Inferring the Ancestral Origin of Sockeye Salmon, *Oncorhynchus nerka*, in The Lake Washington Basin: A Statistical Method in Theory and Application

by

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Abstract

Inferring the Ancestral Origin of Sockeye Salmon, *Oncorhynchus nerka*, in The Lake Washington Basin: A Statistical Method in Theory and Application

by Eric C. Anderson

Chairperson of Supervisory Committee: Associate Professor Thomas Sibley
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It was once held that any native populations of anadromous sockeye salmon in the Lake Washington Basin were extirpated by the changes in the lake following the completion of the Lake Washington Ship Canal in the early 1900's, and were replaced by sockeye planted from Baker or Cultus lakes in the 1930's and 1940's. The authors of two surveys of neutral genetic markers in Lake Washington sockeye populations, however, suggest that the sockeye spawning in Bear Creek and its tributaries are of native origin. I argue that one cannot prove that the fish in Bear Creek are of native origin, but it may be possible to statistically exclude the possibility that sockeye in Bear Creek derive exclusively from Baker Lake or Cultus Lake. I present a likelihood ratio test of the hypothesis that Bear Creek fish could have derived exclusively from Baker (or Cultus) Lake against the alternative hypothesis that at least some of the ancestry of the Bear Creek population must be from a source other than Baker (or Cultus) lake. The test is based on a probability model which includes uncertainty in the data due both to sampling error and to the error due to random genetic drift.

Several different formulations and approximations are used to compute the likelihood ratio as appropriate for four different loci on which data are available.

The method requires independent knowledge of the historical effective sizes of the populations in Bear Creek, Cultus Lake, and Baker Lake. I perform simulations based on very good historical data suggesting that a lower bound on effective size for the Baker Lake population is about 250 individuals and for the Cultus Lake population is about 800. Early run-size data for Bear Creek are not available, so I perform simulations based on a reasonable scenario that show how the Bear Creek population might have grown from the early 1940's so as to have an effective size of 100. If these numbers for effective size are accurate, it is unlikely that the sockeye inhabiting Bear Creek could have descended exclusively from fish introduced from either Baker Lake or Cultus Lake. Unfortunately, this result depends highly on the assumed run sizes in Bear Creek in years with little or no data.

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I began my graduate work at the School of Fisheries with Dr. Robert Naiman. My research interests strayed from his, but I thank him for being supportive throughout in seeing that I received the most of what I wanted from my time at the UW.

Outside of the academic world, I owe much to the extended "Sunnyside House" crew—my support gang of friends. They know who they are. And finally, special thanks to two men from whom I've learned a lot about both mathematics and mountains. Recently Dr. Karel Zikan, a friend, housemate, and mathematician whose generous assessments of my mathematical abilities encouraged me to delve back into quantitative realms. And John Rosendahl, a friend and mentor from my teen years—I still have vivid memories from my junior year in high school, of riding in his old VW van to various rock-climbing destinations discussing Taylor Series or Markov chains, *etc.* I think of him whenever I do mathematical things.

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Chapter 1

INTRODUCTION

This thesis investigates, using allele-frequency data in an hypothesis-testing framework, the origin of sockeye salmon, *Oncorhynchus nerka*, in the Lake Washington basin. Specifically, I test whether the sockeye population in the Bear Creek drainage descended, at least in part, from fish other than those introduced from Baker and Cultus Lakes in the 1930's and 1940's. Previous authors have inferred that one (Hendry *et al.* 1996) or several (Seeb and Wishard 1977) Lake Washington sockeye populations are of "native" origin, but they have not assessed the probability that their conclusions are incorrect (Type I error probability) because there is no straightfoward or "standard" statistical test for doing so. The ability to assign a level of confidence to one's inferences regarding the origin of these sockeye populations, and especially of the Bear Creek population, is desirable in light of recent controversies over hatchery supplementation in the Cedar River and the designation of Evolutionarily Significant Units (ESU's) by the National Marine Fisheries Service (NMFS). Indeed, NMFS recently declared the sockeye of Bear Creek and its tributaries a "provisional" ESU (Gustafson *et al.* 1997).

1.1 Overview and Objectives of the Thesis

There are three chapters of this thesis. This chapter provides an overview, introduces the physical setting of Lake Washington, reviews the history of sockeye salmon in the basin and presents the conclusions of previous genetic work. In Chapter 2, I discuss

which hypotheses about the origin of Bear Creek sockeye are testable. Then I derive an expression for the likelihood of allele frequencies, t generations ago in a population, given sample allele frequencies from that population in the current generation. I use this likelihood in a likelihood-ratio framework for testing hypotheses about ancestral origins. The remainder of the chapter describes ways of calculating or approximating various quantities needed to compute the likelihood, and investigates the distribution of the likelihood ratio test statistic.

In Chapter 3, I apply the statistical test to data from nuclear gene loci, using the data from Hendry *et al.* (1996). The main question concerns the origin of the Bear Creek population, but I also test whether the data available are consistent with an exclusively Baker Lake origin of the sockeye in the Cedar River. Since the sockeye in the Cedar river are almost certainly of Baker Lake origin (based on river and stocking history), testing the origin of the fish from there provides a check on the validity of the statistical method.

1.2 General Introduction to Lake Washington

Figure 1.1 shows a sketch of the Lake Washington basin with the names and approximate locations of the tributaries and other features discussed in the text. The lake has endured considerable anthropogenic disturbance. Most notably, in the late 1800's, canal builders constructed a channel connecting Lake Washington to Lake Union. Later, this channel was widened into what is now the Lake Washington Ship Canal. In 1916, the U.S Army Corps of Engineers began operating the H. M. Chittenden Locks, which connect the Ship Canal to Puget Sound. This caused the mean water level of Lake Washington to drop 2.7 meters and dried out the lake's natural outlet, the Black River, which previously flowed south to the Duwamish River. Water now exits the Lake Washington basin exclusively through the ship canal. Additionally, the Cedar River, formerly a tributary of the Duwamish, was diverted into

Lake Washington to compensate for losses of water due to operation of the locks (Ajwani 1956; Chrzatowski 1983). Urban development in the basin has increased substantially over the last fifty years. Eutrophication due to sewage inputs and the subsequent amelioration of the eutrophic condition following diversion of the sewage in the 1960's significantly altered the biological and chemical conditions in the lake (Edmondson 1991, 1994).

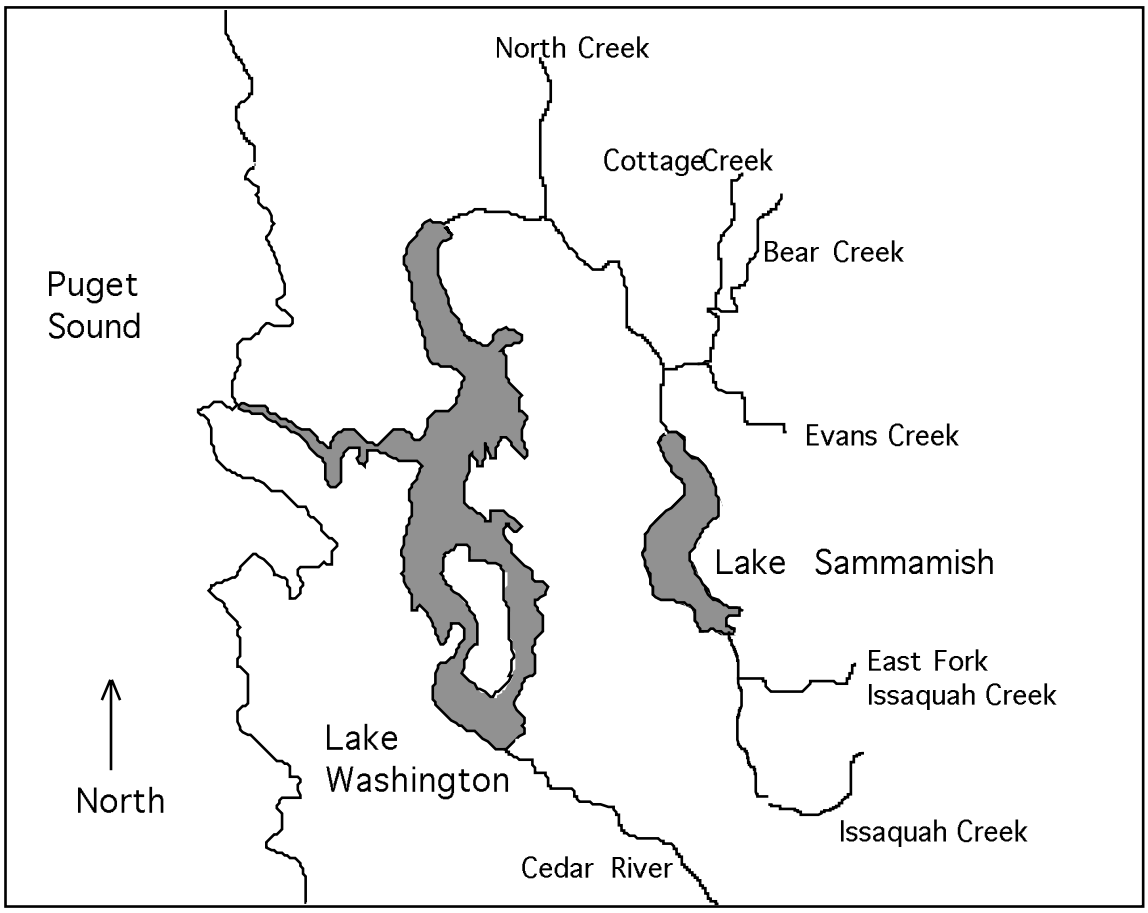


Figure 1.1: Map of the Lake Washington basin showing relevant tributaries.

1.3 History of O. nerka *in the Lake Washington Basin*

The recorded history of sockeye salmon in Lake Washington is incomplete. Prior to the construction of the Lake Washington ship canal, it is likely the lake was inhabited by indigenous kokanee which have persisted to the present day (Pfeifer 1992). It has also been suggested that small numbers of anadromous sockeye inhabited the drainage at that time (Evermann and Goldsborough 1907). These anadromous fish, however, were generally believed to have become extinct with the completion of the ship canal and the drying of the Black River—several reports from the 1920's and 1930's indicate that the Skagit River was the only river of Puget Sound supporting a sockeye run (Cobb 1927; Rounsefell and Kelez 1938), and a very limited (two days in the first week of September, 1930) survey of Lake Washington tributaries found no sockeye (State of Washington Department of Fisheries and Game 1932a). Little else is known regarding the fate, or existence, of Lake Washington's sockeye populations before the 1930's.

Starting in 1917, *O. nerka* from various sources outside of Lake Washington were introduced to tributaries in the Lake Washington drainage (Table 1.1). Fisheries agencies planted kokanee from Lake Whatcom, Lake Stevens, and other unknown sources (Pfeifer 1992). They planted sockeye from Cultus Lake between 1944 and 1954; from Baker Lake between 1937 and 1945; and from an unknown source presumably in the Lower Fraser drainage in 1917 (see review in Hendry *et al.* 1996). After the initial plantings throughout the basin, adult sockeye returning to Issaquah Creek between 1945 and 1963 were spawned at the Issaquah State Fish Hatchery to enhance the newly established populations in the Cedar River and Issaquah Creek (Kolb 1971). In tributaries to the Sammamish River, 576,000 Baker Lake sockeye fry were released to Bear Creek in 1937 and 24,000 Cultus Lake sockeye fingerlings were released to North Creek in 1944.

In 1940, when the fish from the 1937 plantings were four years old, an estimated

9,099 sockeye salmon returned to Issaquah Creek and 400 to the Cedar River. The State Department of Game also caught two sockeye in a fish rack on Bear Creek, but it is unknown how much fishing effort they expended (Royal and Seymour 1940). There is thus some evidence that sockeye inhabited Bear Creek in the early 1940's and that they could have descended from the 1937 Baker Lake plants. Very little is known, however, of the size of the population in Bear Creek.

Small populations of kokanee presently live in various tributaries in the drainage, though their numbers have declined rapidly in some creeks in the last 10 or 20 years [*e.g.*, Bear Creek (Doug Weber, Bear Creek Fish Surveyor, 18000 Bear Creek Road, Woodinville, WA 98072, pers. comm.) and Issaquah Creek (Ostergaard 1995)]. It is unknown which of these populations include kokanee of native origin and which are predominantly transplants, but Pfeifer (1992), comparing spawning times and looking at population abundance trends, has concluded that the early run of Issaquah Creek kokanee is the only kokanee population which can be confidently called "native." All the other historical, indigenous populations have likely been either well-mixed with or replaced by transplants from Lake Whatcom (Bob Pfeifer, Washington Department of Fish and Wildlife (WDF&W), Mill Creek Office, 16018 Mill Creek Blvd, Mill Creek, WA 98012, pers. comm.)

Currently, anadromous sockeye spawn at several sites in the Lake Washington drainage, including the Cedar River, Issaquah Creek, Bear and Cottage creeks, and at beach spawning sites in Lake Washington itself. Run sizes in these populations have fluctuated considerably over the last fifteen years (Figure 1.2). The origin of these sockeye populations has been in question, as fish from at least two different sources (Baker Lake or Cultus Lake) may have contributed to most of them, and Bear and Cottage creeks may have had sockeye of indigenous origin which contributed to the present-day populations (Hendry *et al.* 1996).

Table 1.1: Transplants of sockeye salmon into the Lake Washington drainage basin (taken from Hendry 1995). Transplants from Baker Lake were taken from the U.S. Bureau of Fisheries Hatchery on Grandy Creek (Royal and Seymour 1940). Transplants from Cultus Lake (on the Chilliwack River, a tributary of the Fraser) probably originated from beach spawning populations in the lake (Woodey 1966).

Year	*Receiving Waters*	*Number (1,000's)*	*Age*	*Planted From*
1917[a,d]	Lk. Washington	20	fry	Unknown
1937[a,b,c]	Bear Creek	576	fry	Baker Lake
1937[a,b,c]	Cedar River	656	fry	Baker Lake
1937[a,b,c]	Issaquah Creek	1,257	fry	Baker Lake
1942[b]	Lk. Washington	41	fingerling	Baker Lake
1943[a,b]	Cedar River	227	fingerling	Baker Lake
1943[a,b]	Issaquah Creek	254	fingerling	Baker Lake
1944[b]	Cedar River	54	yearling	Baker Lake
1944[a,b]	Issaquah Creek	42	yearling	Baker Lake
1944[a,b]	North Creek	24	fingerling	Cultus Lake
1945[b]	Cedar River	32	yearling	Baker Lake
1950[b]	Issaquah Creek	6	fingerling	Cultus Lake
1954[b]	Issaquah Creek	54	yearling	Cultus Lake

Sources:
[a] Woodey (1966)
[b] Kolb (1971)
[c] Royal and Seymour (1940)
[d] State of Washington Department of Fisheries and Game (1919b)

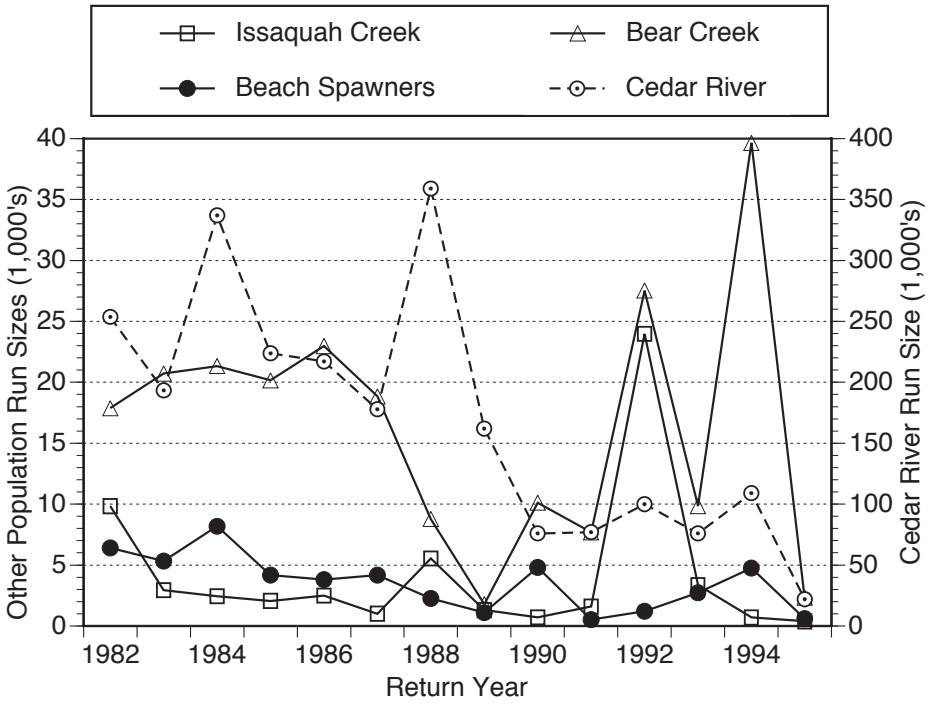


Figure 1.2: Estimates of run sizes of Lake Washington sockeye populations, 1982 to 1995. Cedar River run size given on right vertical axis. All other population sizes given on left vertical axis: Bear Creek System, Issaquah Creek, and Beach Spawners. (Source: WDF&W).

1.4 Recent Genetic Work in Lake Washington

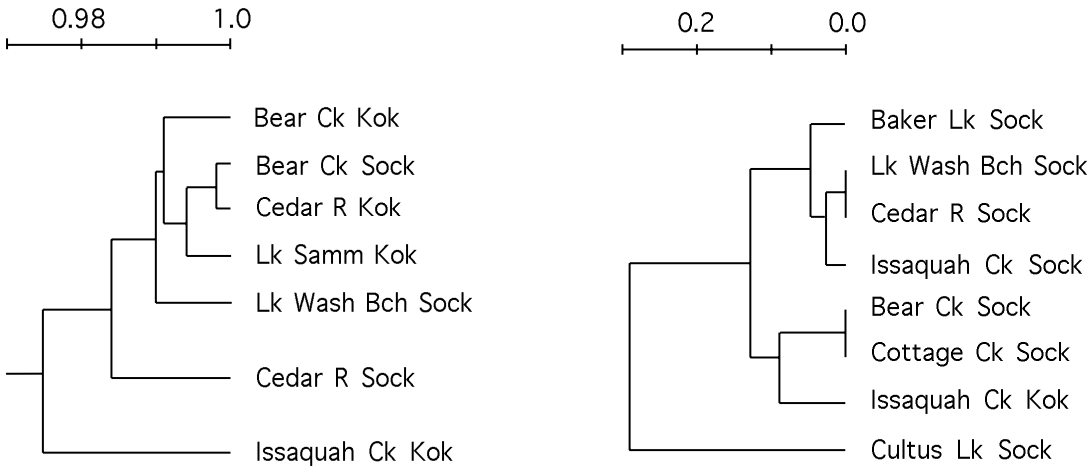
Seeb and Wishard (1977) and Hendry *et al.* (1996) surveyed electrophoretically detectable allozymes from Lake Washington sockeye populations and from Baker and Cultus lakes. Both studies suggest that while many of the sockeye in the lake seem to have descended from Baker Lake plants, one or several of the populations may have descended, in part, from sockeye of indigenous origin that somehow persisted after the completion of the Ship Canal.

In 1976 and 1977, Seeb and Wishard (1977) obtained sockeye tissue samples

from Bear Creek, Lake Sammamish, Cedar River, Lake Washington beach spawners, Baker Lake, and Cultus Lake. They obtained kokanee samples from Bear Creek, Issaquah Creek, Cedar River, and Whatcom Lake. They surveyed 16 loci and found that six had a sample frequency q of the variant allele greater than or equal to .05 in at least one of the populations. The other 10 loci were monomorphic among the sockeye populations. Hendry *et al.* (1996) surveyed 22 loci in fish from the same sockeye populations surveyed by Seeb and Wishard (1977), except that they took spawning sockeye from Issaquah Creek (rather than presmolts from Lake Sammamish), and, due to low returns of kokanee, they obtained data from only 13 kokanee, all of them early spawners from Issaquah Creek. Seven loci were polymorphic (four with $q > 0.05$ and three with $0 < q < 0.05$) in all of the populations sampled. The other 15 loci were monomorphic.

Both of the studies agree that Baker and Cedar sockeye are genetically similar, and infer that the Cedar River sockeye are primarily descended from the Baker River plants. Seeb and Wishard (1977) further conclude that the Bear Creek sockeye, Lake Washington beach-spawning sockeye, and the fish from Lake Sammamish appear to be "primarily remnant native stocks" because they were all "genetically distinguishable" from Cultus Lake, Baker Lake, and Cedar River sockeye.

Hendry *et al.* (1996), by contrast, did not find marked gene frequency differences between Lake Washington beach spawners, and Issaquah Creek, Cedar River, and Baker Lake sockeye. Like Seeb and Wishard (1977), though, they found Bear and Cottage Creek sockeye to be genetically distant from the rest of the sockeye populations, though more closely related to the Issaquah Creek kokanee (Figure 1.3). Hendry *et al.* (1996) report that allele frequency differences were statistically significant between all possible pairs of populations except for Cedar River/Lake Washington beach sockeye and Bear/Cottage creek sockeye, and they suggest that the sockeye populations they surveyed from the Lake Washington basin, except those from Bear and Cottage Creeks, descended primarily from Baker Lake sockeye, whereas



(a) Seeb and Wishard (1977)

(b) Hendry *et al.* (1996)

Figure 1.3: UPGMA dendrograms (a) from Seeb and Wishard (1977) using genetic similarity and showing only the stocks from Lake Washington, and (b) from Hendry *et al.* (1996) showing Nei's (1978) unbiased genetic distance between Lake Washington stocks and the putative donor stocks

Bear/Cottage sockeye and Issaquah kokanee descended from a common ancestor, indigenous to the Lake Washington basin.

In 1996, the WDF&W reported the results from a similar, though more comprehensive, electrophoretic analysis performed by NMFS on Hendry's samples as well as on additional collections of sockeye from Baker Lake, the Cedar River and Bear Creek (Shaklee *et al.* 1996). Their findings support the conclusions of Hendry *et al.* (1996), however the data from these new surveys have not yet been published, so for the analyses in Chapter 3 of this thesis I will use the data from Hendry *et al.* (1996).

An interesting finding in Hendry *et al.* (1996) is that Bear and Cottage Creek sockeye have a $\approx .25$ population frequency of the **500* allele at the *LDH–A1** lo-

cus. This allele is also found at very high frequency in the Issaquah Creek kokanee population, but had not been reported in a previous electrophoretic survey of 80 *O. nerka* populations throughout Canada (Wood *et al.* 1994). Since 1994 the allele has only been detected in kokanee populations in Oregon and Idaho and anadromous sockeye populations from Lake Washington and from the Kamchatka Peninsula, Russia. (Paul Aebersold, NMFS Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA 98112, pers. comm.). The allele has never been been reported in Baker or Cultus Lake, which would seem to argue strongly for a Lake Washington origin of the sockeye in Bear Creek. Unfortunately **500* is not expressed codominantly on electrophoresis gels: *LDH–A1** and *LDH–A2** must be assayed together on the same gel, and **500* has a mobility identical to that of the common allele of *LDH–A2** making **500* reliably detectable only in homozygous phenotypes (see Utter *et al.* 1987). Because of this it is very difficult to detect in populations where it exists at low frequency. For example, if the **500* allele were present in the Baker Lake population at a frequency of $q = .10$, then, only one in 100 fish would be expected to be homozygous for **500*. And so the allele could be present in the population, but with reasonable probability might not be detected in Hendry *et al.*'s sample of size $n = 120$ fish from Baker Lake. We will pay particular attention in Chapter 2 to the statistical issues raised by such alleles that are detectable only as homozygous phenotypes (referred to hereafter as "recessive" or null alleles).

Dendrograms (Figure 1.3) based on genetic distance provide a convenient graphical display summarizing many data and depicting some measure of "degree of relatedness" between populations. Additionally, a number of researchers have used genetic distance measures to infer the origin of introduced populations or species (Hattemer and Ziehe 1996; Morrison and Scott 1996; Roehner *et al.* 1996; Kriegler *et al.* 1995; Mendel *et al.* 1994; Kambhampati *et al.* 1991). However, distance or similarity measures, as such, do not lend themselves to statistically testing hypotheses about the origin of the salmon populations in question. Neither, of

course, do "significant genetic differences" between two stocks prove that the two stocks arose from separate populations in the past. Significant statistical differences in allele frequencies between two populations indicate only that it is improbable that the two samples were taken from the same population at the time of sampling. Such a test accounts only for the random variation involved in drawing one's samples and not for the random variation due to genetic drift. Considering the effects of genetic drift within a hypothesis-testing framework will allow stronger inferences about the ancestral origins of Bear Creek sockeye and may be a useful approach in related questions regarding the origin of other recently established plant or animal populations.

In the remainder of this thesis, I develop a statistical test that treats both the random variation due to sampling and that due to genetic drift, and I employ this technique with the data of Hendry *et al.* (1996) for sockeye salmon in the Lake Washington basin.

Chapter 2

THE STATISTICAL FRAMEWORK FOR HYPOTHESIS TESTING

The first ingredient of a hypothesis test is necessarily a testable hypothesis; the first section of this chapter explicitly states the sorts of hypotheses that are testable with allele-frequency data, and discusses how to interpret different results. The next section describes a model for the probability of observing our genetic samples given certain unknown parameters. This probability model defines a likelihood function which I use in a likelihood ratio test (Section 2.3). In the rest of this chapter I describe and assess methods for computing the likelihood function, explore the distribution of the likelihood ratio test statistic by computer simulation, and describe ways of dealing with recessive alleles.

In this chapter, I consider testing only whether a population (*e.g.*, Bear Creek) of sockeye in Lake Washington has descended solely from a single donor population (either Baker or Cultus Lake, but not both) or from some other unknown, single population (an unknown planted stock or an ancestral population native to the Lake Washington basin). Eventually one may wish to entertain the hypothesis that the sockeye in Bear Creek could have descended from a mixture of fish from Cultus and Baker lakes, however I do not treat that scenario in this thesis.

2.1 Testable Hypotheses

People curious about the origin of sockeye salmon in Lake Washington might want to answer the question, "Did the sockeye in Bear Creek descend from a remnant native

population." This question could, in fact, be posed as a hypothesis; for example, "Hypothesis One: Bear Creek sockeye descended from fish native to the Lake Washington basin." Unfortunately, armed with this hypothesis we cannot scientifically investigate the original question. First, it is crucial to understand that we are not trying to prove hypotheses, but rather to reject or falsify them. Consequently we must accept that formulating "Hypothesis One" as above will never allow anyone to prove that, "Yes, these fish are native." Second and more importantly, with the sorts of genetic data available, it is not even possible to reject "Hypothesis One." Doing so would require that we had estimates of native sockeye allele frequencies and that those frequencies were substantially different from the gene frequencies in the Bear Creek population. Since no populations of unequivocal native descent exist in Lake Washington, this is not an option. One must choose their hypotheses carefully so they are both testable (falsifiable) and potentially informative with the types of data available.

Our genetic data are allele frequencies from samples at different loci taken during the early to mid-1990's from the sockeye in Baker and Cultus Lakes, and in the tributaries of Lake Washington; with these data we may test the two hypotheses:

1. H_A: Bear Creek sockeye could have descended entirely from fish stocked from Baker Lake into Bear Creek in 1937.

2. H_C: Bear Creek sockeye could have descended entirely from fish stocked from Cultus Lake into North Creek in 1944 or into Issaquah Creek in the 1950's.[1]

We may test each hypothesis against its respective "general alternative hypothesis" which we will call H_G.

