 1979–1984. Temporal variability was measured as the percentage of statistically significant ($p < 0.05$) single-locus χ^2 tests of equality of allele frequencies in juvenile samples taken from 1980 and 1984 brood years.

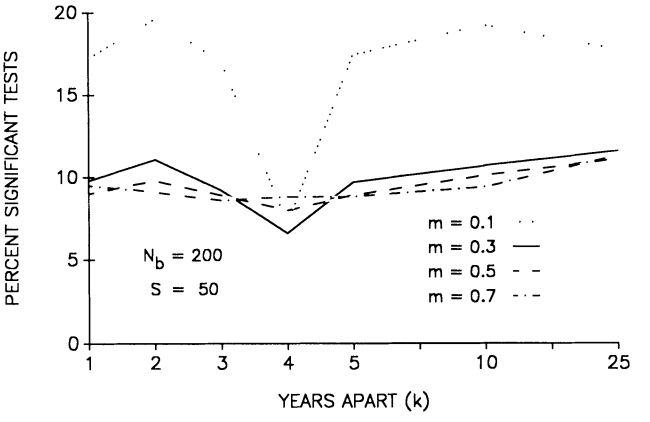

Figure 7. Chinook salmon: effects of gene flow or "migration" between year classes (m) on probability of a significant test of homogeneity of allele frequencies in juvenile samples from the same population taken k *years apart. Results are from simulations with effective number of breeders per year (N_b) set to 200, sample size = 50, and initial allele frequency = 0.5.*

ulation is of finite size and sampling error in choosing allele frequencies in the N_b breeders is inescapable. The similarity of the results for a broad range of migration parameters suggests that they are also qualitatively true for other anadromous Pacific salmonids (sockeye salmon [*O. nerka*], chum salmon [*O. keta*], and steelhead trout [*O. mykiss*, formerly *Salmo gaidnerii*]) with broad overlap in year classes. Quantitatively, the absolute magnitude of allele frequency change will also depend on the average age at reproduction (assumed to be 4 years in the present model). This topic will be addressed in a subsequent paper (Waples, manuscript I, in press).

A dramatic difference in the pattern of change over time was seen if the proportion of gene flow between year classes was as small as 10 percent (Fig. 7). With 90 percent of the spawners returning at age 4, the probability of a significant test for samples not taken exactly 4 years apart was about twice as high as it was for lower migration rates. Such a large preponderance of a single class is unusual in chinook salmon but is typical of coho salmon and some populations of chum salmon. In such cases, interpretation of genetic change requires particular care.

Adult Sampling

Two factors contribute to allele frequency differences in temporally spaced samples: changes in the population frequency and random error in drawing the samples. A juvenile sample of S progeny from brood year *i* is equivalent to a binomial sample of 2S gametes from the N_b spawners in year *i* (allele frequency P_i), so the variance of allele frequency with respect to P_i [$V(X_i|P_i)$] in juvenile samples is just the binomial variance:

$$(\text{juvenile sample})\, V(X_i \mid P_i) = \frac{P_i(1 - P_i)}{2S} \qquad (1)$$

If, as will typically be the case, the effective number of breeders is less than the actual number (N), a juvenile sample still estimates allele frequency (P_i) in the $2N_b$ genes making up the effective breeding population in brood year *i* (with variance still the binomial variance given above). An adult sample, however, provides an estimate of allele frequency (call it P_i') in all the spawning adults subject to being sampled (whether or not they contribute to N_b). The variance of X_i relative to P_i, the allele frequency that is relevant for the evolutionary history of the population, depends on the relationship between N and N_b. It can be shown that this quantity is approximately equal to

$$(\text{adult sample})\, V(X_i \mid P_i) \approx \frac{P_i(1 - P_i)}{2N - 1}\left[\frac{N - S}{S} + \frac{N - N_b}{N_b}\right]. \qquad (2)$$

The sampling variance in equation (2) is less than that for juvenile sampling (equation 1) provided that the ratio N_b/N is larger than 0.5. Under these circumstances, adult sampling can be expected to result in smaller allele frequency differences between samples and fewer statistically significant test results. Because of the dependence on the ratio N_b/N, temporal changes in adult samples are not shown here but they can be modeled for any parameter values desired.

Discussion

The simulation results provide insight into the process of temporal change in allele frequency in Pacific salmon. These insights, in turn, allow an evaluation of the probable causes of the relatively large changes observed in samples from the Oregon hatcheries. Major points to emerge from this analysis include:

1. For populations with broad overlap of year classes, the magnitude of short-term allele frequency change is determined primarily by the effective number of breeders (N_b) per year, rather than the age structure of the spawners. However, in populations or species (e.g., coho salmon) with a large majority of adults spawning at the same age, the magnitude of genetic change is much more sensitive to the elapsed time between the brood years being compared.

