Construction of a Linkage Map for the Pink Salmon Genome

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ABSTRACT

We will complete the analysis of experiments conducted at the Alaska SeaLife Center (ASLC) that use the linkage map we have constructed to test for effects of regions of the genome on traits that are important to recovery of pink salmon (e.g., growth and survival). Sexually mature adults from the 1999 cohorts produced from wild pink salmon collected from Likes Creek are expected to return to Resurrection Bay and the ASLC in August and September 2001. We will compare genotypes in released fry and returning adults to test for genetic differences in marine survival and other life history traits (e.g., body size, egg number, and egg size).

INTRODUCTION

This is the final phase of our research to construct a genetic linkage map for pink salmon (*Oncorhynchus gorbuscha*) and to use this map to evaluate effects of natural selection on the genome of this species. Such a map was proposed initially to provide the necessary platform to identify genetic damage in pink salmon inhabiting oiled streams following the March 1989 *Exxon Valdez* oil spill (EVOS). We have conducted a series of experiments based at the Alaska SeaLife Center (ASLC) to identify regions of the genome that affect various organismal traits and to test for the effects of natural selection on regions of the genome that include markers used to describe genetic population structure. This research will aid recovery efforts with pink salmon, including estimation of straying rates, description of stock structure, and testing if marine survival and other organismal measures of phenotypic variation have a genetic basis.

Genetic linkage maps have provided the necessary information for understanding genetic variation in species since the rediscovery of Mendel's principles early in this century. A genetic map plays a similar role for a geneticist that a geographical map plays for the explorer of new territories. For many years, genetic maps could only be constructed in a very few model species that were suitable for extensive genetic manipulation (e.g., *Drosophila* and mice). Recent advances in molecular genetics now make it possible to uncover enough genetic markers to construct a detailed genetic linkage map in almost any species (Postlethwait et al. 1994).

This project began in FY 96. However, we did not receive authorization to proceed until half-way through FY 96 (March 1996). We have completed our two initial objectives that included identifying several hundred genetic markers and using them to construct a linkage map. We have used the ASLC for experiments that apply the linkage map to an understanding of the fundamental population biology and genetics of pink salmon.

This work was originally designed to support work with pink salmon under the project *Oil-Related Embryo Mortalities* (Restoration Study \191A). The objective of that project was to identify germline mutations in pink salmon exposed to oil. Genetic damage induced by oil may either be small changes in nucleotide sequence (microlesions) or large-scale changes in chromosome structure (macrolesions). A detailed genetic map for pink salmon would have been invaluable for interpreting the results of Restoration Study \191A in several ways. First, it would be possible by following the inheritance of any DNA lesions to determine if they are micro- or macro-lesions. Second, these lesions could be mapped to determine if they are randomly spread throughout the genome or if they occur at mutational "hot spots" that are susceptible to oil induced damage. However, Restoration Study \191A is no longer ongoing, and thus our future work will concentrate on our original Objectives 5 and 6 as described in this proposal.

We modified our proposal last year because we found high mutation rates at two microsatellite loci. These mutations are not randomly distributed among families, but some are "clustered" in certain families. The rates and especially the patterns of mutations that we have observed have provided some important fundamental insights into the evolution of microsatellite loci and their use in population genetic studies. Results with humans have found an increase in mutation rates at minisatellite loci following exposure to radiation following the Chernobyl

incident (Dubrova et al. 1996). Our recent results raise the possibility that microsatellite loci may also be good candidates for monitoring germline mutations in marine species following exposure to oil.

We are continuing our experiments at the ASLC to test for regions of the genome associated with marine survival and fitness. We released nearly 50,000 progeny in spring 1999, and anticipated a 2-5% return rate in our original experimental design. Due to the failure of the fish pass at the ASLC to attract returning marked adults we captured only 36 fish from nearby freshwater streams in Resurrection Bay. The collection of these fish from such a large system indicates that a substantial number of marked fish did survive and return to Resurrection Bay. We were not able to complete any of our objectives with the 1998 cohort because of the poor returns. We have adjusted our plans for recapturing returning adults from the 1999 cohort when they return to Resurrection Bay in the hopes of collecting an adequate sample for analysis.

NEED FOR THE PROJECT

A. Statement of Problem

Elevated embryo mortalities were detected in populations of pink salmon inhabiting oiled streams following the spill. These increased rates of mortality persisted through the 1993 field season, three generations after the oil spill, suggesting that genetic damage may have occurred as a result of exposure to oil during early developmental life-stages. The consequences of the putative genetic damage include impaired physiological function of individuals and reduced reproductive capacity of pink salmon populations (Bue et al. 1998).

The aggregate of evidence from field studies and incubation experiments suggests that embryos exposed to oil in 1989 and 1990 accumulated deleterious mutations in the germline (Bue et al. 1998). However, see Cronin and Bickham (1998) for an alternative interpretation of these data. This hypothesis of genetic damage is consistent with previous field observations and laboratory experiments on the effects of crude oil on early life stages of fish. Long term intra-gravel oil exposures (7-8 months) to freshly fertilized eggs provide embryos sufficient time to accumulate polynuclear aromatic hydrocarbons (PAH's) from very low aqueous concentrations of crude oil. PAH's are abundant in crude oil and are potent clastogens (i.e. capable of breaking chromosomes). Roy et al. (1999) have recently reported evidence of molecular genetic damage to pink salmon embryos exposed to crude oil.

Mironov (1969) observed reduced survival of fish embryos and larvae exposed to very low aqueous doses (1 ul oil/L seawater) of oil. Longwell (1977) reported genetic damage in pelagic embryos affected by the ArgoMerchant oil spill. Moles et al. (1987) confirmed that pink salmon embryos take up PAH's and demonstrated that the uptake was much greater in an intertidal environment than in strictly freshwater conditions. Biggs et al. (1991) found greater numbers of chromosome aberrations in larval herring that incubated in oiled areas than in non-oiled areas. It is likely that the same type of damage may have occurred in pink salmon and other species in Prince William Sound, and this damage could have affected the germline of exposed individuals

(Malkin 1994; Bue et al. 1998).

Molecular genetic techniques have been used extensively to describe population structure of Pacific salmon (Utter et al. 1993; Gharrett and Smoker 1994; Seeb et al. 1998). Genetic divergence among populations has been interpreted as largely reflecting the patterns of exchange of individuals among populations (gene flow) and random changes in frequency of selectively neutral alleles within populations (genetic drift) (Allendorf and Phelps 1981; Waples 1995). This is a useful approach that allows description of the pattern and amount of gene flow among populations.