[1] Note, that this hypothesis requires that fingerlings planted into North Creek survived to adulthood, and, at some point they or their offspring colonized the nearby Bear Creek system; or, even more improbably, that Cultus fish introduced into Issaquah Creek in 1950 and 1954 eventually colonized Bear Creek.

At first glance, the hypotheses H_A and H_C may not appeal to someone trying to make inferences about the origin of Bear Creek sockeye. In particular, nothing in these hypotheses directly asks, "Are these fish native to the watershed?" Nonetheless these hypotheses do shed some light on that question: if one is certain that Baker and Cultus Lakes were the only possible sources for sockeye planted into Lake Washington, then rejecting the hypothesis that Bear Creek fish came exclusively from Baker Lake or Cultus Lake (or some mixture of both) would tell you that some proportion of their ancestry may very well be native. Additionally, for some purposes, like defining related groups of sockeye populations in the lower 48 states of the U.S., the hypotheses address the important question of whether the Bear Creek population's ancestry is significantly different from the other populations introduced to the Lake Washington basin. This may be important because native, non-introduced populations typically enjoy higher status in conservation decisions (Waples 1995).

Finally one must recognize that failure to reject H_A or H_C does not constitute proof that the fish in Bear Creek do not have any native ancestry. The fish in Bear Creek may well be "natives" but still have allele frequencies similar to those in Baker or Cultus lakes. Thus, only rejecting H_A or H_C allows us to make positive statements about the origin of Bear Creek sockeye. With the data available we have only the possibility of concluding that Bear Creek sockeye did not come from Baker or Cultus Lakes. We cannot rigorously conclude that the fish in Bear Creek are surely not native.

2.2 The Probability Model

This model provides a way to compute the probability of drawing the genetic sample allele counts at different loci in two different populations given some stocking history and initial population gene frequencies at the time of stocking. It assumes that the stock of unknown origin (Bear Creek in our specific example, though we will refer to it

generally as Population B) derives either from a single known stock (like Baker Lake in this instance, referred to generally as Population A) or from some single unknown stock.

The scenario is as follows: at a known time $\tau = 0$ in the past, spawners of Population A returned to their natal stream. The following spring, people transferred some of the offspring of those spawners to Creek B where Population B resides in the present. At $\tau = 0$, no one knew if there were fish already living in Creek B, and no one had any genetic information about the fish in Population A; these things are still unknown in the present time. Nonetheless we can say that at $\tau = 0$, Population A had the (unknown) allele frequencies, vector $\mathbf{p}_A = (p_{A1}, \ldots, p_{Ak})$ where p_{Ai} is the frequency of the i^{th} allele, at a locus of interest. (The current discussion deals with a single, k-allelic, codominant locus. Extending the analysis to multiple, independently-segregating loci is a relatively easy matter.) Likewise, the fish in Creek B, if there were any at $\tau = 0$, had the unknown allele frequencies $\mathbf{p}_B$. As time progresses toward the present ($\tau = t$), however, the allele frequencies in the two populations change by genetic drift from their initial values $\mathbf{p}_A$ and $\mathbf{p}_B$. The rate of drift depends inversely on the effective sizes of the populations (Fisher 1930)—a quantity that we must assume to be known from records of the number of returning spawners. We assume that natural selection does not act directly on the genetic loci that we examine and that there is no mutation. By time $\tau = t$ the allele frequencies in the populations have drifted to $\mathbf{q}_A$ and $\mathbf{q}_B$ respectively and we sample n_A and n_B diploid individuals ($2n_A$ and $2n_B$ gene copies) from Populations A and B. These samples yield counts $\mathbf{x}_A$ and $\mathbf{x}_B$ of different types of alleles (*i.e.*, if you find k alleles then $\mathbf{x}_A = (x_{A1}, \ldots, x_{Ak})$ with $\sum_1^k x_{Ai} = 2n_A$). The process described above is illustrated in Figure 2.1.

The probability model for the above scenario expresses the probability of $\mathbf{x}_A$ and $\mathbf{x}_B$ given some value for the allele frequencies $\mathbf{p}_A$ and $\mathbf{p}_B$ at the time of stock introduction. We derive the model by first thinking only of Population A and the probability of $\mathbf{x}_A$ given $\mathbf{p}_A$, which we write $\Pr(\mathbf{x}_A|\mathbf{p}_A)$. We can consider drawing a

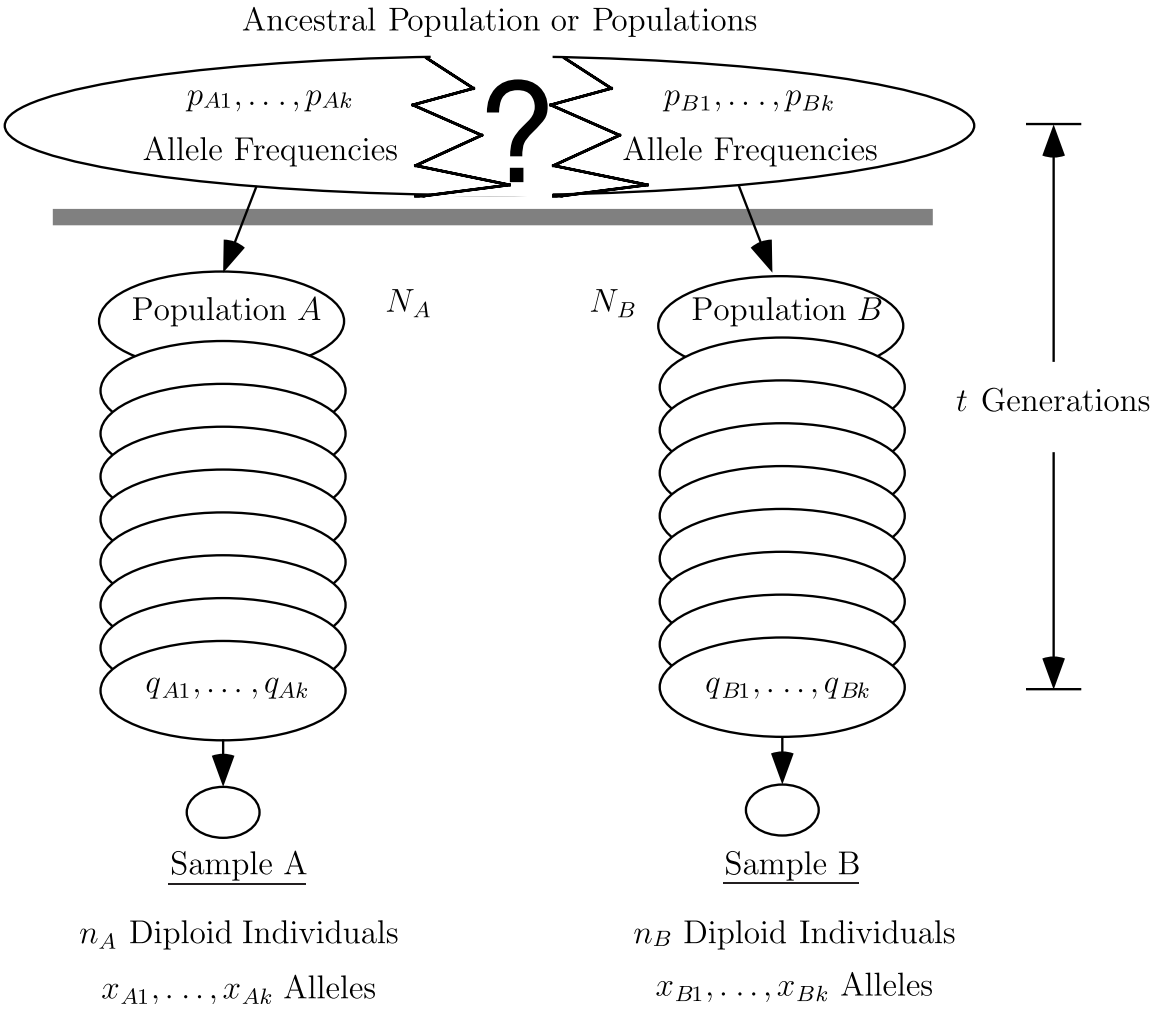


Figure 2.1: Diagmrammatic sketch of the scenario giving rise to the likelihood model. $p_{A1}, \ldots, p_{Ak}$ and $p_{B1}, \ldots, p_{Bk}$ are the allele frequencies in the ancestral populations. In t generations they drift to $q_{A1}, \ldots, q_{Ak}$ and $q_{B1}, \ldots, q_{Bk}$, respectively. $x_{A1}, \ldots, x_{Ak}$ are the counts of alleles of different kinds from a sample taken from Population A. $x_{A1}, \ldots, x_{Ak}$ are the same from Population B. N_A and N_B are the effective sizes of the populations which we must assume to be known from historical population size data. n_A and n_B are the respective number of individuals in each sample. The central question in this study is represented by the large question mark—we do not know if Populations A and B were one in the same t generations ago, or not.

sample of $\mathbf{x}_A$ from a population which started at $\mathbf{p}_A$ to be the result of two events:

Event 1 Population A starts with allele frequencies of $\mathbf{p}_A$ which drift to some new value $\mathbf{q}_A$ in t generations. The probability of this event is $\Pr(\mathbf{q}_A|\mathbf{p}_A)$.

Event 2 We sample n individuals from Population A and get allele counts $\mathbf{x}_A$. This event has probability $\Pr(\mathbf{x}_A|\mathbf{q}_A)$.

The probability that both events occur is $\Pr(1 \text{ and } 2) = \Pr(2|1) \cdot \Pr(1)$ by the law of conditional probability. We obtain $\Pr(\mathbf{x}_A|\mathbf{p}_A)$ by summing the joint probability of $\mathbf{x}_A$ and $\mathbf{q}_A$ given $\mathbf{p}_A$ over all the possible values of $\mathbf{q}_A$ that the population could have drifted to before we sampled from it:

$$\Pr(\mathbf{x}_A|\mathbf{p}_A) = \sum_{\mathbf{q}_A \in \mathcal{Q}} \Pr(\mathbf{q}_A|\mathbf{p}_A) \Pr(\mathbf{x}_A|\mathbf{q}_A) \tag{2.1}$$

where $\mathcal{Q}$ is the set of all possible vectors of allele frequency.

The probability of $\mathbf{x}_A$ for any given $\mathbf{q}_A$ is given by the multinomial probability mass function:

$$\Pr(\mathbf{x}_A|\mathbf{q}_A) = \frac{2n_A!}{x_{A1}!x_{A2}! \cdots x_{Ak}!}[q_{A1}]^{x_{A1}}[q_{A2}]^{x_{A2}} \cdots [q_{Ak}]^{x_{Ak}}. \tag{2.2}$$

Unfortunately, we cannot calculate $\Pr(\mathbf{q}_A|\mathbf{p}_A)$, a genetic drift transition probability, so easily. We will review ways of computing or approximating it in Section 2.5, accepting for now that it will be possible in at least some cases of interest.

Equation 2.1 tells us how to calculate $\Pr(\mathbf{x}_A|\mathbf{p}_A)$ for a sample from a single population. The same argument gives $\Pr(\mathbf{x}_B|\mathbf{p}_B)$. The joint probability of both of our samples is the product of their individual probabilities because they are independent realizations of a random process. Therefore we have that

$$\begin{aligned} \Pr(\mathbf{x}_A, \mathbf{x}_B|\mathbf{p}_A, \mathbf{p}_B) \quad = \quad & \left(\sum_{\mathbf{q}_A \in \mathcal{Q}} \Pr(\mathbf{q}_A|\mathbf{p}_A) \Pr(\mathbf{x}_A|\mathbf{q}_A) \right) \times \\ & \left(\sum_{\mathbf{q}_B \in \mathcal{Q}} \Pr(\mathbf{q}_B|\mathbf{p}_B) \Pr(\mathbf{x}_B|\mathbf{q}_B) \right), \end{aligned} \tag{2.3}$$

giving us our full probability equation. Of course, we don't know what $\mathbf{p}_A$ and $\mathbf{p}_B$ are, though we do know $\mathbf{x}_A$ and $\mathbf{x}_B$. So we consider our probability function as a function of the parameters with the data treated as fixed, and this gives us the likelihood of $\mathbf{p}_A$ and $\mathbf{p}_B$ given $\mathbf{x}_A$ and $\mathbf{x}_B$ (EDWARDS 1992).

2.3 The Likelihood-Ratio Test

With the likelihood $L(\mathbf{p}_A, \mathbf{p}_B|\mathbf{x}_A, \mathbf{x}_B) = \Pr(\mathbf{x}_A, \mathbf{x}_B|\mathbf{p}_A, \mathbf{p}_B)$ we can use an asymptotic likelihood-ratio test for H_A (the hypothesis that Population B descended exclusively from fish planted from Population A) against the general alternative, H_G, that some proportion of the ancestry of B must not be from A. The likelihood ratio test statistic is[2]:

$$\Lambda = 2\log\left(\frac{\sup\limits_{\mathbf{p}_A,\mathbf{p}_B\in\mathcal{P}_G} L(\mathbf{p}_A, \mathbf{p}_B|\mathbf{x}_A, \mathbf{x}_B)}{\sup\limits_{\mathbf{p}_A,\mathbf{p}_B\in\mathcal{P}_A} L(\mathbf{p}_A, \mathbf{p}_B|\mathbf{x}_A, \mathbf{x}_B)}\right) \tag{2.4}$$

where $\mathcal{P}_G$ is the set of values $\mathbf{p}_A$ and $\mathbf{p}_B$ may take under the general alternative hypothesis H_G, and $\mathcal{P}_A$ is the set of values that $\mathbf{p}_A$ and $\mathbf{p}_B$ are constrained to under the more restrictive null hypothesis H_A. Under the general alternative hypothesis $\mathbf{p}_A$ and $\mathbf{p}_B$ may take whatever values they want to so long as they still represent frequencies of alleles (*i.e.*, their components are between 0 and 1 and sum to 1). Under the null hypothesis, however, both Population A and Population B originated from the same population at time $\tau = 0$ and so $\mathbf{p}_A$ and $\mathbf{p}_B$ must be the same under H_A (*i.e.*, $\mathcal{P}_A = \{\mathbf{p}_A, \mathbf{p}_B : \mathbf{p}_A = \mathbf{p}_B\}$).

Since $\mathcal{P}_A$ is a subset of $\mathcal{P}_G$, the numerator in Equation 2.4 will never exceed the denominator, and hence Λ will always be greater than or equal to zero. In fact, when $\mathbf{x}_A = \mathbf{x}_B$, Λ will be zero. However, Λ increases as $\mathbf{x}_A$ and $\mathbf{x}_B$ become more different and we will reject H_A when Λ is sufficiently large[3]. Theory on the asymptotic

[2] For those unfamiliar with it "$\sup_{\mathbf{p}_A,\mathbf{p}_B\in\mathcal{P}_G}$" essentially means "the maximum over values of $\mathbf{p}_A$ and $\mathbf{p}_B$ in the set $\mathcal{P}_G$"

[3] It may be helpful to note that this test statistic is a sort of fancily-dressed G-statistic (SOKAL

distribution of log-likelihood ratios tells us how large Λ should be for us to reject H_A with a Type I error level of α (Kendall and Stuart 1979). If the null hypothesis is true ($\mathbf{p}_A = \mathbf{p}_B$) and if the sizes of Population A and Population B, and the sizes, n_A and n_B, of our samples increase toward infinity, the random variable Λ converges in distribution to a chi-square random variable with ν degrees of freedom, χ^2_ν, where ν is the difference in the number of free parameters under H_G and H_A. For a locus with k alleles, there are $2(k-1)$ free parameters under H_G, and $k-1$ free parameters under H_A, so in this case $\nu = k - 1$.

In Lake Washington, of course, neither the sockeye populations nor our samples are infinite. However, for reasonably large population and sample sizes a χ^2_ν distribution closely approximates the distribution of Λ when H_A is true. (I investigate this through computer simulation in Section 2.9.) Therefore, if H_A is true the probability that we observe a Λ greater than some value, say d is $\Pr(\chi^2_\nu > d)$. If that probability is small, then either 1) H_A is true and a very rare event (observing such a large test statistic) occurred; or 2) H_A is not true so Λ does not have a χ^2_ν distribution, and our observed test statistic is not so out of the ordinary. This, then, gives our test: reject H_A if we observe a $\Lambda = d$ such that $\Pr(\chi^2_\nu > d) \leq \alpha$.

Extending the test to multiple, independently segregating loci is straightforward. For L such loci, indexed by j, the test statistic T_L is the sum of the test statistics for each locus

$$T_L = \sum_{j=1}^{L} \Lambda_j. \tag{2.5}$$

T_L is also chi-square distributed, but with degrees of freedom equal to the sum of the degrees of freedom for each Λ_j.

* * * * *

I have, to this point, presented the statistical method in skeletal form. The logic

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and Rohlf 1981; Zar 1984). In fact, if there were no drift term in the likelihood function, this would boil down to the well-known G-test for multinomial proportions.

behind the test should be clear from the above discussion, even though it remains to fill in some details regarding the computation or approximation of transition probabilities, and routines for maximizing the likelihood function. In the next six sections I address these details, starting with some background on genetic drift and effective population number.

2.4 Genetic Drift and Effective Population Number

In order to compute the transition probability $\Pr(\mathbf{q}_A|\mathbf{p}_A)$ we must adequately model genetic drift, a random evolutionary force acting upon allele frequencies. Genetic drift occurs in populations of finite size because, as a result of random chance, some parents have more offspring than others, thus increasing the frequency of their genes in the following generation. Fisher (1930) and Wright (1931) first described genetic drift mathematically, using an idealized model of a randomly-mating population subsequently known as a "Wright-Fisher" model.

The Wright-Fisher model is a population of constant size and discrete generations (all individuals reproduce at the same age and die after reproduction) with each individual having an equal probability of mating with any other individual or itself. This mating scheme can be visualized for a population of N diploid individuals as follows: 1) individuals produce gametes according to their genotype (so, for example, half the gametes of a heterozygous Aa individual would be a's and the other half A's), 2) at the time of mating, each individual contributes an infinite but equal number of gametes to a "gamete pool," 3) an individual of the next generation is "assembled" by combining two gametes chosen at random from the gamete pool; N such individuals are assembled. Note that as this process continues over generations, alleles may be lost from the population. For example, if in one generation only 3 individuals carry copies of the a allele and none of these three produce offspring, the frequency of a in the next generation will be zero and we say that the population

has become "fixed" for the alternate allele, A. After the a allele is lost, its frequency remains at zero, until it is reintroduced to the population via migration or mutation, two processes we disregard for now.

Such a random mating scheme lacks realism for some organisms, but its simplicity allows a number of important results. In order to understand these, it is necessary to think of allele frequencies in future generations as random variables, and perhaps the easiest way to do this is to think not of the effect of drift in a single population, but rather in an infinite number of initially identical "replicate" populations, any one of which may be an observed or realized population. For the case of two alleles, say A at frequency p_0 and a at frequency $1-p_0$, we see that X_1, the number of A alleles in the next generation, is a binomially distributed random variable: $X_1 \sim \text{Bin}(2N, p_0)$ (*i.e.*, many of the replicate populations will have $2Np_o$ alleles in the next generation, but the others will have more or fewer than that, following a binomial distribution). It follows that the expectation of the frequency of A in our replicate populations after one generation of random mating is $E(\frac{X_1}{2N}) = p_0$ and the variance of the frequency of A across the replicate populations after one generation is $\text{Var}(\frac{X_1}{2N}) = \frac{p_0(1-p_0)}{2N}$. After t generations of drift in a Wright-Fisher population, the expectation and variance of the allele frequency $\frac{X_t}{2N}$ are:

$$E(\tfrac{X_t}{2N}) = p_0 \tag{2.6}$$

$$\text{Var}(\tfrac{X_t}{2N}) = p_0(1-p_0)\left[1-\left(1-\frac{1}{2N}\right)^t\right] \tag{2.7}$$

The expectation is always the initial value p_0, but the variance increases with each generation until, as $t \to \infty$ it reaches its limiting value of $p_0(1-p_0)$. (This limit corresponds to the time when each replicate population has become fixed for either the A or the a allele.) Notice that each generation the variance increases by $1/2N$ of the distance left to its limiting value. In other words, a gene in a Wright-Fisher population has a characteristic rate by which the variance of its frequency (considered as a random variable—as a realization of possible allele frequencies in an infinite

number of replicate populations) increases, and that rate depends on the size of the population. This provides an important way to relate the behavior of the allele frequencies in natural populations to those of the idealized Wright-Fisher model, using the effective population size (also called the effective population number).

Consider any natural or ideal population that violates the Wright-Fisher model (but still with no selection or mutation). It may have two sexes, fluctuating population size, overlapping generations, *etc.* (Felsenstein 1995). Though it may be much more difficult to calculate, the variance of the frequency of a gene in these populations will change through time at some rate. The variance effective size, N_e, of this population is the size of a Wright-Fisher population that would show the same increase in variance of allele frequency in the same amount of time. Many authors have derived expressions for variance effective numbers in populations departing from the Wright-Fisher model. For example, Crow and Denniston (1988) present formulae for populations with two sexes versus a single sex and self-fertilization permitted versus excluded. In general, the derivations for variance effective numbers in different types of populations are more difficult than they are for the more familiar inbreeding effective number which is the size of a Wright-Fisher population that would give the same rate of increase of probability of identity-by-descent of two gene copies taken at random from the population. In many circumstances the variance effective size equals the inbreeding effective size of a population. However, sometimes the two differ. Hereafter, "effective size" will be taken to be the variance effective size, as this is the quantity that is most intimately associated with t-step transition probabilities in the Wright-Fisher model.

In dealing with natural populations, though one could draw from a great many formulae to obtain the effective number for different scenarios, in actual practice it is very difficult to account for all of the ways that a natural population departs from a Wright-Fisher model. Accordingly, since the early 1970's, researchers have empirically estimated the variance effective size of natural populations. They do this

by drawing genetic samples from a population at successive time points and using the information in those samples to estimate the increase in allele frequency variance over time.[4] This increase in variance, then, converts into an effective population number. Krimbas and Tsakas (1971) first employed this method to estimate the effective size of olive fruit fly populations. Several authors have suggested statistical refinements for estimating N_e empirically (Pamilo and Varvio-Aho 1980; Nei and Tajima 1981; Pollak 1983), and more recently Waples (1989) presented a general method that reconciles some of the differences between earlier techniques. Jorde and Ryman (1995) proposed a method specifically for populations with overlapping generations. Salmon researchers commonly use the methods of Waples (1989) to estimate the effective number of spawners in salmon populations. Later, I will discuss how to combine empirical estimates of effective number of spawners over many years to estimate N_e for the Bear Creek Problem (Section 3.2).

One of the great advantages of converting the actual size of a population to its effective size is that almost all of the theory on the behavior of genes and allele frequencies has been formulated in reference to the Wright-Fisher model. Using the effective size allows one to access many useful results. For the present problem, using the effective size will allow us to compute genetic drift transition probabilities using formulae that have been developed for the Wright-Fisher model.

2.5 t-Step Transition Probabilities in the Wright-Fisher Model

Genetic drift may cause allele frequencies to change each generation. The probability that an allele frequency takes a particular value in the next generation depends only on the population's size and on the allele frequency in the current generation. In other words, given the current allele frequency, the frequency in the next generation does

[4] Such a technique is called a temporal method. Another approach sometime used with salmon populations is the disequilibrium method (Bartley *et al.* 1992).

not depend on the frequencies in any of the previous generations. This property makes genetic drift in a Wright-Fisher model a Markov stochastic process. In particular it is a discrete time (the generations are discrete), finite (there are a finite number of values the allele frequency may take) Markov chain (see KARLIN and TAYLOR 1975). As such, one can compute t generation transition probabilities exactly, but, in some cases this requires very many computations. Some authors have developed diffusion approximations to the process which are, unfortunately, difficult to use in their most accurate forms. However, under restricted conditions, simpler approximations are valid and useful for statistical inference. I treat each of these topics in the subsections below, outlining a sort of transition probability "toolkit" to have at our disposal for Chapter 3.

2.5.1 Drift as a Markov Process

Given a Wright-Fisher population with N diploid individuals and two alleles A and a at a locus, there are $2N + 1$ allele frequency states that the population may be in: it may have no copies of the A allele, 1 copy, 2 copies, and so forth up to $2N$ copies. Since the number of A alleles in the next generation is binomially distributed, the probability that a population with i copies of the A allele in the current generation has j copies in the next generation is

$$P_{i,j} = \left(\frac{2N!}{j!(2N-j)!}\right)\left(\frac{i}{2N}\right)^{j}\left(1-\frac{i}{2N}\right)^{2N-j}. \tag{2.8}$$

We can arrange these probabilities into a one-step transition probability matrix, $\mathbf{P^{(1)}}$:

$$\mathbf{P^{(1)}} = ||P_{i,j}|| = \begin{pmatrix} P_{0,0} & P_{0,1} & \cdots & P_{0,2N} \\ P_{1,0} & P_{1,1} & \cdots & P_{1,2N} \\ \vdots & \vdots & \ddots & \vdots \\ P_{2N,0} & P_{2N,1} & \cdots & P_{2N,2N} \end{pmatrix}. \tag{2.9}$$

This matrix is essentially just a table where the entry in the i^{th} row and the j^{th} column is the probability of drifting from i to j copies of the A gene in one generation.

However, with $\mathbf{P^{(1)}}$ we can easily obtain $\mathbf{P^{(t)}}$, the t-generation transition probability matrix, as the matrix product of $\mathbf{P^{(1)}}$ with itself t times:[5]

$$\mathbf{P^{(t)}} = \underbrace{\mathbf{P^{(1)}}\mathbf{P^{(1)}}\mathbf{P^{(1)}}\cdots\mathbf{P^{(1)}}}_{t \text{ times}}. \tag{2.10}$$

In fact, the matrix $\mathbf{P^{(t)}}$ would give us the values we need for $\Pr(\mathbf{q}_A|\mathbf{p}_A)$ and $\Pr(\mathbf{q}_B|\mathbf{p}_B)$ in Equation 2.3 so long as there were only two alleles at the locus in question in Populations A and B. If Populations A and B were Wright-Fisher populations these transition probabilities would be exact and would properly reflect the way that probability mass accumulates at the boundaries due to allele fixation in the populations. Even if A and B are not Wright-Fisher populations (as salmon populations certainly are not), using the effective size to determine the $P_{i,j}$ and the number of rows and columns in $\mathbf{P^{(1)}}$ should yield a reasonably accurate $\mathbf{P^{(t)}}$ by (2.10). For modestly-sized Wright-Fisher populations (say $N < 250$) with only two alleles, multiplying the matrices is manageable. However, with more alleles the number of computations required becomes prohibitively large.