2. The magnitude of sampling error in estimating population allele frequencies is different for juvenile and adult samples of Pacific salmon. Therefore, the life history stage sampled must be taken into consideration when interpreting allele frequency data. Interpretation of results for juvenile samples is more straightforward because sampling error is not a function of the ratio N_b/N, as it is for adult samples.

3. Because genetic drift causes annual fluctuations in allele frequency in Pacific salmon populations, comparisons of samples from different brood years have an inflated probability of being statistically significant. This is not an artifact of small sample size; in fact, it increases with the ratio S/N_b and is therefore more pronounced for larger samples. This has important implications for the field of conservation genetics. Interest typically focuses on populations with reduced effective size, in which case statistical tests of allele frequency change are more likely to be significant. Conclusions based on such tests can be seriously misleading if they do not take the confounding effects of genetic drift into consideration. In the context of the results presented here, it is possible to determine whether observed changes are large enough that it is necessary to consider explanations other than genetic drift and sampling error (selection, genetic admixture, etc.).

Identifying the cause (or causes) of the genetic changes observed in the hatchery populations of chinook salmon is the necessary first step before strategies to minimize rapid changes can be effective. Additional simulations, performed in this study to model natural selection and previously (Waples & Smouse 1990) to

model genetic admixture, indicate that it is difficult to explain a substantial portion of the changes by either of these factors. In contrast, all available evidence is consistent with the conclusion that the effective number of breeders per year averaged 50 or less at most of the hatcheries. This suggests that more serious changes such as inbreeding depression and erosion of genetic variability may also be occurring. Although such changes do not necessarily characterize hatchery populations in general (cf. Utter et al. 1989), they have been documented in a number of cases (reviewed by Allendorf & Ryman 1987).

An important question thus becomes, "Can we expect serious problems from inbreeding in Pacific salmon populations in which N_b is as small as 25 or 50 per year?" In attempting to answer this question, we again face a problem posed by the unusual life history features of Pacific salmon. Most (if not all) discussions of the deleterious effects of inbreeding (e.g., Franklin 1980; Simberloff 1988) refer to effective population size per generation (N_e), whereas we have shown that short-term genetic change in Pacific salmon populations is a function of the effective number of breeders per year (N_b). Because it is not clear exactly how to relate these two effective numbers, it is difficult to adapt results from the extensive literature on conservation genetics for use with Pacific salmon.

The second paper in this series (Waples, manuscript I, in press) addresses this problem. It establishes the relationship between N_b and N_e, evaluates the rate of loss of genetic variability in Pacific salmon populations, and suggests guidelines for a minimum effective number of breeders per year. The minimum acceptable number varies somewhat depending on management goals and other considerations, but an N_b of 25–50 would appear to be too small for long-term genetic health under any circumstances. A variety of measures, apart from simply using more fish, can be adopted to increase the effective number of breeders in hatcheries (see also Simon et al. 1986; Allendorf & Ryman 1987). Differences among individuals in reproductive success can be minimized by using equal numbers of each sex in pairwise spawnings and fertilizing approximately the same number of eggs from each female. If a fixed number of eggs are needed for the next brood year, taking relatively few eggs from each of a large number of females increases the effective number of breeders. Choosing breeders from throughout the spawning run helps to preserve genetically based behavior patterns related to time of spawning. If eggs or progeny are exported, they should be a random sample from the entire brood stock rather than derived from a limited number of individuals.

Measures such as these can do a great deal to prevent the erosion of genetic variability in Pacific salmon hatcheries, but even if they are implemented there is no guarantee that N_b will be as large as desired. Because most mortality occurs after release from the hatchery, a major factor in reducing N_b below the census size (high variance in reproductive success among individuals) can only be evaluated through elaborate tagging programs. Therefore, indirect estimates of N_b can be very valuable in monitoring the success of efforts to minimize inbreeding. For the Oregon hatchery populations, the observed percentage of significant χ^2 tests was used to obtain a crude estimate of N_b. Further analysis has indicated that a more powerful approach is available, analogous to methods that have been used for organisms with discrete generations (Krimbas & Tsakas 1971; Nei & Tajima 1981, Waples 1989*a*). The third paper in this series (Waples, manuscript II, in press) deals with this topic.

Although this study explicitly modeled the unique life history pattern of Pacific salmon, many other organisms with overlapping generations present some of the same problems in understanding possible causes of genetic change. In particular, the ways in which the samples for genetic analysis and the breeding population at a given point in time are drawn from the population as a whole can profoundly affect the perceived rate of change. Computer simulations that incorporate realistic values for life history parameters can contribute substantially to our understanding of genetic changes (e.g., Harris & Allendorf's 1989 study modeling a grizzly bear population).