This approach to describe population structure is based upon the assumption that the pattern and amount of divergence observed is not affected by natural selection or mutation. However, even weak natural selection may have a substantial effect on the pattern of genetic divergence among populations (Allendorf 1983). In addition, different mutation rates at marker loci may also effect the amount of genetic differentiation between populations, in particular if mutation rates at some loci are high (e.g., Jin and Chakraborty 1995). Thus, the high frequency of mutations that we have detected may also have a substantial effect on the amount and pattern of genetic divergence at some loci.

Molecular markers may be affected by natural selection even if the markers themselves are not the target of selection. Loci that are selectively neutral and have no effect on the phenotype are expected to be affected by the action of natural selection at closely linked loci (Slatkin 1995). Apparent heterozygous advantage ("associative overdominance") can result at neutral loci by linkage disequilibrium with nearby loci that are affected by natural selection (Pamilo and Pálsson 1998). Zhivotovsky et al. (1994) have recently questioned the description of genetic population structure of pink salmon and suggested that natural selection may have an important effect on allozyme frequency divergence in pink salmon.

It has been notoriously difficult to detect and measure the effects of natural selection in natural populations (Lewontin 1991). Comparing the distribution of genotypes in a single cohort followed through different life history stages is the most powerful method to detect natural selection (p. 303, Lynch and Walsh, in preparation). The facilities at the ASLC provide an exceptional opportunity to measure lifetime fitness from fertilization to sexual maturity of molecular genetic markers spread throughout the genome identified in previous years of this project.

B. Rationale/Link to Restoration

The recovery objective for pink salmon is healthy and productive populations that exist at prespill levels or levels in unoiled areas. An indication of recovery is when egg mortality in oiled areas match prespill or levels in unoiled areas. A genetic map would be essential for detecting and understanding causes of reduced egg and embryo survival in oiled areas (Bue et al. 1998). The genetic damage caused by exposure to oil may persist longer in populations of pink salmon than in other vertebrates because of the tetraploid nature of the salmonid genome. Salmonid fishes went through a tetraploid event some 25 million years ago that duplicated their entire genome

(Allendorf and Thorgaard 1984). The extra genes in pink salmon may mask the effects of mutational damage caused by recessive deleterious alleles. The effects of these deleterious mutations may be uncovered in subsequent generations.

This research will provide a powerful test of the assumption of the absence of natural selection affecting molecular markers. This assumption is the foundation of interpreting patterns of genetic divergence among populations as reflecting patterns of genetic exchange. Evidence of natural selection affecting the molecular markers would cause a major change in the interpretation of genetic variation in natural populations of pink salmon and other species. This will be true whether the selection is acting on the markers themselves or chromosomal segments linked to the markers. Recent results from molecular studies of the genome suggest that natural selection may play a greater role than previously thought in determining the structure of the genome, including the organization of genes and chromosomes, as well as the patterns and amounts of genetic variation present (Hurst 1999).

C. Location

Gametes for the inheritance studies and linkage map were collected from Prince William Sound in collaboration with the project Oil-Related Embryo Mortalities (Restoration Study \191A). Embryo incubation took place at the Genetics Lab facilities of ADFG. The laboratory analyses were done at the University of Montana and the ADFG genetics lab in Anchorage.

We began in FY 1998 to use the ASLC Research Facilities at Seward for experiments designed to test for natural selection at loci throughout the genome of pink salmon. Sexually mature pink salmon used in the experimental matings in 1998 and 1999 were collected from Likes Creek in Resurrection Bay. The progeny were marked with an adipose fin clip and released into Resurrection Bay. Due to the failure of the fish pass at the ASLC to attract returning adults we have adjusted our plans for recapturing returning adults from the 1999 cohort to include sampling in upper Resurrection Bay.

COMMUNITY INVOLVEMENT

This is a specialized project that will not benefit directly from the knowledge of local/traditional people. We will hire local residents when possible for assistance (e.g., collecting and maintaining fish). We have developed two computer interactive educational games to be incorporated in displays describing our project at the ASLC ("Lost Child" and "Whose Your Father?"). Amy Haddow, ASLC Education Director, is currently developing a display based upon these games. In addition, we have taken opportunities to explain our research in different Alaska high schools. Kathy Knudsen gave a presentation on this project to the freshman biology class at Kenai High School, and Kate Lindner discussed this study with high school students in Kongiganak.

We are attempting to involve the community of Seward in our project and different aspects of the study such as collecting the returning adults. A lottery was conducted during the 2000 field

season as an incentive to recreational fisherman to turn in any marked pink salmon they caught. This resulted in 18 recreational fishermen turning in 22 marked fish. We are also interested in suggestions of other opportunities for informational meetings in the communities of Prince William Sound, and articles in the Trustee Council newsletter.

PROJECT DESIGN

A. Objectives

Our initial primary objective was to construct a detailed genetic linkage map for pink salmon by analyzing the genetic transmission of several hundred DNA polymorphisms. Pink salmon have 26 pairs of chromosomes (2N=52; Allendorf and Thorgaard 1984), and, therefore, should have a total of 27 linkage-groups: 25 autosomes, an X-chromosome, and a Y-chromosome. We planned to map enough variable markers so that a new marker can be assigned with high probability to one of the 27 linkage groups. It was impossible to know how many markers this would require because we did not know the total length of the pink salmon linkage map. The linkage map of the zebrafish (*Danio rerio*) has been estimated to be 2900 centimorgans (cM; Johnson et al. 1996) and that of the medaka (*Oryzias latipes*) to be 2480 cM (Wada et al. 1995). There currently are efforts to include zebrafish among genome projects of model species sponsored by the National Institutes of Health under the Human Genome Project (Roush 1997). Such a massive effort in zebrafish would provide extremely helpful information for understanding the genome of salmonid fishes.

We expected the pink salmon map to be large because of the polyploid ancestry of salmonids and due to the fact it is female based. Young et al. (1998) recently have published a rainbow trout (*Oncorhynchus mykiss*) linkage map based upon recombination rates in males and estimated the total map to be 2628 cM. However, the linkage map in males will be shorter than in females because of the reduced recombination rate in male salmonids (Johnson et al. 1987a). We initially anticipated that it would be necessary to map over 500 markers to ensure that new markers can be assigned to an existing linkage group with high probability (Van der Beek and Van Arendonk 1993). For example, 99% of all loci in the zebrafish were estimated to be located within 20 cM of a marker on the map based upon an earlier report using 414 markers (Postlethwait et al. 1994).

This project originally had the following overall specific objectives:

1. Develop several hundred variable DNA markers in pink salmon and test them for Mendelian inheritance.

2. Construct a linkage map based upon joint segregation patterns of the DNA polymorphisms detected in previous objective.