If there are more than two alleles at a locus (*i.e.*, if the vectors $\mathbf{p}_A$ and $\mathbf{p}_B$ have three or more components), drift in a Wright-Fisher population is still a Markov process but the number of states (combinations of allele frequencies) increases rapidly. As before, computing the one-step transtition probability is easy; it is a multinomial probability. Thus if there are k alleles $A_1, A_2, \ldots, A_k$, and in the current generation there are b_1 alleles of type A_1, b_2 of type A_2 and so forth, then the probability that there are c_1 type A_1 alleles, c_2 type A_2 alleles and so forth in the next generation is:

$$\Pr(c_1, \ldots, c_k|b_1, \ldots, b_k) = \frac{2N!}{c_1!c_2!\cdots c_k!}\left(\frac{b_1}{2N}\right)^{c_1}\left(\frac{b_2}{2N}\right)^{c_2}\cdots\left(\frac{b_k}{2N}\right)^{c_k}. \tag{2.11}$$

[5] The reader may recognize that this says that $\mathbf{p^{(t)}}$, a row vector of transition probabilities, may be obtained by $\mathbf{p^{(t)}} = \mathbf{p^{(0)}}\mathbf{P^{(t)}}$ (where $\mathbf{p^{(0)}}$ is the vector of the starting state) and hence may wonder why one doesn't just find all the eigenvalues and left eigenvectors of $\mathbf{P}$ and compute $\mathbf{p^{(t)}}$ by such a spectral resolution approach. Unfortunately no one has discovered expressions for all but two of the left eigenvectors of $\mathbf{P}$ (Felsenstein 1995).

Table 2.1: Number of allele frequency states for a diploid population of size N individuals with 3,4,5, or 6 alleles. The number of states was obtained by direct evaluation of the sum in (2.12) via a short, recursive, computer program.

	k, the number of alleles			
N	3	4	5	6
25	1,326	23,426	316,251	3,478,761
75	11,476	585,276	22,533,126	698,526,906
150	45,451	4,590,551	348,881,876	21,281,794,436

These one-step transition probabilities may also be arranged into a matrix, but there are very many states to consider. For example, with three alleles and $2N = 200$ a population may have no A_1 alleles, no A_2 alleles, and 200 A_2 alleles—a state that can be denoted (0,0,200). Of course it could also be in state (1,2,197) or (1,3,196) or (24,26,150). In fact there are 20,301 possible states, which is the number of ordered triplets of whole numbers whose sum is 200. In general, for a population of N diploid individuals and k alleles the number of states equals the number of ordered k-tuples whose sum is $2N$. This is the number of terms in the multinomial expansion of $(a_1 + a_2 + \cdots + a_k)^{2N}$ and can be found as the sum:

$$\sum_{i_1=0}^{2N} \left(\sum_{i_2=0}^{2N-i_1} \left(\sum_{i_3=0}^{2N-i_1-i_2} \cdots \sum_{i_{k-1}=0}^{2N-\xi} 1 \right) \right), \tag{2.12}$$

where $\xi = i_1 + i_2 + \cdots + i_{k-2}$. I have computed the number of states for several values of k and N (Table 2.1). Even for modest values of k and N the size of the matrix is too large to realistically compute the t-step transition probability matrix by Equation 2.10. For example, with $N = 75$ and $k = 4$, just the memory required to store all the entries in $\mathbf{P^{(1)}}$ as 32-bit floating points would require over 1,370 gigabytes of computer memory. There is little hope of obtaining the exact transition probabilities from (2.10) for all but the smallest cases; instead we must use approximations.

2.5.2 Diffusion Approximations to Genetic Drift

Kimura (1955a, 1955b, 1956) derived approximations to genetic drift transition probabilities by considering the allele frequencies as continuous random variables rather than as discrete ones existing only in multiples of $1/2N$. Other authors have refined these approximations (Littler and Fackerell 1975; Griffiths 1979), but their results are difficult to implement. I have encountered no cases of statistical inference using the complete density expressions from the above papers. However, Cavalli-Sforza and Edwards (1967) noted from Kimura (1955a) that to a first approximation, the transition probability density for multiple alleles is multinomial in shape. This led to a useful approximation for drift in a multiallelic locus of allele frequencies under stereographic projection.

2.5.3 Brownian Motion and Stereographic Projection

Edwards (1971) showed that drift can be approximated by Brownian motion in a curved space which may be projected into a Euclidean space, giving very nice properties. This approach forms the basis of the well known Cavalli-Sforza and Edwards "Arc" genetic distance, and allowed Thompson (1973) to compute likelihoods involving drift transition probabilities.

The essence of this approximation lies in recognizing the geometrical interpretation of the arc-sine square-root transformation on multinomial proportions, and using the geometry to find a projection of the transformed allele frequencies into a Euclidean space where multinomially distributed random variables are "uncorrelated." It is impossible to visualize the geometric interpretation for the case of more than three alleles, because it involves more than three dimensions. Nonetheless the main points of the result can be visualized and drawn for two or three alleles as done below. Here, I sketch some of the reasoning behind this remarkable result which was proved by Thompson (1972).

Normal approximation and angular transformation. Start with a single locus having k alleles in the frequencies $\mathbf{p} = (p_1, \ldots, p_k)$. The numbers of each type of allele $(c_1, \ldots, c_k)$, after one generation are multinomially distributed. For reasonably large N and small t, however, Edwards (1971) and Cavalli-Sforza and Edwards (1967) quoting a result in Kimura (1955a) indicate that after t generations the allele frequencies (*i.e.*, the $c_i/2N$, which we will denote q_i) are still approximately multinomially distributed, but with variance after t generations given by $\mathrm{Var}(q_i) \approx (1 - e^{-t/2N})p_i(1 - p_i)$. The first order Taylor approximation of $e^{-t/2N}$ is $1 - t/2N$, so to further approximation,

$$\mathrm{Var}(q_i) \approx \frac{tp_i(1-p_i)}{2N} = \frac{p_i(1-p_i)}{2N/t}. \tag{2.13}$$

Notice that this is exactly the variance of a proportion arising from $2N/t$ multinomial trials[6] with cell probabilities $(p_1, \ldots, p_k)$. Thus, the q_i are approximately distributed as the random variable $\frac{\mathbf{X}}{2N/t}$ where $\mathbf{X} \sim \mathrm{Multinomial}(2N/t, \mathbf{p})$.

By the Central Limit Theorem the allele frequencies after t generations converge in distribution as $2N/t \to \infty$ to a multivariate normal random variable in $k-1$ dimensions:

$$\sqrt{2N/t}\Big((q_1, \ldots, q_{k-1}) - (p_1, \ldots, p_{k-1})\Big) \xrightarrow{D} \mathrm{MVN}(\mathbf{0}, \mathbf{V}) \tag{2.14}$$

where $\mathbf{0}$ is the zero vector $(0, \ldots, 0)$ and $\mathbf{V}$ is the variance-covariance matrix having diagonal elements $V_{ii} = p_i(1-p_i)$ and off-diagonal elements $V_{ij} = -p_i p_j$ for $i \neq j$. And so, $(q_1, \ldots, q_{k-1})$ is approximately multivariate normal:

$$(q_1, \ldots, q_{k-1}) \sim \mathrm{MVN}\left((p_1, \ldots, p_{k-1}), \frac{t}{2N}\mathbf{V}\right). \tag{2.15}$$

We now have a normal approximation to the joint distribution of the q_i, but the variance of each q_i is a function of its mean [by the $p_i(1-p_i)$ term in the V_{ii}.] So,

[6] We assume here that $2N/t$ is an integer—an innocuous assumption as we will soon be taking the limit as $2N/t \to \infty$ and losing the discrete character of the multinomial distribution altogether.

apply the angular transformation, obtaining the new random variables $\theta_i = \sin^{-1}\sqrt{q_i}$ for $i = 1, \ldots, k-1$. Under this transformation, our vector of initial allele frequencies $(p_1, \ldots, p_{k-1})$ becomes $\mathbf{\Phi} = (\phi_1, \ldots, \phi_{k-1})$ where $\phi_i = \sin^{-1}\sqrt{p_i}$, and by the Delta-method we obtain:

$$(\theta_1, \ldots, \theta_{k-1}) \sim \text{MVN}(\mathbf{\Phi}, \mathbf{V}^*) \tag{2.16}$$

with the elements of $\mathbf{V}^*$ given by the standard formulas:

$$V^*_{ii} = (\partial\theta_i/\partial q_i)^2 \frac{t}{2N} V_{ii} = \left(\frac{1}{2\sqrt{p_i}\sqrt{1-p_i}}\right)^2 \left(\frac{tp_i(1-p_i)}{2N}\right) = t/8N \tag{2.17}$$

$$V^*_{ij} = \text{Cov}(\theta_i, \theta_j) = \frac{\partial\theta_i}{\partial q_i}\frac{\partial\theta_j}{\partial q_j} V_{ij} = -\frac{t}{8N}\tan\theta_i \tan\theta_j, \quad \text{for } i \neq j \tag{2.18}$$

Notice that the variances of the θ_i are the same no matter "where they start from" (*i.e.*, they are not functions of the initial allele frequencies, $\mathbf{\Phi}$), and the correlation between θ_i and θ_j is $-\tan\theta_i \tan\theta_j$.

The geometric interpretation of these θ_i is important. Consider first the case of $k = 2$ alleles so that the subscripts may be dropped and we have $q \sim \text{N}(p, tp(1-p)/2N)$ which implies $\theta \sim \text{N}(\phi, t/8N)$. We may represent these quantities on the portion of the unit circle in the first quadrant of the Cartesian plane. Starting from the origin, if you go up the y-axis $\sqrt{p}$ units and then to the right $\sqrt{1-p}$ you hit the unit circle at a point that is ϕ units of arc-length (radians) along the unit circle from the x-axis (Figure 2.2). Likewise, the point on the unit circle corresponding to a height of $\sqrt{q}$ is θ radians along the circle. The distance along the circle between these points (call them P and Q) is the arc-length $\theta - \phi$. Since θ is normally distributed around ϕ, the density function of θ depends on the square of the arc-length between P and Q. That is:

$$f(\theta; \phi) = (2\pi t/8N)^{-1/2} \exp\left\{\frac{-(\theta-\phi)^2}{2t/8N}\right\}. \tag{2.19}$$

It follows that the log of the density function equals

$$\log f(\theta; \phi) = -\frac{1}{2}\log(\pi t/4N) - \frac{(\theta-\phi)^2}{t/4N}, \tag{2.20}$$

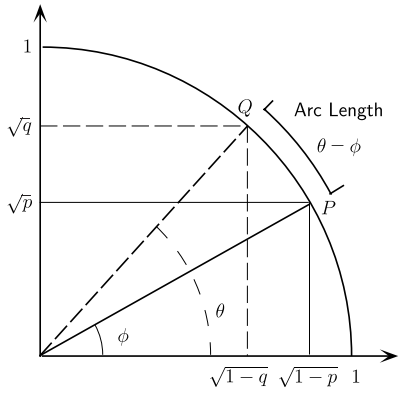


Figure 2.2: A section of the unit circle. The horizontal and vertical axes give the square root of the frequencies of the two alleles at a diallelic locus, where p is the initial frequency of one of the alleles which drifts to a frequency of q. θ and ϕ are arc lengths along the perimeter of the unit circle. ϕ is the mean about which θ is normally distributed with variance independent of θ. The deviation due to drift is the change in arc length, $\theta - \phi$.

and so the difference in log-density at the mean (Point P) and at any other point Q is $-(\theta-\phi)^2/(t/4N)$. And it should also be apparent that the difference in log-likelihood between the maximum likelihood $\hat{\phi}$ and any other estimate of ϕ, say $\tilde{\phi}$, is equal to $-(\hat{\phi} - \tilde{\phi})^2/(t/4N)$.

With $k = 3$ alleles, we can represent a population's allele frequencies as points on the surface of a unit sphere (Figure 2.3), and with $k > 3$ we can plot the allele frequencies on the surface of a k-dimensional hypersphere. Edwards (1971, p. 875) uses the fact that the correlation between any θ_i and θ_j is $-\tan\theta_i \tan\theta_j$ to demonstrate that the level-curves of log density around a mean point P are $k-1$-dimensional hyperspheres in the curved space centered on P. (The same is true for level curves of log-likelihood around the point of maximum likelihood.) Thus, the log of the density between any point Q on the surface of the hypersphere and the mean point P is a

function of the great-circle distance between Q and P. Finally, since the difference in the log of the joint density of $(\theta_1, \theta_2, \ldots, \theta_{k-1})$ between the point $(\phi_1, \phi_2, \ldots, \phi_{k-1})$ and the point $(l - \phi_1, \phi_2, \ldots, \phi_{k-1})$, which is l radians away, is easily seen to be $-l^2/(t/4N)$ it follows that the change in log-density from the mean P to any point Q, is, in fact, proportional to the square of the great-circle distance from P to Q.

Stererographic projection. The above shows that a population's initial allele frequencies may be represented as a point on the surface of a hypersphere. Genetic drift is then a force that makes the population jiggle away from those initial values. Its jiggles are equally likely in any direction on the surface of the hypersphere, and the distance that it travels from its starting point is normally distributed. This is like the diffusion of very small particles outward from their source in a still medium; hence the name "Brownian motion approximation." However, the process takes place in a curved space, and it is desirable to be able to represent the points P and Q

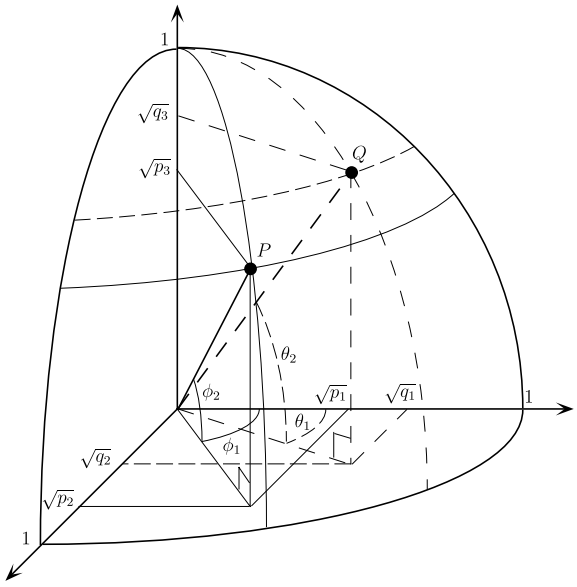


Figure 2.3: Geometric interpretation of the angular transformation for three alleles. The axes give the square roots of the allele frequencies for each of the three alleles.

in a Euclidean space. The solution is the same that cartographers have used for centuries—project the curved space into a "flat" one. Ideally one could find a projection such that shapes were preserved (orthomorphy) and the arc length from P to Q in the curved space was the same as the distance between the corresponding points P' and Q' in the projected space. That is not possible, but the stereographic projection is orthomorphic and lengths are only slightly distorted. Because of the orthomorphy, things that are hyperspheres (*i.e.*, the level curves of equal log-density or log-likelihood) in the curved space will be hyperspheres in the new, Euclidean space. And because the size of things is not too greatly distorted, the difference in log-density (or likelihood) between P and Q in the curved space will be approximately equal to the Euclidean distance between P' and Q', because

$$\frac{(\text{great circle distance from } P \text{ to } Q)^2}{t/4N} \approx \frac{(\text{Euclidean distance from } P' \text{ to } Q')^2}{t/4N}.$$

The plane of projection is a hyperplane of $k-1$ dimensions which exists in k dimensions (see Figure 2.4 for $k=2$). Any point on the plane may thus be specified by k Cartesian coordinates.[7] The i^{th} Cartesian coordinate of the point Q' is given in the appendix[8] of Edwards (1971) as

$$q_i' = \frac{2\left(\sqrt{q_i} + \sqrt{1/k}\right)}{1 + \sum_1^k \sqrt{q_i/k}} - \frac{1}{\sqrt{k}}, \tag{2.21}$$

and the i^{th} Cartesian coordinate of the point P' is

$$p_i' = \frac{2\left(\sqrt{p_i} + \sqrt{1/k}\right)}{1 + \sum_1^k \sqrt{p_i/k}} - \frac{1}{\sqrt{k}}. \tag{2.22}$$

[7] Thompson (1973) actually redefines a new set of $k-1$ axes that span the hyperplane of projection, and defines the coordinates of the projected points on that set of axes. For our current purposes the result is similar.

[8] The formula also appears on page 877 in the text of Edwards (1971) but contains a typographical error.

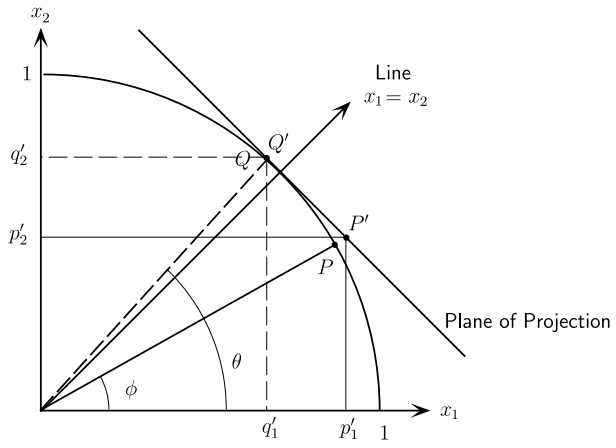


Figure 2.4: Stereographic projection for a diallelic locus (an heuristic diagram, not drawn to scale). The points P and Q are projected into P' and Q' on the "plane" of projection (a line when $k = 2$) which is tangent to the circle at its intersection with the line $x_1 = x_2$. The axes show the allele frequency coordinates, q_1', q_2', p_1', p_2', under stereographic projection given by (2.21) and (2.22).

Finally since the Euclidean distance between P' and Q' is given by

$$\left((q_1' - p_1')^2 + (q_2' - p_2')^2 + \cdots + (q_k' - p_k')^2\right)^{\frac{1}{2}},$$

the log-density at Q, which is the log-density that a population starting with allele frequencies $\mathbf{p} = (p_1, \ldots, q_k)$ drifts to have allele frequencies $\mathbf{q} = (q_1, \ldots, q_k)$ is approximately

$$-\frac{k}{2}\log(2\pi t/8N) - \frac{\sum_{i=1}^{k}(q_i' - p_i')^2}{t/4N}, \tag{2.23}$$

which is exactly the joint log-density we would expect to get for $(q_i', \ldots, q_k')$ if each component q_i' were independently a $\mathrm{N}(p_i', t/8N)$ random variable. So, to compute a drift transition density for $\mathbf{q}$ starting from $\mathbf{p}$, we need only transform $(q_1, \ldots, q_k)$ to $(q_1', \ldots, q_k')$ and $(p_1, \ldots, p_k)$ to $(p_1', \ldots, p_k')$ by Equations 2.21 and 2.22, and then treat each q_i' as if it were distributed normally with mean p_i' and variance $t/8N$, independently of the other q_i'. (Of course, the q_i' are not independent; it is easy to

show that they must sum to $\sqrt{k}$. However, in the present application, this does not matter. We are just using the q_i' to compute the log of the transition density from $\mathbf{p}$ to $\mathbf{q}$).

2.5.4 Other Approximations to Drift Probabilities

Thompson (1973) used the approximation just described to analytically obtain a maximum likelihood solution to the proportion of Norse ancestry in the people of Iceland. With the recent availability of computers, however, analytical solutions are no longer so crucial. Numerical integration and maximization routines available with many computer packages allow us to deal with such things as the covariance between allele frequencies at a locus. Two such approaches that use computation in place of analytical ingenuity are immediately apparent from the previous section. The first method uses the normal approximation in Equation 2.15 to the density of $(q_1, \ldots, q_{k-1})$. This we will call the "Normal" method. The second method (call it the "Angular" method) involves doing almost the same thing as the first, but uses the angularly transformed variables (the θ_i's) and the relation in (2.16).

Another possibility for approximating likelihoods would be to use Monte Carlo simulation (Diggle and Gratton 1984), and if one were to do this it would be best to simulate collecting genetic samples [the $\Pr(\mathbf{x}_A|\mathbf{q}_A)$ term] in addition to the genetic drift. This would involve simulating many replicates of genetic drift and sample collection in two populations starting from allele frequencies $\mathbf{p}_A$ and $\mathbf{p}_B$ and then using the proportion of outcomes with allele counts $\mathbf{x}_A$ and $\mathbf{x}_B$ to estimate the likelihood $L(\mathbf{p}_A, \mathbf{p}_A|\mathbf{x}_A, \mathbf{x}_B)$. But, since we ultimately shall wish to maximize this likelihood over different values of $\mathbf{p}_A$ and $\mathbf{p}_B$, we would have to repeat the Monte Carlo simulations starting from a number of different $\mathbf{p}_A$'s and $\mathbf{p}_B$'s and compare the Monte Carlo estimate of the likelihood from each of these separate simulations. Doing so requires another Monte Carlo simulation for each different set of starting allele frequencies, and so would be called a "many samples" approach to maximizing the

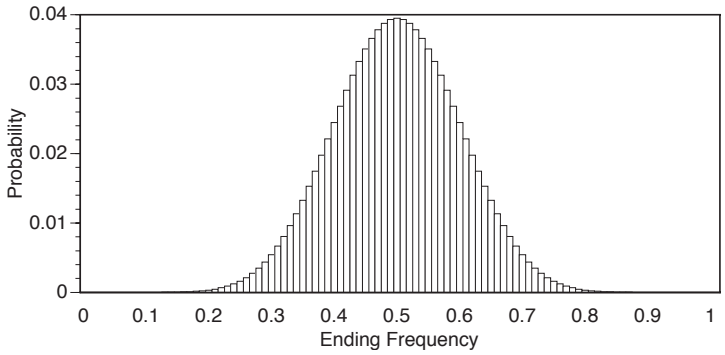
likelihood (Geyer 1996). Such a technique would require a great deal of computation for loci with many alleles because 1) there are so many possible final allele frequency states (Table 2.1) that a good estimate of the probability would require very many Monte Carlo replicates within any one sample, and 2) there are also very many initial allele frequency states to consider maximizing the likelihood over, so one may have to try many initial frequencies. It may be possible to make the second problem more manageable by Markov Chain Monte Carlo. I do not pursue such an approach here.

2.6 Assessing the Approximations

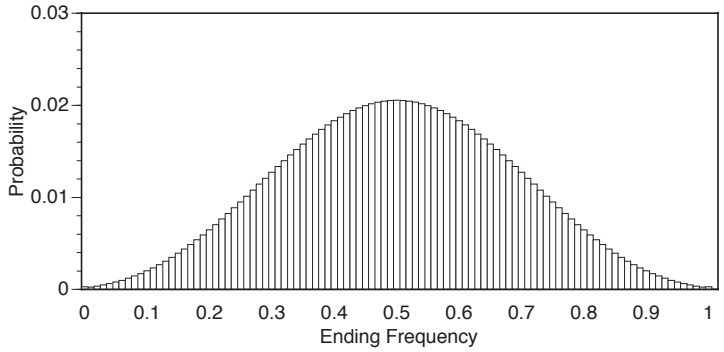
In the last section we learned of four methods for computing transition probabilities in a Wright-Fisher model—one exact method that is infeasible for complex cases, and three related, approximate methods that rely on the asymptotic relationships between transition probabilities, the multinomial distribution, and the normal distribution. These approximations will be best for large population sizes and short time spans (small $t/2N$), however we may want to apply these approximations to small populations as well. This section explores how the approximations break down for small populations. I compare the Normal and the Angular approximations[9] to the exact probabilities in small, computable cases (diallelic loci in small Wright-Fisher populations). The results warn us of situations where the approximations are inappropriate.

Figure 2.5 shows the distributions of allele frequency for an allele starting at a frequency of 0.5 and drifting for four and fourteen generations. (I have chosen fourteen generations for these simulations because that is roughly the number of sockeye salmon generations that passed between the introduction of Baker Lake sockeye to Lake Washington and Hendry *et al.* (1996)'s genetic sampling.) These transition

[9] I do not also compare the approximation from stereographic projection, because for a diallelic locus it is quite close to the Angular method, differing only by the distortion due to the projection.



(a) 4 Generations

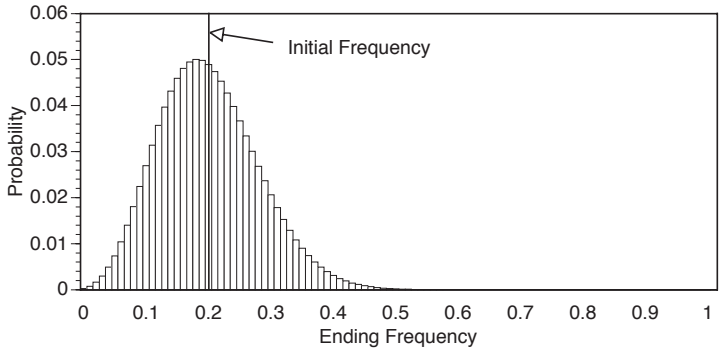


(b) 14 Generations

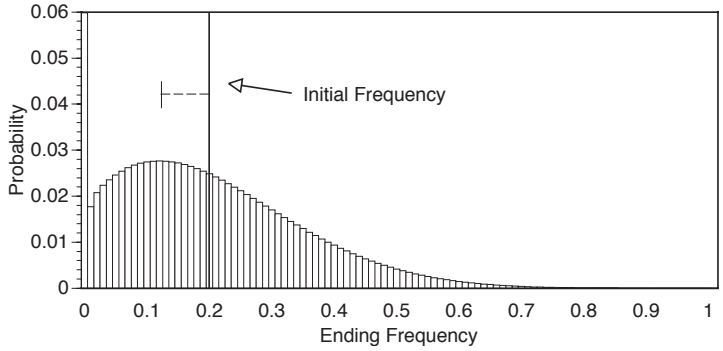
Figure 2.5: Probability distribution of allele frequency (initially 0.5) in a Wright-Fisher population of size $N = 50$ after 4 and 14 generations, computed by Equation 2.10

probabilities look very much like binomial distributions with variance increasing over time [just as Cavalli-Sforza and Edwards (1967) say they should for small $t/2N$]. Accordingly, we expect that any of the approximations would work well here.

However, when we change the starting conditions we get a very different result. Figure 2.6 shows the distributions for another population of size $N = 50$ with a starting allele frequency of 0.2. The distribution in Figure 2.6(b) no longer looks



(a) 4 Generations



(b) 14 Generations

Figure 2.6: Probability distribution of allele frequency in a Wright-Fisher population of size $N = 50$ after 4 and 14 generations, having started from a frequency of 0.2. In (b) note all the probability mass piled up at zero.

binomial. Most notably, after fourteen generations the probability that the frequency is zero (*i.e.*, that the allele has been lost from the population) is much higher than the probability that only one copy of the allele remains, and the peak of the distribution is not at the starting value of the allele frequency (it is shifted over by the length of the dashed line). Both of these features present trouble for the approximations, as we can see if we plot the approximations on the same axes as the exact probabilities

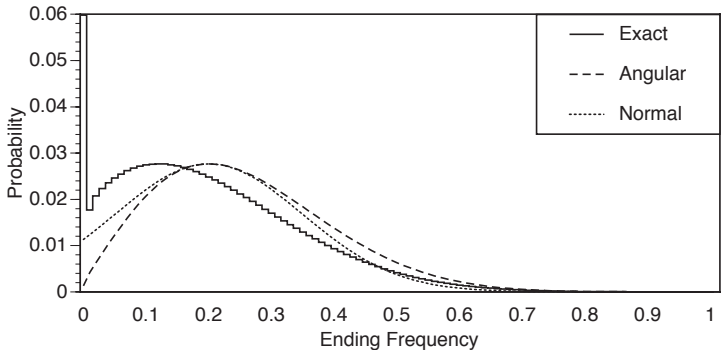


Figure 2.7: Normal and Angular approximations (probability density curves with heights scaled to match the exact probability distribution) for an allele starting from a frequency of 0.2 in a population of $N = 50$ and undergoing drift for 14 generations. The stepped, solid line shows the exact probability. Note that the approximations are shifted horizontally, and furthermore do not capture the probability accumulated at zero.

(Figure 2.7).

