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Inbreeding and Its Consequences for Genetic Variation and Early Life History in Chinook Salmon

Shizhen Wang

A dissertation submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

University of Washington

2001

Program Authorized to Offer Degree: Aquatic and Fishery Sciences

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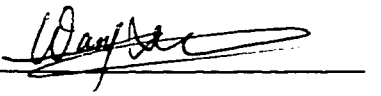
Signature 

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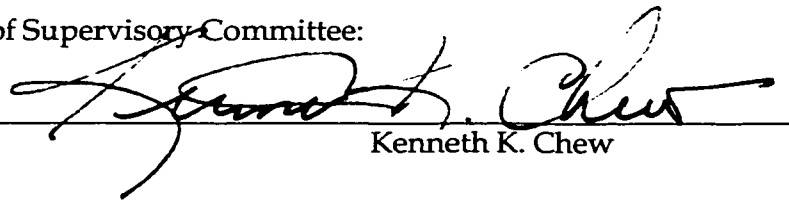

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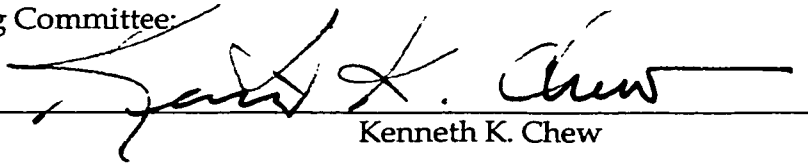

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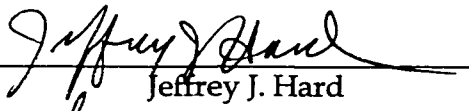

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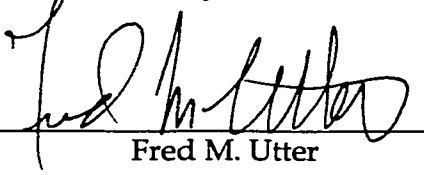

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Abstract

Inbreeding and Its Consequences for Genetic Variation and Early Life History in Chinook Salmon

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As a necessary background for my experimental studies, I reviewed inbreeding and its consequences and the relationship between genetic variability and fitness in salmonid fishes. Inbreeding directly reduces individual heterozygosity and can reduce genetic variation within populations through the loss of genotypes. Although inbreeding occurs naturally, much of the evidence for inbreeding stems from direct or indirect results of human activity. The potential consequences of inbreeding highlight the importance of maintaining genetic diversity in salmonid populations. A relationship between genetic variability and fitness varies both among and within species. The genetic bases of inbreeding depression and the relationship between genetic variability and fitness are complex, and quantification of the genetic effects of inbreeding over the entire life cycle remains challenging.

My experimental studies examined the effects of inbreeding in chinook salmon bred and reared under controlled cultured conditions. Using progeny of unrelated, half-sib and full-sib mating designs, data were collected on genotypic and allelic variation and survival rates for two different broods. Data on a measurement of developmental stability, fluctuating asymmetry (FA), were collected for one brood. The average

heterozygosity at 10 DNA microsatellite loci per family decreased significantly with inbreeding in both broods and changes were in accordance with theoretical expectations. No different viability between homozygotes and heterozygotes by the stage of sampling was detected. The average number of alleles at all loci per family also decreased with inbreeding although the decreases were significant for only one brood. Survivals to eyeing, hatch and alevin were higher in offspring from unrelated matings than from both half-sib and full-sib matings although the differences were significant between only unrelated and half-sib matings in one brood. No linear relation between depression in survival rate and levels of inbreeding was detected. Consistent increases with inbreeding in FA were detected for three out of eight meristic characters. These changes were, however, not significant. Although low power due to limited number of families within each mating group appeared to contribute to the failure to detect an effect of inbreeding on FA, the trends suggest that developmental instability is likely to increase under inbreeding. These initial studies provide a foundation for insights into the effects of further inbreeding through subsequent inbred generations of these lineages.

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ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to Drs. Ken Chew, Bill Hershberger, Jeff Hard, Fred Utter, Paul Bentzen, and Walt Dickhoff for advice and especially to Drs. Jeff Hard and Fred Utter for helpful reviews of earlier drafts of this dissertation. The author also wishes to thank Jerry (Jianrong) Chang, Ted Parker, Kathleen Neely, Vicki Hawk, Linda Park, Brandon Chasco, Mark Tetrick, Glenn Yokoyama, Na (Michael) Li, Wei Xia, Pam Jensen, Mike Canino, personnel in Marine Molecular Biotechnology Laboratory and personnel in the Grovers Creek Hatchery for assistance with the experiment and data collection, and Qingyuan Lou for help in data analyses. This study was supported by funding from the Joint Institute for the Study of the Atmosphere and Ocean, Lauren R. Donald scholarships, Bonneville Power Administration, and National Marine Fisheries Service. This dissertation would never have been completed without the encouragement and devotion of my wife, Ping Yuan.

CHAPTER 1: LITERATURE REVIEW

Part 1: Salmonid Inbreeding

Introduction

A fundamental assumption underlying the potential importance of genetic diversity in conservation of endangered species and captive populations is that loss of genetic diversity reduces the capability for adaptation and increases the risk of extinction (e.g., Frankham 1995a; Caughley and Gunn 1996; Avise and Hamrick 1996; Landweber and Dobson 1999). Inbreeding directly decreases individual heterozygosity and can reduce genetic variation within populations through the loss of genotypes. In particular, increased inbreeding is often associated with a reduction in mean phenotypic value of one or more traits with respect to fitness. This phenomenon, known as inbreeding depression, is generally considered to be a serious threat to the viability of small captive populations (Ralls and Ballou 1983; Charlesworth and Charlesworth 1987; Lande and Barrowclough 1987; Ralls et al. 1988; Hedrick and Miller 1992; Sheffer 1999). Thus, avoidance of inbreeding and maintenance of genetic variation are considered primary genetic issues within conservation biology (Loeschcke et al. 1994).

The declining runs and reductions in abundance of wild salmonids represent a conservation crisis of enormous biological, economic and political significance in the western United States (Hedrick and Miller 1994). These downward trends have led to listings under the U.S. Endangered Species Act (ESA) of several salmonid species involving chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*Oncorhynchus nerka*), steelhead (*Oncorhynchus mykiss*) and recently bull trout (*Salvelinus confluentus*) and cutthroat trout (*Oncorhynchus clarki*) in a variety of regions (Knudsen et al. 2000). The problem is far from limited to Pacific salmonids and the western United States, with many native salmonid populations and species presently threatened with extinction throughout the northern hemisphere (Allendorf and Waples 1996; Laikre 1999). Although preservation of threatened salmonids depends primarily on the protection of the species and

maintenance of adequate natural habitat, long-term survival also depends on a diverse and rich store of genetic variation because of their complex life histories (NRC 1996). Maintenance of genetic diversity has therefore been one of the main goals in salmonid fishery management and conservation (Waples 1990a, b; Ryman 1991; Ryman and Laikre 1991; Waples and Do 1994; Ryman et al. 1995; Allendorf and Waples 1996). With a focus on salmonids, this paper addresses theoretical bases and empirical evidence for inbreeding, and the maintenance of genetic diversity in wild and cultured populations.

Concept and Measurement of Inbreeding

Inbreeding in population genetics has different meanings corresponding to different situations (Templeton and Read 1994). At the individual level, inbreeding, as indicated by the word, simply means the mating of relatives (or one individual in the case of self-fertilization). At the population level, inbreeding is defined as the mating of individuals that are more closely related than they would be if they had been chosen at random from the population. Inbreeding can occur in populations either as a directed or a random process, and it occurs in populations of all sizes.

Related individuals have one or more recent ancestors in common, thus sharing that proportion of their genes received from these common ancestors. Genes descended from the same gene copy are often called identical by descent (IBD). The level of inbreeding of an individual is usually measured by the inbreeding coefficient (f), the probability that two alleles at a locus are IBD in that individual (e.g., Gall 1987). When detailed information on the relationships of individuals over generations and the pathways of inheritance are available, the respective f values of individuals can be calculated by pedigree analysis. Various methods for computing inbreeding coefficients from pedigrees are available. One of the commonly used techniques is path-analysis. From Wright (1951), the general formula for the coefficient in inbreeding of any individual I is

$$f_I = \Sigma\left[\left(\frac{1}{2}\right)^n (1 + f_A)\right] \qquad (1)$$

where n is the number of individuals in the pathway between individual I and common ancestor A (including the parents but not I itself), and f_A is the inbreeding coefficient, summed over all independent pathways. Note that the coefficient of inbreeding of an individual is dependent upon the amount of inbreeding that has occurred in previous generations. A detailed introduction to calculating f by path-analysis can be found elsewhere (e.g., Ballou 1983; Boyce 1983). The method of path-analysis is fast, but becomes complicated for complex expanded pedigrees and when many inbreeding coefficients need to be calculated (Ballou 1983; Hedrick and Miller 1992).

A second technique often used to calculate inbreeding coefficients is the additive matrix method (Quass 1976; Ballou 1983; Boyce 1983). This method is considerably easier for resolving complex pedigrees. It is useful for breeding plans because of its capabilities for calculating inbreeding coefficients for large numbers of hypothetical offspring and for determining the proportion of founder genes present in descendants (Ballou, 1983). With the rapid development of computers and software, it is also possible to estimate inbreeding coefficients for complicated pedigrees with Monte Carlo estimation (Thompson 2000). These methods for computing inbreeding coefficients from pedigrees assume selective neutrality so that the transmission probabilities of genes can be calculated from Mendelian ratios (Charlesworth and Charlesworth 1987).

The analysis of pedigrees has important implications in the genetic management of captive or hatchery populations. In addition to calculating levels of inbreeding of individuals, founder contributions in a current population and the probability of each possible distribution of founder alleles in the descendant population can also be calculated through pedigree analysis (Ballou 1983; Thompson 1986). Individuals that carry founder alleles within captive populations (Ballou and Lacy 1995) or individuals

with high probabilities of possessing single-copy alleles (Geyer et al. 1989) can be identified through pedigree analysis. With this genetic information of the population, subsequent breeding recommendations can be made to maximize the survivorship of founder alleles - an important goal of any captive-breeding program (Lacy et al. 1995). Moreover, it is possible to determine the inbreeding effective size (N_e) more accurately with pedigree data on a managed population (Templeton and Read 1994).

However, a major problem when investigating inbreeding and its effects in salmonid populations is that information on the inbreeding history is generally inadequate. This limitation often makes the use of pedigree analysis infeasible for calculating inbreeding coefficients of individuals within the population (Hard and Hershberger 1995). As such, an average inbreeding coefficient (average probability of IBD), F, in a population is often used in practice instead. Formuli found in the literature for calculating average inbreeding coefficients in a population are derived from an "idealized" finite population of N diploid individuals, equal numbers of males and females that all have equal reproductive capacity, random mating, constant abundance over time and discrete generations (e.g., see details in Gall 1987; Templeton and Read 1994; Caughley and Gunn 1996). According to Falconer and Mackay (1996), the average inbreeding coefficient of a population at generation t is given by

$$F_t = \frac{1}{2N} + F_{t-1}\left(1 - \frac{1}{2N}\right) \qquad (2)$$

By assuming F of the base population is zero and recurring over generations, formula (2) gives the form:

$$F_t = 1 - \left(1 - \frac{1}{2N}\right)^t \qquad (3)$$

Formula (3) simply means that the expected increase of inbreeding per generation in a population of constant size, ΔF, is $1/2N$. For real populations (non-idealized conditions) in which the above presumptions rarely apply, it is necessary to substitute N with inbreeding effective population size, N_e; i.e., that of an ideal population which would have the same expected increase of inbreeding as the real population (Crow and Kimura 1970). Effective population size provides a means to measure the rate of loss of genetic variation due to genetic drift (see discussion below) and inbreeding and is a key parameter in evolutionary and quantitative genetics (Caballero 1994).

A variety of ways exists for real populations to depart from the ideal populations and, hence, to affect N_e. These include unequal sex ratios, differential reproductive success, producing variation in family size, overlapping generations, fluctuations in population size (e.g., Lande and Barrowclough 1987; Felsenstein 1995; Ryman 1997), as well as dispersal distances and patterns that can affect the randomness of breeding (Caughley and Gunn 1996). An additional factor that also controls the random component of inbreeding and influences N_e is assortative mating (preferential mating based on phenotypic traits, Waser 1993a). Moreover, migration, mutation, selection and linkage of the locus in question to nearby loci subject to selection also change N_e (e.g., Felsenstein 1995). The methods of calculating N_e are described in details elsewhere (e.g., Crow and Kimura 1970; Caballero 1994; Felsenstein 1995). Estimates of inbreeding through estimates of N_e are highly sensitive to departure from ideal conditions, a fact that generates skepticism of the value of this procedure (e.g., Caughley and Gunn 1996). But, in the absence of pedigree records or breeding practices that can be used to reconstruct them, this method of approximation provides valuable tools for estimating levels of inbreeding and the rate that inbreeding accumulates in a population (Hard and Hershberger 1995).

As noted in the above discussion, f is used when a reference is made to a particular individual within a population, and F is used when a reference is made to a population of individuals. In applications, f is the expected proportion of all loci of that

individual which are IBD homozygous and F is the expected proportion of individuals in the population that are IBD homozygous at a particular locus (Franklin 1977, cited by Gall 1987). The accuracy of estimates of f depends on the number of loci measured and the linkage relationships among these loci. The accuracy of estimates of F depends upon the number of individuals surveyed. Although the inbreeding coefficient in all its applications has a single theoretical basis (Franklin 1977), F and f are two distinct meanings of the inbreeding coefficient and should be used accordingly (Gall 1987, Templeton and Read 1994). However, with additional information, pedigree-based fs may be used to make inferences about the population. Gall (1987) argued that the average of f-values calculated for all individuals in a population could be used as an estimate of inbreeding for the population, F. In addition, an empirical study shows that coefficients of inbreeding may not be consistent for different alleles or for different loci (Brown et al. 1975). This variability is likely caused by the differential responses of different alleles or loci to natural selection. For a similar reason, inbreeding coefficients estimated from molecular markers may not be consistent with those computed from pedigrees owing to the assumption of selective neutrality underlying the computations from pedigrees (e.g., Hershberger et al. 1990).

Inbreeding caused by population subdivision is attributable to the combined effects of mating between close relatives and restricted population number (Crow and Kimura 1970). In the case of population subdivision, a hierarchical series of f values, namely f_{IS} and f_{ST}, is often defined to estimate contributions to inbreeding from consanguineous mating (mating of close relatives) within subpopulations and from division of the population into subpopulations, respectively. Here f_{IS} is simply the inbreeding coefficient of individual I within a subpopulation. Its inbreeding coefficient within the whole population, f_{IT}, is given by

$$f_{IT} = f_{ST} + (1 - f_{ST}) f_{IS} \qquad (4)$$

More details on this topic can be found in Crow and Kimura (1970).

The basic impact of inbreeding on the genetic constitution of a population is an altered distribution of genotypes toward fewer heterozygotes and more homozygotes. The main effect of inbreeding related to conservation biology is, however, inbreeding depression since it increases the risk of short-term extinction (Frankham 1995a; Saccheri et al. 1998; Bijlsma 2000) as well as reduces the long-term viability in the absence of effective migration. Inbreeding reduces genetic variation through selection and inbreeding depression is a result of the rearrangement of alleles from the heterozygous to the homozygous state following inbreeding. Thus, inbreeding is significant to the conservation of endangered species and populations.

Since inbreeding increases the chance of sharing of parental genes, it is expected that inbreeding increases the amount of homozygosity and decreases the amount of heterozygosity. Single-locus neutralist theory says that the expected heterozygosity (H_t) retained within a population after t generations is

$$H_t = (1 - F_t)H_0 \qquad (5)$$

where H_0 is the initial heterozygosity of the population (Crow and Kimura 1970). This equation indicates that heterozygosity is expected to decline linearly with the average inbreeding coefficient. Furthermore, replace F_t with equation (3), and we have

$$H_t = (1 - \frac{1}{2N_e})^t H_0 \qquad (6)$$

Thus, heterozygosity of a population in each generation decreases by a fraction of $1/2N_e$. This reduction is in concert with the conclusion we made from equation (3) that the expected increase of inbreeding per generation, ΔF, is $1/2N_e$. Equations (3) and (6) are also measures of the evolutionary impact of genetic drift upon a population (Templeton and Read 1994). Genetic drift is a random change in the frequencies of alleles or

haplotypes due to accidents of sampling caused by random variation in rates of survival or reproduction by different genotypes (Futuyma 1997).

The differences and relationship between inbreeding and genetic drift have been discussed in many publications (e.g., Hartl and Clark 1997; Futuyma 1997). In a subdivided population, inbreeding alters genotypic frequencies of the total population, but does not alter allele frequencies, whereas random genetic drift changes allele frequencies. The change in genotype frequencies by inbreeding is temporary and can be reversed by outbreeding among subpopulations in subsequent generations. Loss of alleles caused by random genetic drift is permanent, however, and cannot be recovered barring mutation and exogenous gene flow. Notwithstanding these differences, equations (3) and (6) show that inbreeding and random genetic drift occur through the same process. In random mating populations both mechanisms occur simultaneously, along with concomitant changes of both genotypes and allele frequencies.

Equation (6) suggests that the magnitude of effective population size determines the effects of both random genetic drift and inbreeding and, in addition, optimizing effective population sizes can effectively minimize an immediate loss of heterozygosity or genetic variation. Adequate effective population size is required for endangered species not only to minimize the effects of random drift and inbreeding over the short term, but also to retain evolutionary potential in the long term (Caballero 1994; Franklin and Frankham 1998). However, there is controversy over the effective population size required for such purposes, with proposed sizes ranging from 50 to 5,000 (see Frankham 1995a; Franklin and Frankham 1998) or 10% of census population sizes (Frankham 1995b). In salmonid fishes, corresponding to the concerns of effective population sizes arising from the practice of fish culture and supplementation programs, minimums of 100 (Kincaid 1983) and 200 (Allendorf and Ryman 1987) parents (1:1 sex ratio) per generation have been proposed for these programs. Similarly, a minimum effective number of breeders per year of 100 has been suggested to reduce the rate of loss of rare alleles (Waples 1990a).

Mendelian markers can be used to calculate the expected heterozygosity in a system of mating where inbreeding coefficients can be readily computed, as is the case for regular systems of inbreeding (Crow and Kimura 1970; Gall 1987). In a system of mating within a population, the expected heterozygosity at a single diallelic locus (in the absence of mutation, migration and selection) is

$$H = 2pq(1 - F) \qquad (7)$$

where p and q are allelic frequencies (Crow and Kimura 1970). F measures the average decrease in heterozygosity of the population. When F equals to zero, we can recognize H in equation (7) as the expected frequency of the heterozygote under single-locus Hardy-Weinberg equilibrium. Equation (7) also indicates that, unlike inbreeding caused by restricted population sizes where the loss of heterozygosity within the population is permanent, as soon as random mating occurs, F returns to zero and the original heterozygosity is restored (Crow and Kimura 1970). In real populations, natural selection may modify the distribution of predicted genotypic frequencies. Theoretical studies have shown that inbreeding can result in a level of heterozygosity higher than predicted through heterozygote advantage (Hayman and Mather 1953; Reeve 1955), associative overdominance (i.e., linkage to overdominant loci, Bartlett and Haldane 1935; Reeve and Gower 1958), or linkage to recessive deleterious alleles (Ohta 1971; Wang and Hill 1999). Slower than predicted declines in heterozygosity under inbreeding have been observed in chickens (Mina et al. 1991), fruit flies (Frankham et al. 1993; Rumball et al. 1994; Latter et al. 1995), flour beetles (Goodwill and Enfield 1971; Yeh and Scheinberg 1972), mice (Connor and Bellucci 1979), mosquitoes (Munstermann 1994) and rats (Eriksson et al. 1976), as well as in plants (Strauss 1986). Empirical monitoring of systematic inbreeding with Mendelian markers has been very limited in salmonids (Hershberger et al. 1990), and little is yet known of the relationship of observed to expected heterozygosity in populations undergoing systematic inbreeding.

The genetic basis for the phenomenon of inbreeding depression has been explained by two competing hypotheses (Charlesworth and Charlesworth 1987). The dominance hypothesis argues that inbreeding depression is caused by the expression of recessive deleterious alleles in homozygous individuals. Such alleles arising from mutation are commonly masked in expression by dominant alleles in the heterozygous state, and are expected to persist at low frequencies unless mutation rate is very high (Lynch and Walsh 1998). Inbreeding increases the occurrence of homozygous individuals which are purged through selection, and severely inbred populations surviving this purging are expected to be resistant to further inbreeding impacts. This process can be an effective tool for maintaining long-term fitness (Lacy 1992; Hedrick 1994), and has been shown to be most effective under slow rates of inbreeding (Ehiobu et al. 1989; Latter et al. 1995).

However, purging has not been effective for all animals (e.g., Ballou 1997) and plants (reviewed by Byers and Waller 1999) or in all environments (Bijlsma et al. 1999; Dahlgaard and Hoffmann 2000). Its use has been proscribed for species with high numbers of lethal equivalents (see Charlesworth and Charlesworth 1987 for a definition; Oosterhout et al. 2000). Factors underlying the complex basis of inbreeding depression including rate of inbreeding and genetic drift, pattern and strength of selection, reproductive capacity and genetic structure of the species reflect the presently incomplete understanding of the process of purging (Hedrick 1994; Wang 2000). Furthermore, enforced purging increases the probability of extinction when the genetic load reflects deleterious alleles of small effect (Hedrick 1994; Wang 2000). These combined complications suggest that additional factors may contribute to inbreeding depression, and mandate caution when considering purging as a conservation measure for endangered species.

This caution leads to a second explanation for inbreeding depression of overdominance or heterozygote advantage. As noted above (equation (7) and related discussion), higher fitness of heterozygous individuals increases heterozygous

frequencies above expected values. Contrary to a purging effect under dominance scenarios, allelic loss for overdominant loci lowers the mean fitness of the populations (Charlesworth and Charlesworth 1987), where the concept of overdominance extends to favorable epistatic interactions among loci (i.e., coadapted gene complexes) as well. These two very different evolutionary implications for inbreeding impact are further confounded by the difference in the contribution of individual loci. The loss of fitness imposed by inbreeding for only a few overdominant loci equals the gain arising from a much larger number under dominance (Lynch and Walsh 1998). A problem in determining which, if either method would be applicable to a particular breeding program is the need for additional information (e.g., mutation rate to deleterious alleles, estimates of overdominance from inbred lines). Although the advent of molecular genetic markers has provided valuable new tools for probing inbreeding depression (Karkkainnen et al. 1999; also see Lynch and Walsh 1998 for a more complete review), the unambiguous distinction of overdominant or dominant mechanisms of inbreeding depression remains challenging (Lynch and Walsh 1998; Bierne et al. 2000).

The phenomenon of outbreeding depression reflects breakdown of local adaptations through hybridizations. Two types of outbreeding depression may affect hybridized progeny from parents of diverged lineages. The first category concerns local adaptations that result in native populations being better adapted than are either introduced or hybridized groups. The second category concerns the interactions of sets of genes affecting a specific adaptation that may differ between two diverged lineages (Templeton 1986; Lynch 1991; Lynch and Walsh 1998). However, the loss of favorable overdominant alleles or breakdown of advantageous epistatic interactions under inbreeding depression indicates a connection of inbreeding depression to the second kind of outbreeding depression.