3. Map putative lesions identified in Restoration Study \191A.

4. Test for Mendelian inheritance of markers throughout the genome in progeny of fish exposed to oil. Regions that show aberrant segregation ratios in progeny of fish exposed to oil and

normal 1:1 ratios in fish not exposed to oil would be candidates for oil-induced lesions.

5. Test for regions of the genome that are associated with traits of adaptive significance (e.g., marine mortality or run-timing).

6. Test if protein markers (allozymes) are under natural selection such that they may not provide accurate information about the genetic structure and amount of gene flow among populations.

We have completed Objectives 1 and 2. We cannot pursue Objective 3 because Restoration Study /191A did not identify any putative lesions for mapping. At present, we do not intend to pursue Objective 4 because Restoration Study \191A is no longer ongoing. However, this type of experiment to detect oil-induced lesions could be pursued in the future at the ASLC. The primary focus in FY 02 will be Objectives 5 and 6; we propose to use the linkage map to test if there are phenotypic effects and adaptive significance of different classes of molecular markers.

B. Methods

OBJECTIVES 1 & 2

Our initial map was constructed using gynogenetic haploid and gynogenetic diploid progeny from an odd-year individual female (95-103). This is the same procedure that has been used to build the zebrafish linkage map (Postlethwait et al. 1994). Stanley (1983) reported that haploid embryos of Atlantic salmon will develop until just prior to the stage of hatching if development of the eggs is activated by sperm in which the DNA has been inactivated by UV-radiation. We have used this technique routinely with fishes of the genus *Oncorhynchus* (Forbes et al. 1994; Spruell et al. 1999). This allows us to follow the segregation and linkage relationships in haploid progeny from females. The use of haploid progeny avoids possible difficulties of dominance with some types of DNA markers because recessive alleles are not obscured by their dominant alternatives in haploids (Lie et al. 1994). Our odd-year map is primarily based on 603 segregating markers in 94 haploid progeny from a single pink salmon female (A95-103) that returned to Armin F. Koernig hatchery in Prince William Sound in August 1995. We have placed a number of so-called "anchor" loci on this map.

In addition we have initiated the construction of a linkage map based on the segregation pattern of 90 haploid individuals in an even-year female (V96-13). Odd- and even-year pink salmon are reproductively isolated due to the fixed two-year life cycle of this species (Aspinwall 1974). Beacham et al. (1988) report substantial allozyme and morphological evidence for differentiation of alternate brood years. In addition, Phillips and Kapuscinski (1988) and Phillips et al. (1999) detected chromosomal rearrangements between odd- and even-year populations that occur in the same geographical area. Furthermore, in a recent experimental study, Gharrett et al. (1999) demonstrated outbreeding depression in crosses between the two year classes. Together, these finding suggest that the alternate brood years are reproductively isolated and genetically distinct. Having linkage data from both odd- and even-year individuals will make it possible to map more markers and will allow us to determine whether linkage relationships are conserved between the reproductively isolated year classes.

Differences in meiosis between male and female salmonids have been found in all species that have been examined (Allendorf and Thorgaard 1984; Johnson et al. 1987a). There generally is greater recombination in females than in males (Johnson et al. 1987a; Allendorf et al. 1994). In addition, only disomic inheritance has been reported in females. However, in males some loci show patterns of segregation that approach those expected with tetrasomic inheritance (Allendorf and Thorgaard 1984). We will have to test for segregation and linkage in males as well as females because of these sex-specific differences.

Construction of a full linkage map is a large task. We developed as many time and labor saving procedures as possible. Our linkage map was constructed by computer assisted analysis (MapMaker, Lander et al. 1987). We have been assisted by Mark Daly of the Whitehead Institute at MIT in using this program. We will compare the recombination rates based upon this map to rates of selected pairs of loci in males using families produced for the 1998 cohort. The reduced recombination rates in salmonid males means that it will be easier to assign new markers to a linkage group using male parents. We will test joint segregation of individual markers from different linkage groups identified in females to determine if some of these separate linkage groups in females are linked in males and are therefore syntenic (on the same chromosome).

A useful genetic map contains genetic markers that are abundant, randomly distributed throughout the genome, highly polymorphic, and readily detectable in many laboratories (Jacob et al. 1995). We began using random amplified polymorphic DNA (RAPD) markers because they fit these criteria and they have been used successfully in constructing linkage maps in zebrafish and medaka (Johnson et al. 1996; Wada et al. 1995). We have switched to two other types of genetic markers that are superior to RAPDs in this work.

PINEs: There are a variety of repetitive DNA elements that are scattered throughout the genome of salmonid fishes. Greene and Seeb (1997) have described a technique that uses the sequences from a SINE (short interspersed nuclear element) and a transposon to detect many DNA polymorphisms. They have called this technique SINE-printing. We have modified this technique using other types of repetitive elements for our mapping study to detect a class of molecular markers that we call PINEs (paired interspersed nuclear elements; Spruell et al. 1999).

Kido et al. (1991) described 3 SINEs in salmonid fishes. They documented the presence of two such elements, *Hpa*I and *Sma*I, in pink salmon. Spruell and Thorgaard (1996) subsequently reported the presence of the 5'-end of the third element, *Fok*I, in pink salmon. Goodier and Davidson (1994) confirmed that salmonids also contain the transposon *Tc*1, a member of another class of repetitive elements. Both SINEs and transposons occur in high copy number and are believed to be ubiquitously dispersed throughout the genome, making them ideal candidates for genomic mapping efforts.

We have used DNA sequences from four types of repetitive elements as polymerase chain reaction (PCR) primers to generate multiple DNA fragments from a single PCR reaction in pink salmon. The theoretical basis for this procedure is similar to the use of the human SINE *Alu*I to identify human chromosomes in somatic cell hybridization experiments (Nelson et al. 1989).

Primers complementary to one end of the element are oriented such that they initiate DNA synthesis from the end of the element, progressing into the surrounding genomic DNA. A single primer or combinations of primers may be used to generate multilocus patterns. Greene and Seeb (1997) used this technique to confirm the parentage of pink salmon fry, demonstrating the potential utility of including these fragments in our mapping study. We have used 12 different pairs of PINE primers to detect 162 segregating markers in our reference family.

AFLPs: Amplification fragment length polymorphisms have been used extensively in the construction of genomic maps in plants (Maheswaran et al. 1997; Becker et al. 1995). The AFLP technique is especially advantageous for two reasons. First, many bands are produced per reaction and, therefore, more polymorphic loci are produced per unit effort. Second, the selective amplification step uses a subsample of the PCR products of the preamplification. Up to 133 selective amplifications can be completed from a single pre-amplification that originally used only 0.5 μg of genomic DNA. Much less genomic DNA is needed to produce more bands than using other methods such as RAPDs. This is an important consideration when dealing with the limited amount of tissue available from haploid embryos.