Since Cavalli-Sforza and Edwards introduced the Brownian motion approximation, they and other authors have remarked on its inaccuracy when the probability of gene fixation is high. They (1967, p. 557) write, "Since this [gene frequency space] is finite, with known bounds [zero and one], the Gaussian approximation to the gene frequency distribution will only hold if the variance is sufficiently small and the population sufficiently far from the edge of the space for edge effects to be neglected." Thompson (1972) notes these same problems at the boundary of the space, and Felsenstein (1985) puts it clearly:

> Such a transformation still does not succeed in preserving the full statistical behavior of the process. The gene frequencies reach 0 or 1 in a finite amount of time, but the Brownian motion in the new coordinates has no such bounds. Thus, we might expect the approximation to break

> down at extreme gene frequencies. There simply is no way to fix the transformation so as to make the Brownian motion have exactly the same statistical properties as the genetic drift. The question that then arises is whether the process of Brownian motion is a good enough approximation for practical purposes. (p. 302)

Since we are primarily interested in the portion of the Normal approximation density from 0 to 1, or the Angular approximation density from 0 to $\pi/2$, we might hope that the probability beyond those bounds would be close to the true fixation probability. For example, if $\theta \sim \mathrm{N}(\phi, t/8N)$ approximated the distribution of the allele frequency q given p, then fixation would not worry us so much if $\Pr(\theta \leq 0) \approx \Pr(q = 0)$. Unfortunately that is not the case, and there seems to be no simple, reliable relationship between $\Pr(\theta \leq 0)$ and $\Pr(q = 0)$.

Ultimately, we should avoid using the approximations in instances where the probability of allele fixation is greater than some amount (0.01 seems as good as any other arbitrary value). In practice, this means computing transition probabilities for small populations and noting which starting frequencies yield small fixation probabilities. I have done this for several populations from size $N = 50$ to $N = 200$ drifting for fourteen generations (Figure 2.8). One conservative result is clear: for $N \geq 200$, $t = 14$, and starting allele frequencies p_i such that $0.1 \leq p_i \leq 0.9$, the approximations should work reasonably well.

Though many authors have commented on the problem that fixation poses to the approximations, I am not aware of any reports on the second problem: that the peak of the exact transition probability distribution and the peak of the approximate density do not match. Fortunately we could solve this second problem by changing the mean of the approximating distribution by an amount such that the two distributions will line up (Figure 2.9). So, for example, if we knew that the difference between the allele frequency at the exact peak and at the approximate peak was δ, then we could

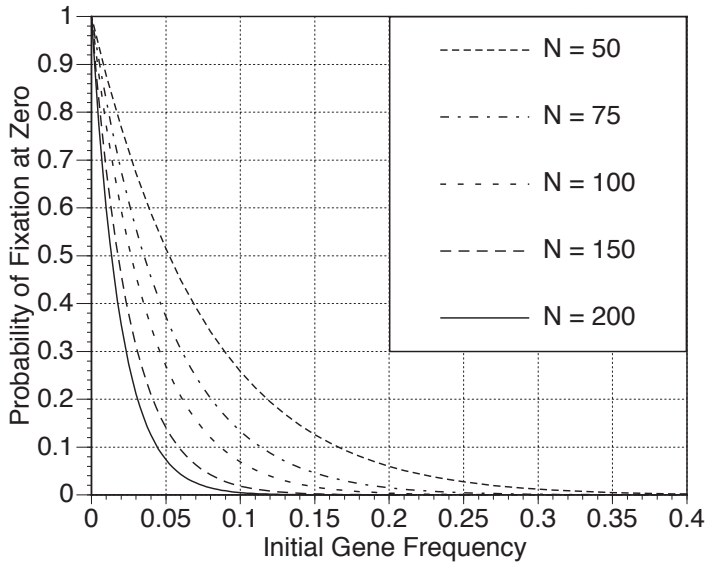


Figure 2.8: Probability that allele frequency drifts to zero in 14 generations for five different population sizes and various initial frequencies.

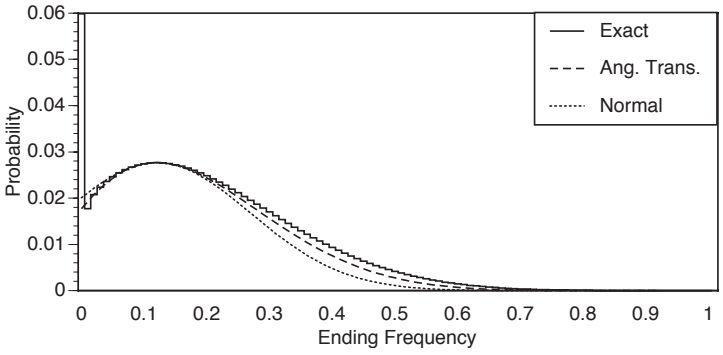


Figure 2.9: Normal and Angular approximations shifted so that the peaks match the peak of exact probability for an allele starting from a frequency of 0.2 in a population of $N = 50$ undergoing drift for 14 generations.

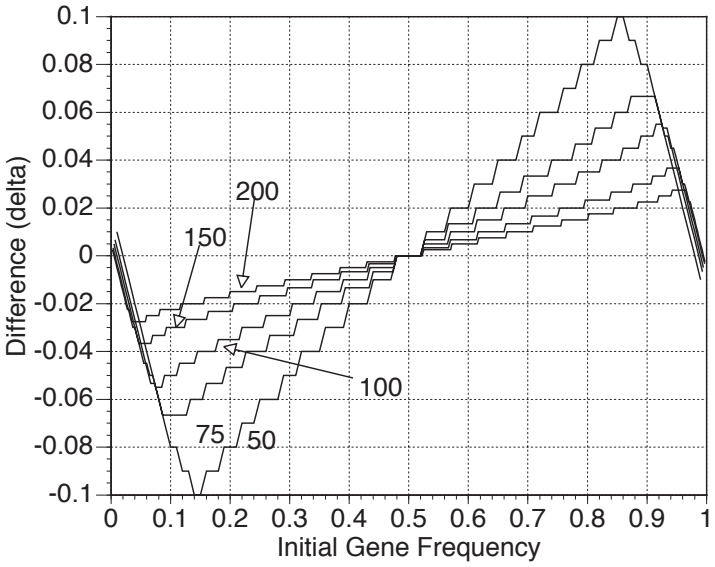


Figure 2.10: Values of δ, the horizontal distance between the peak of the exact transition probability distribution and peaks of the Angular and Normal approximation densities for $t = 14$ generations. Numbers next to the lines indicate population sizes N; the x-axis is the starting allele frequency.

define $\theta \sim \mathrm{N}(\sin^{-1}\sqrt{p+\delta}, t/8N)$ so that the peak was in the right place. Of course, to do this we must know what δ is, and, currently, that means computing the transition probabilities exactly and seeing how far the peak of the distribution is from its mean. (By that time one may just wish to use those exact probabilities, for a diallelic locus, at least.) I have calculated δ after fourteen generations for several population sizes, up to $N = 200$, from all the possible starting allele frequencies (Figure 2.10). From the graph it is apparent that δ may be quite large for small N, but for $N \geq 200$ with a starting frequency between 0.1 and 0.9, δ will not be greater than about 0.02 in magnitude. Furthermore we will see in Section 2.9 that the δ-shift seems to have very little effect on the value of the test statistic, Λ.

On a final note, Figure 2.9 shows that the Angular approximation is closer to

the exact probability than is the simple Normal approximation. This seems to be the case for most starting frequencies between zero and one. (Though the Normal approximation appears better for an initial allele frequency of 0.5, the difference is not so great). I prefer the Angular approximation for this reason.

2.7 Adding the Sampling Step

The last two sections showed ways to compute t-generation transition probabilities. We need those techniques to calculate the $\Pr(\mathbf{q}_A|\mathbf{p}_A)$ and $\Pr(\mathbf{q}_B|\mathbf{p}_B)$ terms in the joint likelihood equation (2.3 on Page 17). But now, we must use those transition probabilities (or the approximations) to obtain the probability of a sample of genes from the population. There are two ways to go about this. The first follows Equation 2.1, in spirit, and returns a discrete probability for the sample. I call this a *sample mass method*. The second method returns a probability density for the sample. Such a *sample density method* requires less computation, but may be inappropriate in some circumstances.

2.7.1 Sample mass methods

With exact transition probabilities. It is not difficult to add the sampling step to transition probabilities computed exactly by Equation 2.10. Since it is only feasible to use (2.10) for diallelic loci, we will only use this method to find $\Pr(x|p)$, where x, the number of alleles of a particular type in a sample of n individuals, and p, the gene frequency t generations ago, are scalars (not vectors as they are for multi-allelic loci). Having computed $\mathbf{P^{(t)}}$, our t-step transition probability matrix (see Section 2.5.1), we can express the sum in (2.1) compactly in matrix notation. Define a $2N+1$-dimensional column vector $\mathbf{c^{(1)}}$ of binomial probabilities corresponding to $\Pr(x|q)$ (remember, x is a known quantity, here) for different values of q such that the i^{th}

component of $\mathbf{c^{(1)}}$ is $\Pr(x|q = \frac{i-1}{2N})$:

$$\mathbf{c^{(1)}} = \left(\Pr(x|\tfrac{0}{2N}), \Pr(x|\tfrac{1}{2N}), \Pr(x|\tfrac{2}{2N}), \ldots, \Pr(x|\tfrac{2N}{2N})\right)^T, \tag{2.24}$$

where the superscript T means matrix or vector transpose. Then, compute

$$\mathbf{c^{(t)}} = \mathbf{P^{(t)}}\mathbf{c^{(1)}}. \tag{2.25}$$

The column vector $\mathbf{c^{(t)}}$ then has $2N+1$ components, and its i^{th} component is $\Pr(x|p = \frac{i-1}{2N})$. Hence the maximum likelihood is just the value of the largest component of $\mathbf{c^{(t)}}$. Given our two samples, A and B, we may find $\mathbf{c^{(t)}}_A$ and $\mathbf{c^{(t)}}_B$, having i^{th} components c^t_{Ai} and c^t_{Bi} respectively (the superscript t denotes "at time t", not "to the t^{th} power."). We can also define a joint likelihood column vector $\mathbf{c^{(t)}}_J$ with components $c^t_{Ji} = c^t_{Ai}c^t_{Bi}$. The likelihood ratio of (2.4) for a single locus is then

$$\Lambda = 2\log\left(\frac{\max\limits_i c^t_{Ai} \;\times\; \max\limits_i c^t_{Bi}}{\max\limits_i c^t_{Ji}}\right). \tag{2.26}$$

When the sizes of Populations A and B are equal and small, this is a very fast method once $\mathbf{P^{(t)}}$ has been computed. When the populations are not of the same size, however, some sort of interpolation of likelihoods becomes necessary and the method loses much of its simplicity.

With the Normal approximation. Even if we use a continuous approximation to the transition probability like our Normal approximation, we can still obtain a discrete probability for our sample by integrating over the approximate density for $\mathbf{q}$. If we substitute the appropriate expressions into (2.1), drop the A subscripts (for notational hygiene), and recognize that the discrete sum in (2.1) becomes an integral over the continuous variable $\mathbf{q}$, we get

$$\Pr(\mathbf{x}|\mathbf{p}) = \int_0^1 \cdots \int_0^1 f\left((q_1, \ldots, q_{k-1}); \mathbf{p}, \frac{t}{2N}\mathbf{V}\right) \cdot \frac{2n!}{x_1! \cdots x_k!}\left(\prod_{i=1}^{k} q_i^{x_i}\right) dq_1 \cdots dq_{k-1} \tag{2.27}$$

where f is the joint density function for the multivariate normal distribution. The second half of the integrand is the multinomial probability of the sample, and $q_k = 1 - \sum_1^{k-1} q_i$. The above integral is analytically intractable, and with large k may be difficult to evaluate numerically.

With the Angular approximation. An expression similar to (2.27) may be derived from the joint distribution of the θ_i for the case of k alleles. For the special case of a diallelic locus the sample mass approximation is

$$\Pr(x|\phi) = \int_0^{\pi/2} f(\theta;\phi) \cdot \frac{2n!}{x!(2n-x)!}(\sin^2\theta)^x(\cos^2\theta)^{2n-x}d\theta. \tag{2.28}$$

Here, $\phi = \sin^{-1}\sqrt{p}$ is our parameter, f is the univariate normal density of θ given ϕ and the second half of the integrand is a binomial probability (see that $p = \sin^2\theta$ and $1 - p = \cos^2\theta$).

Computing $\Pr(\mathbf{x}|\mathbf{p})$ by a sample mass method with the approximation in the stereographically projected space is difficult and of limited utility, and we will not consider it here.

2.7.2 Sample Density Methods

Rather than compute a discrete probability for our sample, we could compute a density for it. This requires approximating the multinomial sampling of our genetic samples with a normal distribution. In this way, drawing our samples is like one more generation of drift. In other words, if $\mathbf{x} = (x_1, \ldots, x_k)$ are the allele counts at a locus in a sample of n diploid individuals from a population that had allele frequencies $\mathbf{p}$ at some "starting" time in the past, then the sample allele frequency of each allele, $x_i/2n$, $i = 1, \ldots, k$, has mean p_i with an error due to drift and an error due to sampling:

$$x_i/2n = p_i + \epsilon_{di} + \epsilon_{si}. \tag{2.29}$$

Here, ϵ_d is the deviation due to genetic drift, and ϵ_s is the deviation due to sampling.

Such a scheme works well with the stereographically projected space (Thompson 1973). If instead of dealing directly with the $x_i/2n$ we use their coordinates in the projected space [*i.e.*, substitute $x_i/2n$ for q_i in (2.21) to get $(x_i/2n)'$], then the log-density of these transformed sample allele frequencies is, analogously to (2.23),

$$-\frac{k}{2}\log(2\pi(t/8N+1/8n))-\frac{\sum_{i=1}^{k}[(x_i/2n)'-p_i']^2}{t/4N+1/4n}. \tag{2.30}$$

This works because the angular transformation makes the variance of each $(x_i/2n)'$ independent of its mean and the stereographic projection takes care of the correlations between alleles.

We could also use the Normal approximation (2.15) and Angular approximation (2.16) to yield a sample density method. However, doing so in the case of multiple alleles is much clumsier than using the stereographically projected space, because there are conditional variances to get straight with the Normal approximation (*i.e.*, the variance added in the sampling step depends on how far the allele frequencies have drifted from $\mathbf{p}$) and covariances to worry about with the Angular approximation.[10] Fortunately, for a diallelic locus, we may find the density for an angularly transformed sample frequency, $\sin^{-1}\sqrt{x/2n}$; it is approximately $\mathrm{N}(\phi, t/8N+1/8n)$. This should be slightly more accurate than using (2.30) as it does not include the distortion of the stereographic projection. Such a sample density method using the Angular

[10] Long (1991) uses an iteratively reweighted least squares method to estimate admixture proportions in human populations. He considers the sample allele frequency to depend on a drift error term and a sampling error term (*i.e.*, $x_i/2n = p_i + \epsilon_{di} + \epsilon_{si}$), but he does not state exactly how these errors are distributed. Given the close correspondence between iteratively reweighted least squares and the EM algorithm with a normal model, Long seems to be implicitly adopting a multivariate normal approximation to allele frequency drift. However, he assumes that the ϵ_d and ϵ_s are independent. (In practice, so long as the sample size is large, this is a fair assumption. See the footnote on Page 49.)

approximation gives the test statistic, after a good deal of simplification, of

$$\Lambda = \frac{\left(\sin^{-1}\sqrt{x_A/2n_A} - \sin^{-1}\sqrt{x_B/2n_B}\right)^2}{\sigma_1^2 + \sigma_2^2} \tag{2.31}$$

where $\sigma_i^2 = t/8N_i + 1/8n_i$.

2.8 Sampling with Recessive or "Null" Alleles

Up to this point we have assumed that all alleles are codominant; that they are detectable in both heterozygous or homozygous form. When this is the case, then a sample of n diploid, Wright-Fisher individuals is equivalent to a sample of $2n$ gene copies from a population. Sometimes, however, recessive alleles at a locus are not observable in all phenotypes and we are forced to estimate the frequency of such an allele by the proportion of certain classes of phenotypes that we can observe in the sample. Such a situation occurs, for example, with the *LDH–A1** locus in some Lake Washington sockeye populations. At that locus, heterozygotes of the common (**100*) allele and the **500* allele [called **NULL* in Hendry (1995)] are indistinguishable from **100* homozygotes. Nonetheless **500* homozygotes are detectable and the sample proportion of such homozygotes contains information about the frequency of the allele in the population.

For loci with recessive alleles we must adjust the probability function for our samples. In general, the recessive nature of an allele does not affect the rate at which it drifts in a Wright-Fisher population. Therefore, only the sampling step is affected by lack of codominance, and so we need only redefine the probability of our samples given q, the present-day population allele frequency of the recessive allele. The following treats only diallelic loci with one dominant and one recessive allele. This treatment will suffice for the data of Hendry *et al.* (1996).

First, rather than observing allele counts x, we now observe only the number y of recessive homozygotes in our sample. Assuming Hardy-Weinberg equilibrium, the

probability of drawing a recessive homozygote is q^2. Thus the probability of drawing y such recessive homozygotes in our sample of n individuals is:

$$\Pr(y|q) = \frac{n!}{y!(n-y)!}\left(q^2\right)^y\left(1-q^2\right)^{n-y}. \tag{2.32}$$

We can immediately use this in our sample mass methods. If using the exact transition probabilities from (2.10) then we may compute the likelihood ratio exactly as in (2.26) except that we must now define $\mathbf{c^{(1)}}$ in (2.25) to be a 2N+1 dimensional column vector with the i^{th} component given by $\Pr(y|q)$—a binomial probability from the distribution $\text{Bin}(n, (\frac{i-1}{2N})^2)$.

Adjusting the sample mass method under the Angular or Normal approximations is similar. All that changes is the term in the integrand corresponding to the sampling step. The Angular approximation sample mass method gives:

$$\Pr(y|\phi) = \int_0^{\pi/2} f(\theta;\phi)\cdot\left(\frac{n!}{y!(n-y)!}\right)(\sin^4\theta)^y(1-\sin^4\theta)^{n-y}d\theta. \tag{2.33}$$

(Compare to Equation 2.28 on Page 44.)

2.8.1 A Sample Density Method for Null Alleles

Thompson (1973) notes the problem that recessive alleles pose to the Brownian motion approximation under stereographic projection. She writes,

> In fact this [likelihood model] applies only in the case where there are sufficient antisera for all genotypes to be identifiable and the sample gene frequencies known, but it remains a good approximation in all cases provided the gene frequencies used are the maximum likelihood estimates from phenotype data. (p. 72)

Indeed, the approximation is quite good for those cases where the frequency of the recessive allele is not very low. However, when the recesssive allele is at a low frequency, the approximation deteriorates mildly. I derive a more accurate Normal

approximation to the sample density for a diallelic locus with one recessive allele, by noting that the asymptotic distribution of the maximum likelihood estimator (mle), $\hat{q}$, of q given y recessive homozygotes gives the asymptotic distribution of the sample statistic $\sqrt{y/n}$ given q. We then can argue that the marginal distribution of $\sqrt{y/n}$ (*i.e.*, its distribution given p) will be approximately normal with a variance that can be computed from its conditional variance and its conditional expectation given q.

Suppose that q is the frequency of allele A in a population at time t, and let each individual in our sample represent an independent random variable W_i, $i = 1, \ldots, n$. $W = 1$ if the individual is a recessive homozygote, and $W = 0$ otherwise. Assuming Hardy-Weinberg equilibrium, the probability of drawing a homozygous, AA individual is q^2. Hence the probability of $\mathbf{W} = (W_1, \ldots, W_n)$ given q is

$$f_{\mathbf{W}}(\mathbf{w}; q) = \left(q^2\right)^{\sum W_i} \left(1 - q^2\right)^{n - \sum W_i} \tag{2.34}$$

Writing $\sum W_i$ as y gives the log of the probability of the n-sample as

$$\log f_{\mathbf{W}}(\mathbf{w}; q) = 2y \log q + (n - y) \log(1 - q^2). \tag{2.35}$$

It is clear that the maximum likelihood estimate $\widehat{q^2} = y/n$. Hence, by the invariance of maximum likelihood estimators to transformation, $\hat{q} = \sqrt{\widehat{q^2}} = \sqrt{y/n}$, and asymptotically, $\hat{q}$ will be normally distributed with mean q and variance $1/I_n(q)$ where $I_n(q)$ is the Fisher Information for q in an n-sample and may be obtained from

$$I_n(q) = -E\left(\frac{\partial^2}{\partial q^2} \log f_{\mathbf{W}}(\mathbf{w}; q)\right) = \frac{4n}{1 - q^2}. \tag{2.36}$$

Immediately we have that $\sqrt{y/n}$ is distributed $\mathrm{N}\left(q, (1 - q^2)/4n\right)$, asymptotically.[11] And so, letting $v = \sqrt{y/n}$ for notational ease, $E(v|q) = q$ and $\mathrm{Var}(v|q) = (1 - q^2)/4n$.

[11] In fact, I realize now that this is more easily derived by the Delta-method: $y/n \xrightarrow{D} \mathrm{N}(q^2, q^2(1 - q^2)/n)$ implies by the Delta-method that $\sqrt{y/n} \xrightarrow{D} \mathrm{N}(q, (q^2(1-q^2)/n) \times (1/4q^2))$ giving $\sqrt{y/n} \xrightarrow{D} \mathrm{N}(q, (1 - q^2)/4n)$.

That gives us $v|q$ but we'd like the marginal distribution of v which we can write "$v|p$" to emphasize that this is the distribution of v given p. We have already seen that given p, $q \sim \mathrm{N}(p, tp(1-p)/2N)$, approximately. So, as before, if we let $\epsilon_d = q - p$ and $\epsilon_s = (v|q) - q$, then both ϵ_s and ϵ_d are normally distributed. It follows that $v = p + \epsilon_d + \epsilon_s$, being the sum of normals, is also normally distributed. We can find the variance of v from the conditional variance, $\mathrm{Var}(v|q)$, and the conditional expectation, $E(v|q)$. By the customary relation,

$$\mathrm{Var}(v|p) = E\Big(\mathrm{Var}(v|q)\Big) + \mathrm{Var}\Big(E(v|q)\Big), \tag{2.37}$$

we find that,

$$\mathrm{Var}(v|p) = \left(1 - \frac{1}{4n}\right)\frac{tp(1-p)}{2N} + \frac{1-p^2}{4n}. \tag{2.38}$$

And if our sample size is large,[12] then $1 - 1/4n \approx 1$ and we have that

$$v = \sqrt{y/n} \sim \mathrm{N}\left(p, \frac{tp(1-p)}{2N} + \frac{1-p^2}{4n}\right), \tag{2.39}$$

which gives us our sample density for a diallelic locus with one recessive allele. Note that there are no transformations that do not involve N and n which will stabilize the variance in this distribution. Therefore it is not possible to continue from here to the angular transformation and stereographic projection. However, with computers and numerical maximization routines this is a manageable approximation for testing H_A and H_C.

2.9 The Distribution of the Test Statistic

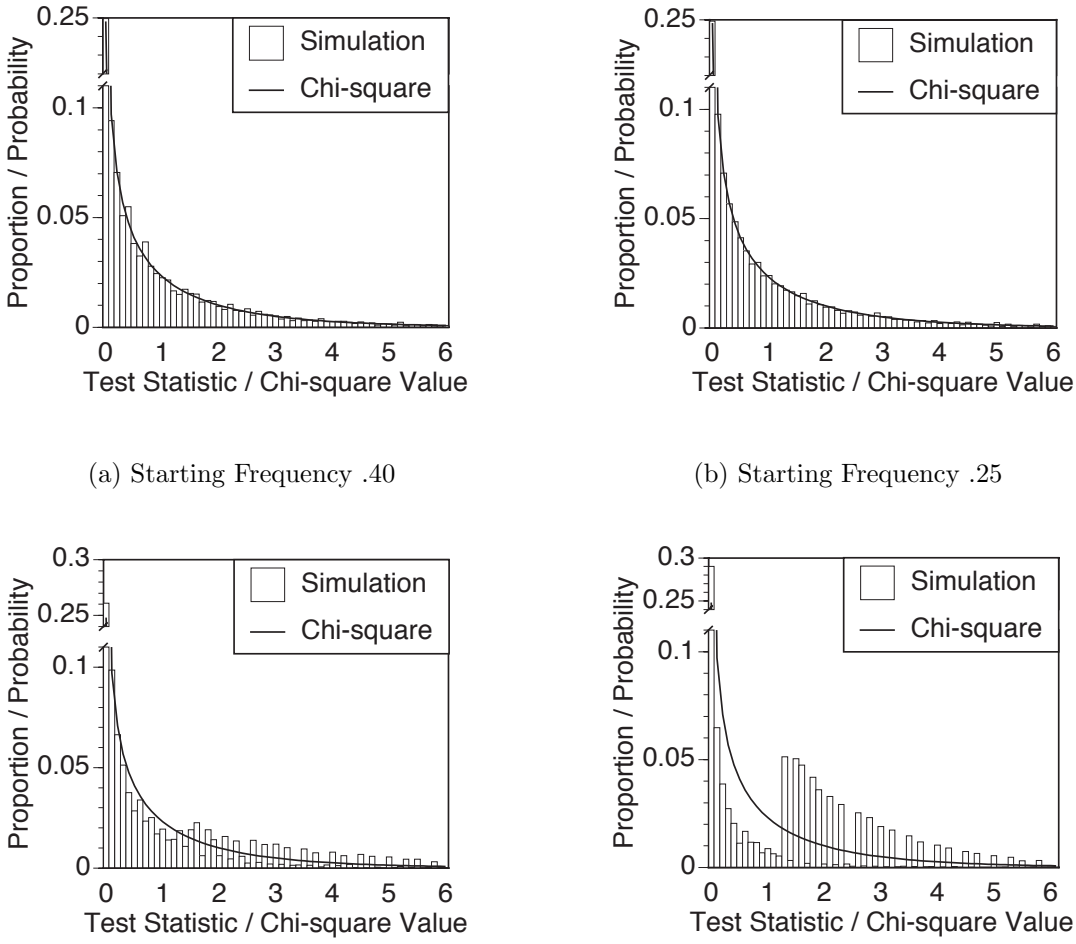
As noted much earlier in this chapter (Page 18), the distribution of the test statistic Λ is asymptotically that of a chi-square random variable. Of course, our populations and samples are not infinite, so I have performed a series of simulations to determine

[12] This is essentially the argument that validates the assumption by Long (1991) that ϵ_d and ϵ_s are independent (see my footnote on Page 45).

how well the chi-square distribution fits the empirical distribution of Λ. I simulated drift in two Wright-Fisher populations, A and B, each of size $N_A = N_B = 100$, and each starting with the same allele frequency in a diallelic locus (.40, .25, .10, or .05, depending on the simulation). After simulating drift in each population for fourteen generations, I drew samples of size $n_A = n_B = 50$ from each population and then computed the test-statistic Λ by both Equation 2.26 (Figure 2.11) and by Equation 2.31 (Figure 2.12). An effective size of 100 is relatively small as are samples of size 50. I chose these values to explore scenarios in which the asymptotic distribution of the test statistic might not be a good approximation. The approximation will improve if N and n are larger. I performed 50,000 replicates for each of the four starting frequencies.