Empirical Observations

Behavioral mechanisms

Inbreeding in wild populations generally occurs either as a consequence of adaptive response to local conditions or from non-adaptive constraints arising from genetic, morphological, behavioral, ecological or combined factors (Shields 1993). In salmonids, innate behavioral characteristics such as strong homing and distinct timings of spawning provide good opportunities for inbreeding through restricting sizes of spawning aggregations (Allendorf and Waples 1996). Some degree of inbreeding in salmonids arising from these tendencies presumably plays a useful role optimizing local adaptations. Indeed, close inbreeding could be an inevitable mechanism for the development and preservation of highly adapted gene complexes (Shields 1982; Selander 1983; Partridge 1983; but see Hedrick 1984). These behaviors are reflected in the often substantial genetic differences among conspecific salmonid populations representing different localities (Ryman 1983; Ståhl 1987; Foote et al. 1989; Utter et al. 1989; Myers et al. 1998; Teel et al. 2000) or run timing (Tallman 1986; Phelps et al. 1994; Myers et al. 1998).

Reinforcement of isolation in ensuing generations is initiated through mechanisms of kin recognition (e.g., odors) that promote aggregations such as schooling (Courtenay et al. 2001). Separation is reinforced through positive assortative matings among individuals sharing a phenotypic trait. Increased levels of inbreeding arising from such matings also increase the frequencies of advantageous alleles affecting adaptive traits (Foote 1989). Size assortative mating has been observed under natural conditions in brook trout (*Salvelinus fontinalis*) (Blanchfield and Ridgway 1998), coho salmon (Chebanov 1990) and sockeye salmon (Foote and Larkin 1988), as well as experimentally in chum salmon (Chebanov 1979), pink salmon (*Oncorhynchus gorbuscha*) (Chebanov 1984) and sockeye salmon (Henson and Smith 1967). The concept of assortative mating also extends to the above noted subgroup formations in time and space where recently colonized regions have provided new insights into the dynamics of

subdivision formation and maintenance (Hendry et al. 2000; Quinn et al. 2000; Utter 2000).

The above examples support some advantages of low levels of inbreeding in subdivided populations (Chesser and Ryman 1986) through balances between (1) offspring viability and increased genetic variability, and (2) the costs of inbreeding depression (discussed below). To counteract the cost of inbreeding while not losing its benefits, the idea of an optimal balance between inbreeding and outbreeding (Shields 1982; Bateson 1983; Waser 1993b) is attractive for salmonids. Early maturing males that are smaller than normally matured males are well known in most salmonid species. Using alternative mating tactics in competition with normal males, successfully spawning early maturing males counteract the cost of inbreeding resulting from size-assortative mating (Foote et al. 1997; Thomaz et al. 1997). Limited gene flow among local subgroups provides another balancing mechanism between excessive inbreeding and breakdown of local adaptations (e.g., Hendry et al. 2000). The ratio between intra- and inter-population components of gene diversity is thought to be maintained at an optimized stable level (Altukhov 1995). Such equilibrium may play an important role in the persistence of a metapopulation (a demographically closed unit that contains multiple open local populations (see NRC 1996 for further details).

Restricted effective population size

Despite well established theory, direct empirical evidence documenting inbreeding in salmonids from cumulative historical declines in natural population numbers is rare (e.g., Campton and Utter 1987). Reasons explaining such limited information include (1) the gradual occurrence of inbreeding over many generations, (2) unavailability of historical data, and (3) confounding effects of inbreeding with other factors such as selection and gene flow. Conversely, much evidence exists on the widespread decline of wild salmonid populations (NRC 1996). The complex life histories and long-range migration behaviors of anadromous salmonids make them particularly vulnerable to natural or human-caused stresses (Allendorf and Waples

1996). In the Pacific Northwest, overfishing and loss of spawning and rearing habitats were cited as the primary causes for the extinction of an estimated 100 stocks of anadromous salmonids and the at a risk status of another 200 stocks (Nehlsen et al. 1991).

Such declines have promoted supplementation through artificial propagation to reestablish or increase the abundance of naturally reproducing populations to mitigate these losses of natural salmonid populations (Hard et al. 1992). A consequence of supplementation is supportive breeding when a fraction of the wild parents (or their offspring) is brought into captivity for reproduction or preferential survival, and the offspring are released into the natural habitat where they mix and randomly breed with their wild counterparts (Ryman and Laikre 1991). A risk accompanying supportive breeding is the reduction in post-supplementation effective population size that results from an inflated variance of family size of the combined wild and cultured population. Risks from supportive breeding are particularly high when enhancement is successful due to the higher rate of inbreeding resulting from the decreased inbreeding effective size of the post-supplemented population (Ryman 1994). Simulations by Waples and Do (1994) indicated that all genes in a successful and continuing supplementation population will ultimately be derived from captive broodstock (see also Ford in press). The consequences of such preferential augmentation of a small portion of the total population were anticipated in a restoration program for winter-run chinook salmon in the Sacramento River, California (Hedrick and Hedgecock 1994); consequently a breeding program seeking to maximize the effective size by equalling contributions of captive spawners did not decrease effective size of total wild-culture population (Hedrick et al. 2000). Effects of supportive breeding have also been reported in land-locked Atlantic salmon (*Salmo salar*) (Tessier et al. 1997).

Many data indicate that hatchery fish make up the majority of many salmon runs (e.g., NRC 1996). Factors in addition to supportive breeding that relate to numbers of fish released and interactions between cultured and wild fish contribute to the reduction

of wild populations and their displacement with hatchery fish (Hard et al. 1992; see also Wertheimer et al. in press; Hilborn and Eggers in press). Despite hatchery-reared fish possibly being generally less adapted to the natural environment than wild fish (e.g., Shrimpton and Randall 1992; Jonsson 1997; Unwin 1997), massive releases and resulting high returns create the potential for the displacement of wild populations by hatchery fish. In addition, the changed patterns of competition, predation, disease, overharvest of wild populations caused by cultured release have also contributed to the reduction of natural populations (Waples 1991; Utter 1998). Given the limited capacity of spawning and rearing habitat (Cooney and Brodeur 1996; Heard 1998), a considerable likelihood exists for the reduction of natural populations as a result of combined effects from all of these factors. Because a considerable degree of uncertainty exists over the effects of hatchery programs on wild fish, it is essential that salmon managers take a proactive and risk-averse approach to managing wild salmon populations in the presence of artificial propagation (Hard 1995a; Busack and Currens 1995). The goal of artificial propagation should be clearly defined at the outset of any propagation program (Waples 1999).

Genetic population structure indicated by molecular markers in numerous studied species differs along broad-scale geographic gradients because of historic patterns of dispersal and gene flow (Avise 1994). Natural salmonid populations can subdivide into genetically different groups because of prolonged isolation as a result of historical biogeographic and demographic events (e.g., Wood 1995; Burger et al. 1997). Such geographic structuring is further influenced where these subdivisions have been affected by human-imposed fragmentations arising from migratory, rearing and spawning restrictions. A notable example in the Pacific Northwest is the construction of hydroelectric dams on the Columbia River that impede or totally block several salmonid runs, with a resultant loss of diversity within and among populations (e.g., Utter et al. 1995). Random genetic drift has played an important role in the process of genetic divergence within these subpopulations along with other evolutionary forces of selection, migration and mutation. As with other situations involving restricted

population sizes, addressing issues of inbreeding is challenging in these subdivisions because of its gradual occurrence coupled with non-existing or very limited data. The largely unknown effects of selection within subdivisions complicate interpretations of inbreeding (Gall 1987). Despite the potential importance of inbreeding in these hierarchical structures of salmonid populations, its effects remain poorly understood.

Operational problems in aquaculture

Often, the potential occurrences of inbreeding in salmonids come from management and operational practices of aquaculture, where inbreeding may arise from almost every operational step of hatchery or captive broodstock programs (Campton 1995; Hard and Hershberger 1995). Major factors that can give rise to inbreeding include insufficient number of breeders, selection of "superior" (e.g., large body size as in the case of positive assortative matings), similar selection of a segment of the total run time (Kincaid 1983; Flagg et al. 1995), inappropriate spawning practices such as pooling of gametes (Tave 1986), and unequal parental representation of progeny released to the wild or samples as broodstock (i.e., variance of family size, Simon 1991; Kincaid 1995). Salmon breeders often use very low numbers of broodstock to produce the next generation, simply because of the high reproductive rate of captive breeders. This practice often leads to an increase in levels of inbreeding in the ensuing generations compared with wild populations (e.g., McElligott et al. 1987; Verspoor 1988).

The spawning procedures used in hatcheries also affect the inbreeding levels of the ensuing generations through alteration of N_e. One way to maintain an effective population size equal to the broodstock number is to spawn randomly one female with one male. However, this rule is often not followed in many hatcheries (Kincaid, 1995) due to reasons such as difficulty in obtaining broodstock, unbalanced sex ratios, and limitation of facilities and resources including management costs. Consequently, eggs are often combined with semen from several males, pooled either sequentially or prior to fertilization. Several studies have shown that both pooling techniques can reduce N_e (Gharrett and Shirley 1985; Withler 1988). Differences in potency (fertilizing ability)

among males lead to unequal parental representation (Gile and Ferguson 1990) and a greater variance among males than would result from single pair crosses (Withler and Beacham 1994). In contrast, a factorial mating design in which each male fertilizes an equal share of egg batches from each female is recommended to minimize inbreeding effects (Withler and Beacham 1994; Waples and Do 1994). Simulations of cultured salmonid populations under selection (McKay and McMillan 1991) demonstrated that factorial matings always produce less inbreeding than either sperm pooling or pairwise matings.

Hatchery operations may cause genetic changes to hatchery fish themselves as well as to wild populations. These possible genetic changes include loss of genetic variation in hatchery populations (see discussion below), loss of overall-population (hatchery and wild) genetic variation due to interbreeding between hatchery and wild fish following supplementation, and reduced fitness in supplemented populations. These effects are closely correlated with potential inbreeding during hatchery operations. Other effects of hatchery operations that may be less related to inbreeding are loss of among-population variability due to egg and fish translocations and interbreeding and domestication due to artificial selection and cultivation. A detailed discussion of these effects can be found in Busack and Currens (1995), Campton (1995) and Reisenbichler and Rubin (1999). However, studies of effects of hatchery fish on wild populations are often difficult due primarily to natural spawning by hatchery fish and interbreeding between hatchery and wild fish (Campton 1995) and lack of historical data on wild populations prior to hatchery operations. Even though there are some studies available (see Hindar et al. 1991; Waples 1991; Utter et al. 1993a; Campton 1995; Utter 1998), our knowledge of the effects of hatchery fish on wild populations is still shallow. Further studies are required to adequately understand the genetic interactions between wild and hatchery fish.

The harmful effects of uncontrolled inbreeding do not warrant concluding that inbreeding should always be avoided. Inbreeding is actually one of the most valuable

tools in controlled breeding programs (Tave 1986, 1991). One use of controlled inbreeding is line breeding where an outstanding individual (usually a male) is mated with its descendants in order to increase its contribution to the gene pool. A second use is to create inbred lines that will be hybridized to produce F_1 hybrids to fix certain alleles of interest. Inbred lines can also be used for other purposes such as mapping and characterizing quantitative trail loci (QTLs, Lynch and Walsh 1998). In addition, controlled inbreeding provides a valuable tool in experimental studies of genetic effects of inbreeding. For example, Kincaid (1976a, 1976b, 1983) and Gjerde et al. (1983) have used half-sib and full-sib matings in order to study the effects of inbreeding of different levels on the performance of rainbow trout (*Oncorhynchus mykiss*).

Monitoring loss of genetic variation

Reduced heterozygosity correlated to finite population size has been found in many salmonid populations. Major data came from the practices of introduction of new species or populations to new areas or establishment of cultured populations from the wild. For example, hatchery stocks of Atlantic salmon have been reported to have, on average, up to 20-30% less heterozygosity than wild populations based on allozyme data (Cross and King 1983; Ståhl 1983; Verspoor 1988). A study of hatchery cutthroat trout showed a similar reduction (Allendorf and Phelps 1980). Furthermore, as discussed above, under certain conditions gene flow from hatchery to native populations can lead to a rapid decrease in effective population size. Consequently, ultimate erosion of genetic variation in the mixture of hatchery and wild populations could be observed in supplementation is based on insufficient numbers of adults (e.g., Vuorinen 1982). A similar effect of inbreeding and random genetic drift due to historically insufficient population number was also found in native populations. For example, Prodoehl et al. (1997) have shown that, contrary to the reported high levels of polymorphism from a wide range of distributions, two populations of brown trout (*Salmo trutta*) from northwest Scotland are genetically completely monomorphic as a result of continual low effective population sizes or severe repeated bottlenecks.

Heterozygosity is often regarded as a relatively insensitive indicator of loss of genetic variability, compared with the loss of rare alleles or allele frequency fluctuations between cohorts when average heterozygosity levels were the same for comparable wild and hatchery groups. For example, a few studies have revealed that reduced genetic variability in cultured populations compared with the wild may be reflected in allelic diversity rather than overall heterozygosity (Danielsdottir et al. 1997; Tessier et al. 1997; Norris et al 1999; Winkler et al. 1999). A population's heterozygosity is primarily a reflection of alleles of high frequency with minimal contribution by low frequency alleles (e.g., Ryman et al. 1995), while low frequency alleles are most susceptible to loss through genetic drift (e.g., Waples 1990a). These tendencies are moderated under overdominance where allelic losses are retarded through greater fitness imparted by heterozygous advantage (Mitton 1993a, b). Heterozygosity and number of alleles are qualitatively similar as measures of the genetic variation of a population only if the number of progeny is Poisson distributed (Campbell 1995). Under such a distribution, heterozygosity is closely related to the breeding structure (inbreeding) of the population, whereas the number of alleles is more sensitive to the variation of the sampling process (genetic drift).

Most of the above-noted examples of reduced genetic variability are from hatchery populations because of their high potential for random genetic drift due to small effective population sizes (Busack and Currens 1995; Campton 1995). Except for remnant groups isolated by natural barriers where excessive drift has been reported (e.g., Campton and Utter 1987), evidence for reduced variability in wild salmonids is rare because of potential gene flow from other wild or hatchery populations.

Evidence of retained genetic variation in salmonids has reflected the failure to detect genetic losses in cultured broodstocks compared with source populations. For example, Ferguson et al. (1991) reported allelic and heterozygosity retentions at allozyme loci in rainbow trout and brown trout broodstock founded and propagated since the early 1980s. Similar findings were reported in seven out of eight rainbow trout

broodstocks (Allendorf and Utter 1979), and in European rainbow trout (Koljonen 1986) and Atlantic salmon (Crozier and Moffett 1989). Busack et al. (1979) interpreted retained polymorphisms in four California strains as a consequence of historical mixing of strains and possibly balancing selections (heterozygote advantage). These interpretations sound attractive since interbreeding following mixing of populations would lead to increased heterozygosities which would be maintained for extended periods than expected by drift through balancing selection (Hartl and Clark 1997).

A number of procedures for optimizing effective population size have been proposed (e.g., Simon 1991, Kincaid 1995). However, because effective population size can be affected by a number of factors, obtaining precise estimates of this parameter is often difficult. The development of molecular markers has promoted new processes for estimating effective population sizes of natural populations (Jorde and Ryman 1995; Neigel 1996; Schwartz et al. 1998) which have been applied to salmonids (e.g., Waples 1990b). The magnitude of detected reduction in genetic variability differs among genetic markers where, for instance, microsatellite loci with higher numbers of alleles (and thus heterozygosities) than allozymes are more sensitive to changes in effective population size (e.g., Mjolnerod et al. 1997; Reilly et al. 1999). Regardless of resolution, not all changes to the genetic structure of populations can be detected by molecular markers because of different evolutionary processes affecting adaptive traits (e.g., run timing, migration distance) and more neutral molecular markers (e.g., Grant et al. 1999; Allendorf and Seeb 2000).

Inbreeding depression

Inbreeding depression is the decline in the mean value of one or more traits with respect to fitness as a direct consequence of inbreeding. Similar to the hierarchical measurement of inbreeding, inbreeding depression can be measured by comparing the average phenotypic values between an inbred population and the base population from which it was derived (Lande and Schemske 1985; Gall 1987) or by comparing the average phenotypic values in offspring from matings between relatives with those from

random matings within the same population (Lynch 1988). Inbreeding depression is typically a laborious quantitative genetic problem to investigate because of the difficulties in establishing pedigrees. Consequently, an alternative method in which heterozygosity measured by genetic markers is correlated with a trait related to fitness is often used. The most common estimates of inbreeding depression involve traits that are closely related to fitness, such as life-history traits (e.g., viability of young, fecundity, hatchability) and morphological traits (e.g., body size, meristic counts of gillrakers). These traits are often connected with reproductive capacity or physiological efficiency and are most strongly affected by inbreeding (Falconer and MacKay 1996). A study on a wide variety of animal species including rainbow trout and channel catfish has shown that life-history traits show greater inbreeding depression than morphological traits (DeRose and Roff 1999). Numerical studies have reported inbreeding depression across a wide variety of species, from plants (Charlesworth and Charlesworth 1987) to animals (Ralls et al. 1988; Thornhill 1993). Inbreeding depression has been found in laboratory, zoo and other domesticated populations (see Shields 1993) and in the wild (Crnokrak and Roff 1999).

In salmonids, detection of inbreeding depression under controlled levels of inbreeding has been reported in rainbow trout, brook trout and Atlantic salmon (see Gall 1987; Waldman and McKinnon 1993; Hard and Hershberger 1995 for reviews). Recently, inbreeding depression in chinook salmon for early survival and early growth after one generation of close inbreeding has also been found by Hard and Hershberger (in press). Even though data on inbreeding depression have been accumulating (e.g., Su et al. 1996; Rye and Mao 1998; Pante et al. 2001), they remain inadequate to permit generalizations about all salmonid fishes owing largely to their different life histories. Indeed, results from different studies of the same species are not necessarily entirely agreeable even for a common increase in the level of inbreeding. For example, Su et al. (1996) observed no significant inbreeding depression in body weight of rainbow trout at early stages in agreement with Aulstad and Kittelsen (1971), Aulstad et al. (1972) and Gjerde et al. (1983). However, Kincaid (1976a,b, 1983) reported a moderate depression in

body weight at various age stages. In addition, a discrepancy was observed in egg survivorship among these studies.

Several points should be noticed from the previous studies on salmonids. First, all studies except Su et al. (1996) and Pante et al. (2001) have used methods that yield high levels of inbreeding in a single generation (e.g., half-sib or full-sib matings). Inbreeding depression may otherwise be undetectable under slow rates of inbreeding which is more typical in wild populations. Slow rates of inbreeding may account for the failure to detect the depression in body weight of early age stages in rainbow trout in Su et al. (1996). Experiments with fruit flies showed that slower rates of inbreeding lead to less inbreeding depression in populations at the same level of inbreeding (Ehiobu et al. 1989). In addition, a threshold relationship on the basis of inbreeding depression for fitness components may exist (Soulé 1980; Frankham 1995c). Thus, one should not expect inbreeding depression until inbreeding coefficients reach high levels or relatively high levels at rapid rates in salmonids. This conclusion is further supported by a comparison between studies by Gjerde et al. (1983) and Pante et al. (2001) in which slower inbreeding rates produced lower inbreeding depression in body weight of rainbow trout (see Pante et al. 2001 for details).

Second, the variability in estimates from different experiments probably results from the diverse background and genetic histories of the stocks studied (Gall 1987). The diverse backgrounds and genetic histories of individuals or stocks may affect the experiments in two ways. (1) Inbreeding coefficients are commonly estimated by assuming ancestors or base populations are completely outbred ($F_0 = 0$). Consequently, estimates of inbreeding coefficients may not reflect the cumulative inbreeding levels if the backgrounds and genetic histories of the stocks are very different. (2) The diversified regime of natural selection in different populations can produce different responses to inbreeding. Populations that survived substantial inbreeding are largely resistant to further inbreeding depression because of the purging of genetic load (Charlesworth and Charlesworth 1987; Barrett and Charlesworth 1991). These situations can apply to

individual populations of a metapopulation (Gall 1987) and different lineages within a single population (e.g., Pray and Goodnight 1995).

Third, the relationship between inbreeding depression and coefficient of inbreeding may vary among species and traits. For example, the relationship between inbreeding depression and coefficient of inbreeding was close to linear for body weight (Kincaid 1976a,b; Kincaid 1983; Su et al. 1996; Pante et al. 2001). The linear decline in the mean value of a fitness trait with an increase in the inbreeding coefficient has been observed in many other animals such as fruit flies (e.g., Latter and Robertson 1962), mice (e.g., Lacy et al. 1996), flour beetles (e.g., Pray and Goodnight 1995), and Speke's gazelle (Tempelton and Read 1983). But the relationship was non-linear for survival at an early stage (Kincaid 1976a, b; Kincaid 1983; Gjerde et al. 1983). In fact, the relationship between observed survival and inbreeding coefficient differed from the prediction of a general negative exponential model (see Kalinowski and Hedrick 1998). Perhaps this poor fit is not a surprise because the model was initially used for the viability from egg to adulthood (Morton et al. 1956). However, since survival rates up to adulthood were not given in these experiments, it is difficult to tell whether it was the poor fit of the model for rainbow trout or incomplete survival data that caused this deviation.

Fourth, inbreeding depression is believed to be environmentally dependent such that a population may suffer from inbreeding depression in one environment but not another (Pray et al. 1994). Jimenez et al. (1994) found more inbreeding depression on survivorship of mice reintroduced into a natural habitat than in the laboratory. Similarly, with fruit flies Latter et al. (1995) showed that under optimal conditions the estimated loss of fitness due to homozygosity throughout the entire genome was only about one third of the amount under competitive conditions. Typically, inbreeding depression is more severe in harsher environments (Miller 1994; Bijlsma et al. 1999) because of higher selective pressures. The impact of environmental stress becomes significantly greater for higher inbreeding levels (Bijlsma et al. 2000). Crnokrak and Roff (1999) also showed that estimates of inbreeding depression under natural conditions are

higher than in captivity following an investigation from a wide variety of species (but see Armbruster et al. 2000). However, due mainly to the complicated life history, studies of inbreeding in salmonids in the natural habitat are very difficult. The only available study in nearly natural habitat was done by Ryman (1970) in which he recaptured fewer inbred juvenile *Salmo salar* than outbred individuals from Swedish rivers into which they had been released. In addition, for similar reasons, studies of inbreeding in salmonids have largely been limited to nonanadromous fish (Hard and Hershberger 1995).

Finally, it is important to note that all studies in salmonids have considered one or more correlates of fitness, but not fitness. Frankham (1995b) suggested that studies that are based on individual fitness correlates underestimate the full effect of inbreeding depression as it is approximately three times greater for total fitness than for it components. On the other hand, genotypes that have an advantage in some fitness traits may have disadvantages for other traits affecting fitness (Allendorf and Leary 1986). Thus, inbreeding depression with respect to total fitness must be a synergistic outcome of all components of fitness. It is generally difficult to measure the total fitness and to estimate differences in fitness among genotypes in natural populations (Allendorf and Leary 1986). As a compromise, the effects of inbreeding on total fitness can be monitored with a trait that is highly correlated with overall fitness (e.g., lifetime breeding success, Slate et al. 2000). However, for populations with complex life histories (e.g., anadromous salmonids), this method is also difficult. Despite the difficulties in their measurement, correlations between heterozygosity and total fitness along with the differences in fitness among genotypes can contribute information valuable to the genetic conservation of endangered species. Management strategies based on observation of merely a single or few components of fitness may be inappropriate and may even potentially threaten the populations under consideration.