Gene-Centromere Map

We estimated recombination rates between 312 loci and their centromeres using half-tetrad analysis in a recently published manuscript (Lindner et al. 2000). We produced the half-tetrads by initiating development with irradiated sperm and blocking the maternal second meiotic division. AFLPs were significantly more centromeric than loci identified by three other techniques (allozymes, microsatellites, and PINEs). The near absence of AFLPs in distal regions could limit their utility in constructing linkage maps. A large proportion of loci had y values approaching 1.0, indicating near complete crossover interference on many chromosome arms. As predicted from models of chromosomal evolution in salmonids, all duplicated microsatellite loci that shared alleles (isoloci) had y values of nearly 1.0. This is consistent with previous data from allozyme loci.

Odd-Year Linkage Map

We have described the segregation of 590 markers in haploid progeny from female A95-103; we have also mapped 13 allozyme loci in the same female (Table 1, Figure 1). We assigned 546 of the 590 markers to one of 44 linkage groups covering a distance of 4559 cM (Table 2). Given the haploid number of 26 chromosomes for pink salmon, our mapping efforts produced 18 extra linkage groups. Taking into account the extra linkage groups and 43 unassigned markers as well as the distance to the telomeres we estimate the size of the pink salmon genome to be 6691 cM. The haploid pink salmon genome is approximately 2.72 million kilobase pairs (kpb; Johnson et al 1987b); thus, we estimate approximately 406 kbp/cM. These results are consistent with our expectations when comparing to maps constructed in other fishes (Table 3).

Table 1. Summary of Marker Types on the Odd-Year Pink Salmon Map

	Number of polymorphic loci	Number of markers unlinked	Percent assigned to linkage group
AFLPs	393	36	91
PINEs	162	7	96
Microsatellites	35	0	100
Allozymes	13	0	100
Total	603	43	93

Table 2. Summary of Odd-Year Pink Salmon Linkage Groups

Number of Markers	Number of groups	Average size (cM)
1-5	10	23.26
6-10	14	53.51
11-15	9	118.50
16-20	6	181.27
21-25	1	189.20
26-30	1	243.70
31-35	2	263.05
36-40	0	--------
41-45	0	--------
46-50	0	--------
over 50	1	457.40

[FIGURE 1 in WORD files (fig-1a.doc; fig-1b.doc; fig-1c.doc)]

[TABLE 3 in WORD file (table-3.doc)]

Putting "Anchor Loci" on the Map

We are still in the process of placing additional loci on the map to aid in consolidation and to make the map useful to other genetic investigators working with salmonids. In particular, it is important to include common markers that can serve as references between maps from divergent taxa (O'Brien et al. 1993). The primary types of so-called "anchor loci" we have used are allozymes and microsatellites that are currently being used in salmonid population genetic studies, including investigations of pink salmon. We will also map other loci that are available and of special interest and usefulness (e.g., growth hormone loci, Forbes et al. 1994, and the major histocompatibility complex, Katagiri et al. 1996; Shum et al. 1996; Miller and Withler 1998). These anchor loci will be used to test for differences in the linkage map between odd- and even-year pink salmon. In addition, we will test for differences in recombination rates, crossover interference, and residual tetrasomic inheritance between males and females (Allendorf and Danzmann 1997).

We have placed 35 microsatellite loci on the odd-year map in collaboration with Drs. Roy Danzmann, Moira Ferguson, and Takashi Sakamoto at the University of Guelph in Ontario. These microsatellite loci are found in 17 linkage groups. We have also placed 13 allozyme loci that are polymorphic in Prince William Sound pink salmon (Seeb et al. 1996; Habicht et al. 1998) on the map using gynogenetic-diploids from female A95-103 and several normal diploid families (Table 4) in collaboration with the ADFG Genetics Lab.

Table 4. Summary of linkages in normal diploid families between allozymes and microsatellites. The sex of the informative parent is given in the third column (F = female, M = male). Rate of recombination between loci is indicated by r.

Loci	Family	Informative Parent	N	r	Chi-sq (1 df)
sAAT3 - FH	A95-14	F	86	0.337	9.12
sAAT3 - sMDHB1,2	A95-14	F	89	0.112	53.49
sAAT4 - STR60	A95-104	F	21	0.238	5.76
ADA2 - PGDH	A95-120	M	56	0.125	31.50
ADA2 - SSA197	A95-103	F	42	0.024	38.10
	A95-120	M	18	0.111	10.89
CKC2 - STR60	A95-103	F	46	0.348	4.26
FH - MDHB1,2	A95-14	F	86	0.291	15.07
bGALA - G3PDH1	V96-2	M	75	0.346	7.05
GDA1 - PEPD2	A95-8	M	82	0.012	78.05
	A95-20	M	95	0.105	59.21
	A95-29	M	45	0.000	45.00
G3PDH1 - PEPLT	V96-5	M	75	0.240	20.28
GPIB1,2 - PEPD2	V96-2	M	75	0.013	71.05
sIDHP2 - OTS1	A95-29	M	41	0.366	2.95
	A95-104	F	33	0.303	5.12
PGDH - SSA197	A95-120	M	20	0.050	16.20

Even-Year Linkage Map

We have completed initial construction of a linkage map for even-year pink salmon from Prince William Sound. We have analyzed the segregation pattern of 85 loci in an even-year family (V96-13) and assigned 63 of 85 loci to one of 22 linkage groups (Table 5, Figure 2). One gene of known function, *MHCB$^{\alpha}$2*, is assigned to a linkage group that consists of one microsatellite and two PINE loci (Figure 2).

Preliminary analysis has not found any differences in location of loci on the maps or recombination rates between the odd- and even-year maps. After the addition of more markers to the even year map we plan to complete the comparative analysis of the odd- and even-year maps, and submit a publication.

Table 5. Summary of Marker Types on the Even-Year Pink Salmon Map

	Number of polymorphic loci	Number of markers unlinked	Percent assigned to linkage group
Microsatellites	19	6	68
PINEs	65	17	74
MHCB$^{\alpha}$2	1	0	100
Total	85	23	73

[FIGURE 2 in WORD file (fig-2)]

OBJECTIVES 5 & 6

The completion of a genome map for pink salmon provides important information for addressing genetic issues related to two other Components of the Pink Salmon Restoration Program. The numerous genetic markers identified in the course of this study will provide greatly increased power and resolution to identify stocks of pink salmon on a very fine scale (Stock Separation and Management). In addition, understanding the process of mutation will help identify appropriate markers for use in stock identification. The genome map also provides a platform to test for the presence of genes having major effects on traits of importance for the management of pink salmon, and to test for phenotypes associated with specific combinations of multilocus genotypes (Lander and Schork 1994). These genetic markers will be of great value in genetically identifying fish from supplementation programs (hatchery-raised stocks) and detecting their ecological and genetic interactions with wild fish (Supplementation).