Under this simulation scheme, both populations start with the same allele frequency, and so the null hypothesis for our test statistic ($p_A = p_B$) holds. Thus the distribution over the 50,000 replicates of the observed Λ's should be approximately chi-square with one degree of freedom. Since the asymptotic distribution of likelihood ratios is closely related to the asymptotic distribution of maximum likelihood estimates (Kendall and Stuart 1979, p. 247), we expect that the chi-square approximation will fail in those same instances where the Normal Approximation to transition probabilities fails. In fact, this is what we observe. At low starting frequencies, (.05 and .10) where we expect the probability of allele fixation to be high and the distribution of allele frequencies after genetic drift to be non-normal, the empirical distribution of Λ strays considerably from that of a χ^2_1 random variable. However, for starting frequencies of .25 and .40 (from which the probability of allele fixation in 14 generations for a population of $N = 100$ is negligible—see Figure 2.8) the observed distribution of Λ computed both by the exact method and by the Angular approximation sample density method is extremely close to that of a χ^2_1 random variable (Figures 2.11 and 2.12).

And, in fact, Figures 2.11(d) and 2.12(d) are somewhat unfair to our test statistic.



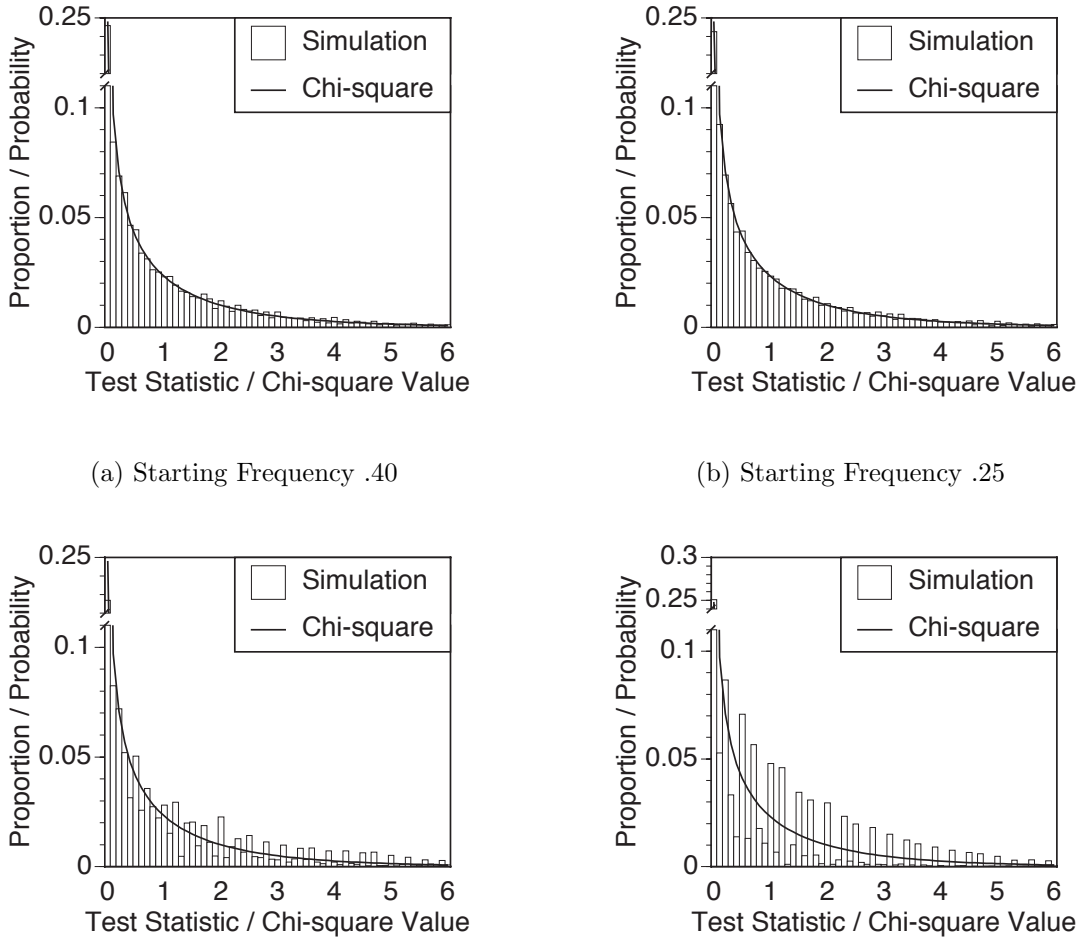
(a) Starting Frequency .40

(b) Starting Frequency .25

(c) Starting Frequency .10

(d) Starting Frequency .05

Figure 2.11: Simulated test statistic (Λ) values compared to the chi-square distribution with one degree of freedom. Λ was computed by the exact method (2.26). Columns are a histogram of simulated Λ's over 50,000 replicates. Figures a–d are results for different starting frequencies. (See text for further explanation of simulation methods.) Note that the test statistic is very closely chi-square distributed except when the starting frequency is such that the probability of allele fixation is high (see Figure 2.8).



(a) Starting Frequency .40

(b) Starting Frequency .25

(c) Starting Frequency .10

(d) Starting Frequency .05

Figure 2.12: Simulated test statistic (Λ) values compared to the chi-square distribution with one degree of freedom. Λ was computed by the sample density method of (2.31). Columns are a histogram of simulated Λ's over 50,000 replicates. Figures a–d are results for different starting frequencies. (See text for further explanation of simulation methods.) Note that even with the sample density method the test statistic is very closely chi-square distributed except when the starting frequency is such that the probability of allele fixation is high (see Figure 2.8).

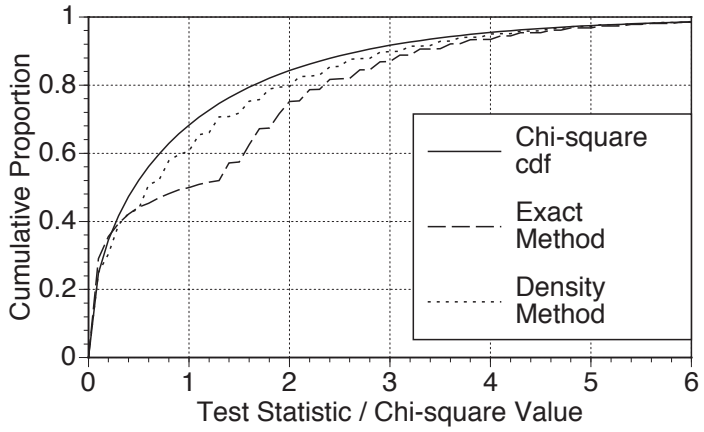


Figure 2.13: Cumulative proportion of test statistics (computed either by the exact method of Equation 2.26 or by the Angular approximation sample density method of Equation 2.31) versus the cumulative distribution function for a χ^2_1 random variable. 50,000 replicates with a starting frequency of .05.

Due to the discrete nature of the drift process, there are only certain values that the test statistic may take. Accordingly there are big spikes and empty columns in the graph. Comparing the observed cumulative proportion of Λ to the cumulative distribution function of a χ^2_1 gives a better sense of the how well the approximation fits (Figure 2.13). This clearly shows that the distribution of the test statistic is skewed toward larger values (to the right) relative to the chi-square distribution. Perhaps even more remarkable, though, is the fact that the distribution of Λ computed by the sample density method using the Angular transformation, is much closer to the chi-square distribution than the distribution of Λ computed exactly. This certainly argues for the routine use of the sample density method and Angular or stereographic projection approximation for loci with codominant alleles.

A final observation is that the horizontal distance (δ—see Page 39) between the peak of the Angular and Normal approximations and the peak of the exact transition probability does not seem to affect the distribution of Λ when the starting frequency

is either .4 or .25, even though for $N = 100$, $t = 14$ and a starting frequency of .25, δ is as large as .03 (see Figure 2.10). Thus it appears that we need worry little about δ in most cases; so long as the probability of allele fixation is not high, it seems to have little effect on the distribution of Λ.

2.10 Review of Assumptions

The proposed statistical technique makes a number of assumptions. It is important to clarify these assumptions in the context of the biology of the situation and to address how robust the method is to violations of them.

To start with, we have made the "standard" genetics assumptions: the markers we use 1) are not subject to mutation, 2) are not subject to selective pressure, and 3) are independently segregating. The first is a reasonable assumption for allozyme markers on the time scales we are interested in, as the mutation rate of allozyme markers is low (Nei 1987). The second assumption is also reasonable; the allozyme loci typically used in salmon population genetic studies appear to be selectively neutral (Utter *et al.* 1987). Finally, since sockeye salmon possess a large number (56 to 58) of chromosomes (Allendorf and Thorgaard 1984) we are unlikely to violate the assumption of independent segregation between loci.

Next, we have made several assumptions regarding the populations involved. First, each of Populations A and B is assumed to be panmictic (well-mixed during reproduction) among their N_A and N_B members (*i.e.*, there is not population subdivision). In practice this assumption is closely linked to how one converts historical spawner counts into an effective population size. We also assume that A and B are reproductively isolated from one another. That is, since the time of possible introduction of individuals of A into the locale of Population B, there has been no migration between the two populations (or other populations). In the case of Bear Creek, it is unlikely that fish from either Baker Lake or Cultus Lake have strayed

to the Bear Creek system in recent years because sockeye home to their natal lake system with great fidelity (Wood *et al.* 1994). It may not be so unlikely that descendants of Baker Lake fish in the Cedar River or Issaquah Creek have strayed into Bear Creek, as straying between tributaries within natal lake systems seems to occur at a higher rate than straying between lake systems (McCart 1970; Wood *et al.* 1994). However, any such straying, if it resulted in successful gene flow, would only make the hypothesis test more conservative; migration would only reduce the allele frequency differences between populations, thus decreasing the test statistic and reducing the probability of rejecting the null hypothesis.

We also assume that our samples are representative of the population. This assumption is related to the panmixia assumption above.

Finally, the heaviest assumptions that we require are those regarding the effective sizes of Populations A and B as inputs to the likelihood model. We assume that historical population size data are available and that such data may be reliably converted into effective sizes. Furthermore, once in the model, we treat those effective sizes as known without error, when, in fact, they are estimates themselves which carry some uncertainty. In practice, one would hope to be able to use a reliable lower bound on N in the model (since the test is more conservative for smaller N). For Baker and Cultus Lakes, where comprehensive population data are available, this is possible. Unfortunately, for the possibly introduced population (B in the model; Bear Creek in the particular example) the very nature of the problem is such that it is unlikely that there will be good population size data for the period soon after introduction of individuals from A. This is certainly the case for Bear Creek, where reliable population estimates exist only after 1981. Nonetheless it is possible to make reasonable guesses at the historical effective size in Bear Creek given some scenarios of its population history. I discuss this in the next chapter.

Chapter 3

THE STATISTICAL METHOD IN PRACTICE

The previous chapter described several ways to compute the likelihood ratio statistic for the hypotheses that the sockeye in Bear Creek descended exclusively from fish planted from Baker Lake (H_A), or exclusively from Cultus Lake (H_C). In this chapter, we test H_A and H_C using the previously-collected data of Hendry *et al.* (1996). The first section introduces the genetic data and tells how we combine them into a form that we can readily use. Next we must use computer simulations to determine the historical effective size of the salmon populations in Baker Lake, Cultus Lake, and Bear Creek from records of spawner return number. Finally we conduct the tests, and discuss the results.

3.1 Data for Baker and Cultus Lakes and Bear Creek

In 1992 and 1993, Andrew Hendry collected tissues from anadromous sockeye in seven populations and performed gel electrophoresis on those samples. His data, at the four loci which contained an alternate allele at a sample frequency $q \geq .05$ in at least one of the populations, for Bear and Cottage creeks and Baker and Cultus lakes appear in Table 3.1. The sample frequencies for all four loci in the populations sampled did not differ significantly between the two years (Hendry 1995, p. 26). So, for the present analyses, we will consider the two samples from two years to be one large sample from a single year as shown in the "pooled data" of Table 3.1. I have also pooled the data from Bear and Cottage creeks together, treating the fish that spawn in those creeks as part of a single, panmictic "Bear Creek System" population. This is reasonable

Table 3.1: Sample allele frequencies from Hendry *et al.* (1996) at four loci in Baker and Cultus Lakes, and Bear and Cottage Creeks. n is the sample size in number of diploid individuals for each locus. The frequency of the alternate (**100*) allele at each locus is not listed, but is 1 minus the sum of the frequencies of the other alleles at the locus. *POOLED DATA* are the allele frequency estimates after pooling the data between years and combining Bear and Cottage Creeks into a single "Bear Creek System" population. *COUNT DATA* are the actual counts, x, of codominant alleles out of $2n$ gene copies, or the counts, y, of recessive homozygote phenotypes out of n individuals in the pooled samples.

		*ALAT**			*PGM–1**		*PGM–2**		*LDH–A1**	
Popln.	*Year*	n	**91*	**95*	n	**NULL*	n	**136*	n	**500*
Baker	1992	40	0.563	0.075	39	0.320	56	0.170	56	0.000
	1993	43	0.512	0.047	40	0.387	64	0.117	64	0.000
Cultus	1992	40	0.050	0.000	40	0.962	40	0.175	64	0.000
Bear	1992	40	0.225	0.150	40	0.671	63	0.127	40	0.224
	1993	43	0.279	0.163	40	0.592	52	0.154	12	0.408
Cottage	1992	40	0.375	0.113	40	0.632	52	0.144	40	0.158
	1993	40	0.213	0.088	40	0.689	40	0.138	38	0.281
↓ *POOLED DATA* ↓										
Baker	92,93	83	0.537	0.060	79	0.356	120	0.142	120	0.000
Cultus	1992	40	0.050	0.000	40	0.962	40	0.175	64	0.000
Bear-Cot	92,93	163	0.273	0.129	160	0.647	207	0.140	130	0.248
↓ *COUNT DATA* ↓										
Popln.	*Year*	$2n$	**91*	**95*	n	**NULL*	$2n$	**136*	n	**500*
Baker	92,93	166	88	10	79	20	240	34	120	0
Cultus	1992	80	4	0	40	37	80	14	64	0
Bear-Cot	92,93	326	89	42	160	67	414	58	130	8

as Bear and Cottage creeks are adjacent tributaries, and the gene frequencies in the two populations are not significantly different.

Underlying the sample estimates of allele frequency are the counts, x, of codominant alleles for *PGM–2** and *ALAT** or counts y of recessive homozygotes for the loci with recessive alleles (*PGM–1** and *LDH–A1**). These counts appear in the table as well.

3.2 Determining Effective Sizes of the Populations

We defined the statistical method of Chapter 2 in reference to a Wright-Fisher population of size N. To apply the method to a real population we must determine its variance effective size, N_e, from historical census data, and use that in place of N in all the equations. Sockeye salmon populations depart from the Wright-Fisher model at two different levels. At the first level are those differences that occur in a single year of reproduction: salmon on spawning grounds are not random-mating, random-surviving individuals. For example, larger females generally produce more eggs, and, presumably, then produce more offspring (Burgner 1991). Additionally, some pairs of parents will have more offspring than others which return to reproduce in future years, due to factors other than random chance (Waples 1990). Consequently the effective number of spawners, N_s, in a particular year is less than the number of individuals N_i counted on the spawning grounds. Researchers using the temporal method and the disequilibrium method estimate that N_s is between $0.2N_i$ and $0.7N_i$ for six chinook salmon, *O. tshawytschwa*, populations in the Snake River basin (Waples *et al.* 1993). The lower end of those estimates (*i.e.*, $N_s/N_i \approx 0.2$ to 0.33) are probably more reliable (Robin Waples, National Marine Fisheries Service, Northwest Fisheries Science Center, pers. comm.). Equivalent numbers for sockeye salmon are, not available, but we assume that they are close to those for chinook.

At the second level of departure from the Wright-Fisher model, sockeye salmon

populations have overlapping year classes, so that the fish on the spawning grounds in any one year represent only a part of the whole population, and the run sizes differ each year (Figure 3.1). There is currently no analytical framework to find the variance effective size of a population with both fluctuating year-class size and overlapping year classes or generations (Joe Felsenstein, University of Washington, Department of Genetics, pers. comm.). However, given data on salmon run-sizes and age composition of a salmon population over some time period, one can simulate the process of drift, estimate the increase in allele frequency variance from the results and use that to estimate the effective size of the population. Such simulations are similar to those in Waples (1990) except that the present ones incorporate fluctuating population size.

Given the situation in Figure 3.1, it would be easy to simulate the progression of the population through time, if at every year along the way we knew how many of the spawners were three, four, or five years old. Though such information is seldom available, we can usually make an educated guess about the proportion of individuals in each year class that will reproduce at age three, four, or five. These proportions are the quantities α_3, α_4, and α_5 in Figure 3.1. The α's and several assumptions

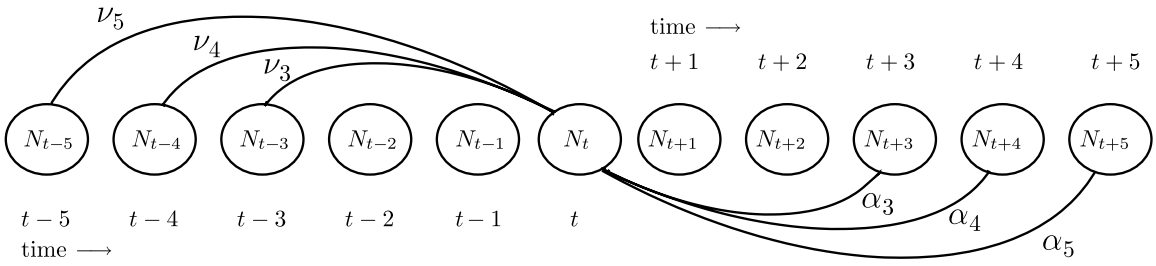


Figure 3.1: A population with overlapping year classes and fluctuating population size. At time t there are N_t effective spawners made up of ν_5 individuals from time $t-5$, ν_4 from $t-4$, and ν_3 from $t-3$. The offspring of the spawners at time t mature at three different ages; a proportion α_3 mature at three years, α_4 at four years, and α_5 at five.

about survival rates of fish from different year classes allow some simulation results.

3.2.1 Baker Lake Simulations

For Baker Lake, I have performed simulations based on a survival scheme in which, if there are N_t effective spawners at year t, then ν_i, the number of effective spawners that were born i years before, is given by $N_t \frac{\alpha_i N_{t-i}}{\bar{\nu}}$ where $\bar{\nu} = \sum_{i=3}^{5} \alpha_i N_{t-i}$. This is equivalent to assuming that each fish which is "destined" to either die before reproducing or to reproduce in year t, experiences the same probability of survival to reproduction, and that probability is just what is needed for N_t effective spawners to return at year t. This scheme tends to minimize the effects of the occasional very small run size, because, though reproduction of the fish in that year will result in a high degree of drift, their offspring will not contribute greatly to future generations because there are very few of them (*i.e.*, N_{t-i} in the $N_{t-i}\alpha_i$ term will be small). These simulations also assume that three-, four- and five-year-olds have the same reproductive potential. This is perhaps incorrect: older females generally have higher fecundity (Burgner 1991), and larger (older) males typically assume more dominant positions in mating hierarchies (Hanson and Smith 1967), (though note that Foote *et al.* (1997) report that the spawning success (based on electrophoretic analysis of offspring) of three-year old jacks was not significantly different than that of large males). Nonetheless, to the accuracy of the other assumptions in these simulations, age-specific reproductive success differences will have little effect, especially when α_3 and α_5 are small compared to α_4. Without other information regarding survival rates, this is a reasonable scheme as it accounts for the fact that, on average, the number of offspring produced decreases as the number of parents does.

The Washington Department of Fish and Wildlife has an excellent record of sockeye run sizes in Baker Lake from the late 1800's to the present. The run sizes from 1932 to 1993 are of interest to us (Figure 3.2) as these are the population sizes which affect the amount of allele frequency drift in the population starting from 1937 (the

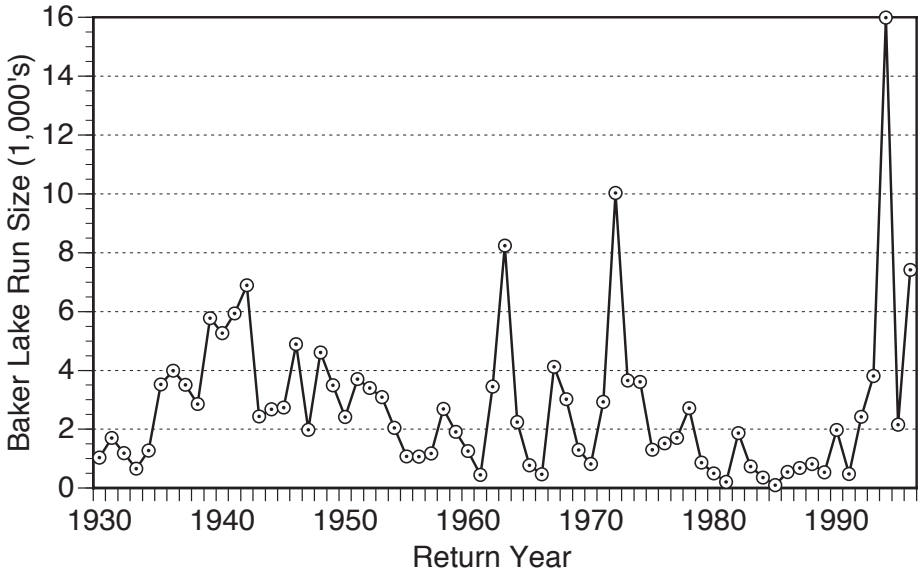


Figure 3.2: Sockeye salmon run sizes in Baker Lake, 1930–1996. (Source: Washington Department of Fish and Wildlife)

year fish were transferred to Bear Creek). The run size estimates are generally quite accurate as most were obtained in the process of transporting fish around a dam. From 1896 until 1947, some of the fish returning to Baker Lake were propagated at a hatchery for local release (Shaklee *et al.* 1996)

Hendry (1995) reports the ages of Baker Lake spawners from samples taken in 1992 and 1993. In 1992, out of 40 adults, only one (3%) was five years old and 39 (97%) were four year-olds. In 1993, however, of 43 adults, 13 (31%) were five year-olds and 30 (69%) were four year-olds. With only these two data points, and no knowledge of survival rates, it is difficult to estimate the proportion of salmon that typically mature at age three, four, or five (the α_i's) in Baker Lake. However, it seems reasonable to imagine that, typically a very small proportion (close to zero) of fish mature at three years, a proportion between .70 and .95 at four years, and between .3 and .05 at five years.

I simulated genetic drift in a model Baker Lake population with a number of different maturity schedules from "greatly overlapping" ($\alpha_3 = \frac{1}{3}, \alpha_4 = \frac{1}{3}, \alpha_5 = \frac{1}{3}$), to "barely overlapping" ($\alpha_3 = 0, \alpha_4 = .95, \alpha_5 = .05$). Each such maturity schedule gives its own "average generation length"—between 3.8 years and 4.3 years for the maturity schedules I simulated from. For each maturity schedule I initialized the allele frequency in the spawners of years 1932 to 1936 to the same value ($.1, .2, \ldots, .9$, over different simulations), then, starting at year 1937 I drew $2N_s$ new gene copies by the scheme described above, to produce the spawners in that year. This was repeated for 1938, '39, '40, and so on until obtaining an allele frequency at year 1993. This whole process was repeated 20,000 times for each starting frequency, and the variance of simulated allele frequencies in 1993 computed.[1] This variance then translates (by solving for N in Equation 2.7) into an effective size defined to be the size of a Wright-Fisher population with a generation length equal to the average generation length for the maturity schedule being simulated that would give the same increase in variance of gene frequency in 56 years.

I used two different values for N_s/N_i, the number of effective spawners per fish counted in the run-size data. In one simulation I took N_s to be 0.16 of the number of spawners counted by WDF&W, and in a second simulation I chose $N_s/N_i = 0.32$. I chose the low value of 0.16 because some of the fish counted at the dam trap on Baker Lake fail to reach the spawning grounds. The simulations using .16 should be fairly conservative (*i.e.*, they ought not result in an overestimate of the effective population size) and the simulations that use 0.32 should provide a good "middle-ground" estimate of the effective size.

The results of the simulations are shown for $N_s/N_i = 0.16$ [Figure 3.3(a)], and

[1] In retrospect, I could have taken the simulated gene frequencies from both 1992 and 1993 (the years that Hendry *et al.* collected samples from Baker Lake) and used both to estimate the variance of the drift component of error in the samples. Note that using 1993 is conservative; it will generally result in a low N_e because of the low returns in 1989, 1985, and 1981.

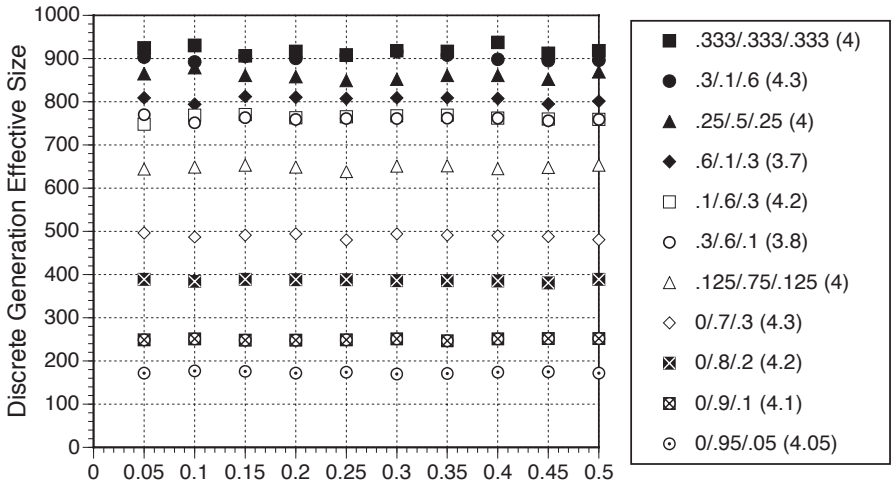
$N_s/N_i = .32$ [Figure 3.3(b)]. As one would expect, those maturity schedules with more overlap yield higher effective sizes (the separate year classes are closer to being one, larger, well-mixed population). A maturity schedule between 0/.8/.2 and 0/.9/.1 is probably close to that of Baker Lake sockeye. Thus our "low" estimate of Baker Lake's historical effective size over the time period of interest is about 250 individuals drifting for 14 generations. A non-conservative estimate is about 600 individuals.

(An additional item we learn from these simulations is that the result is not sensitive to the initial allele frequency. This is to be expected since the $p(1-p)$ term is included in the expression for variance (2.7). For future simulations, though, we may simulate from only a few initial allele frequencies, knowing that our results are still general.)

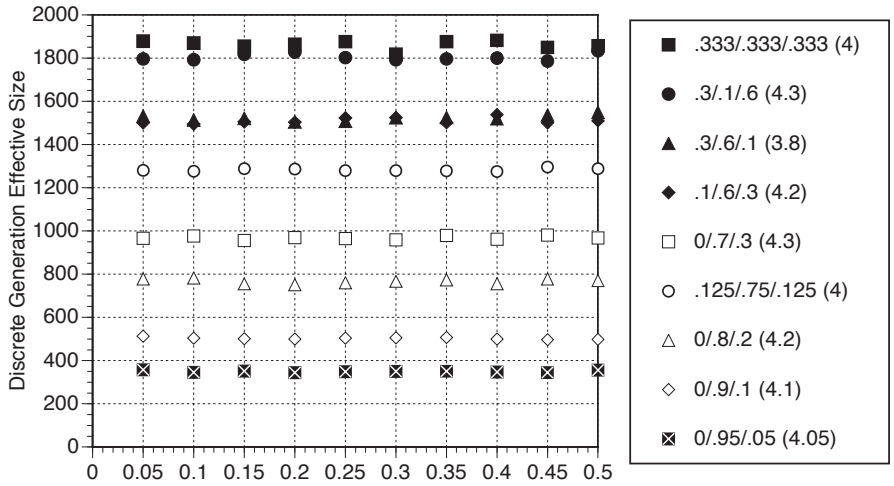
3.2.2 Simulations for Cultus Lake

The historical census records for Cultus Lake are also very good. The escapement estimates for the years 1942–1993 are shown in Figure 3.4. From the return years 1953 to the present, estimates of the age composition of the returning fish are also available. Of all returning fish over that time period, 2% were estimated to be three year-olds, 95% four year-olds, and 3% five year-olds.