In salmonids, their tetraploid ancestry has made prediction of inbreeding depression even more complicated. Although duplicate genes have often lost their

expression (Allendorf and Thorgaard 1984), tetrasomic loci are "buffered" against the effects of genetic drift by having more gene copies (Allendorf and Thorgaard 1984; Allendorf and Waples 1996). The loss of heterozygosity due to inbreeding is also slower at a tetrasomic locus than at a disomic one (Allendorf and Thorgaard 1984). Hence, one would expect that salmonids should be relatively insensitive to inbreeding depression compared with other diploid fishes, although comparison across fish species would be difficult because of their distinct life histories and genetic backgrounds. On the other hand, if their tetraploid ancestry truly helped salmonids buffer against the loss of genetic diversity and inbreeding depression, the same logic suggests that purging a salmonid population of recessive deleterious alleles would have been more difficult at tetrasomic than at disomic loci. Thus, one would expect that salmonids might have relatively high genetic load, compared with diploid fishes. Consequently, purging a salmonid population of its genetic load should not be considered an effective conservation strategy.

Although it may be possible to predict the changes of fitness in the ensuing generations derived from parents from different origins (Emlen 1991), empirical reports of outbreeding depression are rare compared to those of inbreeding depression. Outbreeding depression in salmonids has been observed in crosses between even and odd year pink salmon of Auke Creek, Alaska in which the survival of F-2 hybrids was significantly reduced from that of the parental lines (Gharrett and Smoker 1991; Gharrett et al. 1999). However, outbreeding depression may be pervasive in crosses involving major lineages of anadromous salmonids. The strong resistance of indigenous populations to introgressions despite long histories of extensive introductions from exogenous lineages, including inadvertant crosses among them, has been interpreted as a manisfestation of outbreeding depression (Utter 2000).

Concluding Remarks

A number of sources of inbreeding in salmonids have been identified. While some inbreeding occurs naturally, most positive evidence has been related to direct or

indirect results of human activity. These genetic losses, in turn, indicate a general underestimation of the importance of genetic diversity in salmonid conservation. In particular, the lack of knowledge on interactions between cultured and wild fishes has resulted in genetic deterioration of natural populations. The overall productivity of salmonid fishes continues to decline in spite of increased releases of cultured fish (e.g., Hilborn and Winton 1993; Hilborn and Eggers 2000). While the decline is likely a synergistic outcome of many demographic (e.g., disturbance caused by overfishing, size-selection in salmonid fisheries), environmental (e.g., deterioration of rearing and spawning habitat) and genetic factors (e.g., inbreeding depression), genetic variation provides the basis for the potential of adaptation and, therefore, avoidance of inbreeding and maintenance of genetic variability should be taken into consideration in conservation and fishery management of salmonid as well as all other fishes.

Previous studies in salmonids have rarely attempted to address the genetic basis of inbreeding depression. Perhaps inbreeding depression should never be attributed exclusively to either the dominance or the overdominance hypotheses. As Mitton (1993b) pointed out, it is most likely that inbreeding depression is caused by a combination of mechanisms. In fact, the situation may be more complicated than can be predicted by either of these hypotheses. In contrast to the conventional interpretation of genetic basis of inbreeding depression based on the interactions within loci, Templeton and Read (1994) and Charlesworth (1998) pointed out that data from *Drosophila* have suggested that part of inbreeding depression may also result from synergistic or epistatic interaction among loci. Consequently, the effect of synergistic epistasis should also be considered when models of inbreeding depression and its elimination are considered.

Fitness of an individual is often sensitive to environmental changes (e.g., inbreeding depression may be observed in one environment but not necessarily in another). This phenomenon could compromise goals for conservation that are based on fitness alone (e.g., Kapuscinski and Lannan 1984, 1986; see Ryman 1991 for a discussion).

Genetic and phenotypic variation in quantitative traits determines the evolutionary significance of adaptive differences among units of intraspecific variation and thus are both important with respect to the conservation of intraspecific diversity (Hard 1995b). However, the genetic basis for maintaining the potential for adaptation is often more complex than what seems to be apparent. Precise quantification of genetic impacts in terms of fitness depression remains difficult. Consequently, even under the circumstances that lack a profound relationship between heterozygosity and fitness, maintenance of genetic variation between as well as within populations should be considered the primary goal in conservation and fishery management (Ryman 1991; Busack and Currens 1995).

CHAPTER 1: LITERATURE REVIEW

Part 2: Genetic Variation and Fitness in Salmonids

Introduction

A fundamental tenet of conservation of threatened and endangered populations and species is that loss of genetic diversity reduces adaptive capacity and increases risk of extinction (Franklin 1977; Soulé and Wilcox 1980; Frankel and Soulé 1981; Soulé et al. 1986; Soulé 1987; Loeschcke et al. 1994; Frankham 1995a; Caughley and Gunn 1996; Avise and Hamrick 1996; Landweber and Dobson 1999). Inbreeding leads to an immediate decrease in heterozygosity, reducing genetic variation within populations by eliminating genotypic combinations. Increased inbreeding is often associated with a reduction in mean phenotypic value of one or more traits with respect to fitness or performance, a phenomenon known as inbreeding depression. This reduction is thought to reflect increased homozygosity at loci influencing these traits, which typically include survival, growth, or reproductive success (Ralls and Ballou 1983; Ralls et al. 1988; Charlesworth and Charlesworth 1987; Lande and Barrowclough 1987; Hedrick and Miller 1992; Sheffer et al. 1999; Hedrick and Kalinowski 2000). Recognition of the consequences of inbreeding for fitness is at the foundation of attempts to conserve threatened and endangered salmonid populations (Waples 1990a,b; Ryman 1991; Ryman and Laikre 1991; Waples and Do 1994; Ryman et al. 1995; Allendorf and Waples 1996). However, a direct link between genetic variation and population viability and extinction risk is difficult to establish and, consequently, exceedingly rare for natural populations (e.g., Saccheri et al. 1998). Thus, geneticists must usually rely on indirect approaches.

The evidence that reduced genetic variability, as measured by heterozygosity, is inversely related to fitness or performance is mixed (Britten 1996; Mitton 1997). Several factors probably contribute to the lack of a definitive pattern. Such a relationship is likely to be detected if measured multi-locus heterozygosity reflects heterozygosity at the examined loci are known to influence these fitness-related characters, but whether this is actually the case is seldom known. Available patterns between heterozygosity

and fitness are likely to be biased toward positive relationships: most findings that fail to detect such patterns may not be reported (but see Whitlock 1993). Finally, most studies are of captive or laboratory populations, and there is reason to expect that natural populations may differ from these groups in their responses to inbreeding (Crnokrak and Roff 1999).

The loss of fitness due to inbreeding and erosion of genetic variation is commonly inferred by comparing the average phenotypic values for one or more traits between an inbred population and the base population from which it was derived (Lande and Schemske 1985; Gall 1987; Lynch 1988), or by comparing the average phenotypic values in offspring from matings between relatives with those from random matings within the same population. Although this approach is appealing because of its conceptual simplicity, it is challenging for most populations because of the difficulty in determining pedigrees. Consequently, geneticists typically adopt an alternative method in which heterozygosity measured by genetic markers is correlated with a phenotype related to fitness or performance. Few investigators appear to question whether this method is useful.

My objective in this chapter is two-fold: (1) to briefly review the evidence for correspondence between genetic variability and correlates of fitness in salmonids, and (2) to discuss the value of this relationship for understanding genetic constraints on salmonid viability. Over the past two decades, many studies have attempted to clarify this relationship. I describe the results of these studies, briefly evaluate the approaches that have been taken, and offer some recommendations for future investigations.

Genetic variation and fitness

In the absence of known pedigrees, genetic markers are required in studies of correlations between heterozygosity and fitness-related traits in order to estimate individual or population heterozygosity. However, individual heterozygosity of the entire genome is difficult to characterize (Mitton 1993a,b). Even extensive estimates

based on any single class of markers (such as allozymes and microsatellites) may inadequately characterize genome-wide heterozygosity (Avise 1994). Thus, even if they exist, correlations between heterozygosity and fitness traits will not be detectable unless the examined loci can detect the regions of the genome that influence fitness traits or are themselves directly related to fitness.

Numerous studies have attempted to correlate genetic variability, as revealed by protein or DNA polymorphisms, with measures of fitness or performance. The primary question asked by these investigators appears to be: Does a reduction in mean phenotype with respect to fitness or performance correspond to lower genetic variability? The correlation between heterozygosity and fitness is often investigated at two levels: comparison of individual heterozygosity (the number of heterozygous loci per individual) to fitness (or, more often, a correlate of fitness) within a population, and comparison of mean population heterozygosity and fitness among two or more populations. The majority of studies in salmonids have focused on the correlation between individual heterozygosity and mean fitness or performance measures within a population. Such studies have involved examination of morphological and life history traits in Atlantic, chum, pink, and sockeye salmon, and brook, cutthroat, and rainbow trout (Table 1). Examination of these studies reveals mixed (in sign as well as magnitude) correlations between individual heterozygosity and fitness characteristics. The correlation varies among species but also among conspecific populations.

For example, significant negative correlations between heterozygosity and fluctuating asymmetry (FA) in bilaterally symmetric traits such as gill raker number, paired fin ray counts, and lateral line scales have been detected in some cases but not others, and the reasons for this variation are seldom understood. Negative correlations between heterozygosity and FA have been detected in brook and cutthroat trout (Leary et al. 1984) and rainbow trout (Leary et al. 1983, 1984, 1985a, 1987, and 1992) but not in pink (Beacham and Withler 1987; Beacham 1991) or chum salmon (Beacham and Withler 1987). Negative correlations between heterozygosity and FA within Atlantic salmon

populations were found by Blanco et al. (1990) but not by Crozier (1997) or Vollestad and Hindar (1997). Significant positive correlations between heterozygosity and growth in rainbow trout were found by Ferguson et al. (1985) and Danzmann et al. (1987, 1988) but not by Koljonen (1986). The size and direction of correlations between individual heterozygosity and hatching time has been found to vary among selected strains of rainbow trout (Danzmann et al. 1985). Correlations may vary even between different samples and sexes of the same population. For example, underyearling brook trout sampled in June showed a positive correlation between heterozygosity and growth but no such pattern in samples taken a month earlier (Liskauskas and Ferguson 1991). Liskauskas and Ferguson (1991) also found that the correlation between heterozygosity and size at maturation in brook trout was positive in females but negative in males.

Variability in correlations between individual heterozygosity and fitness traits both within and among species has been observed for numerous taxa (David 1998), and this variability reflects the influence of environmental factors, age, and differing genetic backgrounds. In salmonids, quantitative genetic studies have shown that developmental stability (measured by FA or morphological variation) has a large environmental component (see Allendorf and Leary 1986; Mitton 1993a; Leung et al. 2000). In addition, Leary et al. (1991) showed that rainbow trout reared at high density had greater FA than those at low density. Beacham (1990) has shown that FA in chum salmon increases as rearing temperature increases from 6°C to 14°C. Vollestad and Hindar (1997) have also found that recently established hatchery Atlantic salmon populations had significant greater FA than other populations with a ten-year history of hatchery culture. Kartavtsev (1998) suggested that fitness of pink salmon declines under environmental stress. The effects of environmental factors and genetic background on correlations between individual heterozygosity and fitness traits as well as on inbreeding depression appear to be qualitatively similar (see Chapter 1, Part 1).

The study of rainbow trout by Leary et al. (1992) provides evidence of the importance of environmental factors and genetic background on the association between

genetic variation and developmental stress. In this study, no significant association was detected between heterozygosity and FA among populations, but a significant negative association was found within populations. Although the authors attributed this disparity to differing influences of environmental variability, the possible contribution of different genetic backgrounds in these populations cannot be dismissed. Resolving this issue would require a much more complete understanding of the genetic architecture of these populations.

At different life stages, salmonids seem to produce different patterns in the relationship between heterozygosity and fitness traits. For example, Danzmann et al. (1987, 1988) found a positive correlation between individual heterozygosity and growth in rainbow trout fry, whereas Koljonen (1986) detected no such correlation in adults, and Ferguson (1990) observed a negative correlation at the onset of maturation. However, these comparisons overlook the effects of environmental factors and genetic background, which, along with age variation, may have contributed to the differences in relationship between individual heterozygosity and growth. Thus, an examination of the effect of age on the association between heterozygosity and fitness traits is problematic unless the monitoring is made within individuals of a population with the same genetic background and in a homogeneous environment. In a more rigorous examination, age affected both the strength and direction of the association between multilocus heterozygosity and fork length in rainbow trout originating from the same population and with repeated measures on the same fish (Ferguson 1992).

Since phenotypic plasticity can obscure the responses of life-history and behavioral traits to selection (Via and Lande 1985; Falconer and Mackay 1996), a correlation between heterozygosity and these traits can be difficult to detect. Meta-analysis of published correlation coefficients between multilocus heterozygosity and growth rate as well as FA has shown that the strength of these correlations is generally weak (Britten 1996). A large sample size (thousands of individuals) is generally required in order to detect even weak correlations between heterozygosity and

phenotype (David 1998; Van Dongen 1999). Because it is challenging to analyze even a few hundred samples or more, and the family composition is often not known beforehand, investigators should conduct prospective power analyses to help improve the ability to detect correlations. It would also be useful to conduct retrospective power analyses for previous studies that failed to detect correlations between individual heterozygosity and fitness traits (Hard 1995a). Of course, statistical significance does not necessarily connote biological significance. Because the correlations are mixed in sign and magnitude and studies vary in additional factors that affect them, future studies should focus on the relationship between genetic variation and variation in phenotypes that affect fitness while limiting the effects of confounding factors.

Heterozygosity and consequences of loss of genetic variation

The expectation of a positive correlation between heterozygosity and fitness measures is closely related to the genetic basis of inbreeding depression (Mitton 1993b; David 1998; Lynch and Walsh 1998). There are two primary competing hypotheses for the genetic basis of inbreeding depression (Lande and Schemske 1985; Charlesworth and Charlesworth 1987); both are based on the depression in fitness resulting from increased homozygosity due to inbreeding . The dominance (or partial dominance) hypothesis proposes that inbreeding depression reflects the expression of recessive deleterious alleles in homozygous individuals. Recessive deleterious alleles that arise from mutation are commonly masked in expression by the dominant alleles in the heterozygous state and are expected to be maintained at low frequency by selection unless the mutation rate is very high (Lynch and Walsh 1998).

A second explanation for inbreeding depression is overdominance or heterozygote advantage: heterozygotes are more fit than either corresponding homozygote. The two mechanisms for inbreeding depression have very different evolutionary implications and predict differing resistance to the effects of further inbreeding (Charlesworth and Charlesworth, 1987; Chapter 1, Part 1). Unfortunately, it is often difficult to discriminate between these mechanisms empirically because of the

difficulty in distinguishing unambiguously between functional overdominance and associative overdominance or pseudo-overdominance due to linkage of marker loci with recessive deleterious alleles (Lynch and Walsh 1998; Bierne et al. 2000). One would expect a positive correlation between heterozygosity and fitness traits if the marker loci and the loci that influence fitness are the same. However, the situation becomes more complicated when the two "loci" are distinct. Linked loci carrying partially recessive deleterious alleles in disequilibrium will lead to the appearance of overdominance (Zouros 1993; David 1998; Lynch and Walsh 1998; Bierne et al. 2000). Thus, the same problem that has made drawing a conclusion about the genetic basis of inbreeding depression difficult also hinders investigation of the correlation between an individual's degree of heterozygosity and its fitness.

The tetraploid ancestry of salmonids further complicates interpretation of the correlation between heterozygosity and fitness in these fish. Although an estimated 50% of duplicated genes have lost their expression (Allendorf and Thorgaard 1984), remaining tetrasomic loci may be "buffered" against the effects of genetic drift by having more gene copies than disomic loci (Allendorf and Thorgaard 1984; Allendorf and Waples 1996). In addition, the loss of heterozygosity due to inbreeding is slower at tetrasomic than at disomic loci (Allendorf and Thorgaard 1984). Hence, one might predict that salmonids should be relatively less sensitive to inbreeding depression or express a weaker correlation between heterozygosity and fitness than diploid fishes, although comparison across fish species would be problematic because of their distinct life histories and genetic backgrounds. On the other hand, it would be more difficult to purge deleterious recessive alleles for tetrasomic loci, which should foster accumulation of greater genetic load. The experimental results summarized in Part 1 of this chapter show appreciable inbreeding depression in growth and survival, at least in freshwater resident species.

Sensitivity to inbreeding depression in anadromous salmonids remains largely unexplored (but see Ryman 1970). Research on this topic is warranted: evolutionary

"tension" between inbreeding depression and outbreeding depression may result from the unique life histories and mating systems of these species. There may be ample opportunity for inbreeding to accumulate in small salmon populations that exhibit high homing fidelity or spawn in habitats that limit effective gene flow, and the consequences of inbreeding have important implications for conservation and recovery measures (Allendorf and Waples 1996). If inbreeding depression in such fish is low or offset by other processes, it is not yet known whether resistance to inbreeding depression or behavioral or physiological mechanisms to avoid mating with close relatives is responsible. However, elevated or sustained interbreeding between locally adapted populations that might occur through habitat changes, fish introductions, and overharvest, etc. may also reduce fitness of endemic salmonids (NRC 1996).

Interpretation of the correlation between heterozygosity and fitness in salmonids may also be complicated by the presence of null alleles. The existence of null allele polymorphism in salmonids is considered evidence of the loss of duplicated gene expression (Stoneking et al. 1981). Null alleles in microsatellite or allozyme loci have been observed in several salmonid species (Stoneking et al. 1981; Knudsen et al. 1984; Ferguson et al. 1988; Banks et al. 1999; Spruell et al. 1999). Although the performance of fitness traits can be reduced in null allele heterozygotes and homozygotes relative to fish lacking null alleles (Knudsen et al. 1984; Ferguson et al. 1988), null alleles may cause underestimates of heterozygosity and consequently may obscure the correlations between heterozygosity and fitness under certain circumstances (David 1998). A more comprehensive discussion of null alleles and fitness can be found in Eanes (1987) and David (1998).

In addition, different types of genetic markers that are used to measure heterozygosity may also yield different results in the relationship between heterozygosity and fitness. This contrast is demonstrated by a recent study in rainbow trout by Thelen and Allendorf (2001) in which they found that condition factor (calculated by weight and length) was positively associated with heterozygosity at

allozyme loci rather than at microsatellite loci. Allozymes are protein-coding markers and are presumably to be more subject to selection than non-coding DNA markers (Ward and Grewe, 1994). Based on this assumption, a heterozygosity and fitness correlation is not expected if there are no other alleles or loci influencing fitness linked to the examined loci from DNA markers. However, previous studies of heterozygosity and fitness correlations in salmonids have exclusively relied on allozyme markers. Whether results for DNA markers similar to those of Thelen and Allendorf (2001) can be obtained for other salmonid species or populations is not yet known, and warrants investigation.

Heterozygosity and heritability

Most of the studies of the relationship between heterozygosity and fitness in salmonids (Table 1) have relied on comparisons of heterozygosity and average performance. Few studies have examined the more germane relationship between genetic variation (e.g., heterozygosity) and the inheritance of phenotypic variation (e.g., Beacham and Withler 1985a,b; Kartavtsev 1992). Knowledge of the relationship between molecular genetic variation and average performance may be useful for situations such as aquaculture where one or more defined phenotypes are the primary performance objectives. However, an examination of the relationship between molecular genetic variation and genetically based phenotypic variation in quantitative traits is more relevant for conservation because both forms of variation contribute to the generation and maintenance of intraspecific diversity (Utter et al. 1993b; Lynch 1996; Grant et al. 1999; Crandall et al. 2000). Indeed, molecular genetic variation alone usually provides little insight into adaptive variation of phenotypes (Hard 1995b; Lynch 1996; Storfer 1996). The relationship between genetic and phenotypic variability can be evaluated by examining the correlations of heterozygosities and heritabilities (Hard 1995b; Lynch et al. 1999). For a given trait, heritability is the proportion of total phenotypic variance contributed by additive genetic variance. The additive genetic variance is the variance of breeding values, which reflects the variance of the average effects of expressed alleles (Falconer and Mackay 1996). Because it describes the genetic resemblance between

relatives and determines short-term response to selection (Lynch and Walsh 1998), the additive genetic variance is the most important component of the total genetic variance. Factors that influence the additive genetic variance may influence heterozygosity in similar ways and to similar degrees (Caughley and Gunn 1996), but these factors may be difficult to detect in surveys of only a few traits.

Inspection of the relationship between heterozygosity and heritability provides a more direct means to test the assumption that molecular genetic variation is a reasonable proxy for quantitative genetic variation (Hard 1995b). However, inferential power is low and these studies are difficult to implement, especially for natural populations. Nevertheless, only a few studies have been done on the relationship between molecular variation and heritability (see reviews by Hard 1995b; Lynch 1996), and none of them involved salmonids. Hard (1995b) found no evidence for concordance of heterozygosity and heritability within populations in nine studies of various taxa.

Fitness and its correlates

In examining the relationship between heterozygosity and fitness, all studies in salmonids have considered one or more correlates of fitness, but not fitness itself. Studies that estimate the relationship between genetic variation and fitness (e.g., Gustafsson 1986, Kruuk et al. 2000) are extraordinarily difficult and time consuming to conduct for most organisms. In the absence of estimates of total fitness, Frankham (1995b) suggested that studies that are based on individual fitness correlates underestimate the full effect of inbreeding depression, as it is approximately three times greater for total fitness (measured as lifetime reproductive success) than for its components. On the other hand, genotypes that confer higher fitness with respect to some fitness traits may have reduced fitness for others (Allendorf and Leary 1986), probably due to antagonistic pleiotropy in expression of genes affecting fitness (Service and Rose 1985; Barton and Turelli 1989). Thus, inbreeding depression with respect to total fitness is a synergistic outcome of all its components.

Unfortunately, it is difficult to measure fitness and to estimate differences in fitness among genotypes in natural populations (Allendorf and Leary 1986; Endler 1986). As a compromise, the effects of inbreeding on total fitness can be monitored with a trait that is highly correlated with overall fitness, such as lifetime reproductive success (Slate et al. 2000). However, for populations with complex life histories like anadromous salmonids, even this method is especially challenging to implement. Despite the difficulties in measuring them, correlations between heterozygosity and fitness along with demonstrable differences in fitness among genotypes would provide information valuable for the conservation of genetic resources. Management strategies based on observation of single or few components of fitness may be misguided because they do not consider potential trade-offs between fitness components, potentially placing the populations under consideration at even greater risk.

Finally, individual fitness is sensitive to environmental variation. For example, inbreeding depression may be observed in one environment but not necessarily in another (Pray et al. 1994), and these disparities may reflect differential responses to environmental stress. This effect could compromise goals for conservation that are based on mean fitness alone (Kapuscinski and Lannan 1984, 1986; see Ryman 1991 for discussion). As mentioned above, genetic and phenotypic variation in quantitative traits determines the evolutionary significance of adaptive differences among units of intraspecific variation; therefore, both are important for the conservation of intraspecific diversity. However, the genetic basis of adaptation is likely to be very complex. For example, Armbruster et al. (1997) showed in natural mosquito populations that nonadditive genetic effects such as epistatic interactions among loci contributing to fitness variation can produce opposing patterns of differentiation at structural genes and quantitative traits that are not predicted from consideration of population genetic processes alone. The resulting patterns of differentiation reflected by adaptation to ecological habitats can contrast sharply with expected differentiation through isolation and drift.