This aspect of the research is being performed at the ASLC research facilities. Approximately 50,000 and 24,000 marked fish were released in spring of 1999 and 2000 respectively. We will collect surviving individuals when they return to upper Resurrection Bay at sexual maturity. A sample of the fish were collected at release and will be analyzed so that their genetic characteristics prior to the marine phase of the life cycle can be compared to the returning adults. We will test for genetic effects on phenotypes of special importance by comparing the genotypes of the released fish with the genotypes of the returning fish. This will allow us to test for genes with a major effect on marine survival. We will test for loci or regions of the genome that have a large effect on phenotypes of interest, so-called quantitative trait loci (QTL's). For example, Jackson et al. (1998) recently have presented evidence for QTL's that affect upper temperature tolerance in rainbow trout linked to two of 24 polymorphic loci that they examined. Mousseau et al. (1998) have used a similar approach to estimate heritabilities for weight, length, and age at sexual maturation in chinook salmon.

Previous work has demonstrated genetic differences in run-timing has a genetic basis (McGregor et al. 1998; Smoker et al. manuscript). We will compare the genotypes of fish returning to the facility at different times to test for genes with a major effect on run timing. We will use a suite of genetic markers spread uniformly throughout the genome. Regions of the genome that show major associations with run-timing can then be examined in more detail by comparing additional markers within that region. A similar approach using only 10 protein markers in hatchery rainbow trout revealed several regions of the genome associated with time of spawning (Leary et al. 1989). Sakamoto et al. (1999) have reported similar results on the basis of 54 microsatellite loci.

Karl and Avise (1992) reported concordant patterns of genetic differentiation for mitochondrial DNA and four nuclear DNA loci in the American oyster (*Crassostrea virginica*) along the east coast of North America. In contrast, previous allozyme studies had not detected these genetic differences among these same populations. Karl and Avise concluded that the pattern observed for the DNA markers reflected the historical patterns of isolation and gene flow among these populations while this pattern is obscured in the allozymes because of "balancing selection" at the allozyme loci. Similar results have been reported in the Atlantic cod (Pogson et al. 1995). These results provide an important challenge to the generally accepted utility of allozyme markers for describing historical patterns and amounts of gene flow between populations. That is, if allozymes are under strong natural selection then they may not provide accurate information about the genetic structure and amount of gene flow among populations.

Restoration Projects 95320D and 96196 have described the genetic population structure in Prince William Sound (PWS) odd- and even-year fish at allozyme loci and mitochondrial DNA (mtDNA) (Seeb et al 1996; Habicht et al. 1998). These studies reported small but statistically significant genetic allele frequency differences among streams, and concluded that pink salmon in PWS should be managed taking into account subpopulation structure rather than as a single panmictic population. As is usually done in such studies, these authors assumed that the genes they examined were selectively neutral (that is, not affected by natural selection). However, the estimates of these authors could be severe overestimates of the actual amount of gene flow if "balancing" selection is maintaining similar frequencies (Karl and Avise 1992; Pogson et al.

1995). That is, there may be much less gene flow among populations than is suggested by these studies.

Zhivotovsky et al. (1994) have reviewed population genetic data of pink salmon and concluded that the interpretations concerning amounts and patterns of gene flow are questionable because even weak natural selection could have a major effect on genetic divergence among populations of pink salmon. A series of papers by Altukhov and his colleagues has provided evidence for phenotypic and fitness effects of genetic variation at allozyme loci in pink salmon (Altukhov 1990; Altukhov et al. 1987, 1989; Dubrova et al. 1995; Kartavtsev 1992). These papers argue that genotypes at allozyme loci have a significant effect on marine survival, growth rate, and several other important factors.

The clearest and perhaps most important effects have been demonstrated on marine survival and growth rates. Pink salmon that are more heterozygous at allozyme loci have greater viability and growth rates than more homozygous individuals (Altukhov et al. 1991; Zhivotovsky et al. 1987; Kartavtsev 1992). Table 6 shows the distribution of individual heterozygosities at four allozyme loci in fry before release into salt water and returning adult spawners in odd-year pink salmon from the Sakhalin Island (Altukhov et al. 1987). We would expect the heterozygosities in fry and adults to be similar if the genotypes at these loci are not associated with survival. The significantly higher heterozygosity in the returning adults (0.619) than in the fry (0.424) indicates that individuals that were more heterozygous at the four loci had greater marine survival.

Altukhov et al. (1991) found a significant positive regression ($r=0.14$; $P<0.01$) between individual heterozygosity at these same four allozyme loci and body length of fry immediately preceding downstream migration from a hatchery on the Sakhalin Island. Kartavtsev (1992) reported a similar relationship in a different experiment with pink salmon from Sakhalin island ($r=0.23$; $P<0.001$). Previous studies with salmonids have found that size has an important effect on survival (Hunt 1969).

Table 6. Distribution of Heterozygosity at Four Allozyme Loci in Pink Salmon from Sakhalin Island

	Number of heterozygous loci*			
Age-class	0	1	2-4	Average Heterozygosity
Fry	0.620 (559)	0.336 (302)	0.044 (40)	0.424 (901)
Adults	0.495 (300)	0.391 (237)	0.144 (69)	0.619 (606)

$\chi^2 = 37.3$
d.f. = 2
P<0.001

* values are the frequencies (and number) of individuals with the indicated number of heterozygous loci.

Similar results have been reported in other salmonid species for many phenotypes of evolutionary importance (e.g., developmental rate, egg size, and disease resistance; reviewed by Ferguson 1992). Positive associations between heterozygosity at allozyme loci and important phenotypic characters, such as growth rate, survival, fertility, disease resistance, developmental rate, and developmental stability, have been described in many organisms (reviewed by Zouros and Foltz 1986; Allendorf and Leary 1986).

The mechanism underlying these associations remains unknown. The most likely explanations are (1) the associations are the consequence of heterozygosity at the loci examined, or (2) the loci examined may be in linkage disequilibrium with other loci that affect the traits being studied (associative overdominance; Leary et al. 1987).