Finally, it should be stressed that regardless of the organism, results of standard statistical tests comparing temporally spaced samples should be interpreted with caution. Even if nothing but stochastic factors are involved, the probability of a significant test statistic is an increasing function of S/N_b. Conservation biologists, who typically are interested in small populations, can therefore expect to detect "significant" genetic changes fairly often. It is important to realize that such results are sensitive to sampling effort as well as population size and may not reflect any directional evolutionary forces.

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Literature Cited

Abrahamson, P. 1988. A detailed listing of the liberations of salmon into the open waters of the state of Washington during 1987. Progress Report No. 267. Washington Department of Fisheries, Olympia, Washington. 447 pp.

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. NOAA Technical Report **61**:1–19.

Allendorf, F. W., and S. R. Phelps. 1981. Use of allele frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Sciences **38**:1507–1514.

Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery stocks. Pages 141–159 in N. Ryman and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle, Washington.

Allendorf, F. W., and G. H. Thorgaard. 1984. Tetraploidy and the evolution of salmonid fishes. Pages 1–53 in B. Turner, editor. Evolutionary genetics of fishes. Plenum Press, New York.

Aspinwall, N. 1974. Genetic analysis of North American populations of the pink salmon (*Oncorhynchus gorbuscha*): possible evidence for the neutral mutation–random drift hypothesis. Evolution **28**:295–305.

Eanes, W. F. 1987. Allozymes and fitness: evolution of a problem. Trends in Ecology and Evolution **2**:44–48.

Fraidenburg, M. E., and R. H. Lincoln. 1985. Wild chinook salmon management: an international conservation challenge. North American Journal of Fisheries Management **5**:311–329.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–150 in M. E. Soulé and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer, Sunderland, Massachusetts.

Fredin, R. A. 1980. Trends in North Pacific salmon fisheries. Pages 59–119 in J. W. McNeil and D. C. Himsworth, editors. Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, Oregon.

Harris, R. B., and F. W. Allendorf. 1989. Genetically effective population size of large mammals: an assessment of estimators. Conservation Biology **3**:1–11.

Hill, W. G. 1979. A note on effective population size with overlapping generations. Genetics **92**:317–322.

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 C. C. Cockerham. 1959. The goodness-of-fit test for detecting natural selection in randomly mating populations. Evolution **13**:561–564.

Lister, D. B., D. G. Hickey, and I. Wallace. 1981. Review of the effects of enhancement strategies on the homing, straying, and survival of Pacific salmonids. Volume I. Department of Fisheries and Oceans, Vancouver, British Columbia.

Nei, M., and W-H. Li. 1973. Linkage disequilibrium in subdivided populations. Genetics **73**:213–219.

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

Nicholas, J. W., and D. G. Hankin. 1988. Chinook salmon populations in Oregon coastal river basins: description of life histories and assessment of recent trends in run strengths. Oregon Department of Fish and Wildlife, Information Report **88-1**:1–359.

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

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations. Pages 27–160 in R. C. Simon and P. A. Larkin, editors. The stock concept of Pacific salmon. H. R. MacMillan Lectures in Fisheries. University of British Columbia, Vancouver.

Simberloff, D. 1988. The contribution of population and community biology to conservation science. Annual Review of Ecology and Systematics **19**:473–511.

Simon, R. C., J. D. McIntyre, and A. R. Hemingsen. 1986. Family size and effective population size in a hatchery stock of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences **43**:2434–2442.

Utter, F., P. Aebersold, and G. Winans. 1987. Interpreting genetic variation detected by electrophoresis. Pages 21–45 in N. Ryman and F. Utter, editors. Population Genetics and Fishery Management. University of Washington Press, Seattle, Washington.

Utter, R., G. Milner, G. Stahl, and D. Teel. 1989. Genetic population structure of chinook slamon in the Pacific Northwest. Fisheries Bulletin **87**:239–263.

Waples, R. S. 1988. Estimation of allele frequencies at isoloci. Genetics **118**:371–384.

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

Waples, R. S. 1989*b*. Temporal stability of allele frequencies: testing the right hypothesis. Evolution **43**:1236–1251.

Waples, R. S. Conservation genetics of Pacific salmon. II. Effective population size and the rate of loss of genetic variability. (Manuscript I, Journal of Heredity, in press.)

Waples, R. S. Conservation genetics of Pacific salmon. III. Estimating effective population size. (Manuscript II, Journal of Heredity, in press.)

Waples, R. S., and P. E. Smouse. 1990. Gametic disequilibrium analysis as a means of identifying mixtures of salmon populations. American Fisheries Society Symposium **7**:439–458, in press.

Williams, J. G. 1989. Snake River spring and summer chinook salmon: can they be saved? Regulated Rivers **4**:17–26.

Withler, R. E. 1988. Genetic consequences of fertilizing chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture **68**:15–25.

Wright, S. 1938. Size of population and breeding structure in relation to evolution. Science **87**:430–431.