Precise quantification of genetic impacts in terms of fitness depression or enhancement remains difficult. Consequently, even when a heterozygosity and fitness correlation is not evident and the genetic basis of putatively adaptive phenotypic variation has not been demonstrated, maintenance of genetic variation between as well as within populations should be a primary goal of conservation and fishery management (Ryman 1991; Busack and Currens 1995). The relationship between molecular genetic and quantitative genetic differentiation among populations would haveserious implications for efforts to conserve or recover wild populations with artificial propagation. A lack of molecular divergence between a population requiring conservation and another to be used to augment productivity of the conserved population provides no assurance that negative genetic interactions will not occur upon reproduction between these populations in the wild (e.g., Krueger and May 1991, Utter 2000). Consequently, such a strategy should be considered as a conservation tool only after options requiring less intervention have been exhausted, and only if adequate monitoring and evaluation of interactions is assured. Preserving natural population connectivities and maintaining high-quality habitat are essential components of conservation strategies in the absence of more definitive information on the genetic basis of adaptive variation.

Conclusions

Although correlations between heterozygosity and fitness traits vary among salmonid species and populations and the overall concordance between heterozygosity and fitness is weak, there are several examples showing positive correlations. These correlations can be affected by environmental factors, age, and genetic backgrounds, and depend on the markers and phenotypes involved. The difficulty in distinguishing empirically between functional overdominance and associative overdominance and in the adequate characterization of the relationship between heterozygosity and fitness makes it difficult to determine the genetic mechanisms of adaptive variation. Furthermore, it remains unclear whether describing the relationship between effectively neutral genetic variability and variation in traits that have an unknown effect on fitness

deserves the degree of attention it has attracted. However, the uncertainty in this relationship suggests that direct investigation of genetic variation in fitness is also an important avenue for investigation. Evidence for a relationship between molecular genetic variation and correlates of mean fitness does not preempt the need for quantitative genetic evaluation of variation in fitness (measured as lifetime reproductive success) and its relationship to molecular variation (Hard 1995b).

Future work in salmonids should include more intensive investigation of the relationship between molecular variation and heritability. These factors are direct, complementary estimates of genomic variation. While there is good reason to expect that they are correlated, at present the evidence is ambiguous, and for salmonids, no direct evidence is yet available. Characterizing this relationship is needed to understand the relative roles of adaptive and passive evolutionary processes in producing genetic and phenotypic variation in nature.

Genetic and phenotypic variation in quantitative traits determine the evolutionary significance of adaptive differences among populations and, therefore, both are necessary to maintain to effectively conserve intraspecific diversity. In the absence of a more complete understanding of the relationship between genetic variation and fitness, such maintenance of genetic and phenotypic variation within and among populations should be considered a primary goal of conservation and management of salmonid populations.

Table 1.1. Summary of studies on correlations between fitness trait value and heterozygosity in salmonid populations. The symbols +, -, = indicate a positive correlation, negative correlation and no correlation, respectively. Russian language publications in *Genetika* have not been included.

Trait	Observation	Species	Correlation	References
Viability	up to 410 days	*Oncorhynchus gorbuscha*	=	Beacham (1991)
	embryo and alevin	*Oncorhynchus gorbuscha*	=	Beacham and Varnavskaya (1991)
	up to fry	*Oncorhynchus gorbuscha*	+	Kartavtsev (1992)
	up to fry under rearing density stress	*Oncorhynchus gorbuscha*	+	Kartavtsev (1998)
Disease resistance	bacterial gill disease	*Oncorhynchus mykiss*	+	Ferguson and Drahushchak (1990)
Fecundity	egg size	*Oncorhynchus mykiss*	+	Danzmann, et al. (1988, 1989)
	egg number and ovulated egg weight	*Salvelinus fontinalis*	+	Liskauskas and Ferguson (1990)
Growth and Developmental rate	Hatching and emergence time	*Oncorhynchus gorbuscha*	=	Beacham and Varnavskaya (1991)
	Length at fry	*Oncorhynchus gorbuscha*	=	Dubrova et al. (1995)
	Length at fry	*Oncorhynchus gorbuscha*	+	Kartavtsev (1992)
	Length at fry under rearing density stress	*Oncorhynchus gorbuscha*	+	Kartavtsev (1998)
	Hatching time	*Oncorhynchus mykiss*	+	Ferguson, et al. (1985)
	Length and weight at maturation	*Oncorhynchus mykiss*	-	Ferguson (1990)
	Length at 6 months	*Oncorhynchus mykiss*	=, + (varies among groups)	Ferguson (1992)
	Length at 1 year	*Oncorhynchus mykiss*	-	Ferguson (1992)
	Length and weight at 2 and 2.5 years	*Oncorhynchus mykiss*	=	Koljonen (1986)
	Length or weight up to 6 months	*Oncorhynchus mykiss*	+	Danzmann, et al. (1987, 1988)
	Hatching time	*Oncorhynchus mykiss*	-, + (varies among strains)	Danzmann, et al. (1985)
			+	Danzmann, et al. (1986)
			+	Danzmann, et al. (1989)

Table 1.1 continued

Trait	Observation	Species	Correlation	References
Growth and Developmental rate	Hatching time	*Oncorhynchus mykiss*	=	Danzmann and Ferguson (1988)
	Length and weight up to 465 days	*Salmo salar*	+	Blanco, et al. (1998)
	Length and weight up to yearlings	*Salvelinus fontinalis*	= (Sampled in May) + (Sampled in June)	Liskauskas and Ferguson (1991)
	Size at maturation (3 and 4 year-old)	*Salvelinus fontinalis*	- (Male) + (Female)	Liskauskas and Ferguson (1991)
Physiological efficiency	oxygen-consumption rate up to 6 months	*Oncorhynchus mykiss*	-	Danzmann, et al. (1987, 1988)
Developmental homeostasis	Morphological variability (MV)	*Oncorhynchus gorbuscha*	=	Beacham and Withler (1985a)
	MV	*Oncorhynchus gorbuscha*	+	Kartavtsev (1992)
	MV	*Oncorhynchus keta*	=	Beacham and Withler (1985b)
	Fluctuating asymmetry (FA)	*Oncorhynchus gorbuscha*	=	Beacham and Withler (1987) Beacham (1991)
	FA	*Oncorhynchus keta*	=	Beacham and Withler (1987)
	FA	*Oncorhynchus mykiss*	-	Leary, et al. (1983, 1984, 1985a) (1987, 1992)
	FA	*Oncorhynchus mykiss*	-, +, = (varies among strains)	Ferguson (1986)
	FA	*Salmo clarki lewisi*	-	Leary, et al. (1984)
	FA	*Salmo salar*	-	Blanco, et al. (1990)
	FA	*Salmo salar*	- (between pop.) = (within pop.) - (heterozygotes vs homozygotes in hatchery pop.)	Crozier (1997)
	FA		=	Vollestad and Hindar (1997)
	FA	*Salvelinus fontinalis*	-	Leary, et al. (1984)

CHAPTER 2: EFFECTS OF INBREEDING ON GENETIC VARIATION

Introduction

Inbreeding leads to an immediate decrease in heterozygosity in inbred individuals and can also reduce genetic variation within populations through the loss of genotypes from selection and drift. In particular, increased inbreeding is often associated with a decrease in mean phenotypic value of one or more traits with respect to fitness, a phenomenon known as inbreeding depression (Hard and Hershberger 1995). Thus, inbreeding has been a topic of considerable concern in conservation and fishery management (e.g., Nelson and Soulé 1987; Leberg 1990; Hindar et al. 1990; Waples 1990; Ryman 1991; Allendorf and Waples 1996). Inbreeding is also a concern in aquaculture where the growth and survival rates are often of primary interest to the farmers (Tave 1991). In addition, concerns of inbreeding also come from supplementation of depleted natural populations of fishes (e.g., salmonids) commonly implemented with cultured fishes (Ryman and Laikre 1991; Ryman 1991; Waples and Do 1994; Utter 1998).

Inbreeding can be defined as the mating of relatives. The level of inbreeding of an individual is usually measured by the inbreeding coefficient (f), the probability that two alleles at a locus are identical by descent (IBD) in that individual. The general formula for the coefficient of inbreeding of any individual I is

$$f_I = \Sigma\left[\left(\frac{1}{2}\right)^n (1 + f_A)\right] \qquad (1)$$

where n is the number of individuals in the pathway between individual I and common ancestor A, and f_A is the inbreeding coefficient (Wright 1951). The summation is over all independent pathways. The coefficient of inbreeding of an individual is dependent upon the amount of inbreeding that has occurred in previous

generations. From a noninbred population, f_I equals 0, 0.125, and 0.25 for offspring from unrelated, half-sib, and full-sib crosses, respectively.

Single-locus neutralist theory says that the expected heterozygosity retained within a population (the proportion of individuals that are heterozygous at a locus) after t generations is

$$H_t = (1 - F_t)H_0 \qquad (2)$$

where H_0 is the initial heterozygosity of the population and F is the average inbreeding coefficient which is the expected proportion of individuals in the population that are IBD homozygous at the examined locus (Crow and Kimura 1970; Gall 1987). Equation (2) indicates that population heterozygosity is expected to decline linearly with the average inbreeding coefficient.

Population heterozygosity can be averaged over loci to provide a genome-wide estimate of genetic variation of that population (Lacy 1995). From equation (2), for a population of individuals with equal f s, the expected average population heterozygosity over a number of loci after t generations is

$$\bar{h}_t = (1 - f_t)\bar{h}_0 \qquad (3)$$

where $\bar{h}_0$ is the initial average heterozygosity of the population and $\bar{h}$ is defined as

$$\bar{h} = \frac{\sum_j H}{L} \qquad (4)$$

where L is number of loci examined and $j = 1,2,...,L$. By its definition, the average population heterozygosity, $\bar{h}$, is the proportion of heterozygotes within a population

per locus averaged over the total number of loci examined. Another way to interpret $\bar{h}$ is that $\bar{h}$ is the proportion of heterozygous loci per individual averaged over the total number of individuals of the population surveyed. In the latter sense, $\bar{h}$ can be termed as average individual heterozygosity. Correspondingly, the proportion of heterozygous loci in an individual is termed as individual heterozygosity (denoted by h). The parameter h provides a genome-wide estimate of genetic variation in that individual. It is easy to show that $\bar{h}$s are the same for these two different interpretations. Thus, $\bar{h}$ is often referred to average heterozygosity (Hartl and Clark 1997).

In a system of mating within a population, the expected single-locus heterozygosity of a population with inbreeding coefficient f, H_f, is given by

$$H_f = \sum_{i \neq j} p_i p_j (1 - f) \qquad (5)$$

where p_i and p_j are the frequencies of allele i and j, respectively, and the summation is over all combinations of values of i and j except when these are equal (Crow and Kimura 1970).

An important difference between equations (2) or (3) and (5) is that equations (2) and (3) are concerned with the changes of heterozygosities in populations across generations, whereas equation (5) is concerned with a deviation of observed from expected heterozygosity under random mating ($f = 0$) in populations undergoing systematic inbreeding within a particular generation. For example, by equation (1), (2) and (3), in one generation population heterozygosity and average heterozygosity are expected to decrease by 12.5% for a half-sib cross and 25% for a full-sib cross relative to a cross of unrelated individuals. However, heterozygosities estimated from equations (2), (3) or (5) may be influenced by selection, mutation or gene flow. In real populations,

natural selection may modify the distribution of genotypic frequencies predicted by theory. Consequently, empirical estimates of changes in heterozygosity under inbreeding may depart from predictions of the theory. In fact, theoretical studies have shown that inbreeding can result in a level of heterozygosity higher than predicted as the result of overdominance (Hayman and Mather 1953; Reeve 1955), or as the result of associative overdominance due to linkage of genetic markers to overdominant alleles at a single locus (Bartlett and Haldane 1935; Reeve and Gower 1958) or to recessive deleterious alleles at different loci (Ohta 1971; Wang and Hill 1999). Slower decline in heterozygosity than theory predicts at a single locus under inbreeding has been observed in chickens (Mina et al. 1991), fruit fries (Frankham et al. 1993; Rumball et al. 1994; Latter et al. 1995), flour beetles (Goodwill and Enfield 1971; Yeh and Scheinberg 1972), mice (Connor and Bellucci 1979), mosquitoes (Munstermann 1994), oysters (McGoldrick and Hedgecock 1997; Bierne et al. 1998) and rats (Eriksson et al 1976), with very few exceptions (Morrow et al. 1974; Lacy and Horner 1996). The opposite has been observed in wild butterflies (Saccheri et al. 1999).

The majority of these above results reflect a deviation of observed heterozygosities under random mating, i.e., calculated by equation (5). Others, e.g., McGoldrick and Hedgecock (1997) and Bierne et al. (1998) reflect comparisons of observed genotypic frequencies with their expectations from Mendelian segregation ratios. However, to my knowledge an empirical examination of equations (2) and (3) has not been reported by these and any other studies. Empirical monitoring of systematic inbreeding with Mendelian markers has been very limited in salmonids (Hershberger et al. 1990), and little is yet known of the relationship of observed to expected heterozygosity in populations undergoing systematic inbreeding.

By their meanings, equations (2) and (3) can be examined by comparing sequential generations originated from the base population in which the level of inbreeding increases and heterozygosity decreases in each generation. However, since different environmental conditions in each generation may influence the distribution of

genotype frequencies of that generation, interpretation of the results may become difficult in the presence of non-neutrality of some genetic markers or linkage of genetic markers to loci subject to selection. In addition, it takes more than one generation in this approach and therefore precludes studies that are constrained by time and other resources. Alternatively, equations (2) and (3) can be examined by comparing different levels of inbreeding in a single generation in which different inbreeding levels are achieved by applying different breeding designs to progeny of the same base population. A conventional method is to employ a hierarchical half-sib/full-sib mating design (see Hard et al. 1999; also Fig. 2.2). This approach can effectively reduce the possible effects of environmental variation between generations through using parallel line(s) and allow a rapid comparison between different levels of inbreeding since it establishes groups of different inbreeding levels within a single generation. However, parental differences in genotypic distribution between mating groups resulting from sampling errors rather than siblingships can confound interpretations of patterns of genetic variation in this approach. The problem can be minimized by increasing the number of random families in each mating group or the number of alleles in common between mating groups. Evaluating multilocus instead of single-locus heterozygosity and increasing the number of loci screened can also effectively reduce sampling error. Equation (3) uses multilocus heterozygosity. In addition, for empirical data equation (3) apparently holds if equation (2) holds for all loci examined. However, for loci under selection the reverse may not be true because the direction and magnitude of selection at different loci are likely to be different even for the same increase in the level of inbreeding and these differences may lead to different changes in heterozygosity at these loci.

The purpose of this study is to compare observed rates of decline in individual heterozygosity with their predictions from equations (2) and (3) under two different rates of inbreeding compared to a control line that is composed of noninbred individuals. To accomplish this objective, individuals of chinook salmon were inbred in a single generation with half-sib and full-sib matings. I then measured heterozygosities

at 10 DNA tetra-nucleotide microsatellite loci, and compared the decreases in heterozygosities and number of alleles in inbred offspring relative to noninbred control offspring. I compared viability of heterozygotes and homozygotes with segregation analysis.

Material and Methods

Establishment of experimental groups

Adult fall chinook salmon returning to the Suquamish Tribe's Grovers Creek Hatchery near Kingston, Northwestern Puget Sound (Fig. 2.1), were spawned during October 3-21, 1994 to establish a conventional half-sib/full-sib family breeding design (Fig. 2.2). A total of 30 males and 120 females (grandparents) were chosen from the central 56% of the run with a random numbers table to produce 30 half-sib and 120 full-sib families of offspring with 4 full-sib families per half-sib family (Hard et al. 1999). This design provided a convenient means of establishing different levels of inbreeding in experimental groups within the population as well as an opportunity to estimate genetic components of variation in quantitative characters (Hard et al. 1999).

The Grovers Creek Hatchery fall chinook salmon broodstock was founded from the Washington Department of Fish and Wildlife's Green River Hatchery in eastern Puget Sound between 1978 and 1981 (Table 2.1). Chinook salmon were first produced from Grovers Creek Hatchery in 1981 and have been a self-sustaining stock since then. The base population for the experiment was established from the 1994 return.

Between 4 and 26 April 1995, 257, 093 F_1 chinook salmon smolts, representing 96 full-sib and 30 half-sib families, were marked by removing adipose fins and inserting family-specific coded-wire tags (CWTs) at Grovers Creek Hatchery by Northwest Indian Fisheries Commission personnel. Up to 410 fish from each of the 96 families were retained at the hatchery to be monitored for tag retention after 30 days. The remaining fish were released from a common pond into the stream connecting Miller Bay in northwestern Puget Sound.

From 6-8 June 1995, 50 fish from each of the 96 full-sib families were individually injected with passive integrated transponder (PIT) tags by the National Marine Fisheries Service (NMFS) personnel. These 4, 800 fish were combined after marking into a circular concrete rearing pond at the hatchery and then were transferred after 10 days to seawater net-pens for rearing, growth and maturation at the NMFS Marine Experimental Station at Manchester, Washington in southwestern Puget Sound.

To establish the experimentally inbred lines, two F_2 broods from 1994-brood adults maturing between 1997 and 1998 were created. Each brood was composed of fish reflecting three different mating schemes, i.e., derived from parents related as full siblings, half siblings and unrelated individuals. The first brood (1997) was derived from three-year-old captive 1994-brood adults maturing in net-pens at the Manchester Station. The second brood (1998) was derived from corresponding four-year-old fish maturing in 1998 and returning to the Grovers Creek Hatchery from the ocean. Both broods were established at the School of Aquatic and Fisheries Sciences Hatchery of the University of Washington after transfer of unmixed gametes from either Grovers Creek Hatchery (returning fish) or the Manchester Station (captive fish) (Fig. 2.1). These groups developed to the juvenile stage in isolated full-sib family groups at the hatchery until they could be identifiably marked with either family-specific CWTs or PIT tags, at which time they were pooled into common raceways until transfer or release to sea water.

Sampling

Twelve F_2 full-sib families from each brood were used in this study. Each experimental group included 4 full-sib families from same type of mating (full-sib, half-sib and unrelated). All full-sib families were produced by pairwise matings (one female with one male). The 12 full-sib families for 1997 brood originated from 8 grandsires and 16 granddams, whereas the 12 full-sib families for 1998 brood originated from 7 grandsires and 15 granddams. Three full-sib families, each being produced by a

different type of mating, were chosen for every shared grandsire. Such a design can help minimize the possible effect on offspring of parental differences in genotypic distribution between mating groups from sampling by increasing the number of alleles in common between mating groups. Since this study focuses on variation of heterozygosity and allelic diversity among different mating groups, the sharing of grandparents in this way should not bias the results.

For the purpose of DNA analysis, random samples of 34 individuals (F_2) from each of the 24 full-sib families were taken and preserved in 100% alcohol at the alevin stage. Tissues (fin clips) from all parents (F_1) of the 24 full-sib families were also taken, labeled, and preserved in 100% alcohol at the time of spawning. Muscles from all but one grandparent (missing) were stored at -80°C and then transferred into 100% alcohol.

Genotyping

Genotyping was conducted in the Marine Molecular Biotechnology Laboratory (MMBL) of the University of Washington. Total genomic DNA was extracted from alcohol-preserved fin clips (F_1), muscles (grandparents) or tail cuts (F_2) using a Puregene™ DNA isolation kit from Gentra Systems Inc. (Minneapolis, MN). Precipitated DNA was hydrated in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) and heated at 65°C for approximately 1 hour. The DNA concentration was measured by spectrophotometry with GeneQuant (Pharmacia) and diluted with sterilized nanopure H_2O 100 ng/μL for use in the polymerase chain reaction (PCR).

10 tetra-nucleotide microsatellite loci were amplified for grandparents and for the 34 F_2 individuals and their parents F_1 of each family by the PCR using primer pairs developed from salmonids (Appendix A). PCR primers were custom made by Operon Technologies Inc. or Gibco, BRL. All PCRs were carried out with 384-well plates using Techne (Techne [Cambridge], Ltd.) in a 10 μL volume containing 1μL 10× *Taq* PCR buffer (Promega); 1.5 mM $MgCl_2$ (Promega); 0.8 mM dNTPs; 0.5 U *Taq* DNA polymerase (Promega); 1.1 μL Tartrazine-ficoll PCR additive; 0.3 μM each PCR primer; and 100ng

DNA template. PCR temperature cycles were preceded by a denaturation incubation for 2 min at 94°C. The cycle denaturation was 1 min at 94°C for the first seven cycles and 30 sec at 94°C for the remaining 18 cycles. The extension step was 15 sec at 72°C for all cycles. The annealing step was 30 sec for all cycles, but the annealing temperature varied for different loci. An annealing temperature of 54°C was used for *Ots101*, *Ots102*, *Ots107*, and *Ssa197*; 50°C was used for *Ots104* and *Ots108*; 58°C was used for *Ots100*; 56°C was used for *Ots103*; 59°C was used for *Ogo1a*; and 60°C was used for *Oki3a*.

PCR products were size fractionated on 6% nondenaturing polyacrylamide (19:1 acrylamide to bisacrylamide) gels. Of 25 wells on each gel, 20 wells were loaded with 5 µL of PCR product from each fish, and five equally spaced wells were loaded with a mixture of 20- and 100-bp marker (GenSura Labs Inc.) for sizing microsatellite alleles. Gels were run with 1× TBE buffer for approximately 2 hours at 200 V. Following electrophoresis the contents of each gel was stained with a 1:10, 000 solution of SYBR Green 1 nucleic acid gel stain (Molecular Probes, Inc.) and 1× TBE buffer for 20-30 min and scanned on the FluorImager 575 (Molecular Dynamics, Sunnyvale, CA) at a PMT voltage of 550-650.

To increase the precision of sizing different alleles, PCR products of F_2 and their corresponding F_1 from each family were run on the same gels when feasible. However, the lower sizing precision of tetra-nucleotide alleles should not affect genotyping accuracy because most alleles differed by four bases which allows for non-contiguous allele categories (Olsen 1999).

Data Analysis

The number of alleles at each locus was calculated for F_2 and F_1 fish in each group and for corresponding grandparents using Genepop version 3.2a (Raymond and Rousset 1995). Tests for conformity to Hardy-Weinberg expectation (HWE) and analyses of genotypic linkage disequilibrium were performed for the base population (grandparents) using Genepop. For each brood, pairwise comparison of genetic

structure of F_1 of the three different groups was performed using Genepop. In addition, differences in mean heterozygosity and the mean number of alleles across these groups were evaluated with Kruskal-Wallis rank sum tests.

Kruskal-Wallis rank sum tests were also performed to detect differences in average heterozygosity and mean number of alleles over the 10 loci across mating groups in F_2 offspring. If a difference across groups was detected, this test was then followed by a nonparametric Dunnett-type multiple comparison (Zar 1984) of the unrelated mating group with either the half-sib group or the full-sib group. Linear regression analysis was used to describe the relationships between observed heterozygosities and levels of inbreeding for each brood. Working-Hotelling confidence bands (Neter et al. 1996) were calculated to determine the concordance of observed heterozygosity with the expected values under different levels of inbreeding.

Segregation analysis was made to families whose number of genotypes should have Mendelian ratios 1:1 (parental genotypes AA/BB and AB) and 1:2:1 (parental genotypes AB and AB) in their offspring. The difference between the number of heterozygotes and homozygotes in offspring was examined using binomial tests by grouping different offspring genotypes into either homozygotes or heterozygotes. A sign test for the direction of the difference between the number of heterozygotes and the number of homozygotes was performed to determine the likelihood that the differences arose by chance across loci and families.

An examination of equation (5) was performed to compare the results from those of equations (2) and (3) as well as the results from the segregation analysis. Offspring from different families within the same group were pooled together in this examination. However, offspring from the two broods were analyzed separately because of heterogeneity in their genotypic distributions (not shown). Chi-square tests were performed for the deviation of observed heterozygosities from HWE as well as from expectations from equation (5). A sign test for the direction of the deviations in the latter

was performed in order to test for the significance of these deviations across loci and groups.