It has been argued that these relationships between multiple locus heterozygosity and phenotypes have been found with allozymes because these loci are important in ATP production and protein catabolism (Koehn et al. 1988). We propose to distinguish between these hypotheses by using the linkage map to compare the effects of different markers on marine survival and other traits. If the enzyme loci themselves are responsible for this effect, then we would expect to find an association between enzyme genotypes and survival, but not between genotypes at DNA markers spread throughout the nuclear genome. However, if we find a similar association using DNA markers, this would suggest that the effect is due to chromosomal segments and not the enzyme loci themselves.

We believe that it is unlikely that the enzyme loci themselves are responsible for the observed

relationships. Nevertheless, regardless of the underlying mechanisms of these associations, even weak heterozygous advantage (or associative overdominance) would act to maintain similar allele frequencies in different populations in the absence of significant gene flow (Allendorf 1983). This could cause a large overestimation of the actual amount of gene flow among Prince William Sound pink salmon populations. For example, just a 10% selective advantage of heterozygotes will cause a 10-fold over estimation of the amount of migration in the case where local populations have an effective size of 100 and an average 0.5 migrants per generation (Allendorf 1983). Altukhov et al. (1987) have estimated an average selective advantage of approximately 25% at four allozyme loci in pink salmon.

We will ask a series of questions in this aspect of the research. The primary question is are there regions of the genome that have a significant effect on survival during the marine phase of the life cycle? Secondarily, we will ask if allozyme markers tend to occur in those regions that affect survival. We will also determine if selection favors heterozygotes.

Marine Survival and Fitness Experiment: 1998 cohort

In August 1998, 150 (75 male and 75 female) mature pink salmon were collected from Likes Creek, Resurrection Bay, and transported to the ASLC for controlled matings. We made 75 families of full-sibs by crossing one male and one female. One hundred progeny from each family were collected to test marker inheritance for parentage analysis. We then selected 50 of these families on the basis of egg number and survival during incubation for the release experiment. These families were pooled together into a single tank in March shortly after hatching. In May 1999, approximately 1,500 progeny from each of these 50 single-pair mating families were marked and released from the ASLC facility.

Progeny from this experiment returned in August 2000. We had anticipated a return rate of 2%, for a total of 1,000-2,000 individuals expected to be recovered for genetic and morphological analyses (approximately 30 fish per family). However, no fish returned to the ASLC fish pass. We did capture a total of 36 fish from 30 families throughout Resurrection Bay (Table 7, Figure 3). Based on the number of fish collected with our limited resources it is clear that a large number of marked fish did survive and returned to Resurrection Bay. We were not able to achieve any of our objectives with this small number of fish. It is interesting to note, however, that fish from the same family did tend to return at the same time, indicating a genetic component to time of return (Table 7).

[TABLE 7 in WORD file (table-7.doc)]

[FIGURE 3 in WORD file (fig-3.doc)]

Marine Survival and Fitness Experiment: 1999 cohort

We repeated this experiment with odd-year pink salmon in August 1999. We collected 68 adults (34 females and 34 males) from Likes Creek, and released their marked progeny from the

ASLC in May 2000. This cohort should return in the summer of 2001. We used a different experimental mating scheme with these fish to allow a more powerful genetic analysis of the progeny. Each male and each female was crossed with two individuals in a series of 2 x 2 diallele crosses (Figure 4). Based on results from 2000, we do not expect our fish to return to the ASLC. Therefore, we are planning a more extensive survey of freshwater streams in upper Resurrection Bay.

[FIGURE 4 in WORD file (fig-4.doc)]

Mutation Analysis

Our results have provided exciting and important information about mutation processes in microsatellites which we have described in a manuscript for submission to the journal Molecular Biology and Evolution. Our experimental design depends upon being able to place returning adults into their correct family on the basis of their multiple-locus genotypes. We tested this by examining inheritance data at 11 loci (nine microsatellites and two genes of known function) for 10 progeny from each of the 50 families that were released in spring of 1999. In the process of analyzing the inheritance data, we detected several mutations at two of the microsatellite loci (*SSA408* and *OGO1c*), indicating that these loci have particularly high mutation rates. Furthermore, at *SSA408* the mutations detected were not distributed randomly among families. Rather, clusters of identical mutant alleles were found in certain families, suggesting they may have resulted from mutation events occurring very early in gametogenesis, prior to meiosis.

To further evaluate mutation rates and patterns, we genotyped 35-40 progeny from each of five of the 1998 cohort families at the nine microsatellite loci included in the initial inheritance analysis (Table 8). We combined the data for these progeny and the initial inheritance data for estimation of mutation rates. Sixteen of the individuals in this dataset have genotypes best explained as resulting from mutation events (Table 9a). Five of the mutations were at *OGO1c* and 11 were at *SSA408*. Because each individual inherits two copies of each gene, we estimated mutation rates as the number of mutations/two times the number of individuals genotyped. The resulting mutation rate estimates at *SSA408* and *OGO1c* are 8.5×10^{-3} and 3.7×10^{-3} respectively (Table 8). No mutations were detected at any of the other seven loci, indicating that their mutation rates are lower than could be detected with our data.

[TABLE 8 in WORD (table 8.doc)]

[TABLE 9 in WORD (table 9.doc)]

We also analyzed additional progeny from the two families which had several individuals with identical mutations at *SSA408* in the initial inheritance study. Two more mutant individuals were detected in 36 additional progeny sampled from family 98-23 and five were found in 40 additional progeny from family 98-26 (Table 9b). The fact that the same mutant allele at *SSA408* was transmitted to a total of nine of 50 embryos from family 98-26 and similarly to four of 46 embryos in family 98-23 (Table 10) strongly suggests that these are premeiotic cluster mutations. In sexually reproducing animals, gametes develop from primordial germ cells (PGCs) that differentiate from the somatic cells

very early in development. These cells eventually migrate to the area of the developing gonads where the germ cells are produced. The number of PGCs produced varies widely among organisms (reviewed by Matova and Cooley 2001). In zebrafish (*Danio rerio*), researchers combining morphological and mRNA expression studies using germ line markers have recently determined that by the 5-somite (32-cell) stage and until about the 1000 cell stage there are four PGCs (Braat et al. 1999). During their migration towards the gonads the four PGCs give rise to 20-30 cells that populate the gonad and differentiate into germ cells (Braat et al. 1999). In theory, if there is a mutation in one of the original four PGCs, approximately one out of eight (12.5%) of the progeny should inherit the mutant allele. Assuming gametogenesis is similar in pink salmon, our findings of nine identical mutant alleles out of the 50 transmitted maternally (18%) in family 98-26 and four of 46 identical mutant alleles (8.7%) transmitted paternally in family 98-23 suggest that each of these mutations likely occurred either in one of the four PGCs or in the subsequent one or two generations of cells that populated the gonad.