Since fish planted into North Creek in 1944 would have been the offspring of fish returning to Cultus Lake in 1943, ideally we could find age and escapement data for the years 1938 to the present. However such data are not available, so I filled in the holes as follows: 1) from 1938 to 1941 I took the escapement to be the harmonic mean of the escapement for the years 1943 to 1953, with age composition being .02/.95/.03; 2) for 1942 to 1945 the escapement data are available but I had to assume an age composition of .02/.95/.03 in each return year; 3) though age data are not available for the years 1946–1952, I used the scheme described for Baker Lake to determine the number of three-, four- and five year-olds in each of those return years (again, assuming an age composition of .02/.95/.03. For the years 1952 to 1992, escapement



(a) Simulation Results for $N_s/N_i = .16$



(b) Simulation Results for $N_s/N_i = .32$

Figure 3.3: Simulation results for the effective size of the Baker Lake sockeye salmon population. (a) $N_s/N_i = .16$, (b) $N_s/N_i = .32$. Maturity schedule is given in the legend as $\alpha_3/\alpha_4/\alpha_5$ with the average generation length in parentheses. The age structure of Baker Lake's population is probably somewhere between the maturity schedules 0/.8/.2 and 0/.95/.5. Therefore, the bottom three rows of symbols in each figure are of most interest, here. The other schedules are shown for general interest.

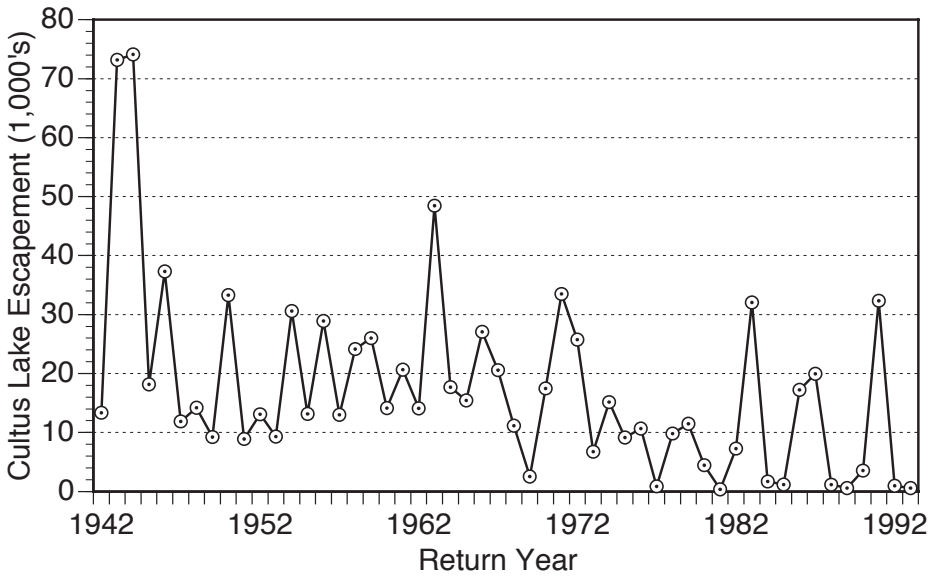


Figure 3.4: Cultus Lake escapement 1942–1993. Data from Michael LaPointe, Pacific Salmon Commission

and age composition data are available. The resulting data are shown in Table A.1 in the Appendix.

I assumed that fish of all ages have equal reproductive success and then simulated allele frequency drift using the values in Table A.1 for the number of fish of each age class amongst the spawners (the ν_i of Figure 3.1). Performing 10,000 replicates for various initial allele frequencies revealed that for $N_s = 0.16N_i$ the Cultus Lake population is much like a Wright-Fisher population of size 800 that has been drifting for 12 generations; for $N_s = 0.32N_i$, its effective size is close to 1640. These are quite large effective sizes which will improve the power of our test of H_C.

One source of concern remains. Because most of the Cultus Lake sockeye mature at four years and there is very little overlap between year classes, we would like to ensure that our present-day samples from Cultus Lake are from fish that returned some multiple of four years after 1943 (the return year whose offspring were planted into North Creek). Somewhat fortuitously, in fact, Hendry *et al.* (1996) collected

juveniles in 1992 that were offspring of the return year 1991, which is on the proper four year cycle (1943, 1947,. . . , 1987, 1991).

3.2.3 Simulations for Bear Creek

It is much more difficult to determine the effective size of the Bear Creek population since 1937 because there is little census data. WDF&W has records for Bear Creek only since 1982, so it may be impossible to get a good estimate of Bear Creek's historical effective size. I have, however, considered a biologically plausible scenario under which Bear Creek's effective size would be about 100. Ultimately one has to decide if that seems reasonable or not given only scarce historical data. Nonetheless, I shall carry out the tests for a Bear Creek N_e of 100 as this is as small as possible without risking breakdown of the asymptotic approximations upon which many of the expressions for Λ in Chapter 2 are based.

For Bear Creek, I performed simulations following the scheme used for Baker Lake, assuming $N_s = .25N_i$. I used a single maturity schedule ($\alpha_3 = .125$, $\alpha_4 = .75$, and $\alpha_5 = .125$) which is close to the average age composition of Bear Creek sockeye that Hendry and Quinn (1997) report from two years of data. To obtain run sizes for 1932–1981, I imagined that the Bear Creek population was of size 25 individuals in 1932 and then grew by the equation

$$N_{\text{year}} = 25 + 17,161 \left(\frac{\text{year} - 1932}{1982 - 1932} \right)^6 . \tag{3.1}$$

Such a 6^{th} degree polynomial relationship gives a population growth curve that starts slowly, and then "rushes up" to meet the population size of 17,186 in 1982 (the open triangles in Figure 3.5). This is a growth pattern that coincides temporally with the increase of sockeye in Lake Washington in the late 1960's (Edmondson 1991). In other words, it assumes that the Bear Creek population was very small until only recently. Simulating over 10,000 replicates with the values from (3.1) yields an historical effective size of 116.

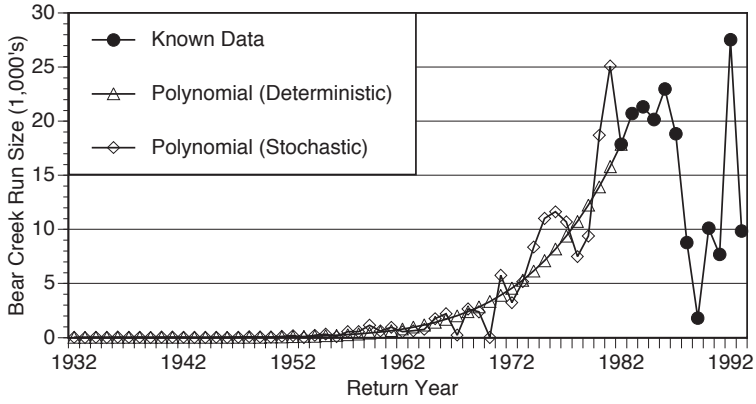


Figure 3.5: Yearly run sizes for Bear Creek for the purposes of genetic drift simulations. Filled circles are WDF&W estimates. Polynomial (Deterministic) are values obtained from Equation 3.1 and Polynomial (Stochastic) are one set of realized values from Equation 3.2. Notice how this assumes that the Bear Creek population was very small for many years, and the rapid growth of the population starting in the 1960's, mirrors the remarkable growth of other Lake Washington populations at the same time (Kolb 1971; Edmondson 1991).

Salmon run sizes, however, seldom follow a smooth curve through time like the one determined by (3.1). Accordingly, I repeated the simulations several times for run sizes determined each year by

$$N_{\text{stoch}} = \begin{cases} y & \text{if } y > 0 \\ 12 & \text{if } y \leq 0 \end{cases} \tag{3.2}$$

where y is the realized value of a normal random variable with mean equal to N_{year} as determined by (3.1) and variance equal to $(N_{\text{year}}/2.5)^2$ (*i.e.*, coefficient of variation equal to 40%). The open diamonds in Figure 3.5 show one realization of run sizes. Over several simulations using run sizes generated by the random model of (3.2) the effective size was around 105. This says that even if there were some early years when very few (say 10 or 12) fish returned to Bear Creek, the effective size could still be close to 100 because the population has grown to be quite sizeable today.

Additional complexity Unfortunately there is a further complication. The historical effective size just given for Bear Creek assumed that the number of spawners in each of the the years from 1932 to the present was a realization from Equation 3.2. This might be reasonable if the Bear Creek population were already established before the introductions from Baker Lake. However if H_A is true, then the Bear Creek population would have started with only a single year class. In effect, under H_A, the adults "returning" to Bear Creek in 1936 were Baker Lake adults spawned at the Birdsview Hatchery whose fry were released into Lake Washington. Since almost 2.5 million fry raised from these adults were released to various Lake Washington locales (see Table 1.1) there was clearly a great number of adults contributing to fry released to Bear Creek—likely at least 800 females, assuming the average female had 2,900 eggs, as suggested by Royal and Seymour (1940). Considering that the number of males spawned may not have been as great, we might make a conservative estimate that there were 100 "effective" Baker Lake adults in 1936 that contributed fry to Bear Creek. Subsequently, there should have been zero fish returning in 1937 and

Table 3.2: An example of Bear Creek run-size history under H_A that gives an historical effective size of about 100 under simulations as described on Page 66. The first 15 years follow a distinct pattern of strong and weak returns as might be expected due to the initial stocking from Baker Lake taking place in only one year. For 1950 to 1981 the runs sizes are a realization of Equation 3.2, and from 1982 to 1993 they are estimates from WDF&W data.

1937	0	1952	156	1967	2,568	1982	17,871
1938	0	1953	140	1968	2,153	1983	20,720
1939	6	1954	231	1969	2,023	1984	21,335
1940	50	1955	163	1970	1,595	1985	20,160
1941	10	1956	155	1971	6,497	1986	22,982
1942	0	1957	534	1972	4,618	1987	18,844
1943	8	1958	263	1973	7,830	1988	8,779
1944	60	1959	580	1974	3,887	1989	1,795
1945	30	1960	94	1975	9,707	1990	10,115
1946	10	1961	254	1976	9,535	1991	7,691
1947	16	1962	664	1977	19,372	1992	27,533
1948	80	1963	1,558	1978	10,428	1993	9,848
1949	66	1964	1,781	1979	14,797		
1950	27	1965	2,811	1980	13,868		
1951	65	1966	2,576	1981	15,231		

1938, very few, if any, in 1939, some four year-olds in 1940 and a few five year-olds in 1941, and then again, not many more fish until 1944 and 1945. Under this scenario, there are fewer fish overall and so the effective size will be smaller. In particular, for the historical effective size of the Bear Creek population to be as large as 100, then there must have been more than 25 fish returning in 1940. A scenario which gives an effective size of about 100 is that of starting with 100 "effective spawners" in 1936, and then having the returns listed in Table 3.2.

A number of simulations verified that the run sizes which would most influence the effective size of the population over the last fifty-six years under this model of population growth, are precisely the earliest ones (for example 1940 and 1944) about which little is known. There is one relevant report, though: Royal and Seymour (1940) write that no fish were observed in the creek in September of 1940, but in

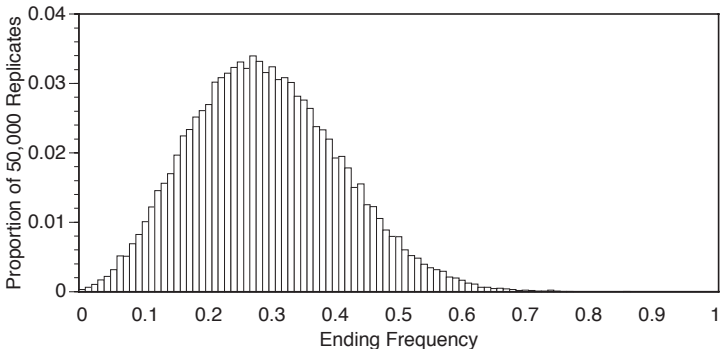
October, two were counted over a rack installed by the State Game Deptartment. Unfortunately there is no indication of how much effort went into the surveys or whether the rack was positioned so as to intercept every fish entering Bear Creek and its tributaries. We are left wondering if as many as 50 fish might have entered the Bear Creek system in 1940 without being caught in the rack.

3.2.4 On the shapes of the distributions

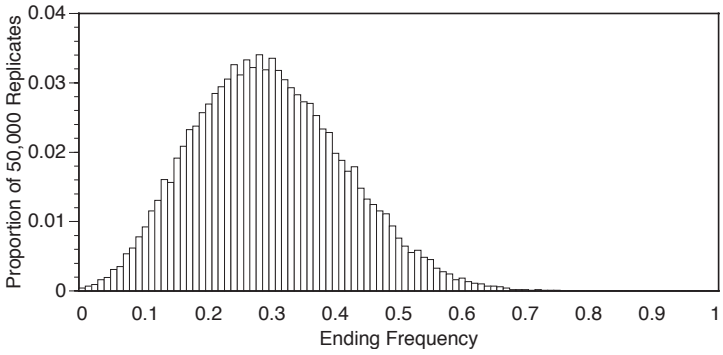
In the above three subsections, I've defined historical effective sizes in terms of the increase in allele frequency variance over time. However, it remains to be shown whether a gene drifting in a population with overlapping year classes will have the same t-generation probability distribution as a gene drifting in an appropriately-sized Wright Fisher population without overlapping generations. When the probability of allele fixation is small (and such cases are the ones we limit ourselves to) the two distributions appear to be very similar. Figure 3.6(a) shows a Monte Carlo approximation to the allele frequency distribution for a Bear Creek population with run sizes as in Table 3.2. These simulations revealed a final allele frequency variance comparable to that of a Wright-Fisher population of 103 individuals drifting for 14 generations. A Monte Carlo approximation to the distribution of allele frequency in such a Wright-Fisher population after 14 generations appears in Figure 3.6(b). Evidently the Wright-Fisher model of the appropriate size is a suitable approximation.

3.3 Computing Likelihood Ratios for H_A

Under H_A, the hypothesis that the Bear Creek population descended exclusively from fish planted from Baker Lake, we can compute a Λ for each of the four loci separately and then add them all together for our final test statistic which will have a chi-square distribution on four (or five, see below) degrees of freedom. We thus get to choose the



(a) Overlapping Year Classes



(b) Wright Fisher Population

Figure 3.6: Simulation results showing that an appropriately-sized Wright-Fisher population is a good approximation for a population with overlapping year classes. (a) The result of 50,000 replicates of drift in a model Bear Creek population with overlapping year classes and run sizes as given in Table 3.2. (b) Simulation results for an appropriately sized Wright-Fisher population drifting for 14 generations. Each simulation used .3 as the initial allele frequency. The two distributions are clearly very similar.

best method from Chapter 2 for each locus. ("Best" here means a sufficiently accurate method that requires the least computation.) In computing the test statistics for H_A, the time t was taken as 14 generations (*i.e.*, $[1993-1936]\div 4$ years average generation length is about 14).

Computing Λ for *PGM–2** is the easiest of the four. Its two alleles are codominant, and the **136* allele appears in the samples from Baker and from Bear at frequencies of .142 and .140 respectively. The maximum likelihood estimate of the starting frequency p of **136* under all hypotheses is then going to be no less than .140. Returning to Figure 2.8 on Page 40, we see that for populations of $N \geq 100$, the probability of an allele starting at .14 drifting to fixation in 14 generations is very small. Hence, the normal approximation to drift transition probabilities will hold, and we can very quickly obtain Λ from Equation 2.31. Appendix B.1 shows an input file to *Mathematica* that will compute this quantity.

Dealing with *ALAT** is more complicated. Since there are three codominant alleles at the locus, we must use the density in the stereographically projected space (Equation 2.30 gives the log of that density) to compute the density for our sample and hence to find Λ. However, the frequency of the **95* allele in the sample from Baker Lake is .06. The maximum likelihood estimate (using the normal approximations that we do here) of the original frequency of **95* in Baker Lake is thus .06. Consulting Figure 2.8 again, we see that such a starting frequency will result in fixation $> 6\%$ of the time in a population of size $N \leq 200$. Consequently, when computing Λ for instances when we take the effective size of Baker Lake to be less than $N_e \approx 300$, we must lump the **95* allele with **91* or **100* and treat the two as one, computing Λ as for *PGM–2**. (The same is true if we take Bear Creek to have an effective size less than ≈ 250.) This lumping throws away information, and one must be wary because lumping **95* with **91* will result in a smaller Λ than will lumping **95* with **100*. I have chosen to always lump in the conservative direction, such that it will yield a smaller Λ. Appendix B.2 shows some input to *Mathematica* that will compute Λ for

a triallelic locus using the coordinates in the stereographically projected space.

For *PGM–1**, which includes the recessive **NULL* allele, we use the sample density for recessive alleles developed in Section 2.8.1. (See Appendix B.3 for some *Mathematica* input.)

Finally, dealing with the *LDH–A1** locus is the most difficult, because it involves a recessive allele that was not detected in any samples from Baker Lake. Under the hypothesis of separate origin, the maximum likelihood estimate of the frequency of the recessive **500* in 1936 in Baker Lake is zero, and the probability of the sample given $p = 0$ is clearly 1. This is a probability mass, and so we need a sample mass method to compute Λ. (I tried various sample density methods, including the sample density for recessive alleles, and they did not perform well.) The Angular approximation sample mass method for recessive alleles (Equation 2.33) works well. The frequency of the **500* is high enough in Bear Creek that the maximum likelihood estimate of its frequency in 1936 under the hypothesis of common origin (H_A) is generally higher than .1 (at least while assuming Bear Creek effective population sizes that are not very, very small) and the probability of fixation in 14 generations for an allele starting at frequency greater than .1 for a population of $N > 100$ is reasonably low. So, likelihoods for *LDH–A1** computed using (2.33) should be quite accurate. Computing Λ by this method requires much more computer time than for the other methods. Appendix B.4 shows a *Mathematica* input for computing this likelihood ratio.

3.4 Computing Likelihood Ratios for H_C

For H_C, similarly, we must choose how to compute Λ for the different loci. We can compute Λ for *PGM–2** and *LDH–A1** in the same way we did for H_A. For *PGM–1** as well, we can use the sample density for recessive alleles developed in Section 2.8.1, but only because the effective size of Cultus Lake has been so large

that the probability that an allele at frequency .962 (the maximum likelihood estimate of the initial frequency of **Null* under the alternative hypothesis) drifts to fixation (frequency 1.0) is small. Finally, since the **95* allele does not appear in the sample from Cultus Lake, we must treat *ALAT** as a diallelic locus, lumping **95* with **100* to be conservative. And here, even though the mle of the initial frequency of **91* in Cultus Lake under the alternative hypothesis is .05, the large effective size ensures that the Angular method approximation will work well.

For testing H_C, t is 12 generations, since the introduction from Cultus Lake occurred eight years after the introduction from Baker Lake.

3.5 Testing the Cedar River

In order to assess how well the statistical method performs in a case where the null hypothesis is known to be true, we may try to reject the hypothesis that the sockeye in the Cedar River descended from the Baker Lake introductions (call that H_R). Since the Cedar River contained no lake-rearing habitat for juvenile sockeye before it was diverted into Lake Washington, and because the only transplants made to the Cedar were from Baker Lake (outside of any survivors of the Cultus transplants to Issaquah Creek that might have later been transferred to the Cedar River when some sockeye offspring from the Issaquah Hatchery were planted into the Cedar River), the Cedar River population is almost certainly from Baker Lake. Thus, we should fail to reject H_R for all reasonable effective sizes of the Cedar population. Carrying out this test, I assumed that the Cedar population's history up to 1993 could be adequately represented by a Wright-Fisher population of size N_R drifting for 14 generations, even though some plantings from Baker Lake occurred as late as 1944. This is allowable since t and N always occur together as t/N, in the transition densitities, so any dicrepancy in t can is made up for by exploring different values of N.

It is difficult to determine an effective size for the Cedar River population. The

population was not consistently surveyed until 1967, shortly after it began growing rapidly. In the ten years before 1993, the run size was never below 76,000 individuals (Figure 1.2). However, in 1962, the run size estimate was as low as 2,100, and in 1961 it was 9,900 (Kolb 1971). In fact, Kolb (1971) states that the returns of sockeye to Lake Washington "fluctuated at low levels after 1940 and general interest diminished until... the early 1960's" (p. 3). It is therefore unlikely that the run sizes to the Cedar River were larger than a few thousands of fish each year before the mid-1960's. In Appendix A.2 I describe how I combined run size data from different sources and performed simulations to determine the approximate effective size of the Cedar River population. These simulations suggest that if the annual escapement in the Cedar River was 500 between 1940 and 1960, then its historical effective size would be about 1150 individuals. Likewise, if the run sizes were about 2,000 for each year between 1940 and 1960, the effective size would be 4,100. Even if there were as many as 5,000 fish every year between 1940 and 1960, the effective size of the Cedar River would only be about 9,000.

Hendry *et al.* (1996)'s data for the Cedar river is shown in Table 3.3. To compute Λ with these values, and the large effective sizes that we will be assuming, we can use the normal-based approximations for three loci: for *PGM–2**, the standard Angular method; for *ALAT**, the density for three alleles in the stereographically projected space (so long as Baker's effective size is taken to be greater than 300); and for *PGM–1**, the sample density method for null alleles. Using the data from *LDH–A1** is more difficult. Since the observed frequency in the Cedar sample is so low, the mle of the frequency of the **500* allele in Baker Lake under the null hypothesis would be very small, and it would not be possible to accurately obtain a probability mass for the sample due to the high probability of fixation. However, we may assume that for *LDH–A1** the effective sizes in Baker Lake and the Cedar River have been infinite, so that the frequency of **500* does not change due to genetic drift, and the differences in observed frequencies arise only from sampling. Such an assumption gives a larger Λ

Table 3.3: Sample allele frequencies from HENDRY *et al.* (1996) at four loci in the Cedar River. n is the sample size in number of diploid individuals for each locus. The frequency of the alternate (**100*) allele at each locus is not listed, but is 1 minus the sum of the frequencies of the other alleles at the locus. *POOLED DATA* are the allele frequency estimates after pooling the data between years. *COUNT DATA* are the actual counts, x, of codominant alleles out of $2n$ gene copies, or the counts, y, of recessive homozygote phenotypes out of n individuals in the pooled samples.

		*ALAT**			*PGM–1**		*PGM–2**		*LDH–A1**	
Popln.	*Year*	n	**91*	**95*	n	**NULL*	n	**136*	n	**500*
Cedar	1992	76	0.382	0.072	40	0.447	134	0.086	135	0.086
	1993	115	0.361	0.074	40	0.354	115	0.096	117	0.000
				↓ *POOLED DATA* ↓						
Cedar	92,93	191	0.369	0.073	80	0.403	249	0.090	252	0.063
				↓ *COUNT DATA* ↓						
Popln.	*Year*	$2n$	**91*	**95*	n	**NULL*	$2n$	**136*	n	**500*
Cedar	92,93	382	141	28	80	13	498	45	252	1

which is acceptable since the burden, in this case, is to demonstrate that the test does not reject H_R. Since the differences in the samples for LDH–A1* are only zero *500* homozygotes out of 120 individuals (Baker) against one homozygous individual out of 252 (Cedar), there really is very little difference between them. Nonetheless, given some initial frequency p of *500*, the probability of finding y homozygotes in a sample of size n can be obtained quickly by the poisson approximation to the binomial, *i.e.*, if $X \sim \text{Bin}(n, p)$ with $pn \approx 1$ then X is distributed approximately Poisson(np).

Thus, denoting the sample sizes for LDH–A1* from Baker and Cedar to be n_A and n_R, respectively, the joint probability of getting y_A and y_R recessive homozygotes, given initial allele frequencies of p_A and p_R respectively is

$$\left(\frac{e^{-n_A p_A}(n_A p_A)^{y_A}}{y_A!}\right)\left(\frac{e^{-n_R p_R}(n_R p_R)^{y_R}}{y_R!}\right). \tag{3.3}$$

Since $y_A = 0$ and $y_R = 1$, this reduces to $n_R p_R \exp\{-(n_A p_A + n_R p_R)\}$. When p_A and p_R are not constrained to equal one another, this quantity is maximized when $p_A = 0$ and $p_R = 1/n_R$, and it takes the value e^{-1}. This is the numerator of our likelihood ratio. When $p_A = p_R = p$ (as under the null hypothesis) the likelihood can be maximized by setting the first derivative w.r.t. p to zero. This gives the denominator of the likelihood ratio: $e^{-1} n_R/(n_A + n_R)$. And so, for testing H_R we approximate Λ for LDH–A1* as

$$\Lambda_{\text{LDH-A1}} = 2\log\left(\frac{n_A + n_R}{n_R}\right) = 2\log\left(\frac{120 + 252}{252}\right) = 0.779. \tag{3.4}$$

3.6 Results of the Likelihood Ratio Tests

3.6.1 Result for H_A

The likelihood ratio test described above shows that it is unlikely that the sockeye in Bear Creek could have descended exclusively from the Baker Lake plantings. Table 3.4 shows values of Λ_j for each locus, and gives an overall (sum over loci) Λ with a corresponding p-value for Bear Creek effective sizes of 75, 100, and 150, and several

Baker Lake effective sizes from 250 to 600 (which are our conservative and "middle ground" estimates, respectively, from the historical population data). From the table we see that if the effective size of Baker Lake were 250, and that of Bear Creek, 100, then our probability of Type I error in rejecting H_A would still be as low as .07. (There are some caveats in interpreting these p-values explained in the Discussion.)

As can be seen from the differences in p-values for $N_B = 75$, 100, and 150, the result is sensitive to the value one is willing to accept for the effective size of the Bear Creek population. I have included the results for $N_B = 75$ to demonstrate this sensitivity even though the result is likely to be somewhat inaccurate in this case because of the non-negligible probability of allele fixation for alleles starting from a frequency of about .15 (*i.e.*, **96* at *PGM–2** and **500* at *LDH–A1** under the null hypothesis).

An important feature of these results is that the overall test statistic does not rely exclusively on a large contribution from *LDH–A1**. The genetic differences between Baker Lake and Bear Creek at *PGM–1** also contribute substantially to our ability to reject H_A.

3.6.2 Result for H_C

Table 3.5 gives values for the Λ_j and p-values for the test of whether the Bear Creek population could have descended exclusively from the plants from Cultus Lake. The results are for Bear Creek effective sizes of 75, 100, and 150, and for one value of effective size for Cultus Lake, 800, our conservative, low estimate of its historical effective size for the last twelve generations. With such low p-values, it is even more clear in this case that we may reject H_C with very low probability of being incorrect in doing so. This accords well with historical knowledge, as there is no record that Cultus sockeye were ever planted directly into the Bear Creek system, but only to other nearby tributaries of the Sammamish River or Lake Sammamish.