Linear regression analyses (as well as examination of the model assumptions of variance and normality) and all other statistical analyses were carried out with Splus version 2000 (MathSoft, Inc.).

Results

Characterization of the grandparents

Table 2.2 shows heterozygosity and the number of alleles at each locus for 45 grandparents of the 24 full-sib families analyzed in this study (the base population). The average heterozygosity over all 10 loci was 0.72 with SD = 0.15. The number of alleles per locus ranged from 2 at *Ogo1a* to 25 at *Ots102*. Significant heterozygosity deficiency based on Hardy-Weinberg equilibrium expectations was found for 5 out of 10 loci (Table 2.2). No significant linear relationship of heterozygosity with the number of alleles was detected at these loci (adjusted $r^2 = 0.04$, $p = 0.27$).

Characterization of the F_1 parents for the 1997 and 1998 broods

Table 2.3 shows heterozygosity and the number of alleles at each locus for F_1 parents of different groups in both broods. Single-locus heterozygosity and the number of alleles at each locus varied inconsistently across groups at each locus. A consistent decline across groups in average heterozygosity over all 10 loci was observed in the 1997 brood. The average heterozygosities were 0.836 and 0.734 in parents of half-sib and full-sib mating groups, respectively, corresponding to decreases of 2.2% and 14.2% relative to the average heterozygosity in parents of the unrelated mating group. However, the decline across groups was nonsignificant as revealed by a Kruskal Wallis rank test ($\chi^2 = 2.43$, $p = 0.30$). No difference in average heterozygosity of F_1 parents across the same groups in the 1998 brood was detected ($\chi^2 = 3.64$, $p = 0.16$). There were generally downward trends across groups in the mean number of alleles over all ten loci in both

broods. However, none of them were significant ($\chi^2 = 1.21, p = 0.55$ for the 1997 brood and $\chi^2 = 0.84, p = 0.66$ for the 1998 brood).

Significant differences in genotypic distributions were detected between parents of full-sib and half-sib mating groups and between parents of full-sib and unrelated mating groups in the 1998 brood at locus *Oki3a*. Significant differences in genotypic distribution were also found between parents of full-sib and unrelated mating groups in the 1997 brood at locus *Ots108*. No other significant differences in genotypic distribution were detected in either brood. Excluding *Oki3a* in the 1998 brood or *Ots108* in the 1997 brood only slightly changed the observed rates of decline in average heterozygosity and did not affect the overall agreement of these rates to the expected values (see below).

Linkage analysis

Linkage can bias the average heterozygosity distribution by modifying the expected number of double homozygotes and double heterozygotes. Probability tests for genotypic linkage disequilibrium resulted in 4 significant *P*-values among 45 pairwise tests of ten tetranucleotide microsatellite loci within 46 grandparents. These were *Ots103* and *Ots108* ($p = 0.012$), *Ogo1a* and *Ots102* ($p = 0.031$), *Ogo1a* and *Ots103* ($p = 0.004$), *Ogo1a* and *Ots107* ($p = 0.041$). Excluding *Ots103* and *Ogo1a*, which provided a means for discriminating the possible bias, only slightly changed the observed rates of decline in average heterozygosity and did not affect the overall agreement of these rates to expected values for either brood (see below).

Observed heterozygosities at different inbreeding levels

Observed single-locus heterozygosity of a family (the proportion of heterozygous individuals of offspring at each locus calculated for each family) varied appreciably among families and loci. Consistent decline of heterozygosity across mating groups was observed at *Ots100, Ots103, Ots107, Ots108, Ogo1a, Oki3a* and *Ssa197* in the

1997 brood and at *Ots103*, *Ots107*, and *Ssa197* in the 1998 brood (Fig. 2.3 and Fig. 2.4). Heterozygosity averaged over all 10 loci varied across individuals within each family (Table 2.4). When they were averaged across families within the group, overall individual heterozygosities at all 10 loci averaged showed a clear decline with the degree of inbreeding of the group in both 1997 and 1998 broods. The average heterozygosities per family in offspring were 0.712 (SD = 0.057) and 0.513 (SD = 0.135) for half-sib and full-sib mating groups, respectively, corresponding to decreases of 11.4% ($p < 0.05$) and 36.2% ($p < 0.01$) from the heterozygosity in the unrelated mating group (0.804 and SD = 0.065) in the 1997 brood. In the 1998 brood, the average heterozygosities per family in offspring were 0.711 (SD = 0.123) and 0.636 (SD = 0.099) for half-sib and full-sib matings groups, respectively, corresponding to decreases of 14.5% ($p < 0.05$) and 23.6% ($p < 0.01$) from the heterozygosity in the unrelated mating group (0.832 and SD = 0.022).

Comparisons of observed heterozygosities with theoretical expectations

Discrepancies between the single-locus heterozygosities of an average family and the expectations from equation (2) at different levels of inbreeding were found at a number of loci in both 1997 and 1998 broods (Fig. 2.3 and Fig. 2.4). However, the observed average heterozygosity at all 10 loci per family showed clear conformity to expectations from equation (3) (Fig. 2.5). The decreases in average heterozygosity per family showed a clear linear trend across different levels of inbreeding in both broods (Pearson coefficient of correlation $r = 0.82$, $p = 0.001$ for the 1997 brood; $r = 0.70$, $p = 0.01$ for the 1998 brood).

When the offspring from different families were combined within their groups, significant heterozygosity deficiency was, as expected, observed at all or most loci for full-sib and half-sib mating groups in both 1997 and 1998 broods (Table 2.5). In contrast, both significant heterozygosity deficiency and excess were observed in offspring from the unrelated mating in each brood. Significant deviations of observed heterozygosities

from equation (5) expectations were observed at a number of loci in each brood. However, both heterozygosity deficiency and excess were observed in each brood. Heterozygosity deficiency or excess at a particular locus that occurred in one group did not necessarily occur in another group. In addition, a sign test for the direction of the deviation was performed for each brood and both tests yielded non-significant results (one-sided $p = 0.26$ for 1997 the brood and 0.18 for the 1998 brood).

Changes of number of alleles at different levels of inbreeding

Fig. 2.6 shows the number of alleles of an average family within each inbreeding group at each locus for both 1997 and 1998 broods. The number of alleles per family ranged from 2 to 4 at each locus. Lack of obvious decline across mating groups in the number of alleles was observed at a number of loci in both broods. However, the total number of alleles at all 10 loci in an average family showed a trend of decline across mating groups (Fig. 2.7). The total number of alleles of an average family in offspring was 24, 29 and 32 in the full-sib, half-sib and unrelated mating groups, respectively, in the 1997 brood and 26, 29 and 31 in the full-sib, half-sib and unrelated mating groups, respectively, in the 1998 brood. Although the declines from unrelated mating group to full-sib mating group were significant in both broods ($p < 0.01$), the decline from the unrelated mating group to the half-sib mating group was not significant in either of the two broods.

Fig. 2.7 also shows the number of alleles in an average family when the 10 loci were grouped based on whether a heterozygosity deficiency was detected at that locus in the grandparents (Table 2.2, discussed below).

Test for differential viability between heterozygotes and homozygotes

A segregation analysis at different loci to 23 families whose offspring genotypes had a Mendelian ratio of 1:1 or 1:2:1 showed no significant difference between the number of heterozygotes and the number of homozygotes at 8 loci in 21 families (Table 2.6). A lower number of heterozygotes was observed in family 13 at *Ots103* and in

family 104 at *Ssa197*. A sign test for the direction of the difference between the numbers of heterozygotes and homozygotes across families and loci yielded an insignificant result (16 '+', 17 '-', and 8 equals).

Discussion

Concordance of observed decline in heterozygosity and theoretical expectations

Although a consistent decline of single-locus heterozygosity of an average family was observed across different levels of inbreeding at some loci in each brood, a lack of a decline was also observed at a number of loci in both broods. A discrepancy between changes of observed single-locus heterozygosity across all levels of inbreeding and expectations by equation (2) was found at a number of loci in both broods. However, the changes of average heterozygosity over all 10 loci across all levels of inbreeding conformed to the expectations of equation (3). The decline of average heterozygosity was linearly related to the level of inbreeding.

An examination of equation (5) by combining offspring of all families within a group yielded results similar to those from the examination of equation (2) with single-locus heterozygosity in both broods. Heterozygote deficiency or excess did not occur at the same loci in different groups. A deficit of heterozygotes and an excess of heterozygotes were observed 29 and 31 times, respectively, across all loci, groups and broods. These results imply that the observed deficiency or excess occurred more likely by chance than as a result of processes acting at specific loci.

Segregation analysis

Only 2 out of 23 families at two different loci showed a significant difference between the numbers of heterozygotes and homozygotes. In addition to the 8 families with equal numbers of heterozygotes and homozygotes, almost equal numbers of heterozygote excess (16) and deficit (17) were observed across families and loci, suggesting that heterozygote excess or deficit was random across families and loci. On the other hand, on average I analyzed only 34 segregations at each locus. To detect a

10% difference between the numbers of heterozygotes and homozygotes with a binomial test, an estimated 214 segregations at each locus are necessary in order to achieve 80% power at the 5% significance level. Thus, information from a single family at a single locus is less informative in my analysis and was more subject to sampling bias. A combination of all families and loci in both broods yielded 673 heterozygotes and 676 homozygotes. The almost equal numbers of heterozygotes and homozygotes resulting from this combination suggested there was, overall, no difference in the viability between homozygotes and heterozygotes by this stage of sampling, though a similar conclusion for each locus is tenuous. Small numbers of offspring genotyped also prevented the segregation analysis of families with more complicated Mendelian ratios in offspring genotypes.

Single-locus vs. multilocus

Sampling variation rather than differential selection between homozygotes and heterozygotes is more likely responsible for the observed discrepancies between the observed declines of heterozygosity with inbreeding and the expectations from equation (2) or (5). This conclusion is supported by both a comparison of the observed decline of average heterozygosity with equation (3) and the results of the segregation analyses. A comparison of the observed decline of heterozygosity with the expectations of equation (2) or (5) indicates that more families and perhaps more offspring in each family are required at each level of inbreeding to minimize or eliminate the effects of sampling variation. The effects of sampling variation could also be minimized or eliminated by computing average heterozygosity from more loci.

Heterozygosity vs. number of alleles

The relationship of single-locus heterozygosity of an average family to the degree of inbreeding in general agrees with the changes in the observed number of alleles of an average family at each locus under inbreeding. The consistency between the changes across levels of inbreeding in average heterozygosity and the total number of alleles at all 10 loci was more obvious than that revealed for any single locus. As expected,

offspring from unrelated matings had the greatest average heterozygosity and largest total number of alleles while offspring from full-sib matings had the lowest average heterozygosity and fewest total number of alleles.

Multilocus heterozygosity (MLH) is the most commonly used criterion in monitoring the loss of genetic variation. However, heterozygosity is often regarded as a relatively insensitive indicator of loss of genetic variation, compared with the loss of rare alleles or allele frequency fluctuations between cohorts (Waples et al. 1990; Butler and Cross 1996). A few studies have revealed that reduced genetic variability in cultured populations compared with the wild may be reflected in allelic diversity rather than overall heterozygosity (Danielsdottir et al. 1997; Tessier et al. 1997; Norris et al. 1999; Winkler et al. 1999). Heterozygote advantage or associative overdominance could account for maintenance of overall heterozygosity. Heterozygosity and number of alleles are qualitatively similar as measures of the genetic variation of a population only if the number of progeny is Poisson distributed (Campbell 1995). Under such a distribution, heterozygosity is closely related to the breeding structure of the population, whereas the number of alleles is more sensitive to the variation of the sampling process.

In this study, the number of alleles and heterozygosity showed consistent trends under inbreeding and the consistency was more apparent when the total number of alleles and average heterozygosity over all 10 loci were considered. One possible reason for this discrepancy between my study and the others discussed above was that the same numbers of parents and offspring were used in each group in this study. The same numbers of parents and offspring in each group yielded a balanced comparison and reduced the possible effect of sampling variation when a comparison was made between groups. A more likely explanation is that there were few parental differences in genotypic distribution and number of alleles between groups, and genetic variation in offspring of each group might not yet have been reduced relative to the variation in their parents. Inbreeding leads to an immediate decrease in heterozygosity but can only reduce genetic variation within populations through the loss of genotypes. I did not

detect differential selection against homozygotes versus heterozygotes in my study. Neither did any differential selection against different genotypes introduce noticeable changes in the expected genotypic distributions of offspring among the different mating groups. Thus, one can assume that the observed decline in average heterozygosity of offspring across mating groups was initially introduced by the inbreeding implemented in this study. Nevertheless, divergence between multilocus heterozygosity and number of alleles when they are used to characterize subsequent generations of each mating group is also possible, especially if random genetic drift is appreciable during this period.

Effects of heterozygosity-deficient loci in the grandparents on the changes of average heterozygosity and number of alleles with inbreeding

Significant deficiency in heterozygosity was observed at 5 out of 10 loci in the base population of 45 grandparents based on H-W equilibrium. The existence of null alleles is a possible reason for the observed deficiency in heterozygosity. Null alleles were reported at *Ots102* and *Oki3a* in chinook salmon (Olsen 1999). In addition to these two loci, null alleles might also occur at *Ots103* in the Grovers Creek Hatchery population.

When those loci showing heterozygosity deficiencies in the grandparents were analyzed separately from the loci not showing heterozygosity deficiencies in the grandparents (Table 2.2), average within-family heterozygosity at each of the two groups of loci showed, in general, a similar trend across different levels of inbreeding as when the 10 loci were analyzed together for both broods, although the average within-family heterozygosity at the loci showing heterozygosity deficiencies was slightly higher in the half-sib than in the unrelated mating group for the 1998 brood (Fig. 2.8). This higher average heterozygosity observed in the 1998 brood slightly increased the slope of the regression line of average heterozygosities against inbreeding coefficients, but it did not affect the overall agreement of averaged heterozygosities with the expected values when all 10 loci were counted (Fig. 2.5).

Changes in the average number of alleles with the level of inbreeding showed a similar trend as when all 10 loci were counted in the 1997 brood for both groups of loci and in the 1998 for the group of loci not showing heterozygosity deficiencies in the grandparents (Fig. 2.7). The average number of alleles for those heterozygosity-deficient loci in the 1998 brood was, however, slightly higher in the half-sib than in the unrelated mating group although it was lower in the full-sib than in both the half-sib and unrelated mating groups. These changes might explain why the decreases in the average number of alleles with inbreeding for all 10 loci were nonsignificant in the 1998 brood (as discussed above).

DNA markers vs. allozymes

Both the examinations of equation (5) and the segregation analysis in this study revealed no significant or consistent differences in viability between homozygotes and heterozygotes. This result differs from the findings of previous studies in other species in which slower declines in heterozygosity at single loci under inbreeding were observed than were expected. One of the striking differences between my study and others might lie in the difference of genetic marker. Previously reported studies all used allozymes and a level of observed heterozygosity higher than predicted has been presumed to result from overdominance or associative overdominance. However, since non-coding DNA markers are more likely to be selectively neutral than protein-coding allozymic markers (Ward and Grewe 1994), we should not expect a difference in viability between homozygotes and heterozygotes unless the examined loci from DNA markers are linked to alleles or loci subject to selection. Such linkage was not detected in my present study.

Effects of the evolutionary history of salmonids

Salmonids had an ancestral tetraploid event during their evolution (Allendorf and Thorgaard 1984; Allendorf and Waples 1996). The loss of heterozygosity due to

inbreeding is expected to be slower at a tetrasomic locus than at a disomic locus because of duplicated genes present at the former. Gene duplication arising from the tetraploidization of the salmonid genome has been reported extensively at protein loci (Allendorf and Thorgaard 1984). Its presence has also been confirmed by studies of DNA sequences (e.g., Devlin 1993; Kavsan et al. 1993). Gene duplication in salmonids has been found at microsatellite loci (e.g., Spruell et al. 1999). Extensive gene duplication in salmonids could make genetic interpretation of molecular variation more difficult than in diploid species (Allendorf and Waples 1996). I did not, however, explore whether gene duplication exists at any of the loci in my study. In fact, duplicated alleles are often hard to detect, in part because of the complexity of PCR in genotyping. However, as is the case for the effects of null alleles, the effects of duplicated genes (if any) should not affect my study because the analyses are based mainly on comparisons between different mating groups from the same base population. Although gene duplication could possibly affect the results from analyses based on single loci, such an effect is likely only at specific markers and should not bias the overall results.

Limitations and opportunities of the present study

Despite the widespread application of inbreeding theory as well as genetic markers to many aspects of population genetics of salmonids (see Ryman and Utter 1987; Utter 1994), little is yet known of the relationship of observed to expected heterozygosity in populations undergoing inbreeding. As far as I know, my study is the first to describe the molecular genetic changes in response to known levels of close inbreeding in salmonids. Characterizing these changes is important to understand the mechanism of inbreeding and inbreeding depression that are important considerations for conservation and fishery management. However, there are limitations to my study. First, only two inbreeding levels along with the control level were analyzed. A study of subsequent generations would enhance inferential power considerably by providing the opportunity to incorporate a wider range of inbreeding levels. Second, the expensive and time-consuming nature of genotyping limited my study to only four families per

group for each brood. Analysis of more families could dramatically increase the accuracy of these estimates in this study, especially those based on single-locus analysis. Nevertheless, my study provides a glimpse of the relationship between the observed decline of heterozygosity under inbreeding and the theoretical expectations under simplifying assumptions, and provides an important validation of the theory of inbreeding for salmonids using a battery of neutral molecular markers.

Table 2.1. Escapements of fall chinook salmon to Grovers Creek Hatchery (from Hard and Hershberger, *in press*).

Year	No. jacks	No. males	No. females	Total
1981	2	87	110	199
1982	318	707	724	1,749
1983	1,675	582	748	3,005
1984	197	3,075	2,463	5,735
1985	167	907	1,460	2,534
1986	134	1,034	831	1,999
1987	161	1,180	932	2,273
1988	175	1,107	1,015	2,297
1989	47	1,336	1,098	2,481
1990	10	492	666	1,168
1991	88	236	266	590
1992	87	106	61	254
1993	137	701	266	1,104
1994	726	607	701	2,034
1995	918	3,687	1,660	6,265

Table 2.2. Number of alleles and heterozygosity (He) in 45 grandparents (adults spawned in 1994)[1].

Locus	Number of alleles	He
Ots100	19	0.932
Ots101	18	0.711*
Ots102	25	0.591**
Ots103	9	0.558**
Ots104	17	0.911
Ots107	17	0.658*
Ots108	11	0.844
Ogo1a	2	0.511
Oki3a	19	0.644**
Ssa197	21	0.889

[1] Significant heterozygosity deficiency based on Hardy-Weinberg equilibrium expectations is marked with asterisks (* $p < 0.01$, ** $p < 0.001$).

Table 2.3. Observed heterozygosity and number of alleles (in parentheses) in parents (F_1) of different groups and broods.

Locus	1997 brood parents			1998 brood parents		
	Full-sib	Half-sib	Unrelated	Full-sib	Half-sib	Unrelated
Ots100	0.625 (8)	0.875 (9)	1.000 (11)	1.000 (9)	1.000 (10)	0.750 (8)
Ots101	0.833 (8)	1.000 (9)	0.857 (9)	0.875 (11)	1.000 (11)	1.000 (12)
Ots102	0.428 (6)	0.714 (7)	0.714 (8)	0.625 (8)	1.000 (9)	0.714 (10)
Ots103	0.500 (6)	0.571 (5)	0.600 (5)	0.833 (6)	0.857 (7)	0.800 (5)
Ots104	1.000 (8)	0.875 (8)	0.875 (8)	0.750 (8)	0.875 (7)	0.875 (10)
Ots107	0.750 (7)	1.000 (8)	1.000 (7)	0.750 (7)	1.000 (8)	0.857 (9)
Ots108	0.875 (5)	1.000 (8)	1.000 (7)	0.500 (6)	0.750 (6)	0.625 (7)
Ogo1a	0.500 (2)	0.750 (2)	0.500 (2)	0.500 (2)	0.375 (2)	0.750 (2)
Oki3a	0.833 (6)	0.571 (7)	1.000 (7)	0.600 (6)	0.750 (7)	0.857 (7)
Ssa197	1.000 (11)	1.000 (11)	1.000 (11)	0.875 (9)	1.000 (11)	1.000 (10)
Mean* ± SD**	0.734 ± 0.210 (6.7 ± 2.4)	0.836 ± 0.174 (7.4 ± 2.5)	0.855 ± 0.188 (7.5 ± 2.7)	0.731 ± 0.170 (7.2 ± 2.4)	0.861 ± 0.199 (7.8 ± 2.7)	0.823 ± 0.120 (8.0 ± 2.9)

* Multilocus heterozygosity.

** Standard deviation.

Table 2.4. Observed average individual heterozygosity (HE) in offspring within each family.

Brood	Group	Family ID	HE
1997	Full-sib	48	0.490 ± 0.139*
		60	0.376 ± 0.154
		54	0.699 ± 0.171
		21	0.486 ± 0.117
	Half-sib	32	0.784 ± 0.128
		12	0.665 ± 0.100
		37	0.734 ± 0.162
		33	0.667 ± 0.103
	Unrelated	23	0.713 ± 0.096
		44	0.847 ± 0.097
		13	0.804 ± 0.096
		43	0.854 ± 0.118
1998	Full-sib	104	0.625 ± 0.127
		58	0.626 ± 0.152
		106	0.526 ± 0.096
		16	0.767 ± 0.114
	Half-sib	114	0.891 ± 0.101
		102	0.673 ± 0.142
		24	0.611 ± 0.162
		101	0.670 ± 0.124
	Unrelated	64	0.829 ± 0.116
		31	0.803 ± 0.114
		30	0.843 ± 0.105
		66	0.853 ± 0.092

* Standard deviation in individual heterozygosity within family.

Table 2.5. Genotype frequencies for different groups, along with comparisons of heterozygosities with Hardy-Weinberg equilibrium expectations and expectations from equation (5).