[TABLE 10 in WORD (table 10.doc)]

The occurrence of clustered mutations results in non-uniform distributions of novel alleles in a population which, if not identified as such, could influence interpretations of mutation rates and patterns as well as interpretations of patterns of genetic population structure. Woodruff et al. (1996) have shown that mutant alleles that are part of clusters are more likely to persist and be fixed in a population than mutant alleles entering the population independently. In the present study, 13 of the 23 mutant alleles detected (57%) apparently resulted from premeiotic mutations. Jones et al. (1999) similarly found that a high proportion (40%) of new mutants observed in pipefish (*Sygnathus typhle*) occurred in mutational clusters. No other published accounts of cluster mutations in fish microsatellites were found, however these results are similar to the estimates from *Drosophila* of 20-50% reported by Woodruff et al. (1996).

Microsatellite mutations are generally thought to result from DNA polymerase strand slippage (Levinson and Gutman 1987, Weber and Wong 1993). Recent evidence indicates there is a tendency towards upward biases in size change (Amos et al. 1996, Primmer et al. 1996, Wierdl et al 1997). To evaluate whether the mutations we detected reflected size increases or decreases, we assumed the progenitor of the mutant allele was the parental allele that was closest in size (Table 10). Assuming single mutation events account for the mutation clusters at *SSA408* in families 98-23 and 98-26, six of the seven mutant alleles detected involved size increases of four bases. At *OGO1c* three of the five mutations detected resulted in size increases of four bases and two resulted in size decreases of four bases. All of these changes are consistent with single repeat unit addition or deletion mutations at both loci with a bias towards increasing the number of repeats at *SSA408*. Banks et al. (1999) similarly found that the single mutation in their study of the inheritance of microsatellites in chinook salmon was a gain of a single repeat-unit.

These results have important significance for the use of microsatellite loci in management. Mutations are expected to have a substantial effect on the amount and pattern of genetic divergence among populations if the mutation rate approaches the rate of migration among populations (see discussion in Allendorf and Seeb 2000). Not surprisingly, the number of mutations detected was correlated with the number of alleles in the sample (Table 8). We detected mutations at the two loci that have the greatest number of alleles in the parental population (*OGO1c* and *SSA408*, Table 8). The mutation rate estimates at *OGO1c* and *SSA408* (3.7×10^{-3} and 5.4×10^{-3}) are at the high end of the range

of 10^{-3} to 10^{-6} reported for other organisms (Dallas 1992, Weber and Wong 1993, Schug et al. 1997). The variability of these two loci makes them powerful tools for assigning parentage. We were able to unambiguously assign parentage to 35 of the 36 returning fish from the 1998 cohort based on these two loci alone. However, given the high probability of mutation at these loci, our results indicate that it is important to use a combination of low and high variability markers for parentage analysis. Our mutation analysis also suggests that *OGO1c* and *SSA408* are inappropriate as markers for analysis of stock structure in pink salmon.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

The ADFG Genetics Lab is no longer funded to assist us in the work at the ASLC. Therefore, we are currently doing all of the allozyme analysis at the University of Montana.

SCHEDULE

A. Measurable Project Tasks for FY 02 (1 Oct 01 - 30 Sep 02)

1 Oct 01 - 31 Dec 01: Complete genetic analyses of fry from 1999 cohort sampled at time of release from the ASLC.

1 Oct 01 - 31 Dec 01: Perform morphological analysis of returning adults from 1999 cohort.

1 Oct 01 - 31 July 02: Perform genetic analyses of returning adults from 1999 cohort.

1 Dec 01 - 30 Sep 02: Perform data analysis to test for correlations between markers from the linkage map and traits associated with marine survival and fitness in the returns of the 1999 cohort.

1 Oct 01 - 1 Mar 02: Add markers to the even-year linkage map.

1 Jan 02 - 30 Sep 02: Prepare manuscript for publication describing results of marine survival and fitness experiment.

1 Mar 02 - 30 Sep 02: Prepare manuscript for publication comparing odd- and even-year linkage maps.

B. Project Milestones and Endpoints

Objective 1: This objective has been completed.

Objective 2: This objective has been completed.

Objective 3: This objective will not be pursued.

Objective 4: This objective will not be pursued.

Objective 5: This objective will be completed by the end of year 8.

Objective 6: This objective will be completed by the end of year 8.

C. Completion Date

We initially proposed to continue this work for five years. However, our release experiments were delayed until the ASLC facilities were available. The 1998 cohort fish released in the spring of 1999 returned at the end of year five, and the 1999 cohort fish will return at the end of year six. Genetic analysis should be completed by the end of year seven and data analysis and publications completed by the end of year eight.

PUBLICATIONS AND REPORTS

Allendorf, F. W., P. Spruell, K. L. Knudsen, K. R. Lindner and K. L. Pilgrim. 1997. Construction of a Linkage Map for the Pink Salmon Genome, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 97190), University of Montana, Missoula, Montana.

Allendorf, F. W., P. Spruell, K. L. Knudsen, K. R. Lindner, D.J. Reedy, and K. L. Pilgrim.1998. Construction of a Linkage Map for the Pink Salmon Genome, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 98190), University of Montana, Missoula, Montana.

Allendorf, F. W., P. Spruell, K. L. Knudsen, and K. R. Lindner. 1999. Construction of a Linkage Map for the Pink Salmon Genome, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 99190), University of Montana, Missoula, Montana.

Spruell, P., B.A. Greene, C. Habicht, K.L. Knudsen, K.R. Lindner, J.B. Olsen, K.L. Pilgrim, G.K. Sage, J.E. Seeb, and F.W. Allendorf. 1999. Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon (*Oncorhynchus gorbuscha*). Journal of Heredity 90:289-296.

Lindner, K. R., E. K. Steinberg, P. Spruell, K. L. Knudsen, A. Maxwell, and Allendorf, F. W. 2000. Construction of a Linkage Map for the Pink Salmon Genome, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 00190), University of Montana, Missoula, Montana.

Lindner, K.R., J. E. Seeb, C. Habicht, K.L. Knudsen, E. Kretschmer, D. J. Reedy, P. Spruell, and F. W. Allendorf. 2000. Gene-centromere mapping of 312 loci in pink salmon by half-tetrad analysis. Genome 43:538-549.

Steinberg, E.K., K.R. Lindner, J. Gallea, J. Meng, A.Maxwell, and F.W. Allendorf. Rates and patterns

of microsatellite mutations in pink salmon. To be submitted to Molecular Biology and Evolution.

Lindner, K.R., P. Spruell, C. Habicht, J. E. Seeb, H. Zhao, and F. W. Allendorf. In preparation. Estimation of chiasma interference and construction of a linkage map for pink salmon. To be submitted to Genetics.