Table 3.4: Values of Λ at each locus for testing H_A, assuming different effective sizes for Baker Lake and Bear Creek. N_A is the effective size used for Baker Lake and N_B is for Bear Creek. In each of the next four columns are the test statistics computed for the indicated loci computed as described in Section 3.3. $\sum \Lambda_j$ gives the sum of the individual test statistics as per Equation 2.5. The degrees of freedom for comparing the test statistic to a χ^2 random variable are in the *df* column. For the effective sizes considered, the degrees of freedom is 4 (one for each locus) because the three alleles of *ALAT** were lumped into two. The *p-value* is $\Pr(\chi^2_4 \geq \sum \Lambda_j)$.

N_A	N_B	*ALAT**	*PGM–1**	*PGM–2**	*LDH–A1**	$\sum \Lambda_j$	*df*	*p-value*
250	75	1.103	2.737	0.000	3.589	7.430	4	0.114849
250	100	1.344	3.268	0.000	4.049	8.661	4	0.070159
250	150	1.718	4.082	0.000	4.678	10.478	4	0.033096
300	75	1.144	2.826	0.000	3.607	7.578	4	0.108325
300	100	1.405	3.398	0.000	4.091	8.894	4	0.063804
300	150	1.819	4.291	0.000	4.760	10.870	4	0.028066
400	75	1.200	2.947	0.000	3.628	7.775	4	0.100182
400	100	1.490	3.578	0.000	4.143	9.211	4	0.056039
400	150	1.964	4.586	0.000	4.868	11.418	4	0.022250
500	75	1.236	3.026	0.000	3.638	7.900	4	0.095326
500	100	1.546	3.697	0.000	4.173	9.416	4	0.051500
500	150	2.063	4.785	0.000	4.935	11.783	4	0.019041
600	75	1.261	3.081	0.000	3.643	7.986	4	0.092109
600	100	1.585	3.782	0.000	4.192	9.560	4	0.048534
600	150	2.134	4.928	0.000	4.981	12.044	4	0.017029

Table 3.5: Values of Λ at each locus for testing H_C, assuming different effective sizes for Cultus Lake and Bear Creek. N_C is the effective size used for Cultus Lake and N_B is for Bear Creek. In each of the next four columns are the test statistics computed for the indicated loci computed as described in Section 3.4. $\sum \Lambda_j$ gives the sum of the individual test statistics as per Equation 2.5. The degrees of freedom for comparing the test statistic to a χ^2 random variable are in the *df* column. For the effective sizes considered, the degrees of freedom is 4 (one for each locus) because the three alleles of *ALAT** were lumped into two. The *p-value* is $\Pr(\chi^2_4 \geq \sum \Lambda_j)$.

N_C	N_B	*ALAT**	*PGM–1**	*PGM–2**	*LDH–A1**	$\sum \Lambda_j$	*df*	*p-value*
800	75	5.933	9.120	0.090	3.032	18.174	4	0.001141
800	100	7.362	10.292	0.112	3.412	21.178	4	0.000292
800	150	9.696	11.899	0.147	3.932	25.675	4	0.000037

3.6.3 Result for H_R: Making sure this test doesn't reject everything

We test H_R, the hypothesis that the Cedar River sockeye came exclusively from Baker Lake, as a sort of "test of our hypothesis test." It provides a general check on the test, and, more importantly, gives us some information about the values used for effective sizes of the populations involved. I have conducted the test for five values of Baker Lake effective size from 250 to 600, and for values of the Cedar population's historical effective size of 1,200, 5,000, and 10,000. The results appear in Table 3.6, where it is clear that we would not be able to reject H_R "at the .05 level" even if we assumed that the effective size of Baker Lake has been as high as 600 and of the Cedar River as high as 10,000 since the 1930's.

One difficulty with the above analysis, however, is that it may be preferable to exclude *LDH-A1** from the analysis, because variation at that locus between the Cedar River and Baker Lake is so difficult to detect. Such a change, however, alters the results very little. Note that if we drop *LDH-A1** from the analysis and compare the

Table 3.6: Values of Λ at each locus for testing H_R, assuming different effective sizes for Baker Lake and the Cedar River. N_A is the effective size used for Baker Lake and N_R is for the Cedar River population. In each of the next four columns are the test statistics computed for the indicated loci computed as described in Section 3.5. The value in the *LDH–A1** column is the likelihood ratio for that locus assuming infinite Cedar and Baker population sizes (explained in Section 3.5). $\sum \Lambda_j$ gives the sum of the individual test statistics as per Equation 2.5. The degrees of freedom for comparing the test statistic to a χ^2 random variable are in the *df* column. For the effective sizes considered, the degrees of freedom is five—two for *ALAT** and one for the each of the other loci because the three alleles of *ALAT** need not be lumped into two. The *p-value* is $\Pr(\chi^2_4 \geq \sum \Lambda_j)$.

N_A	N_R	*ALAT**	*PGM–1**	*PGM–2**	*LDH–A1**	$\sum \Lambda_j$	*df*	*p-value*
250	1,200	2.987	0.190	0.649	0.779	4.605	5	0.46591
250	5,000	3.281	0.210	0.730	0.779	5.000	5	0.41593
250	10,000	3.399	0.213	0.745	0.779	5.136	5	0.39953
300	1,200	3.420	0.202	0.735	0.779	5.136	5	0.39953
300	5,000	3.714	0.225	0.840	0.779	5.558	5	0.35166
300	10,000	3.832	0.229	0.860	0.779	5.699	5	0.33658
400	1,200	4.202	0.221	0.880	0.779	6.082	5	0.29833
400	5,000	4.496	0.249	1.036	0.779	6.559	5	0.25553
400	10,000	4.614	0.254	1.065	0.779	6.712	5	0.24293
500	1,200	4.890	0.235	0.998	0.779	6.902	5	0.22803
500	5,000	5.183	0.267	1.204	0.779	7.433	5	0.19035
500	10,000	5.302	0.273	1.244	0.779	7.598	5	0.17985
600	1,200	5.500	0.245	1.097	0.779	7.621	5	0.17841
600	1,500	5.542	0.254	1.154	0.779	7.729	5	0.17182
600	5,000	5.793	0.281	1.349	0.779	8.203	5	0.14539
600	10,000	5.911	0.288	1.400	0.779	8.379	5	0.13656

resulting $\sum \Lambda_j$ to a chi-square distribution on four degrees of freedom (four because we lose one degree of freedom when we eliminate the *LDH–A1** locus) the result is very similar. In fact, the resulting p-values are never more than .036 less than those reported in Table 3.6. Thus, the lowest p-value, that for $N_A = 600$ and $N_R = 10,000$ is .10738.

Such a p-value of .10738 does appear disturbingly low, however, and this probably indicates that an estimate of 600 for Baker Lake's effective size is too high, and that our lower bound estimate of 250 is much closer to the true value of Baker Lake's effective size than the non-conservative estimate derived earlier to be 600. Assuming $N_A = 250$ and $N_R = 10{,}000$ yields a p-value of .39.

3.7 Discussion

This final section recaps several features of the likelihood-ratio test and discusses its applicability to other instances of inference on the ancestral origin of introduced populations. It then describes issues in the interpretation of the p-values from the test and offers several conclusions about the Bear Creek sockeye and the information available in genetic data for inferring their ancestral origin. Finally, I offer some suggestions for future work, both statistical and empirical, on the origin of Bear Creek sockeye.

3.7.1 The test and other applications

The test presented here is a generalized likelihood ratio test—a commonly employed method of testing hypotheses. The main difficulty in conducting the test is computing the likelihoods. The methods I used to do so were similar to those used in admixture analyses by Thompson (1973) and Long (1991). They both (either explicitly or implicitly) expressed their likelihood as a probability density for their samples.

One of the conspicuous features of the Bear Creek problem was the moderate

frequency of the **500* allele of *LDH–A1** in Bear Creek, and its absence in samples from Baker and Cultus lakes. This required a novel treatment because of the breakdown of the asymptotic approximations with allele frequencies equal to or near zero. It was necessary to compute probability masses for the samples under H_A and H_C and the general alternative hypotheses corresponding to each. Numerically integrating the binomial sampling probabilities weighted by the appropriate drift transition probabilities (as in Equation 2.33) worked well for this. However, it was quite fortunate that conditions were such that the method worked. Had there been just one homozygous **500* individual in the sample from Baker Lake then (2.33) would not have properly computed the probability mass for that sample under the hypothesis of separate origin. (Since there were, in actuality, no **500* homozygotes in the sample it was easy to compute its probability mass given the most likely initial gene frequency; it was simply unity.) A truly general method would require a different approach to approximating probability masses for samples—one which is still accurate when the probability of allele fixation is non-negligible.

The test requires much information to bound its assumptions regarding historical effective population size. Additionally, since it seeks to say something about the origin of a population by rejecting putative donor populations, the test demands good information about which populations may be donors. It is thus not clear that this test is widely applicable to other problems of inferring the origin of recently established populations. I have identified six recent studies which used genetic analyses to infer the origin of introduced organisms. Hattemer and Ziehe (1996) describe an approach that is similar to the approach taken here, in that they attempted to exclude the possibility that a stand of oddly-shaped beech trees, *Fagus sylvatica* L., originated from any of 22 stands of trees in the Rheinland-Pfalz region of Germany. They did not take a strict hypothesis-testing approach, but they do acknowledge that any inferences they could make were complicated by genetic drift. Unlike the Bear Creek problem, though, they had to contend with the fact that some of their enzyme

markers are well known to experience selective pressures in different environments. For that reason, the test I have proposed would not be suitable for their problem.

Kambhampati *et al.* (1991) studied allozyme frequencies in 57 populations of the Asian-native mosquito *Aedes albopictus* from four different continents. They used both genetic distance analysis and a discriminant analysis to conclude that *A. albopictus* populations in the U.S. and Brazil probably originated from Japan. They note that fixation of some alleles in the Brazilian population suggests a "founder effect" but do not address how that might affect the confidence in their conclusions about the origin of the Brazilian mosquito populations. Roehner *et al.* (1996) studied the distribution of allele frequencies in polychaete populations, concluding that recently established populations in Europe's North and Baltic Seas originated from the eastern seaboard of the U.S. They based their conclusions on Nei's genetic distance measure (Nei 1978), and they proposed that genetic drift was likely not an important factor because the population transfers typically involve large numbers of individuals carried in the ballast water of ocean-going ships. Morrison and Scott (1996) studied the origin of an exotic weed in Australia by allozyme electrophoresis of samples from 54 populations worldwide. Applying the likelihood ratio test of Chapter 2 to the situations studied in any of these three papers would be difficult. Effective sizes are known even less accurately than they are in Lake Washington, and the issue of multiple comparisons would be problematic because there is not a small set of possible donor populations.

Mendel *et al.* (1994) used randomly-amplified, polymorphic DNA (RAPD) markers to determine the origin of a scale insect pest in Israel. RAPD's do not lend themselves to analyses which assume Mendelian inheritance. Finally, Kriegler *et al.* (1995) used allozyme frequencies to classify 38 Tennessee populations of brook trout, *Salvelinus fontinalis*, as being either of hatchery origin, wild origin, or both. They based their classifications on a system devised by McCracken *et al.* (1993) which relies on the fixation of alternate alleles at some loci between hatchery and wild fish. If

researchers wanted to test hypotheses about the origin of particular brook trout populations, the hypothesis test I have presented, since it relies on approximations that are typically inaccurate in the face of allele fixation, would probably have difficulty handling all of the data of Kriegler *et al.* (1995).

3.7.2 Interpretation of p-values

The hypothesis test here requires a number of assumptions, many of which were mentioned in Section 2.10. The heaviest requirement is the assumption that the historical effective sizes of the populations in the test are known without error. If there is some probability that the estimates of N_e used are incorrect, then, of course, the p-value will not accurately reflect the probability of Type I error. I have tried to manage this by choosing N_e's for the populations in question that are probably lower than the true N_e's. It is reasonable to think that this has been accomplished for the Baker and Cultus Lake populations by choosing low values of N_b/N_i (Page 62) and ages at maturity that offered little overlap between years, and by using the good run-size data for the two populations.

I cannot say the same for my estimates of N_e for Bear Creek. I provided an example of the sorts of run sizes in the early 1940's to the early 1980's that would lead to genetic drift comparable to that in a population of $N_e = 100$ for 14 generations, but accurate run-size data for Bear Creek in the years before 1982 do not exist. It is important to realize that the results of the tests presented here are highly dependent on the effective sizes chosen, and, conspicuously, since Bear Creek's population size has likely been smaller than the others', it is Bear Creek's size to which the tests are most sensitive. In a sense, it seems that framing the origin of Bear Creek sockeye in a hypothesis-testing framework has replaced "global ignorance," an inability to statistically assess the origin of the Bear Creek fish because we didn't know how to do so, with "local ignorance," *i.e.*, we know what to do but one piece of the puzzle is missing entirely—knowledge of Bear Creek's historical run sizes. Yet, by casting

the problem in a statistical framework, we have learned a good deal about what knowledge is required to assess inferences in this sort of problem.

Two other important considerations in interpreting our p-values are the related issues of ascertainment and multiple comparisons. The multiple-testing problem arises because we have performed three separate tests (one for each of H_A, H_C, and H_R), and thus the p-value for any one of the tests reflects not only the Type I error probability for that test, but also the fact that we have "given ourselves three chances" to obtain a p-value below any given level.

The ascertainment problem[2] arises because the population in Bear Creek was singled out from the surveys of Hendry *et al.* (1996) and Seeb and Wishard (1977) as having allele frequencies different from those in the three other sockeye populations in Lake Washington and the two putative donor stocks. The problem here may be seen by a simple analogy. Suppose that you wished to test whether a coin was fair (had equal probability of coming up heads or tails) so you flipped it 10,000 times in sets of ten. At the end of that, suppose you noticed that in one of the sets of ten flips the coin came up heads every time. If you based your test on this one set of ten heads without considering that it was only one set of 1,000, you could overwhelmingly reject the hypothesis that the coin was fair, but doing so would obviously be incorrect. The situation in testing H_A and H_C with Bear Creek is similar, yet more intricate since we know *a priori* that some of the populations (*e.g.*, Cedar River) very likely had no spawning sockeye before the plantings from Baker Lake.

One way to treat both the multiple-testing and ascertainment problems would be to use a Bonferroni correction. That is, in order to reject any hypothesis at a prescribed α-level we would require the p-value to be α divided by the number of comparisions we've made (which would be three for the various hypothesis tests, plus two more for looking at the allele frequencies in Issaquah Creek and the beach

[2] I thank Warwick Daw in the Department of Statistics for first pointing this out to me.

spawning population, as well). Doing this, however, would make the test excessively conservative, as the Bonferroni correction is a conservative tool. Since the test is already based on conservative assumptions of no migration between Lake Washington stocks (Section 2.10), lower bound estimates of N_e for Baker and Cultus Lakes, and lumping alleles in the conservative direction (Page 72), it might be desirable to simply allow those conservative assumptions to be, in effect, a correction for the multiple testing and the ascertainment of the Bear Creek sample (Elizabeth Thompson University of Washington, Department of Statistics, pers. comm.).

The final act in interpreting a p-value may be using it to help make a decision. One decision that awaits making is whether the Bear Creek population will be changed from Provisional ESU status to ESU status, thus allowing its listing, if necessary, under the Endangered Species Act (ESA), or to non-ESU status, in which case it could not be listed under the ESA (Gustafson *et al.* 1997). In light of this, I offer two important considerations.

First, the notion of declaring some statistical test "significant" only if it gives a p-value below some previously-determined value (for instance, .05), has fallen into disfavor among many statisticians and fisheries managers (Pat Sullivan, International Pacific Halibut Commission, P.O. Box 95009, Seattle, WA 98145). For this reason, I have provided the actual p-values from the hypothesis test under different assumptions about effective size. It would be unwise to disregard a p-value of, say, .07 or .08, for rejecting H_A, simply because it is greater than the "traditional" .05 level.

Second, any decision-making process which uses statistical significance levels must also consider the Type II error rate of the test; that is, the probability of failure to reject the null hypothesis when it is false. Typically the Type II error rate of a test is explored by considering the power of the test (one minus the Type II error rate) to reject the null hypothesis when it is false to some specified degree. The issue of statistical power is particularly important in those cases when the p-values from a test

are not very low. The Bear Creek situation is one such example, and the assumption of low effective size reduces the statistical power.

I have not undertaken a formal power analysis, but a simple analogy makes clear how low the power in this test is. Imagine that you are trying to carry out some sort of contingency-table test for genetic differences between two salmon populations, *i.e.*, you are conducting a simple G-test, say, to determine if two populations have significantly different allele frequencies at the time of sampling. Then, the sample sizes which would give you statistical power comparable to the test I have presented with $N_B = 100$ and $N_A = 250$ would be about $n = 7$ fish for the sample from one population and $n = 18$ for the other sample.[3] Very few people would give much weight to a "non-significant" result from samples of size $n = 7$ and $n = 18$. And, likewise, failure to reject H_A while assuming small effective sizes would constitute extremely weak grounds for believing that the sockeye in Bear Creek descended from the Baker Lake transplants.

3.7.3 Conclusions on Bear Creek

If we accept that Bear Creek's historical effective size was 100, and if we agree to our conservative estimate of a Baker Lake effective size of 250 individuals, then we may conclude from the data of Hendry *et al.* (1996) that Bear Creek sockeye could not be exclusively derived from the Baker Lake plantings at the $p = .07$ level. This demonstrates that the observed allele frequencies in Bear Creek are very different from those in Baker Lake. For comparison, using the same effective size (250) for Baker Lake and assuming an extremely generous Cedar River historical effective size of 10,000, we would not reject the hypothesis that the Cedar population derived exclusively from Baker Lake ($p = .39$).

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[3] These values may be approximately derived by finding the binomial sample size n such that if $X \sim \text{Bin}(2n, p)$ and if $\sin^{-1}\sqrt{X/(2n)} \xrightarrow{D} \text{N}(\sin^{-1}\sqrt{p}, 1/(8n))$, then $1/(8n)$ will be equal to the variance of θ (see Page 29) which is at least $t/(8N_B)$ (or $t/(8N_A)$).

We can be even more confident in rejecting the hypothesis that Bear Creek's population descended entirely from Cultus Lake. If we assume an effective size of 75 from Bear Creek and use our conservative estimate of 800 for Cultus' effective size, we reject H_C with $p = .001$. This p-value may be slightly high due to the possibility of allele fixation (see, for example, Figure 2.13), but the difference will be slight, and our conclusion the same: there is substantial evidence against an exclusive Cultus Lake origin.

We must consider these results in light of Royal and Seymour (1940)'s observation that the State Dept. of Game captured only two fish in Bear Creek in 1940 (the year the most adults should have been returning from the 1937 releases of Baker Lake fry to Bear Creek). If the Game Dept. was really set up to intercept most of the fish entering Bear Creek, then probably fewer than 50 fish returned in 1940 and the historical effective size of Bear Creek's population would likely be less than 100 due to this extreme founder effect. As the statistical test shows, we cannot say as much from the data of Hendry *et al.* (1996) if this is the case.

The goal of my analysis has not only been to test H_A and H_C, but also to assess how much information regarding those hypotheses may be found in present-day allele-frequency data. In this regard, the analysis has been successful. I have presented a method which makes explicit what must be known if one wishes to statistically characterize the strengths of their conclusions regarding the origin of Bear Creek sockeye. The result that the power of Hendry *et al.* (1996)'s data to reject H_A depends heavily on unknown run sizes, though disapointing, is not unexpected. The awareness that founder effects are bound to affect inferences such as these is not at all new, however, the likelihood ratio test provides a way to judge the magnitude of such effects.

3.7.4 Future work on Bear Creek

Further statistical analysis. The above method, relying as it does on the Brownian motion approximation to genetic drift transition probabilities, has been taken up to its limit. In order to extend the statistical analysis so it may accurately handle smaller assumed effective sizes and the inclusion of data from other datasets, and so as to address the question of whether the sockeye of Bear Creek may have arisen from a mixture of Baker and Cultus Lake transplants, it will be necessary to adopt a very different approach—one which dispenses with the Brownian motion approximation, and instead determines probabilities or test statistics and their distributions entirely by computer simulation.

The first application for such a method should be to use the additional information available in other allele-frequency datasets. For example, including the data of Seeb and Wishard (1977) from Bear Creek, Baker Lake, and Cultus Lake would be valuable. Though the sample sizes of Seeb and Wishard (1977) are not as large as those of Hendry *et al.* (1996), they collected their data from Baker Lake at a time when considerably less genetic drift had occurred in the population since the 1930's, so their data are potentially quite informative. From a statistical standpoint, an interesting challenge in using both datasets comes from the fact that the data of Hendry *et al.* (1996) is not independent of the observations made by Seeb and Wishard, because they are connected in time to one another via a stochastic process. Thus, deriving the joint probability for the two sets of data would require some care.

There have also been other, more recent, collections of fish from Baker Lake (Winans *et al.* 1996; Shaklee *et al.* 1996) which ought to be included in the analysis. Including them in the current analysis is difficult because the increased data at *LDH–A1** in Baker Lake makes the maximum likelihood estimate of the frequency of the **500* allele under the null hypothesis, H_A, low enough that the normal approxi-

mation is no longer accurate. Preliminary work suggests that including such data will only strengthen the case against H_A or H_C. When a good computational method for hypothesis testing is developed and used with these other datasets I expect to observe lower p-values than obtained here, and to be able to reject H_A, even while assuming that the historical effective size of the Bear Creek population is considerably less than 100 individuals.

A computational approach would also lend itself more readily to testing whether Bear Creek sockeye may have descended from a mixture of Baker Lake and Cultus Lake transplants. I have not addressed such a "mixture hypothesis" in this thesis, though I have elsewhere investigated a possible method for testing it. As is apparent from Table 3.1, the allele frequencies of the **91* allele at *ALAT** and the **NULL* allele at *PGM–1** in Bear Creek are intermediate to the frequencies in Baker and Cultus Lake. Accordingly (and this is also borne out in practice) contributions to the likelihood ratio statistic for testing the mixture hypothesis come primarily from *LDH–A1**. Since no *LDH–A1* *500* homozygotes have been reported from any samples from Baker and Cultus lakes, one possibly fruitful, and relatively simple first step would be to estimate, by Monte Carlo simulation, the joint probability of finding no **500* homozygotes in the samples from Baker and Cultus lakes, and the observed number (or more) of **500* homozygotes in the samples from Bear Creek, given some initial frequencies of the **500* allele in Baker and Cultus lakes at the time of the introductions. If this joint probability were very low for all different initial frequencies of the allele in Baker and Cultus lakes, this could be taken as evidence against the mixture hypothesis. Some adjustment for the fact that *LDH–A1** was only one of several loci assayed would probably be appropriate.

Further empirical work Especially given the importance of the *LDH–A1* *500* allele, as I discussed above, some recent developments indicate that more empirical work could be helpful. In particular, some parties have proposed that sockeye

from other sources have been introduced into Lake Washington. In October of 1997, Andrew Hendry received an email message which read:

> . . . also, I KNOW, that Kamchatka Sockeye WERE possibly planted to Lake Washington in the late 60's. I raised them from eggs shipped here, and I know that the person who was supposed to have destroyed them (200,000), later claimed that he didn't (he is now deceased). JM

This assertion is made particularly interesting by the fact that Kamchatka is a region where the **500* allele at *LDH–A1** has been detected in anadromous sockeye populations (Paul Aebersold, NMFS Northwest Fisheries Science Center, pers. comm.). Furthermore, the allele frequencies at *PGM–1**, *PGM–2**, and *ALAT** of sockeye in the Kamchatka River are quite close to those in Bear Creek (Varnavskaya *et al.* 1994). I believe this issue warrants further study, especially since the possibility of a Kamchatka origin could be elegantly and simply tested with molecular markers.

Knowing little more than that some fish from somewhere in Kamchatka may have been released into Lake Washington, hypothesis testing on the basis of allozyme frequencies is an inappropriate tool for exploring this hypothesis. Varnavskaya *et al.* (1994) list the allele frequencies of many sockeye populations in Kamchatka, and some of them are very likely to have allele frequencies close to those in Bear Creek. As mentioned above, the test I have proposed requires that one have a clear idea of which populations may have been donors. It is not intended for surveying many populations and finding one that has allele frequencies similar to those in Bear Creek.

Instead, mitochondrial DNA should be useful in testing the Kamchatka hypothesis. Bickham *et al.* (1995) surveyed sequences from the cytochrome *b* region of mitochondrial DNA from sockeye salmon in North America and Asia. They found that sockeye in the Fraser River, postglacially recolonized by fish from the "Columbia" glacial refuge (Taylor *et al.* 1996), have a high (40%) frequency of an "AC" hap-

lotype that does not occur in populations from Kamchatka. The Fraser populations do not possess the "GC" haplotype which occurrs in the Kamchatka populations at a frequency of 50%. Since Baker Lake (and Lake Washington) were both ancestrally recolonized by fish from the Columbia refuge, any stocks of native or Baker Lake origin in Lake Washington should have a high frequency of "AC" and a low (or zero) frequency of the "GC" haplotype. If a high frequency of "GC" were found in Bear Creek, but in no other populations of sockeye in Washington and the Fraser River, then that would provide good evidence that the sockeye in Bear Creek may have come from Kamchatka. Such information, as well as other data collected in the future on Lake Washington sockeye, should continue to clarify the issue of ancestry of the sockeye in Bear Creek.

BIBLIOGRAPHY

Ajwani, S., 1956 A review of the Lake Washington watershed, historical, biological and limnological. Master's thesis, University of Washington. 3

Allendorf, F. W. and G. H. Thorgaard, 1984 Tetraploidy and the evolution of salmonid fishes. In B. J. Turner (Ed.), *Evolutionary Genetics of Fishes*, pp. 1–53. New York: Plenum Press. 54

Bartley, D., M. Bagley, G. Gall, and B. Bentley, 1992 Use of linkage disequilibrium data to estimate the effective size of hatchery and natural fish populations. Conservation Biology **6:** 365–375. 23

Bickham, J. W., C. C. Wood, and J. C. Patton, 1995 Biogeographic implications of cytochrome *b* sequences and allozymes in sockeye salmon (*Oncorhynchus nerka*). J. Heredity **86:** 140–144.

Burgner, R. L., 1991 Life history of sockeye salmon (*Oncorhynchus nerka*). In C. Groot and L. Margolis (Eds.), *Pacific Salmon Life Histories*, pp. 1–117. Vancouver: UBC Press. 58, 60

Cavalli-Sforza, L. L. and A. W. F. Edwards, 1967 Phylogenetic analysis: models and estimation procedures. Evolution **21:** 550–570. 38

Chrzatowski, M., 1983 Historical changes to Lake Washington and route of the Lake Washington Ship Canal, King County, Washington. Accompaniment to Water Resources Investigation Open-File Report 81–1182, United States Geological Survey. 3

Cobb, J. N., 1927 Preliminary report of fishway work. Transactions of the Amer-

ican Fisheries Society **57:** 181–201. 4

Crow, J. F. and C. Denniston, 1988 Inbreeding and variance effective numbers. Evolution **42:** 482–495.

Diggle, P. J. and R. J. Gratton, 1984 Monte Carlo methods of inference for implicit statistical models (with discussion). Journal of the Royal Statistical Society, Series B **46:** 193–227. 34

Edmondson, W. T., 1991 *The Uses of Ecology: Lake Washington and Beyond*. Seattle: University of Washington Press. 3, 66, 67

Edmondson, W. T., 1994 Sixty years of Lake Washington: A curriculum vitae. Lake And Reservoir Management **10:** 75–84.