Brood	Group	Locus	No. individuals	Observed heterozygosity	$\sum_{i \neq j} p_i p_j$	$\sum_{i \neq j} p_i p_j (1-f)$
1997	Full-sib	*Ots100*	132	0.591	(-)[a] 0.837**	(-)[b, c] 0.628
		Ots101	118	0.636	(-) 0.849**	(-) 0.637
		Ots102	108	0.278	(-) 0.651**	(-) 0.489**
		Ots103	136	0.265	(-) 0.823**	(-) 0.617**
		Ots104	113	0.735	(-) 0.819*	(+) 0.614**
		Ots107	124	0.500	(-) 0.788**	(-) 0.591*
		Ots108	135	0.541	(-) 0.753**	(-) 0.564
		Ogo1a	127	0.543	(-) 0.858**	(-) 0.644*
		Oki3a	134	0.246	(-) 0.501**	(-) 0.376*
		Ssa197	127	0.819	(-) 0.899**	(+) 0.674**
	Half-sib	*Ots100*	136	0.860	(-) 0.869	(+) 0.760*
		Ots101	108	0.741	(-) 0.889**	(-) 0.778
		Ots102	119	0.580	(-) 0.777**	(-) 0.680*
		Ots103	118	0.568	(-) 0.763**	(-) 0.668*
		Ots104	131	0.771	(-) 0.821	(+) 0.718
		Ots107	122	0.803	(-) 0.827	(+) 0.724
		Ots108	136	0.904	(+) 0.840*	(+) 0.735**
		Ogo1a	120	0.583	(-) 0.758**	(-) 0.663
		Oki3a	136	0.382	(-) 0.454	(-) 0.397
		Ssa197	131	0.878	(-) 0.881	(+) 0.771*
	Unrelated	*Ots100*	132	1.000	(+) 0.894**	
		Ots101	118	0.627	(-) 0.847**	
		Ots102	132	0.606	(-) 0.818**	
		Ots103	113	0.637	(-) 0.767*	
		Ots104	135	0.948	(+) 0.827**	
		Ots107	129	0.891	(+) 0.785*	
		Ots108	136	0.971	(+) 0.842**	
		Ogo1a	88	1.000	(+) 0.898*	
		Oki3a	136	0.412	(-) 0.501*	
		Ssa197	125	0.968	(+) 0.895*	

Table 2.5 continued

Brood	Group	Locus	No. individuals	Observed heterozygosity	$\sum_{i \neq j} p_i p_j$ [a]	$\sum_{i \neq j} p_i p_j (1-f)$
1998	Full-sib	*Ots100*	112	0.857	(-) 0.887	(+) 0.665**
		Ots101	128	0.773	(-) 0.867*	(+) 0.650*
		Ots102	116	0.595	(-) 0.798**	(-) 0.599
		Ots103	109	0.569	(-) 0.800**	(-) 0.600
		Ots104	130	0.831	(+) 0.830	(+) 0.622**
		Ots107	127	0.606	(-) 0.746**	(+) 0.560
		Ots108	105	0.571	(-) 0.756**	(+) 0.567
		Ogo1a	91	0.560	(-) 0.775**	(-) 0.581
		Oki3a	131	0.389	(-) 0.502*	(+) 0.376
		Ssa197	130	0.608	(-) 0.877**	(-) 0.657
	Half-sib	*Ots100*	135	0.881	(+) 0.860	(+) 0.753*
		Ots101	113	0.947	(+) 0.896	(+) 0.784**
		Ots102	96	0.750	(-) 0.845*	(+) 0.739
		Ots103	108	0.713	(-) 0.816*	(-) 0.714
		Ots104	135	0.674	(-) 0.794*	(-) 0.695
		Ots107	132	0.879	(+) 0.816	(+) 0.714**
		Ots108	133	0.617	(-) 0.724*	(-) 0.634
		Ogo1a	135	0.556	(-) 0.640*	(-) 0.560
		Oki3a	136	0.235	(-) 0.328*	(-) 0.287
		Ssa197	132	0.939	(+) 0.902	(+) 0.789**
	Unrelated	*Ots100*	130	0.800	(-) 0.853	
		Ots101	132	0.909	(+) 0.891	
		Ots102	92	0.696	(-) 0.885**	
		Ots103	66	0.924	(+) 0.766*	
		Ots104	131	1.000	(+) 0.879**	
		Ots107	130	0.892	(+) 0.889	
		Ots108	126	0.857	(+) 0.809	
		Ogo1a	119	0.790	(+) 0.774	
		Oki3a	134	0.448	(-) 0.499	
		Ssa197	127	1.000	(+) 0.878**	

[a] Direction of deviation of observed heterozygosity from Hardy-Weinberg equilibrium expectations: (-) represents a deficiency of heterozygotes.

* $p < 0.05$; ** $p < 0.001$.

[b] Direction of deviation of observed heterozygosity from $\sum_{i \neq j} p_i p_j (1-f)$ expectation: (+) indicates an excess of heterozygotes.

[c] Sign test revealed no difference between the number of (+) and the number of (-) in each brood.

Table 2.6 Segregation at 10 microsatellite loci in families whose offspring genotypes have Mendelian ratios 1:1 or 1:2:1.

Locus	Cross	Family ID	No. genotypes			No. heterozygotes	No. homozygotes	Sign[a, b]	*p*-value[c]
			AA	AB	BB				
Ots100	FS	21	14	19		19	14	+	NS
		48	16	16		16	16	0	NS
	HS	33	8	19	7	19	15	+	NS
	U	66	17	15		15	17	-	NS
Ots101	FS	21	18	15		15	18	-	NS
Ots102	FS	58	14	12		12	14	-	NS
		106	11	16		16	11	+	NS
	U	13	12	20		20	12	+	NS
		43	13	21		21	13	+	NS
Ots103	FS	104	6	19	8	19	14	+	NS
	U	13	12	22		22	12	+	< 0.05
Ots104	FS	60	7	15	11	15	18	-	NS
		106	14	19		19	14	+	NS
	HS	12	18	15		15	18	-	NS
		24	21	12		12	21	-	NS
		101	4	18	12	18	16	+	NS
Ots107	FS	106	19	14		14	19	-	NS
Ots108	FS	54	7	17	10	17	17	0	NS
		60	9	17	8	17	17	0	NS
	HS	24	19	14		14	19	-	NS

Table 2.6 continued

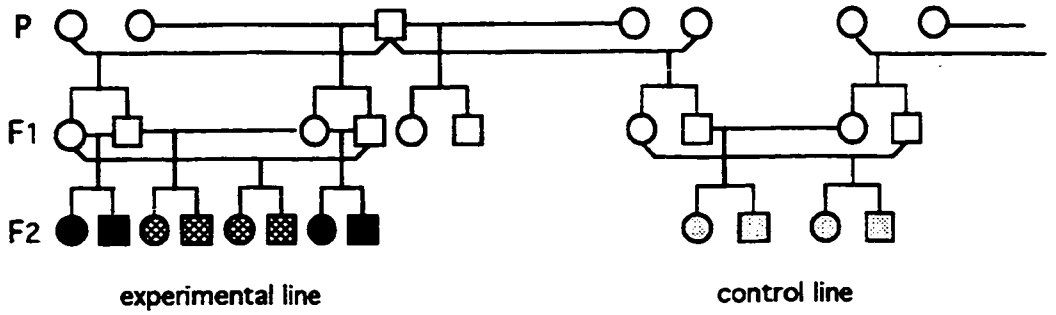
Locus	Cross	Family ID	No. genotypes AA	AB	BB	No. heterozygotes	No. homozygotes	Sign	*p*-value
Oki3a	FS	54	4	14	10	14	14	0	NS
	HS	37	16	18		18	16	+	NS
		102	20	14		14	20	-	NS
Ogo1a	FS	16	9	20	5	20	14	+	NS
		54	8	16	9	16	17	-	NS
		58	14	17		17	14	+	NS
		60	6	17	11	17	17	0	NS
		104	18	14		14	18	-	NS
	HS	12	7	21	6	21	13	+	NS
		33	10	14	10	14	20	-	NS
		37	4	17	13	17	17	0	NS
		114	12	17	5	17	17	0	NS
	U	23	18	16		16	18	-	NS
		30	17	16		16	17	-	NS
		31	10	13	11	13	21	-	NS
		43	16	18		18	16	+	NS
		44	8	22	4	22	12	+	NS
		64	16	18		18	16	+	NS
		66	6	13	14	13	20	-	NS
Ssa197	FS	104	15	6	12	6	27	-	< 0.01
		106	5	17	12	17	17	0	NS

[a] Direction of difference between number of homozygotes and number of heterozygotes.
(+) represents an excess of heterozygotes.
(0) represents equal numbers of homozygotes and heterozygotes.

[b] Sign test showed no difference between the number of (+) and the number of (-).
across all families and loci.

[c] Test for the significance of the observed sign. NS = non-significant.

Figure 2.1. Map of Puget Sound, Washington, indicating the locations of the collection and rearing sites of chinook salmon (from Hard and Hershberger, *in press*).



* Circles represent female fish and squares represent male fish

offspring from unrelated matings

offspring from half-sib matings

offspring from full-sib matings

Some F_1 parents may be used twice.

Figure 2.2. The schematic mating design for the experiment*

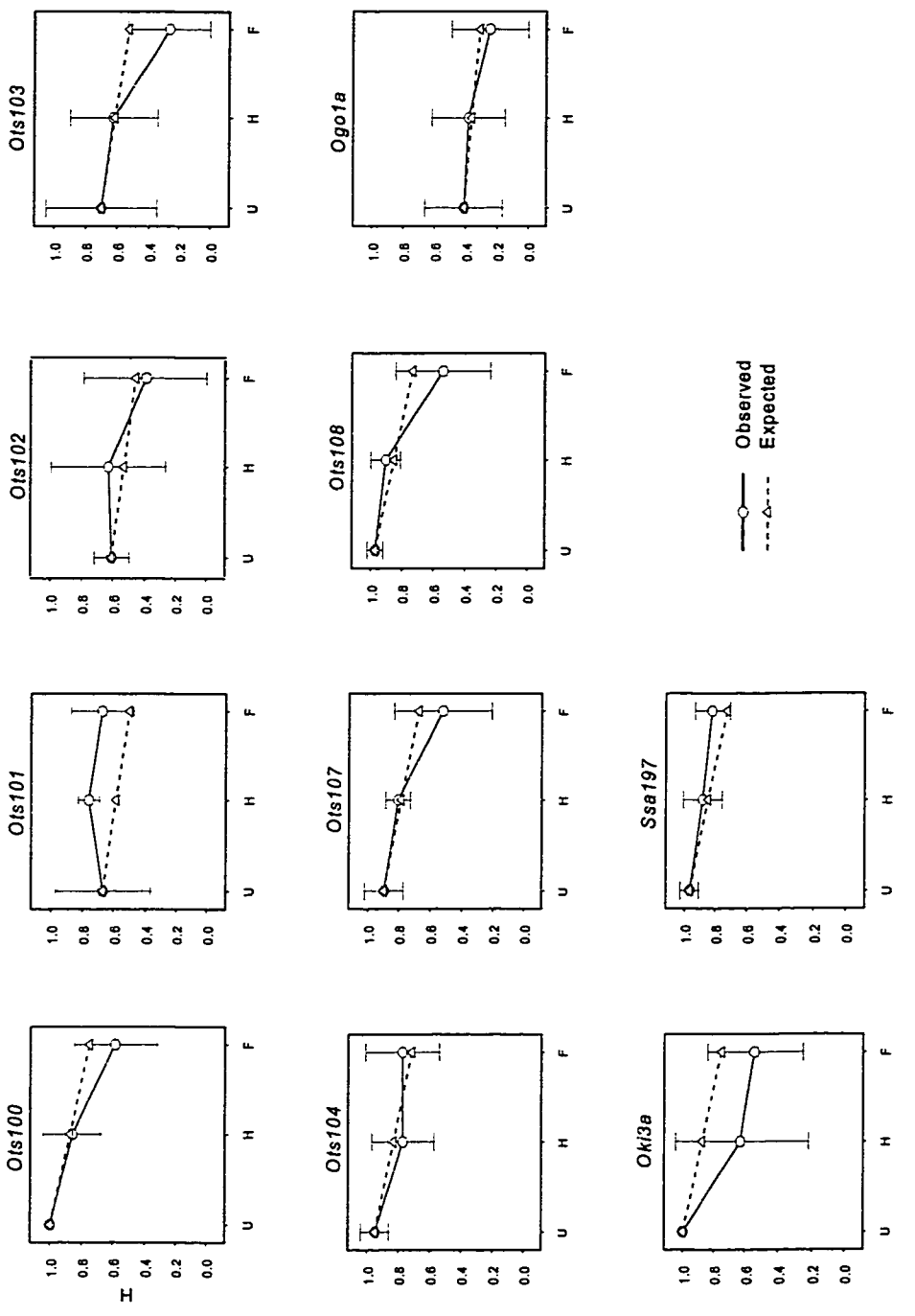


Figure 2.3. Proportion of heterozygotes per family (H) (± SD) observed at each of 10 microsatellite loci, compared to expectations from equation (2) in the 1997 brood (SD = Standard deviation across families within the group).

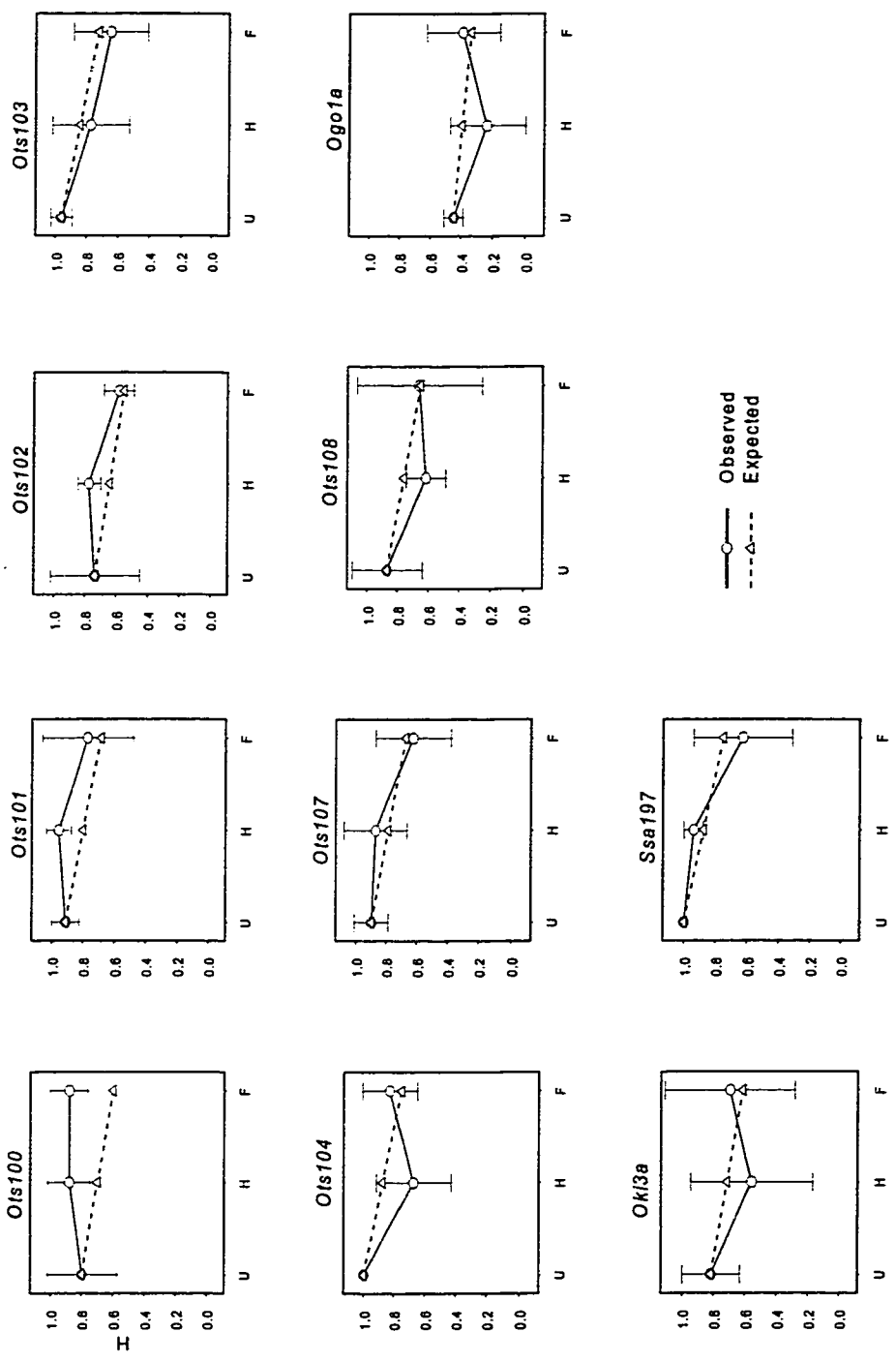


Figure 2.4. Proportion of heterozygotes per family (H) (± SD) observed at each of 10 microsatellite loci, compared to expectations from equation (2) in the 1998 brood (SD = Standard deviation across families within the group).

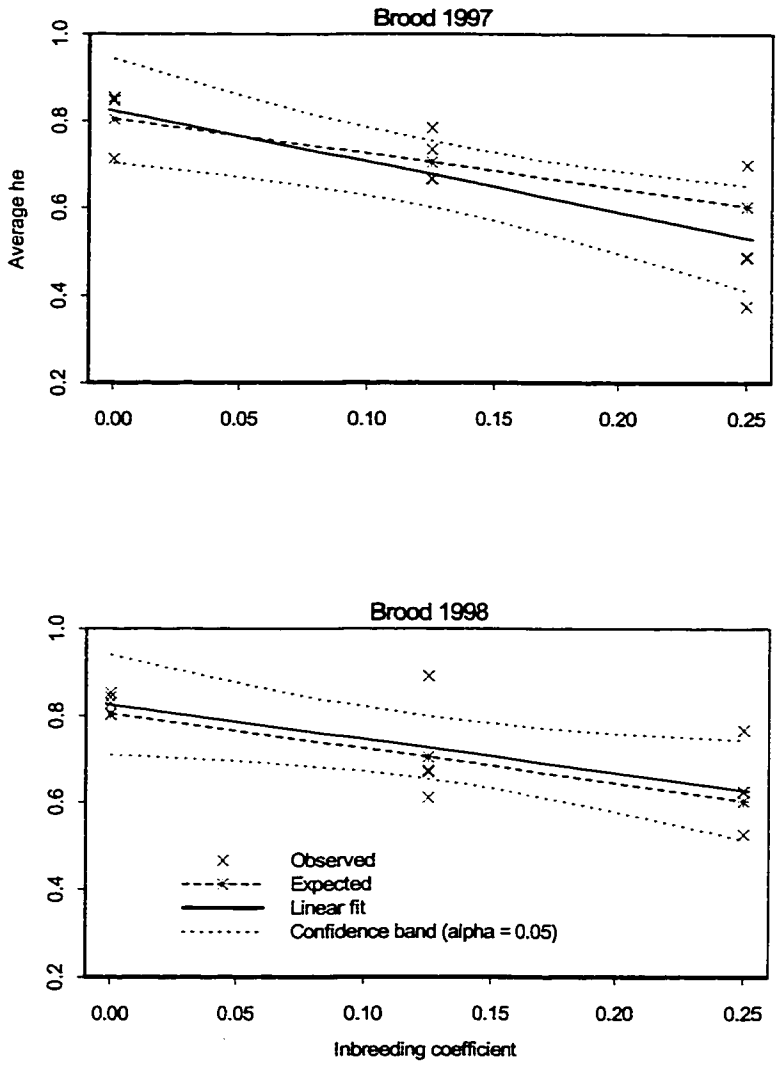


Figure 2.5. Relationship of observed average heterozygosity (He) within family at all 10 loci to the average inbreeding coefficient. Expected values are those predicted from equation (3).

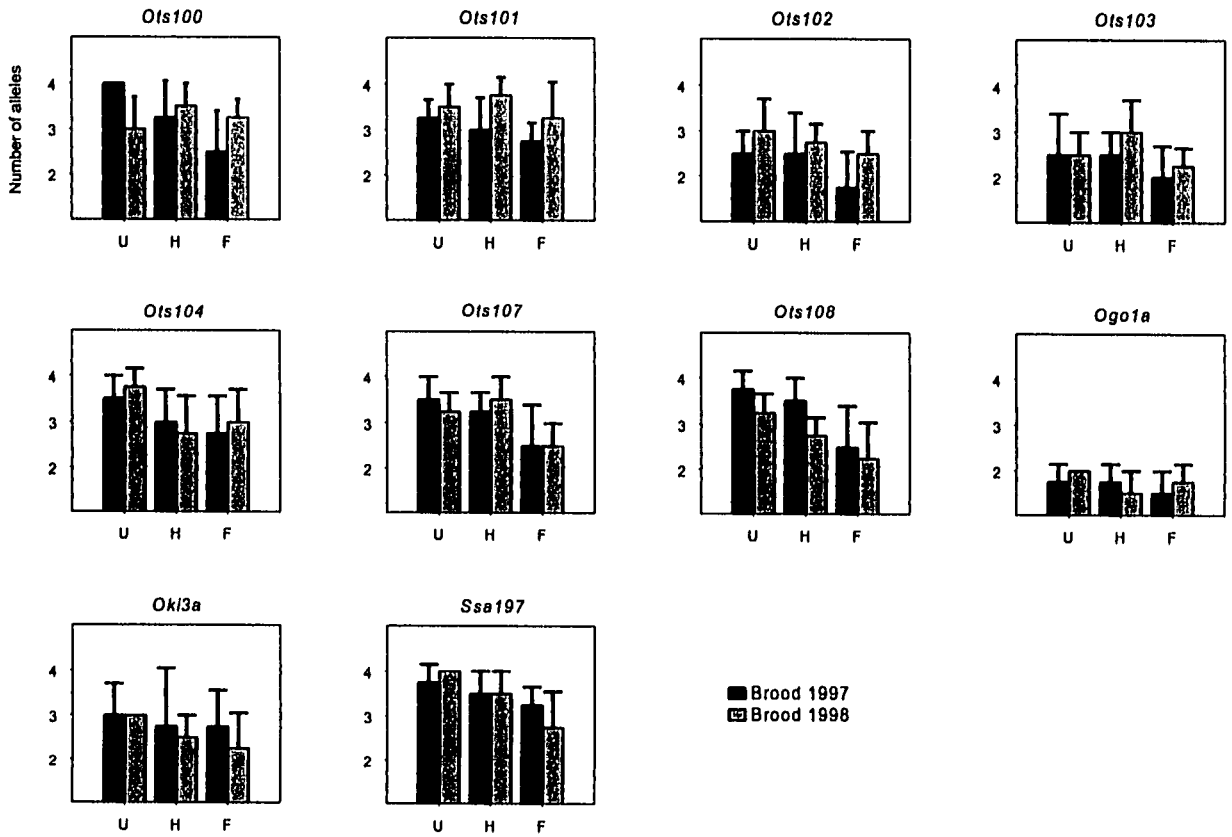


Figure 2.6. Average number of alleles per family (± SD) at each of 10 microsatellite loci for each of the three groups (U = unrelated, H = half-sib cross, F = full-sib cross). SD = standard deviation over families within the group.

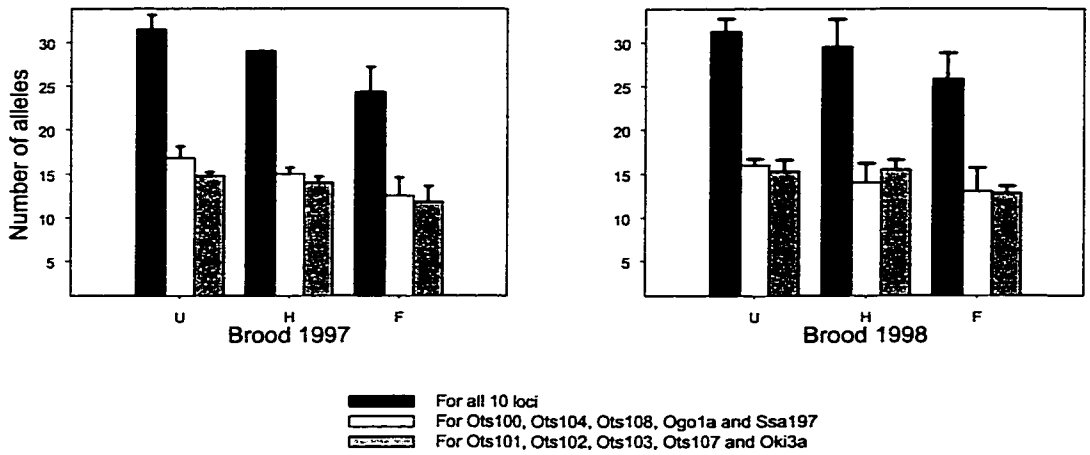


Figure 2.7. The number of alleles in an average family (± SD). (SD = standard deviation over families within the group).

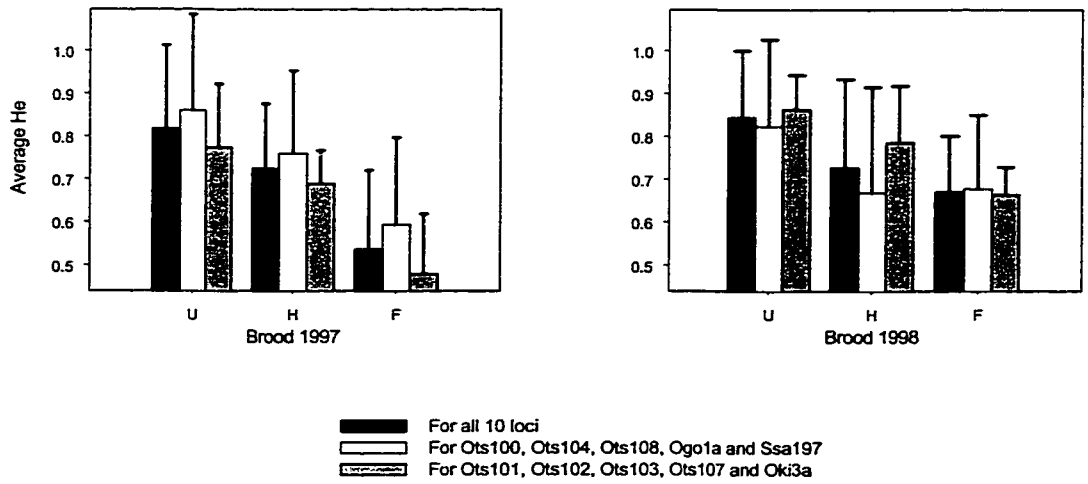


Figure 2.8. Observed average heterozygosity (He) within family (± SD) when the 10 loci were analyzed together and separately based on whether heterozygosity deficiency was detected at the locus in the grandparents.

CHAPTER 3: EFFECTS OF INBREEDING ON EARLY SURVIVAL RATES

Introduction

Inbreeding depression and erosion of genetic variation in adaptive quantitative traits are two major genetic factors that may cause the extinction of a population (Tanaka 1997). Inbreeding depression is the decline of the mean value of one or more traits related to fitness as a direct consequence of inbreeding. Geneticists generally consider inbreeding depression to a serious threat to the viability of small captive populations (Ralls and Ballou 1983; Lande and Barrowclough 1987; Charlesworth and Charlesworth 1987; Ralls et al. 1988; Hedrick and Miller 1992; Sheffer et al. 1999).

Similar to the hierarchical measurement of inbreeding, inbreeding depression can be measured by comparing the average phenotypic values between an inbred population and the base population from which it was derived (Lande and Schemske 1985; Gall 1987) or by comparing the average phenotypic values in offspring from matings between relatives with those from random matings within the same population. The practice of comparisons between inbred and outbred groups as contemporaries minimizes or removes environmental effects on measurements of inbreeding depression of different groups (Kincaid 1976a; Lynch 1988). The most common estimates of inbreeding depression involve traits that are closely related to fitness, such as life-history traits (e.g., viability of the young, fecundity, hatchability) and morphological traits (e.g., growth rate, meristic counts of gillrakers). These traits are often connected with reproductive capacity or physiological efficiency and are most strongly affected by inbreeding (Falconer and MacKay 1996).

Inbreeding depression has been documented repeatedly in many plants and animals (Charlesworth and Charlesworth 1987; Ralls et al. 1988; Thornhill 1993). It has been found in laboratory and zoo populations (Shields 1993) and in wild populations (Crnokrak and Roff 1999). Inbreeding depression found in salmonids includes rainbow

trout (Aulstad and Kittelsen 1971; Aulstad et al. 1972; Kincaid 1976a, b; Kincaid 1983; Gjerde et al. 1983; Su et al. 1996; Pante, et al. 2001), brook trout (Cooper 1961), and Atlantic salmon (Ryman 1970; Rye and Mao 1998). Although data on inbreeding depression have been accumulating, they remain inadequate to permit generalizations about all salmonid fishes owing largely to the marked variation in life histories, genetic backgrounds and living environments. For the same reasons, contrasting results from independent studies of the same species have been reported for same rates of increase in the level of inbreeding. For example, Su et al. (1996) observed no significant inbreeding depression in body weight of rainbow trout at early stages, in agreement with Aulstad and Kittelsen (1971), Aulstad et al. (1972) and Gjerde et al. (1983). However, Kincaid (1976a,b; 1983) reported a moderate depression in body weight at various age stages at equivalent rates of increase in the inbreeding level. In addition, a discrepancy was observed in egg survivorship among these studies. Consequently, further studies on different species and populations are necessary for a better understanding of the relationships between inbreeding and fitness and for identifying factors that influence these relationships in salmonids.

To my knowledge, inbreeding depression has not been documented quantitatively in populations of anadromous Pacific salmon. Precipitous declines in abundance have led several distinct population segments of Pacific Northwest chinook salmon to be listed by the National Marine Fisheries Service (NMFS) under the U.S. Endangered Species Act (ESA) (Myers et al. 1998). A study of the prevalence and severity of inbreeding depression in chinook salmon will be useful in recovery planning and also help to resolve how inbreeding affects population viability for different anadromous salmonid species.

The purpose of my study is to investigate the relationship of different inbreeding rates with survival in the early development of chinook salmon. Survival rates in the early development were measured under two different rates of inbreeding (full-sib and

half-sib matings) and compared to the rates observed for a corresponding control group (unrelated matings).

Material and Methods

Establishment of experimental groups

The establishment of experimental groups was described in Chapter 2.

Sampling

There were 52 full-sib families (18, 19, and 15 for full-sib, half-sib and unrelated mating groups, respectively) in the 1997 brood and 111 full-sib families (38, 37, and 36 for full-sib, half-sib and unrelated mating groups, respectively) in the 1998 brood. However, I did not include in my analysis the families that completely or nearly completely (90% or more) failed before eyeing since unsuccessful fertilization rather than other factors was a possible reason for the failure. As a result, 23 families (11, 8, and 4 for full-sib, half-sib, and unrelated mating groups, respectively) in the 1997 brood and 33 families (13, 12, 8 for full-sib, half-sib, and unrelated mating groups, respectively) in the 1998 brood were removed. The majority of the 1998-brood families that were removed from the study were produced from cryopreserved milt (in liquid N_2). Only 6 families (1, 2, 3 for unrelated, half-sib, and full-sib mating groups, respectively) that were produced from cryopreserved milt had mortality lower than 90% before eyeing. I excluded them from the analysis since it was difficult to discriminate the possible effects of cryopreserved milt on survival rates from those of fresh milt at different developmental stages with only these 6 families. Consequently, 29 full-sib families (7, 11, and 11 for full-sib, half-sib and unrelated mating groups, respectively) in the 1997 brood and 72 full-sib families (24, 23, and 25 for full-sib, half-sib and unrelated mating groups, respectively) in the 1998 brood were actually used for the analysis. All these families were produced from fresh milt.

Measurement of survival rates

Survival rates at eyed, hatch and alevin stages were measured as the proportion of embryos surviving to the first pick-off at each corresponding developmental stage to fecundity.

Statistical analysis

The decrease in survival rate at different developmental stages with inbreeding was estimated by the percent decline in mean survival of inbred relative to unrelated (outbred) fish. Permutation tests (number of permute = 1000, Appendix B) were performed to compare mean survival rates between different mating groups at each developmental stage within each brood. In addition, survival rates were also transformed by $2\arcsin\sqrt{\ }$ as recommended by Neter (1996) and then analyzed using nested ANOVA to show the effects of inbreeding rate (group) and brood. Group was nested within brood and families within group as shown by the nested ANOVA model:

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk} \qquad (1)$$

where

Y_{ijk} is the value of response of the kth family within the jth group within the ith brood,

$\mu_{..}$ is a constant and usually a mean,

α_i is the effect of the ith brood,

$\beta_{j(i)}$ is the effect of the jth group within the ith brood,

ε_{ijk} is the error term,

$i = 1, 2; j = 1, 2, 3,$ and $k = 1, 2, \ldots, n_{ij}$.

F-statistics for brood and family was computed separately using error mean square. All statistical analyses were carried out in Splus 2000 (MathSoft, Seattle).

Results

Mean survival rates in different mating groups

The mean survival rate in the 1997 brood was lower in both half-sib and full-sib mating groups at all stages than in the unrelated mating group (Table 3.1). Compared to the unrelated mating group, mean survival in the half-sib mating group at the eyed, hatch and alevin stages decreased 3% ($p = 0.64$), 6.9% ($p = 0.32$) and 6.9% ($p = 0.38$), respectively, and in the full-sib mating group 1.3% ($p = 0.83$), 8.3% ($p = 0.33$) and 8.8% ($p = 0.31$), respectively. Mean survival rates in half-sib and full-sib mating groups were, however, similar at all stages. The mean survival rate in the half-sib mating group decreased 1.5% ($p = 0.89$) at the hatch stage and 2.0% ($p = 0.85$) at the alevin stage, but increased 1.8% ($p = 0.85$) at the eyed stage relative to the full-sib mating group.

A decrease in the mean survival rate was also observed in both half-sib and full-sib mating groups compared to the unrelated mating group at all stages in the 1998 brood (Table 3.1). Compared to the unrelated mating group, mean survival in the half-sib mating group at the eyed, hatch and alevin stages decreased 10% ($p = 0.01$), 16.6% ($p = 0.01$) and 16% ($p = 0.02$), respectively, and in the full-sib mating group 5.9% ($p = 0.14$), 6.0% ($p = 0.27$), and 6.0% ($p = 0.36$), respectively. However, mean survival rate in the full-sib mating group was higher than in the half-sib mating group at all stages, showing 4.6% ($p = 0.40$), 12.7% ($p = 0.15$) and 12% ($p = 0.22$) increases at the eyed, hatch and alevin stages, respectively.

Nested ANOVA for transformed survival rate revealed that survival rates at each stage were similar for the two broods and that none of changes across groups in the survival rate was significant (Table 3.2).

Variability of survival rate in different mating groups

Both the 1997 and 1998 broods showed an increase in the variance of the survival rate at each stage between families in the half-sib and full-sib mating groups compared to that of the unrelated mating group (Table 3.1). The direction of the difference in the variance of the survival rate between the two inbred groups was, however, inconsistent and the difference was small and not significant at all stages. The differences in the variance of the unrelated mating group with the variance of the half-sib mating group and with the variance of the full-sib mating group increased dramatically subsequent to the eyed stage in both broods. Compared to the variance of the survival rate in the unrelated mating group, in the 1997 brood the variance increased 104% (nonsignificant), 234% ($p = 0.04$) and 224%($p = 0.04$) in the half-sib mating group and 70.6% (nonsignificant), 283% ($p = 0.03$), and 287% ($p = 0.03$) in the full-sib mating group at the eyed, hatch and alevin stages, respectively. The corresponding amount of increase of the variance stages in the 1998 brood at the eyed, hatch, and alevin were 140% ($p = 0.02$), 233% ($p = 0.002$), and 180% ($p = 0.01$) in the half-sib mating group and 266% ($p = 0.001$), 170% ($p = 0.01$), and 178% ($p = 0.01$) in the full-sib mating group.

Discussion

Estimating survival rates

I used initial pick-off at each stage as the endpoint of that stage in terms of calculating survival rates in order to remove the possible effect of different spawning times for each family on survival rates. This effect might occur after initial pick-off at each stage because subsequent pick-offs might occur at the same time for families spawned at different time. Consequently, families spawned earlier might end up with higher mortality at that stage simply because they lived longer. By choosing the initial pick-off as the endpoint of the measurement of a developmental stage, survival differences between families after the initial pick-off would be reflected by the survival difference at the next subsequent stage.

Effects of inbreeding on mean survival rate

Decreased mean survival rate relative to the unrelated mating group was observed at all developmental stages in both half-sib and full-sib groups in the two broods although the effects of inbreeding on survival rate were significant only in the 1998-brood half-sib mating group. Compared to mean survival rate in the unrelated mating group, mean survival rate in the full-sib mating group decreased 8.3% and 8.8% in the 1997 brood and 6.0% and 6.0% in the 1998 brood at the hatch and alevin stage, respectively. The magnitude of the decrease observed in my study was close to those observed for the same level of inbreeding in rainbow trout by Aulstad et al. (1972), who estimated the decrease for survival of eggs at 10.6%, and by Gjerde et al. (1983), who estimated the decrease for survival from the eyed eggs to hatching at 10% and the decrease for survival from hatching to first feeding at 5.3%. Decreases detected in my study were nonsignificant, in disagreement with the results for rainbow trout by Gjerde et al. (1983), but in agreement with the results for rainbow trout by Kincaid (1976a, b), who found no significant effect of inbreeding on egg hatchability at $\Delta F = 0.25$ and $\Delta F = 0.375$.

With the exception of 1998-brood full-sib mating group which showed similar decreases at all stages in survival, a decrease in survival in the inbred groups was more pronounced after the eyed stage. More pronounced decreases in survival at later developmental stages were also observed in rainbow trout by Kincaid (1976a, b), who found nonsignificant reductions in embryos and egg hatchabilities but a significant reduction in percent fry survival under inbreeding. Similarly, Su et al. (1996) also noted a tendency for inbreeding depression for body weight to increase with advancing age, suggesting that close inbreeding might have more severe effects during stressful developmental transitions in salmonids such as the hatching process as in first feeding.

Significant effects of inbreeding on survival rate were found for the half-sib mating group in the 1998 brood by a permutation test. However, the nested ANOVA failed to reveal significant effects of inbreeding among all groups. Low power

due to insufficient number of families within each group may account for the failure to detect significant differences between different mating groups in my study. In fact, retrospective power analyses showed that the greatest power among these three separate nested ANOVAs (Table 3.2) is only approximately 10%, suggesting that a larger number of families within each group are required to detect an effect of inbreeding.

Effects of inbreeding on variability of survival rate

In comparison with that of the unrelated mating group, the variance of the survival rate between families increased at each stage in the half-sib and full-sib mating group in both the 1997 and 1998 broods. The increase was more pronounced after the eyed stage. However, there was no apparent trend for increasing variance of the survival rate with increasing levels of inbreeding at any developmental stage (Table 3.1). As a result, a significant difference in the variance of survival rate between the half-sib mating group and the full-sib mating group was not found at any stage in either brood.

Although it has long been recognized as a possibility (Kimura and Crow 1970; Falconer and MacKay 1996), the effect of inbreeding on the variability of fitness traits has seldom been addressed in most empirical studies. One reason for this 'oversight' is that increased variability of fitness traits was often less of concern than the reduction of their mean values (i.e., inbreeding depression). A second reason is that the variability of fitness traits might be more sensitive than their mean values to the influence of environmental factors. In particular, inbred individuals often show greater environmental variation than non-inbred individuals (Falconer and MacKay 1996). These facts make it difficult to determine whether the increase of variability in inbred fish was a direct result of inbreeding. On the other hand, increase in the variability of fitness traits in the inbred group could also mask the effects of inbreeding on their mean values if a comparison was made of different groups and each group was constituted by a number of independent lines or families, as in my study.

Factors influencing the effects of inbreeding

There was no apparent linear relation between inbreeding depression in survival rate and the level of inbreeding. My results were similar to those obtained from rainbow trout by Kincaid (1976a) and Gjerde et al. (1983). Similarly, there also seemed no apparent linear relation between variance of the survival rate and the level of inbreeding at any developmental stage. Several factors could interfere with the effects of inbreeding and obscure a linear relationship between the effects of inbreeding and the levels of inbreeding. For instance, diverse parental genetic backgrounds may lead to a different response to the same rate of inbreeding in their progeny. The historical tetraploidization in salmonid evolution might also complicate the relationship of survival rate with levels of inbreeding if tetraploidization can "buffer" against inbreeding depression (see Chapter 1, Part 1 and 2).

Although all mating groups were reared under similar environmental conditions in each brood, subtle differences in environmental conditions could still exist at different levels (e.g., difference in rearing density and water supply between trays and stacks that were used to incubate eggs). In general, inbreeding depression is more severe in harsher environments (Bijlsma et al. 1999; Jimenez et al. 1994; Pray et al. 1994). The impact of environmental stress becomes significantly greater for higher levels of inbreeding (Bijlsma, et al. 2000). In fact, environmental stress can be more relevant than inbreeding effects to an immediate decrease in some fitness values. For instance, a study in mosquitofish by Kandl (In press) has shown that inbreeding (ΔF = 0.125, 0.25) did not affect population growth rate in either salinity treatments (6.5 ppt and 0 ppt). However, populations in the 6.5 salinity treatment had significantly lower growth rate, smaller juveniles, larger females and fewer juveniles relative to adults than populations in the 0 ppt salinity. In addition, selection can also counteract inbreeding depression if selection leads to favorable alleles being fixed at more of the loci than would occur by chance (Falconer and MacKay 1996; Gall 1987).

Maternal influences on the effects of inbreeding were found in the study of rainbow trout by Kincaid (1976a), where he indicated that the range of size and general quality of the eggs from different female fish could be a reason for this observed effects. However, my analysis of the 1998 brood, which showed a higher mean survival rate in the full-sib mating group than in the half-sib mating group, failed to detect a significant linear relationship between survival rate and average egg size[1] within any groups (not shown). Despite the lack of a linear relation between survival rate and egg size I noticed that there was a shift from small to large in the selection of egg size across the mating groups in the 1998 brood (Fig. 3.1). In addition, the variability of egg size in the half-sib mating group and full-sib mating group was larger than that in the unrelated mating group in both the 1997 and 1998 broods. Nonetheless, it was difficult to tell from my study whether the increased variability of egg size in the half-sib and full-sib mating groups had contributed to the increased variance between families in the survival rate in these two groups.

[1] Measured by a 6-inch-long stainless steel "pig trough".

Table 3.1. Survival rate at different developmental stages in the 1997 and 1998 broods.

Brood	Developmental stage	Group	N	Survival rates	
				Mean	SD[1]
1997	Eyed	U	11	82.5	9.8
		HS	11	80.0	14.0
		FS	7	81.4	12.8
	Hatching	U	11	81.1	9.3
		HS	11	75.5	17.0*
		FS	7	74.4	18.2*
	Alevin	U	11	80.6	9.5
		HS	11	75.0	17.1*
		FS	7	73.5	18.7*
1998	Eyed	U	25	85.0	8.0
		HS	23	76.5*	12.4*
		FS	24	80.0	15.3*
	Hatching	U	25	79.3	11.5
		HS	23	66.1*	21.0*
		FS	24	74.5	18.9*
	Alevin	U	25	77.3	12.9
		HS	23	64.9*	21.6*
		FS	24	72.7	21.5*

* $p < 0.05$ in comparisons with the unrelated group.

Table 3.2. Nested ANOVA for transformed survival rate at different stages, testing for brood and group effects.

Stage	Source	df	MS	F
Eyed	Brood	1	0.00946	0.09
	Group (Brood)	4	0.13669	1.29
	Error	95	0.10586	
Hatching	Brood	1	0.14336	0.86
	Group (Brood)	4	0.28804	1.73
	Error	95	0.16603	
Alevin	Brood	1	0.22512	1.22
	Group (Brood)	4	0.29809	1.61
	Error	95	0.18474	

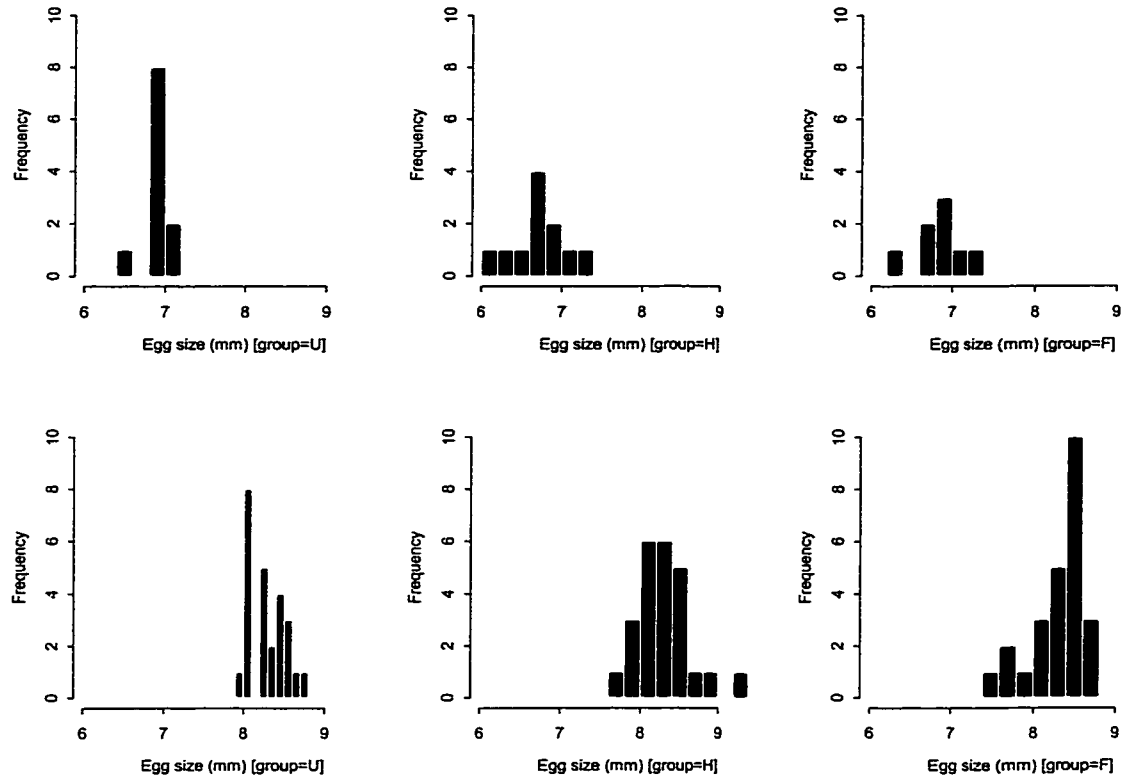


Figure 3.1. The distribution of average egg size of individual female within each group in the 1997 brood (upper row) and the1998 brood (lower row).

CHAPTER 4: EFFECTS OF INBREEDING ON EARLY DEVELOPMENTAL STABILITY

Introduction

Although any trait expressed during development of an individual is expected to have some, usually unknown, underlying genetic bases, its developmental pathway can, nevertheless, be disturbed by internal (e.g., developmental accidents) and external (e.g., environment) factors. The ability of development to produce a genetically targeted phenotype despite potential disturbing factors has been termed developmental stability (Leary et al. 1992). Fluctuating asymmetry (FA), defined as small deviations from perfect symmetry, is commonly used as a measure of the developmental stability of bilaterally symmetrical morphological or meristic traits (Palmer and Strobeck 1986, 1992). Developmental stability is expected to be positively correlated with the overall level of individual heterozygosity (Lerner 1954). As such, FA should decrease with increasing individual heterozygosity within a population and, perhaps, within families. The presence of environmental or genetic stress during ontogeny has been proposed to elevate FA by reducing the efficiency of normal developmental processes (Clarke 1992; see also Leung et al. 2000). Based on these underlying theories of FA, inbreeding may increase FA of individuals either by decreasing individual heterozygosities or by imposing stress as a result of genetic perturbation or both. However, evidence across taxa shows that the negative association of FA with individual heterozygosity is by no means universal (e.g., Polak and Starmer 2001). Neither can a general conclusion with respect to altered FA be reached in individuals after inbreeding or crossbreeding (Lynch and Walsh 1998). Studies of association of FA with individual heterozygosity in salmonids have been found in a variety of species including brook trout, cutthroat trout, rainbow trout, Atlantic salmon, chum salmon, and pink salmon. A summary of these existing results on salmonids indicates that a clear association of FA with individual heterozygosity does not exist (see Chapter 1, Part 2).