Edwards, A. W. F., 1971 Distances between populations on the basis of gene frequencies. Biometrics **27:** 873–81.

Edwards, A. W. F., 1992 *Likelihood*. Baltimore: The Johns Hopkins University Press. 18

Evermann, B. W. and E. L. Goldsborough, 1907 The fishes of Alaska. Bulletin of the U.S. Fisheries Commision **26:** 219–360. 4

Felsenstein, J., 1985 Phylogenies from gene frequencies: a statistical problem. Systematic Zoology **34:** 300–311.

Felsenstein, J., 1995 *Theoretical Evolutionary Genetics*. Seattle: ASUW Publications. 22, 25

Fisher, R. A., 1930 *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press. 15

Foote, C., G. S. Brown, and C. C. Wood, 1997 Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. Canadian Journal of Fisheries and Aquatic Sciences **54:** 1785–1795.

Geyer, C. J., 1996 Estimation and optimization of functions. In W. R. Gilks, S. Richardson, and D. J. Spiegelhalter (Eds.), *Markov Chain Monte Carlo in Practice*, pp. 241–258. New York: Chapman and Hall. 35

Griffiths, R. C., 1979 A transition density expansion for a multi-allele diffusion model. Advances in Applied Probability **11:** 310–325. 27

Gustafson, R. G., T. C. Wainwright, G. A. Winans, F. W. Waknitz, L. T. Parker, and R. S. Waples, 1997 Status review of sockeye salmon from Washington and Oregon. U.S. Dept. Commer., NOAA Tech. Memo. NMFS–NWFSC–33, 282 p. 1, 87

Hanson, A. J. and H. D. Smith, 1967 Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*). Journal of the Fisheries Research Board of Canada **24:** 1955–1977. 60

Hattemer, H. H. and M. Ziehe, 1996 An attempt to infer on the origin of a beech (*Fagus sylvatica* L.) stand in Rheinland-Pfalz (Germany). Silvae Genetica **45:** 276–283. 10

Hendry, A., 1995 Sockeye salmon (*Oncorhynchus nerka*) in Lake Washington: an investigation of ancestral origins, population differentiation and local adaptation. Master's thesis, University of Washington. 6, 56

Hendry, A. P. and T. P. Quinn, 1997 Variation in adult life history and morphology among Lake Washington sockeye salmon (*Oncorhynchus nerka*) populations in relation to habitat features and ancestral affinities. Canadian Journal of Fisheries and Aquatic Sciences **54:** 75–84.

Hendry, A. P., T. P. Quinn, and F. M. Utter, 1996 Genetic evidence for the persistence and divergence of native and introduced sockeye salmon (*Oncorhynchus nerka*) within Lake Washington. Canadian Journal of Fisheries and Aquatic Sciences **53:** 823–832. 1, 4, 5, 10, 62

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.

Kambhampati, S., W. C. I. Black, and K. S. Rai, 1991 Geographic origin of the USA and Brazilian *Aedes albopictus* inferred from allozyme analysis. Heredity **67:** 85–94. 10

Karlin, S. A. and H. M. Taylor, 1975 *A First Course in Stochastic Processes, 2nd Ed.* New York: Academic Press. 24

Kendall, M. and A. Stuart, 1979 *The Advanced Theory of Statistics, Vol. 2.* New York: Macmillan. 19, 50

Kimura, M., 1955a Random genetic drift in multi-allelic locus. Evolution **9:** 419–435.

Kimura, M., 1955b Solution of a process of random genetic drift with a continuous model. Proceedings of the National Academy of Sciences, USA **41:** 144–150.

Kimura, M., 1956 Random genetic drift in a tri-allelic locus: exact solution with a continuous model. Biometrics **12:** 57–66.

Kolb, R., 1971 A review of Lake Washington sockeye (*Oncorhynchus nerka*) age and racial characteristics as determined by scale pattern analysis. Supplemental progess report marine fisheries investigations, Washington Department of Fisheries. 4, 67, 75

Kriegler, F. J., G. F. McCracken, J. W. Habera, and R. J. Strange, 1995 Genetic characterization of Tennessee brook trout populations and associated management implications. North American Journal Of Fisheries Management **15:** 804–813. 10

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.

Littler, R. A. and E. D. Fackerell, 1975 Transition densities for neutral allele diffusion models. Biometrics **31:** 117–123. 27

Long, J. C., 1991 The genetic structure of admixed populations. Genetics **127:** 417–428. 45

McCart, P., 1970 A polymorphic population of *Oncorhynchus nerka* at Babine Lake, B.C. involving anadromous (sockeye) and non-anadromous (kokanee) forms. Ph. D. thesis, University of British Columbia, Vancouver. 55

McCracken, G. F., P. C. R, and S. Z. Guffey, 1993 Genetic differentiation and hybridzation between hatchery stock and native brook trout in the Great Smoky Mountains National Park. Transactions of the American Fisheries Society **122:** 533–542.

Mendel, Z., D. Nestel, and R. Gafny, 1994 Examination of the origin of the Israeli population of *Matsucoccus josephi* (Homoptera: Matsucoccidae) using random amplified polymorphic DNA-polymerase chain reaction method. Annals Of The Entomological Society Of America **87:** 165–169. 10

Morrison, S. M. and J. K. Scott, 1996 Variation of populations of *Tribulus terrestris* (Zygophyllaceae): 3. Isozyme analysis. Australian Journal Of Botany **44:** 201–212. 10

Nei, 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89:** 583–590. 9, 84

Nei, M., 1987 Genetic distance and molecular phylogeny. In N. Ryman and F. M. Utter (Eds.), *Population Genetics and Fishery Management*, pp. 21–46. Seattle: University of Washington Press. 54

Nei, M. and F. Tajima, 1981 Genetic drift and estimation of effective population size. Genetics **98:** 625–640. 23

Ostergaard, E., 1995 Status report: Abundance of spawning kokanee in the Sammamish River basin. Addendum to 1994 status report. Technical report, King County Surface Water Management. 5

Pamilo, P. and S. Varvio-Aho, 1980 Letter to the editor of the estimation of population size from allele frequency changes. Genetics **95:** 1055–1058. 23

Pfeifer, B., 1992 Fisheries Investigations of Lakes Washington and Sammamish—1980–1990. Part V. Wild cutthroat and kokanee in Lakes Washington and Sammamish. Technical report, Washington Department of Fisheries. Unpubl. Draft. 4

Pollak, E., 1983 A new method for estimating the effective population size from allele frequency changes. Genetics **104:** 531–548. 23

Roehner, M., R. Bastrop, and K. Juerss, 1996 Colonization of Europe by two American genetic types or species of the genus *Marenzelleria* (Polychaeta: Spionidae). An electrophoretic analysis of allozymes. Marine Biology **127:** 277–287. 10

Rounsefell, G. A. and G. B. Kelez, 1938 The salmon and salmon fisheries of Swiftsure Bank, Puget Sound, and the Fraser River. Bulletin of the U.S. Bureau of Fisheries **49:** 693–823. 4

Royal, L. A. and A. Seymour, 1940 Building new salmon runs. Progressive Fish Culturist **52:** 1–7. 5, 6

Seeb, J. and L. Wishard, 1977 The use of biochemical genetics in the management of Pacific salmon stocks: genetic marking and mixed fishery analysis. Final Report Service Contract 792, Washington Department of Fisheries. 1, 90

Shaklee, J. B., J. Ames, and L. LaVoy, 1996 Genetic diversity units and major ancestral lineages for sockeye salmon in Washington. Technical Report RAD 95–02, Washington Department of Fish and Wildlife. 9, 61, 90

Sokal, R. R. and F. J. Rohlf, 1981 *Biometry, 2nd ed.* New York: W. H. Freeman and Company. 19

State of Washington Department of Fisheries and Game, 1919b Fifth and sixth annual reports of the state game warden to the to the Governor of the State of Washington March 1, 1917 to February 28, 1917. Wash. Dept. of Fish. Game, Olympia, WA.

State of Washington Department of Fisheries and Game, 1932a Fortieth and forty-first annual reports of State Department of Fisheries and Game, Division of Fisheries, for the period from April 1, 1929, to March 31, 1931. Wash. Dept. of Fish. Game, Olympia, WA. 4

State of Washington Department of Fisheries and Puget Sound Treaty Indian Tribes Northwest Indian Fisheries Commission, 1992 Puget Sound sockeye salmon forecasts and management recommendations. Olympia, WA. 103

Taylor, E. B., C. J. Foote, and C. C. Wood, 1996 Molecular genetic evidence for parallel life-history evolution within a Pacific salmon (sockeye salmon and kokanee, *Oncorhynchus nerka*). Evolution **50:** 401–416. 92

Thompson, E. A., 1972 The likelihood for multinomial proportions under stereographic projection. Biometrics **28:** 618–620.

Thompson, E. A., 1973 The Icelandic mixture problem. Annals of Human Genetics, London **37:** 69–80. 45

Utter, F. M., P. Aebersold, and G. Winans, 1987 Interpreting genetic variation detected by electrophoresis. In N. Ryman and F. M. Utter (Eds.),

Population Genetics and Fishery Management, pp. 21–46. Seattle: University of Washington Press. 10, 54

Varnavskaya, N. V., C. C. Wood, and R. J. Everett, 1994 Genetic variation in sockeye salmon (*Oncorhynchus nerka*) populations of Asia and North America. Canadian Journal of Fisheries and Aquatic Sciences **51(Suppl. 1):** 132–146. 92

Waples, R. S., 1989 A generalized approach for esimating 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. Journal of Heredity **81:** 277–289. 58

Waples, R. S., 1995 Evolutionarily Significant Units and the conservation of biological diversity under the Endangered Species Act. In J. L. Nielsen (Ed.), *Evolution and the Aquatic Ecosystem: defining unique units in population conservation*, pp. 8–27. Bethesda, MD: American Fisheries Society Symposium 17. 14

Waples, R. S., O. W. Johnson, P. B. Aebersold, C. K. Shiflett, D. M. VanDoornik, D. J. Teel, and A. E. Cook, 1993 A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River basin. Annual report of research, Coastal Zone and Estuarine Studies Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA. 58

Winans, G. A., P. B. Aebersold, and R. S. Waples, 1996 Allozyme variability of *Oncorhynchus nerka* in the Pacific Northwest, with special consideration to populations of Redfish Lake, Idaho. Transactions of the American Fisheries Society **125:** 645–663. 90

Wood, C. C., B. E. Riddell, D. T. Rutherford, and R. E. Withler,

1994 Biochemical genetic survey of sockeye salmon (*Oncorhynchus nerka*) in Canada. Canadian Journal of Fisheries and Aquatic Sciences **51(Suppl. 1):** 114–131. 10, 55, 55

Woodey, J. C., 1966 Sockeye spawning grounds and adult returns in the Lake Washington watershed, 1965. Master's thesis, University of Washington. 6

Wright, S., 1931 Evolution in Mendelian populations. Genetics **16:** 97–159.

Zar, J. H., 1984 *Biostatistical Analysis, 2nd ed.* Englewood Cliffs, NJ: Prentice Hall. 19

Appendix A

DETERMINING THE EFFECTIVE SIZES

A.1 Population Sizes and Age Composition of Cultus Lake Sockeye

A.2 Determining Effective Size of the Cedar River Population

To compute an effective size for the Cedar River population I gathered population size estimates from various sources. Escapement estimates for 1961–1969 come from Kolb (1971). For 1970 to 1991 there are sockeye counts from the H. M. Chittenden Locks (State of Washington Department of Fisheries and Puget Sound Treaty Indian Tribes Northwest Indian Fisheries Commission 1992) For the years 1982 to 1993, Ron Egan of the Washington Department of Fish and Wildlife provided me with Cedar River escapement data. The Cedar escapement is lower than the counts at the Locks because of prespawning mortality and the fact that the fish ascending the ladders at the locks are a mixture of fish from all the different populations in Lake Washington, not just the Cedar River. For the period 1982–1991, on average, the Cedar escapement was 78 percent of the counts at the Locks. Therefore, I obtained rough escapement estimates for 1970–1981 by multiplying the counts at the Locks by

Table does not appear here because it is a longtable and apparently not compatible with the current hyperref implentation in Textures. If you really want to see this table you can obtain my thesis from the University of Washington

Table A.1: The table that does not appear here

0.78.

The run-size data for 1970–1991 do include estimates of age composition. These age class counts are for a mixture of all the Lake Washington populations, so they are not entirely representative of the Cedar River population. (In particular there are probably relatively more three year-olds among the mixed group of returning fish than in the Cedar River.) Nonetheless, they are a good approximation. The average proportion of different-aged fish in Lake Washington from 1970 to 1991 was 12% three year-olds, 85% four year-olds, and 3% five year-olds. For the years 1970–1991 I took the number of fish of each age class in the Cedar River to be .78 of the number of fish counted at the locks in that age class. For 1932–1969 I took the proportion of three year olds to be .12 of the total escapement; the proportion of four year-olds to be .85; and the proportion of five year-olds to be .03. For 1992 and 1993, I used the age compositions of Cedar River samples reported by Hendry and Quinn (1997).

We don't know how large the Cedar River population was before 1960. Kolb (1971) says it was small. I performed four different simulations, each time assuming that the escapement was of constant size N_u in each year before 1960. Such a scheme leads to the population numbers shown in Table A.2 which I used in the simulations. I ran simulations for four different values of N_u: 500, 1,000, 2,000, and 5,000.

Table A.2: Cedar River escapements used in the simulations to determine effective size.

Return Year	3 yr-olds	4 yr-olds	5 yr-olds	Total
1932	$.12N_u$	$.85N_u$	$.03N_u$	N_u
⋮	⋮	⋮	⋮	⋮
1960	$.12N_u$	$.85N_u$	$.03N_u$	N_u
1961	1,188	8,415	396	9,900
1962	252	1,785	84	2,100
1963	4,236	30,005	1,412	35,300
1964	8,220	58,225	2,740	68,500
1965	5,760	40,800	1,920	48,000
1966	5,448	38,590	1,816	45,400
1967	2,273	16,099	758	18,940
1968	19,200	136,000	6,400	160,000
1969	14,880	105,400	4,960	124,000
1970	32,794	133,708	8,507	105,734
1971	15,862	676,793	25,430	433,842
1972	26,613	340,997	18,840	233,480
1973	24,689	557,989	4,651	354,843
1974	29,958	178,461	9,415	131,608
1975	17,969	157,831	9,448	111,921
1976	18,738	188,551	24,142	139,823
1977	4,185	729,986	20,604	456,009
1978	5,052	392,719	3,084	242,183
1979	6,043	256,663	32,980	178,643
1980	27,796	609,726	2,378	386,605
1981	57,314	89,495	6,680	92,733
1982	2,839	244,048	6,772	253,658
1983	64,867	125,919	2,551	193,338
1984	55,600	279,945	1,415	336,960
1985	49,044	168,928	5,773	223,745
1986	37,914	176,411	2,808	217,133
1987	60,076	114,360	3,405	177,841
1988	32,867	325,556	576	359,000
1989	103,489	57,935	576	162,000
1990	23,540	51,452	1,008	76,000
1991	14,165	61,544	1,291	77,000
1992	0	94,000	6,000	100,000
1993	760	47,120	28,120	76,000

Appendix B

ROUTINES FOR COMPUTING THE LIKELIHOOD RATIO

Following are a series of sample inputs to *Mathematica* that I used to compute Λ in various cases.

B.1 Diallelic Codominant Loci

I used the following input file to compute Λ for diallelic codominant loci such as *PGM–2** and for *ALAT** when I had lumped the**91* allele together with one of the other alleles, making it appear to be a diallelic locus.

```
(* This Mathematica input defines a function that computes
the likelihood ratio test statistic for a simple diallelic
locus with codominant alleles. It includes the data for
``b'' = Bear Creek and ``a'' = Baker Lake at the PGM-2 locus.
FUNCTION NAME:
     ``LambdaEasy''
VARIABLES/INPUTS:
     t ->time of drift in generations
     na ->effective size of population A
     nb ->effective size of population B
     sa ->  size of sample from population A (# of individuals)
     sb ->  size of sample from population B (# of individuals)
     xa ->  allele count in sample A
     xb ->allele count in sample B
DATA VALUES FOR PGM--2:  *) (
     t  =  14;
     sb  = 207;
     xb = 58;
     sa  = 120;
     xa = 34;
(* FUNCTION DEFINITION *)
```

```
     LambdaEasy[t_,na_,nb_,sa_,sb_,xa_,xb_] := N[
          ( ArcSin[Sqrt[xa/(2sa)]] - ArcSin[Sqrt[xb/(2sb)]] ) ^2   /
          ( t/(8na) + 1/(8sa) + t/(8nb) + 1/(8sb)  ) ];
(*  LOOP TO PRINT VALUES FOR DIFFERENT VALUES OF na AND nb  *)
size = {50,75,100,150,200,250,300,400,500,600,700,800,900,1000};
For[i=1,i<=14,i++, na = size[[i]];
    For[j=1,j<=14,j++, nb = size[[j]];
    PutAppend[{na, nb, LambdaEasy[t,na,nb,sa,sb,xa,xb]}, "pgm2out"  ] ]
]
)
```

B.2 Triallelic Codominant Locus

For *ALAT** with all three of its alleles, the functions defined in the following input file compute Λ using the stereographic projection:

```
(* This Mathematica input defines a function that computes
the likelihood ratio test statistic for a triallelic
locus with codominant alleles, using the density from the
stereographically projected space.  (Actually it uses the
log-density the whole way through. It includes the data for
``b'' = Bear Creek and ``a'' = Baker Lake at the ALAT locus.
I've opted to use the sample gene frequencies rather than the
count data because the counts don't appear anyhwere in the ex-
pressions for this thing.
FUNCTION NAME:
``LambdaFunct''
VARIABLES/INPUTS:
     t ->time of drift in generations
     na ->effective size of population A
     nb ->effective size of population B
     sa ->  size of sample from population A (# of individuals)
     sb ->  size of sample from population B (# of individuals)
     freqa1 ->  frequency of first allele in sample A
     freqa2 ->  frequency of second allele in sample A
     freqa3 ->  frequency of third allele in sample A
     freqb1 ->  frequency of first allele in sample B
     freqb2 ->  frequency of second allele in sample B
     freqb3 ->  frequency of third allele in sample B
DATA VALUES FOR ALAT:  *) (
   t  =  14;
   sa  = 83;
```

```
   sb  = 163;
   freqa1 = .403;
   freqa2 = .537;
   freqa3 = .06;
   freqb1 = .598;
   freqb2 = .273;
   freqb3 = .129;
(* FUNCTION TO COMPUTE THE SUM IN THE TRANSFORMATION  *)
   SumFre[f1_,f2_,f3_] := Sqrt[f1/3] + Sqrt[f2/3] + Sqrt[f3/3];
(* FUNCTION TO TRANSFORM VARIABLES INTO STER PROJ SPACE
RETURNS THEM IN A LIST  *)
ProjectFreq1[f1_,f2_,f3_] :=
     N[(  (2(Sqrt[f1] + Sqrt[1/3]) /
     (1 + SumFre[f1,f2,f3])  ) )  - Sqrt[1/3] ];
ProjectFreq2[f1_,f2_,f3_] :=
     N[(  (2(Sqrt[f2] + Sqrt[1/3]) /
     (1 + SumFre[f1,f2,f3])  ) )  - Sqrt[1/3] ];
ProjectFreq3[f1_,f2_,f3_] :=
     N[(  (2(Sqrt[f3] + Sqrt[1/3]) /
     (1 + SumFre[f1,f2,f3])  ) ) -  Sqrt[1/3] ];
(* ASSIGN THE TRANSFORMED VALUES TO THE VARIABLES wa1,wa2, etc. *)
     wa1 = ProjectFreq1[freqa1,freqa2,freqa3];
     wa2 = ProjectFreq2[freqa1,freqa2,freqa3];
     wa3 = ProjectFreq3[freqa1,freqa2,freqa3];
     wb1 = ProjectFreq1[freqb1,freqb2,freqb3];
     wb2 = ProjectFreq2[freqb1,freqb2,freqb3];
     wb3 = ProjectFreq3[freqb1,freqb2,freqb3];
(* COMPUTE VARIANCES  *)
     TheVar[n_,s_,t_] := N[ ( (t/(4n)) + (1/(4s)) ) ] ;
     vara = TheVar[na,sa,t];
     varb = TheVar[nb,sb,t];
(*  MLE'S OF JOINT DISTRIBUTION ARE WEIGHTED AVERAGES  *)
     p1 = ( varb * wa1 + vara * wb1 ) / (vara + varb);
     p2 = ( varb * wa2 + vara * wb2 ) / (vara + varb);
     p3 = ( varb * wa3 + vara * wb3 ) / (vara + varb);
(*  COMPUTE LAMBDA  *)
LambdaFunct[wa1_,wa2_,wa3_,wb1_,wb2_,wb3_,p1_,p2_,p3_]  := 2 * (
     ( ( (wa1 - p1)^2 + (wa2 - p2)^2 + (wa3 - p3)^2 ) /
     ( (t/(4na)) + (1/(4sa)) ) ) + ( ( (wb1 - p1)^2 +
     (wb2 - p2)^2 + (wb3 - p3)^2 ) / ( (t/(4nb)) + (1/(4sb)) ) )  )
)
(* COMPUTE FOR VARIOUS EFFECTIVE SIZES  *)
(
size = {50,75,100,150,200,250,300,400,500,600,700,800,900,1000};
```

```
For[i=1,i<=14,i++, na = size[[i]];
    For[j=1,j<=14,j++, nb = size[[j]];
    PutAppend[{na, nb, LambdaFunct[wa1,wa2,wa3,wb1,wb2,wb3,p1,p2,p3]},
    "alat.sterout"  ] ]
]
)
```

*B.3 Diallelic Locus With Recessive—PGM–1**

For a locus such as *PGM–1** which has a recessive allele that is detected in ample numbers in both samples we may use the sample density method of Section 2.8.1.

```
(* This Mathematica input  computes
the likelihood ratio test statistic for a diallelic
locus with one recessive allele that appears in both
of the samples  (like the PGM-1 locus for Baker Lake
and Bear Creek). It uses the sample density method
for null alleles, and this file includes the data for
``b'' = Bear Creek and ``a'' = Baker Lake at the PGM-1 locus
VARIABLES/INPUTS
    t ->time of drift in generations
    na ->effective size of population A
    nb ->effective size of population B
    sa ->  size of sample from population A (# of individuals)
    sb ->  size of sample from population B (# of individuals)
    ya ->  # of recessive homozygotes in sample A
    yb ->#  of recessive homozygotes in sample B
    p   -> used by FindMinimum.  It ends up being the
                mle of the ancestral frequency under the
                hypothesis of common origin *)
(
t  =  14;
sb  = 160;
yb = 68;
sa  = 79;
ya = 10;
numera = FindMinimum[-1 *
    ( 2*Pi*(t*p(1-p)/(2na) + (1-p^2)/(4sa) ) )^(-.5)  *
    Exp[ (-(p - Sqrt[ya/sa])^2) /
    (2*(t*p(1-p)/(2na) + (1-p^2)/(4sa) ) ) ],
    {p, {.3,.305}}  ];
numerb = FindMinimum[-1 *
```

```
    ( 2*Pi*(t*p(1-p)/(2nb) + (1-p^2)/(4sb) ) )^(-.5)  *
    Exp[ (-(p - Sqrt[yb/sb])^2) /
    (2*(t*p(1-p)/(2nb) + (1-p^2)/(4sb) ) ) ],
    {p, {.6,.605}}  ];
denom = FindMinimum[-1 *
    ( 2*Pi*(t*p(1-p)/(2na) + (1-p^2)/(4sa) ) )^(-.5)  *
        Exp[ (-(p - Sqrt[ya/sa])^2) /
        (2*(t*p(1-p)/(2na) + (1-p^2)/(4sa) ) ) ] *
    ( 2*Pi*(t*p(1-p)/(2nb) + (1-p^2)/(4sb) ) )^(-.5)  *
        Exp[ (-(p - Sqrt[yb/sb])^2) /
        (2*(t*p(1-p)/(2nb) + (1-p^2)/(4sb) ) ) ],
    {p, {.5,.505}}  ];
loglr = 2*Log[ (numera[[1]] * numerb[[1]]) / (-1*denom[[1]])];
PutAppend[{na,nb,loglr,denom[[2]]},"pgm1xtra"]
)
```

*B.4 Diallelic Locus With Recessive—LDH–A1**

*LDH–A1**, since it includes a recessive allele that was not detected in the samples from Baker Lake or Cultus Lake requires special treatment. The following input will compute Λ by the sample mass method for recessive alleles using the Angular approximation.

```
(* This Mathematica input  computes
the likelihood ratio test statistic for a diallelic
locus with one recessive allele that does not appear in one
of the samples  (like the LDH-A1 locus). It includes the data for
``b'' = Bear Creek and ``a'' = Baker Lake for the LDH-A1 locus.
Note that the variables na and nb must be assigned values before
this is run.
VARIABLES/INPUTS
    t ->time of drift in generations
    na ->effective size of population A
    nb ->effective size of population B
    sa ->  size of sample from population A (# of individuals)
    sb ->  size of sample from population B (# of individuals)
    sahomo ->  # of recessive homozygotes in sample A
    sbhomo ->#  of recessive homozygotes in sample B
    ldhnullt -> used by FindMinimum.  It ends up being the
                mle of the ancestral frequency under the
```

```
                hypothesis of common origin *)
(
t  =  14;
sb  = 130;
sbhomo = 8;
sa  = 120;
sahomo = 0;
ldha = 1 (* THE MAX OF THE LIKELIHOOD FUNCTION IS 1 *)  ;
ldhb = NIntegrate[ ( 2*Pi*(t/(8nb)) )^(-.5)  *
    Exp[-((x-ArcSin[Sqrt[ Sqrt[sbhomo/sb] ]])^2)/(t/(4nb))] *
    Binomial[sb,sbhomo]  *
    ((Sin[x])^(4*sbhomo))  *
    (1- ( (Sin[x])^4 ) )^(sb-sbhomo),
    {x, 0 ,Pi/2},
    WorkingPrecision->5    ]  ;
ldht =  FindMinimum[ -1*
    NIntegrate[ ( 2*Pi*(t/(8na)) )^(-.5)  *
        Exp[-((x-ArcSin[Sqrt[ldhnullt]])^2)/(t/(4na))] *
        Binomial[sa,sahomo]  *
        ((Sin[x])^(4*sahomo))  *
        (1- ( (Sin[x])^4 ) )^(sa-sahomo),
        {x, 0 ,Pi/2},
        WorkingPrecision->5    ]  *
    NIntegrate[ ( 2*Pi*(t/(8nb)) )^(-.5)  *
        Exp[-((x-ArcSin[Sqrt[ldhnullt]])^2)/(t/(4nb))] *
        Binomial[sb,sbhomo]  *
        ((Sin[x])^(4*sbhomo))  *
        (1- ( (Sin[x])^4 ) )^(sb-sbhomo),
        {x, 0 ,Pi/2},
        WorkingPrecision->5    ]  ,
    {ldhnullt, {.1,.11}}    ];
ldhloglr = 2*Log[(ldha*ldhb)/(-1*ldht[[1]])];
PutAppend[{na,nb,ldhloglr,ldht[[2]]}, "ldhnofunout" ]
)
```