Studies of the relationship of FA to genetic perturbation in salmonids are few. Leary et al. (1985b) showed increased FA in all four interspecific hybrids of bull trout

with brook trout and captive rainbow trout with three strains of cutthroat trout relative to either of the pure strains. Ferguson (1986), however, found variable results in the change of FA caused by hybrids between different strains of rainbow trout. Studies were also made to investigate the effects of inbreeding on FA. However, these studies were based on either the comparison of hatchery strains with their wild counterparts (e.g., Leary et al. 1985c; Crozier 1997) or the comparison of different strains or species (e.g., Wagner 1996). The results of these studies showed that the effects of inbreeding on performance of fitness traits as well as the relationship between individual heterozygosities and performance of fitness traits were determined by the genetic background of the populations and subject to the influence of environment (see Chapter 1, Part 1). Thus, the results of these studies were compromised by the substantially different environments experienced by hatchery and wild fishes or by the genetic difference between strains or species.

As suggested by the variable results in the association of FA with individual heterozygosity, such an association may also differ among species and even populations. An accumulation of studies in different species and populations is, however, necessary to gain better understanding of these associations. In the present study, I aimed to investigate the relationship of developmental stability through FA with different levels of inbreeding in chinook salmon. Unrelated, half-sib and full-sib matings were used to produce one outbred group and two inbred groups ($\Delta F = 0$, 0.125 and 0.25, respectively) from the same outbred base population (Chapter 2). Levels of FA in offspring produced from different matings were compared with one another. Offspring from these three mating groups differed significantly in the overall levels of individual heterozygosity based on ten microsatellite loci. Decreases of individual heterozygosity in the inbred groups were in agreement with those expected from the inbreeding rates (Chapter 2).

To my knowledge, association of developmental stability with individual heterozygosity or levels of inbreeding has not yet been reported for chinook salmon.

One unique feature of the present study is that it controlled the confounding effects of environment by rearing offspring of different mating groups under similar environmental conditions and the effects of genetic background by producing groups of different levels of inbreeding from the same base population. In addition, individual heterozygosity in offspring from inbred groups decreased measurably compared to that in offspring from unrelated matings. These changes enhanced the chance of detecting a relationship between developmental stability and individual heterozygosity provided such a relationship exists in the chinook salmon population.

Material and Methods

Establishment of experimental groups

The establishment of experimental groups was introduced in Chapter 2.

Sampling

9 families from unrelated matings, 13 families from half-sib matings and 9 families from full-sib matings were chosen at random from the 1998 brood. 10 to 21 fingerlings were then sampled haphazardly from each family for the assessment of FA.

Measurement of FA and trait size

Fluctuating asymmetry was assessed by counting gill rakers, branchiostegal rays, mandibular pores, pectoral fin rays and pelvic fin rays. Gill rakers on the lower first branchial arch, upper first branchial arch, lower second branchial arch and upper second brachial arch were all measured and denoted as LGR1, UGR1, LGR2 and UGR2, respectively. Mandibular pores, branchiostegal rays, pectoral fin rays and pelvic fin rays were denoted as MP, BR, PEC, and PEL, respectively.

FA was calculated as the value of a given trait on the right side of the body minus its value on the left (R-L). All measures of FA were scaled by trait size so that there was consistency between measures. Using scaled FA can also reduce the effects of trait size on FA. A dependence of FA on trait size can bias the results in studies of

developmental stability (Palmer and Strobeck 1994). Trait size was calculated as the mean number of counts across the two sides of the body ([R+L]/2) for a given trait.

For traits exhibiting FA, the distribution of signed (R-L) values will be normally distributed with a mean of zero (Van Valen 1962; Palmer and Strobeck 1986, 1992, 1994). Departure from a normal distribution in FA can signal directional asymmetry or antisymmetry whose reliability as measurement of developmental stability remains controversial (Palmer and Strobeck 1992, 1994). To test for FA, I calculated mean, skewness, and kurtosis of signed FA (R-L) for each trait by pooling families within each group and by pooling families across groups. The joint use of skew and kurtosis statistics appears to be useful way to detect departures from normality for meristic characters (Palmer and Strobeck 1992).

Statistical analysis

Scaled unsigned FA (|R-L|) data were analyzed using nested ANOVA in which I analyzed the effects of levels of inbreeding (group) and family on each measure of FA. Family was nested within group and individuals within family as shown by the nested ANOVA model:

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk} \qquad (1)$$

where

Y_{ijk} is the value of response of the *k*th individual within the *j*th family within the *i*th group,

$\mu_{..}$ is a constant and usually a mean,

α_i is the effect of the *i*th group,

$\beta_{j(i)}$ is the effect of the *j*th family within the *i*th group,

ε_{ijk} is the error term,

$i = 1, 2, 3; j = 1, 2, \ldots, b_i$ and $k = 1, 2, \ldots, n_{ij}$.

F-statistics for group and family were computed using family and error mean square, respectively. Trait size was entered as a covariate. This analysis is equivalent to ANOVA (without adjusting for trait size) of residuals from regression of FA on trait size. There are two reasons for using trait size as a covariate. First, residuals in FA generated using the size covariate are more likely to be normally distributed than unsigned FA values (see Polak and Starmer 2001). Second, if FA is correlated with trait size (Table 4.3), the size effects on FA caused by the correlation may not be removed even using scaled FA (Palmer and Strobeck 1994). I also tested for the simultaneous effects of levels of inbreeding and family on FAs of all traits using nested ANOVA with trait as a group variable.

Results

Table 4.1 shows the mean size for each trait over all groups. Descriptive statistics for signed FA values (R-L) for each trait within site are presented in Table 4.2. Mean signed FA values (R-L) for BR differed significantly from zero in all groups, suggesting directional asymmetry (DA) in this trait (see Palmer 1994). Mean signed FA values for UGR1 and LGR2 differed from zero only in the full-sib mating group ($p = 0.02$) and half-sib mating group ($p = 0.04$), respectively. Distributions of signed FA were skewed to the left for all traits. However, significant skewness was detected only for UGR2 in the unrelated mating group, MP in the full-sib mating group and PEL in all groups. There was little or no platykurtosis (negative kurtosis) which indicates antisymmetry (see Palmer 1994) although significant leptokurtosis (positive kurtosis) was detected for UGR1, UGR2, MP, BR, PEC and PEL in one or more groups. Leptokurtosis may suggest significant genetic heterogeneity within each group (Palmer and Strobeck 1986; Gangestad and Thornhill 1999; Polak and Starmer 2000).

A significant phenotypic correlation between unsigned FA (| R-L |) and trait size was found for MP in all groups (r = 0.27, 0.18, and 0.31 for the unrelated, half-sib and full-sib mating group, respectively) and PEL in the unrelated mating group (r = 0.41) (Table 4.3). No significant correlation in unsigned FA between traits was detected.

A consistent increase in the mean scaled unsigned FA and proportion of asymmetric individuals with level of inbreeding was observed only for LGR1, UGR1, and UGR2 (Table 4.4). However, nested ANOVA revealed that the effect of inbreeding (group) on FA was nonsignificant for all traits (Table 4.5). This analysis also indicated that family effects on FA were not significant for any of these traits, suggesting homogeneity of FA level of each trait across families. The effect of trait size on FA was highly significant for MP. The significant effect of trait size in MP was in agreement with the significant phenotypic correlations between unsigned FA and trait size for MP in all groups (Table 4.3).

Nested ANOVA with trait as a group variable revealed that the simultaneous effects of inbreeding on FA for all traits were not significant (Table 4.6). Neither were family effects on FA of these traits. Effect of trait on FA of each trait was significant, suggesting that the effects of trait size on FA were different among traits and that some traits were more sensitive to the effects of inbreeding than others (Table 4.6). Removing BR which may show directional asymmetry, or/and PEL whose distribution in FA departed from normality did not change these results.

Discussion

The genetic basis of FA variation in natural populations and knowledge of the degree of genetic covariance between FA and traits reflecting other aspects of fitness are fundamental to predicting the evolution of developmental stability (Polak and Starmer 2001). Negative correlations between level of FA and overall individual heterozygosity have been found in brook trout, cutthroat trout (Leary et al. 1984), rainbow trout (Leary et al. 1983, 1984, 1985a, 1987, 1992) and Atlantic salmon (Blanco et al. 1990, but see

Vollestad and Hindar 1997). However, a significant correlation between FA and heterozygosity was not detected in chum salmon (Beacham and Withler 1987) or pink salmon (Beacham and Withler 1987; Beacham 1991). Studies of the effects of inbreeding on FA so far not found in salmonids have been found in other species such as the fruit fly and house mouse. Variable effects similar to the relationship between individual heterozygosity and FA have been observed (reviewed by Lynch and Walsh 1998).

In my study of the direct relationship between levels of inbreeding and FA in salmonids, consistent increases in the level of FA and the proportion of asymmetric individuals with inbreeding were detected for LGR1, UGR1, and UGR2 although none of these increases were significant. Such consistent increases were not observed for other traits, suggesting that the effects of inbreeding on FA may vary considerably among traits. The same result was also shown by the nested ANOVA when trait was entered as a group variable (Table 4.6).

Diverse environments and genetic backgrounds can influence the effects of inbreeding on FA and the relationship between individual heterozygosity and FA (see Chapter 1, Part 2). In salmonids, elevated FA by environmental stress has been observed in Atlantic salmon (Vollestad and Hindar 1997) and rainbow trout (Leary et al. 1992). A benign hatchery environment might have weakened the relationship between the magnitude of FA and the level of inbreeding in my experiment by minimizing stress during development. Detecting a weak correlation between heterozygosity or the level of inbreeding and FA (see Britten 1996) may require a very large sample size. Due to limitations of family sampling and rearing space, FA was measured in only 9 to 13 families for each mating group and 10 to 21 individuals for each family, values that are far below those recommended in the literature (e.g., David 1998; Van Dongen 1999).

Although family effects on levels of FA were not detected, we can not rule out family variation in genetic diversity or levels of FA obscuring the correlation of levels of FA with low levels of inbreeding. For example, Leary et al. (1992) observed a significant

negative association between heterozygosity and asymmetry within populations but not among populations in rainbow trout. In addition to the diverse genetic backgrounds and environments among populations that were acknowledged by these workers, variation in levels of FA among populations could also have contributed to the failure to detect a relationship between heterozygosity and asymmetry among populations.

In addition to the decrease of heterozygosity, inbreeding may also cause stress to inbred individuals. Such stress may or may not be relevant to the reduction of heterozygosity. For instance, stress may be caused by unmasking of deleterious recessive alleles due to inbreeding (Clarke 1992). Whether a reduction of heterozygosity or a stress during ontogeny is a more immediate reason for elevation of asymmetry remains unknown. Leary et al. (1987) showed that the association between heterozygosity at allozyme loci and developmental stability was as strong among full-sibs as it was among individuals sampled at random from populations of salmonid fishes. As a result, they argued that differences in inbreeding coefficients did not explain the association between heterozygosity and developmental stability. Caution should be applied when concluding these studies because the results from Leary et al. (1987) were not obtained directly from close inbreeding. In addition, recent inbreeding may be more relevant than that occurring many generations past in drawing conclusions about the effects of inbreeding on developmental stability as well as other aspects of fitness.

In addition to diverse genetic background and environment, several other factors can presumably influence the effects of inbreeding on FA and the relationship between individual heterozygosity and FA. These factors can be null alleles, low power (see Chapter 1, Part 2), failure to correct for scaling with trait size, measurement error, and natural selection (see Palmer and Strobeck 1992; Palmer 1994; Lynch and Walsh 1998). Furthermore, associations between FA and fitness components being inconsistent and often weak (see Leung and Forbes 1997; Gangestad and Thornhill 1999; Bourguet 2000) might indicate that one or a few randomly selected traits will be a very poor

predictor of developmental stability unless deviations from symmetry are large. Nevertheless, associations between FA and fitness components have rarely been studied in salmonids. Knowledge of the associations between FA and fitness components of the studied traits would be useful for a comprehensive assessment of the effects of factors that elevate FA (e.g., genetic and environmental stress) because fitness components are more directly relevant than FA to conservation issues.

Retrospective power analyses to the nested ANOVA showed that the largest power for the analyses of group (inbreeding) effects is only approximately 0.35 (for UGR1) among all separate analyses (Table 4.5). Low power in the statistical analyses due to limited number of families within each group appeared to be important for the failure to detect a significant effect of inbreeding on FA. Power will increase as more families are tested. Similarly, significant effects of inbreeding on FA may be detected in an analysis to the subsequent inbred generations since increased level of inbreeding elevates FA. The latter assumption is further supported by a proposed threshold relationship on the basis of inbreeding depression for fitness components which suggests that inbreeding depression become detectable only when the level of inbreeding increases to a certain level (Soulé 1980, Frankham 1995c). Nevertheless, these initial studies provide a foundation for further insights into the effects of inbreeding through subsequent inbred generations of these lineages.

The results of my study imply that establishing cause and effect relationships in genetic study of FA is difficult even under appropriate experimental designs. Results of similar studies may be misleading without considering both effects of genetic background of populations under study and effects of environmental factors. This caution also implies that drawing conclusions about the association of heterozygosity or levels of inbreeding with levels of FA is extraordinarily difficult without large studies on a variety of organisms. Nevertheless, our understanding of these relationships is improving and will continue to improve with the growing number of studies on different populations and species.

Table 4.1. Mean, standard deviation (SD) of size for each trait.

	Trait							
	LGR1	UGR1	LGR2	UGR2	MP	BR	PEC	PEL
Mean	13.3	8.9	12.6	8.0	8.5	15.2	15.5	10.2
SD	0.6	0.7	0.6	0.5	0.7	0.8	0.5	0.4

Table 4.2. Mean signed FA (R-L), skewness and kurtosis for each trait.

Trait	Group	N	Mean (SD)	p[1]	Skewness	Kurtosis
LGR1	U	101	0.0594 (0.772)	0.44	0.103	0.127
	H	141	-0.0284 (0.828)	0.68	0.254	-0.057
	F	113	-0.1504 (0.918)	0.08	0.024	-0.362
	Pooled	355	-0.0422 (0.844)	0.35	0.146	-0.148
UGR1	U	101	0.0297 (0.591)	0.61	0.006	-0.058
	H	141	-0.0851 (0.806)	0.21	0.341	1.307***
	F	113	-0.1786 (0.762)	0.02	0.065	-0.096
	Pooled	355	-0.0819 (0.739)	0.04	0.208	0.783**
LGR2	U	101	-0.0495 (0.766)	0.52	0.085	0.195
	H	141	-0.1286 (0.718)	0.04	0.395	0.225
	F	113	0.0268 (0.741)	0.70	0.178	-0.002
	Pooled	355	-0.0567 (0.740)	0.15	0.163	0.141
UGR2	U	101	-0.0693 (0.515)	0.18	0.557*	2.370***
	H	141	-0.0143 (0.523)	0.75	0.288	1.995***
	F	113	0.0089 (0.593)	0.87	0.002	1.771***
	Pooled	355	-0.0227 (0.543)	0.43	0.017	2.010***
BR	U	101	-1.1485 (0.888)	0.00[2]	0.298	1.197**
	H	141	-1.2269 (0.778)	0.00[2]	0.318	1.304***
	F	113	-1.0540 (0.769)	0.00[2]	0.029	0.300
	Pooled	355	-1.1497 (0.809)	0.00[2]	0.011	1.026***
MP	U	101	-0.0297 (1.000)	0.77	0.121	0.848
	H	141	-0.0213 (0.857)	0.77	0.800***	3.232***
	F	113	-0.0268 (0.854)	0.74	0.214	1.124**
	Pooled	355	-0.0254 (0.898)	0.59	0.262*	1.715***
PEC	U	101	-0.0495 (0.498)	0.32	0.109	1.132**
	H	141	0.0071 (0.387)	0.83	0.069	3.891***
	F	113	-0.0531 (0.440)	0.20	0.270	2.220***
	Pooled	355	-0.0282 (0.437)	0.23	0.145	2.250***
PEL	U	101	0.0396 (0.280)	0.16	1.423***	9.757***
	H	141	-0.0213 (0.348)	0.47	3.803***	43.211***
	F	113	0.04425 (0.338)	0.17	0.777**	5.794***
	Pooled	355	0.01690 (0.327)	0.33	2.277***	24.057***

[1] t-test for H_0 : mean (R-L) = 0.

[2] Values are less than 0.001.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4.3. Phenotypic correlation coefficients between FA (|R-L|) and trait size.

	FA measure							
Group	LGR1	UGR1	LGR2	UGR2	BR	MP	PEC	PEL
U	-0.074	0.019	0.028	0.057	0.136	0.273**	0.160	0.413***
H	-0.077	0.046	0.036	0.019	-0.027	0.185*	-0.107	-0.139
F	0.137	0.113	0.020	0.052	0.107	0.308***	0.046	0.173

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 4.4. Mean and standard deviation (SD) of scaled unsigned FA and proportion of asymmetric individuals for each trait (PA) within each group.

Trait	Group	Mean	SD	PA
LGR1	U	0.0387	0.043	0.475
	H	0.0430	0.046	0.511
	F	0.0514	0.047	0.593
UGR1	U	0.0392	0.054	0.346
	H	0.0563	0.073	0.426
	F	0.0597	0.063	0.500
LGR2	U	0.0395	0.045	0.465
	H	0.0362	0.045	0.421
	F	0.0393	0.044	0.464
UGR2	U	0.0310	0.057	0.238
	H	0.0324	0.057	0.250
	F	0.0394	0.064	0.295
BR	U	0.0816	0.051	0.852
	H	0.0820	0.050	0.894
	F	0.0721	0.047	0.814
MP	U	0.0765	0.085	0.515
	H	0.0614	0.079	0.433
	F	0.0623	0.074	0.469
PEC	U	0.0158	0.028	0.248
	H	0.0096	0.023	0.149
	F	0.0125	0.026	0.195
PEL	U	0.0075	0.026	0.079
	H	0.0079	0.037	0.064
	F	0.0110	0.030	0.115

Table 4.5. Nested ANOVAs for scaled unsigned FA of each trait, testing for group and family effects and adjusted for trait size.

Trait	Source	df	MS	F
LGR1	Size	1	0.003032	1.42
	Group	2	0.004090	2.96
	Family (Group)	28	0.001381	0.65
	Error	324	0.002134	
UGR1	Size	1	0.000001	0.00
	Group	2	0.012947	2.47
	Family (Group)	28	0.005233	1.27
	Error	322	0.004133	
LGR2	Size	1	0.000048	0.02
	Group	2	0.000437	0.31
	Family (Group)	28	0.001430	0.69
	Error	321	0.002077	
UGR2	Size	1	0.000193	0.05
	Group	2	0.002220	0.53
	Family (Group)	28	0.004176	1.19
	Error	321	0.003513	
BR	Size	1	0.000344	0.14
	Group	2	0.003840	1.29
	Family (Group)	28	0.002984	1.24
	Error	322	0.002414	
MP	Size	1	0.072978	11.91***
	Group	2	0.005757	1.06
	Family (Group)	28	0.005406	0.88
	Error	322	0.006128	
PEC	Size	1	0.000011	0.02
	Group	2	0.001156	1.43
	Family (Group)	28	0.000811	1.29
	Error	323	0.000628	
PEL	Size	1	0.000743	0.73
	Group	2	0.000293	0.26
	Family (Group)	28	0.001128	1.11
	Error	232	0.001013	

*** $p < 0.001$.

Table 4.6. Nested ANOVA for scaled unsigned FA, testing for trait, group and family effects and adjusted for trait size.

Source	df	MS	F
Size	1	0.000299	0.11
Trait	7	0.208617	74.99***
Group	2	0.001703	0.57
Family (Group)	28	0.002979	1.07
Error	2794	0.002782	

*** $p < 0.001$.

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Appendix A. Panel of 10 salmonid microsatellite primer pairs.

Locus	Primer sequence (F > Forward, R > Reverse)	Reference	Source
Ots100	F>5'-TGA-ACA-TGA-GCT-GTG-TGA-G R>5'-ACG-GAC-GTG-CCA-GTG-AG	Nelson and Beacham, 1999	*Oncorhynchus tshawytscha*
Ots101	F>5'-ACG-TCT-GAC-TTC-AAT-GAT-GTT-T R>5'-TAT-TAA-TTA-TCC-TCC-AAC-CCA-G	Small, et al., 1998	"
Ots102	F>5'-AGG-ATC-CAA-TAA-GGA-GTG-ATA R>5'-ACT-AGG-TAT-CCC-CTT-AAC-CA	Nelson and Beacham, 1999	"
Ots103	F>5'-AGG-CTC-TGG-GTC-CGT-G R>5'-GAC-ATA-GCG-TTC-AGC-ACA-G	Small, et al., 1998	"
Ots104	F>5'-GCA-CTG-TAT-CCA-CCA-GTA R>5'-GTA-GGA-GTT-TCA-TTT-GAA-TC	Nelson and Beacham, 1999	"
Ots107	F>5'-ACA-GAC-CAG-ACC-TCA-ACA R>5'-ATA-GAG-ACC-TGA-ATC-GGT-A	"	"
Ots108	F>5'-TTT-CTA-TTA-GTC-TGT-CAC-TAC R>5'-TGG-CAA-GGA-GAG-AGA-CAG-AGG-G	"	"
Ogo1a	F>5'-GAT-CTG-GGC-CTA-AGG-GAA-AC R>5'-ACT-AGC-GGT-TGG-AGA-ACC-C	Olsen, 1999	*Oncorhynchus gorbuscha*
Oki3a	F>5'-GAT-CAA-TGG-ACA-ACC-TGT-CAA-A R>5'-AAC-ACA-GGC-ATC-CCC-ACT-AA	Spidle, A. unpulished	*Oncorhynchus kisutch*
Ssa197	F>5'-GGG-TTG-AGT-AGG-GAG-GCT-TG R>5'-TGG-CAG-GGA-TTT-GAC-ATA-AC	O'Reilly et al., 1996	*Salmo salar*

Appendix B. Splus code for permutation test used in Chapter 3.

```
perm.test <- function(x, npermute, theta, group, alt="t")
{
#x       = vector of observations
#npermute = number of times to resample without replacement from x
#theta    = statistic for which permutation distribution will be computed
#group    = vector identifying to which group each observation in x belongs
#alt      = form of alternative distribution
#                  "t" = two-sided
#                  "l" = less than
#                  "g" = greater than

call <- match.call()

#Identify the two populations
grp <- unique(group)

#Compute total number of observations in both populations
n <- length(x)

#Create a vector which will hold the npermute estimates of theta.
thetastar <-  rep(NA, npermute)

#Compute npermute estimates of theta, one at a time.
#These will comprise thetastar, our permutation distribution.
for (i in 1:npermute)
   {
   gstar <- sample(group, n, replace=F)
   thetastar[i] <-  theta(x[gstar==grp[1]]) - theta(x[gstar==grp[2]])
   }

#Compute the observed estimate of theta.
obsdiff <- theta(x[group==grp[1]]) - theta(x[group==grp[2]])

#Compute p-value, depending on the form of alt.
reject <- switch(alt, t=abs(thetastar) >= abs(obsdiff),
                      l=thetastar <= obsdiff,
                      g=thetastar >= obsdiff
          )
p.value <- sum(reject)/npermute

return(thetastar, p.value, obsdiff, call=call)
}
```

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