PROFESSIONAL CONFERENCES

We anticipate presenting our results at professional and scientific meetings.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This work has been done in collaboration with James E. Seeb, Principal Geneticist, ADFG. The inheritance experiments were performed in coordination with the project Oil-Related Embryo Mortalities (Restoration Study \191A). Dr. Seeb is no longer funded to collaborate with us in this Restoration Study.

This work is related to my ongoing genetic research with salmonid fishes that has been supported by the National Science Foundation since 1980. Many of the techniques and approaches proposed here are based upon the results of that research. I also intend to continue seeking support from NSF that will complement the research proposed here. A genetic map for pink salmon will allow us to address a number of fundamental questions in the conservation and genetics of pink salmon and other *Oncorhynchus* species.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The changes in this proposal reflect the discontinuation of Restoration Study \191A, and the decision not to fund our ADFG collaborators on this project. We have made changes in our plans to capture fish returning to Resurrection Bay based upon the lack of any returns to the ASLC in summer 2000.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPLE INVESTIGATOR

FRED W. ALLENDORF: Principal Investigator

BIRTH: 29 April 1947; Philadelphia, Pennsylvania

MILITARY SERVICE: U.S. Army, 1965-1968 (Vietnam, 1966-1967)

EDUCATION: B.S., Zoology, Pennsylvania State University, 1971
M.S., Fisheries, University of Washington, 1973
Ph.D., Genetics and Fisheries, University of Washington, 1975 (co-directors, Joe Felsenstein and Fred Utter)

POSITIONS:

1975-1976 Lektor, Department of Genetics and Ecology, Aarhus University, Denmark
1976-1979 Assistant Professor of Zoology, University of Montana
1978-1979 NATO Fellow, Genetics Research Unit, University of Nottingham, England
1979-1984 Associate Professor of Zoology, University of Montana
1983-1984 Visiting Scientist, Department of Genetics, Univ. of California, Davis
1984-1989 Professor of Zoology, University of Montana
1989-1990 Program Director, Population Biology and Physiological Ecology, National Science Foundation (NSF)
1992-1993 Visiting Professor, University of Oregon
1990- Professor of Biology, University of Montana
1993-1996 Director, Organismal Biology and Ecology Graduate Program, University of Montana
2000-2001 Fulbright Senior Scholar, Victoria University of Wellington, New Zealand

HONORS: NATO/NSF Postdoctoral Fellowship, University of Nottingham, 1978-1979
European Molecular Biology Organisation (EMBO), Fellowship, University of Stockholm, 1979
Distinguished Scholar Award, University of Montana, June 1985
Burlington Northern Faculty Achievement Award for Research, University of Montana, June 1987
Elected Fellow, American Association for the Advancement of Science (AAAS), February 1987
Burlington Northern Faculty Achievement Award for Research, University of Montana, May 1991
Elected Member, AAAS Council (Biological Sciences Division), 1996-1998
President, American Genetic Association, 1997
Fulbright Senior Scholar Award, New Zealand, 2000

MAJOR GRANTS:
National Science Foundation Research Grant, EPSCR, 1980-1983, $70,000

National Science Foundation Research Grant, Population Biology, 1980-1982, $60,000
National Science Foundation Research Grant, 1983-1986, $121,000
National Science Foundation, Faculty Research Opportunity Award, 1986, $10,000
United States Department of Agriculture Grant, Aquaculture, 1983-1985, $43,000
National Science Foundation Research Grant, 1986-1989, $148,000
National Science Foundation, Dissertation Research Grant, 1988-1990, $9,850
National Science Foundation Research Grant, 1989-1993, $150,000
National Science Foundation Research Grant, Conservation and Restoration Biology, 1993-1998, $270,000
National Science Foundation Research Grant, Small Grant for Experimental Research, Population Biology, 2000-2001, $50,000

ASSOCIATE EDITORSHIPS: Evolution (1987-1990)
Journal of Heredity (1986-1989)
Progressive Fish Culturist (1986-1989)
Molecular Biology and Evolution (1994-1996)

EDITORIAL BOARDS: Molecular Biology and Evolution (1983-1989)
Conservation Biology (1990-1993)
Molecular Ecology (1991-present)

PROFESSIONAL SERVICE:

Panel Member, Population Biology and Physiological Ecology, NSF (1987-1989)
Panel Member, International Program, National Science Foundation (1987)
Panel Member, Conservation and Restoration Biology, NSF (1991-1992; 1995)
Council Member, The American Genetic Association (1986-1989)
Genetics Nomenclature Committee, American Fisheries Society (1986-present)
Member, Committee on the Protection and Management of Pacific Northwest Anadromous Salmonids, National Research Council (1992-present)
Chair, Committee of Visitors, Systematic and Population Biology Programs, NSF (1993)

PROFESSIONAL SOCIETIES: Society for the Study of Evolution
American Society of Naturalists
Genetics Society of America
Society for Conservation Biology
American Association for the Advancement of Science
American Society of Ichthyologists and Herpetologists
American Fisheries Society
American Genetic Association
Desert Fishes Council
Ecological Society of America

Montana Native Plant Society
Society of Systematic Biologists
Society for Molecular Biology and Evolution

CONSERVATION GENETIC BIBLIOGRAPHY:

I have compiled a bibliography focussed on genetics and conservation that contains over 40,000 references. This bibliography can be searched on the World Wide Web (http://www.lib.umt.edu/guide/allendorf.htm). The primary focus of this bibliography is genetic variation in natural populations of animals, plants, and microbes. The secondary focus has been conservation, with an emphasis on the application of genetic principles to conservation. There is a taxonomic bias towards fish, especially salmon, trout, and their kin. There has been no effort to exclude papers dealing with other taxa, but there has been an effort to include more papers on the general biology and natural history of salmonid fishes.

BOOK CHAPTERS:

Utter, F.M., H.O. Hodgins, F.W. Allendorf, A.G. Johnson, and J.L. Mighell. 1973. Biochemical variants in Pacific salmon and rainbow trout: their inheritance and application in population studies. In: Genetics and Mutagenesis of Fish, pp. 329-339. Springer-Verlag, Berlin.

Utter, F.M., H.O. Hodgins, and F.W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations. In: Biochemical and Biophysical Perspectives in Marine Biology, Vol. l, pp. 213-238.

Allendorf, F.W., F.M. Utter, and B.P. May. 1975. Gene duplication in the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. In: Isozymes IV: Genetics and Evolution, pp. 415-432. Clement L. Markert, editor. Academic Press, New York.

Allendorf, F.W., and F.M. Utter. 1979. Population genetics. In: Fish Physiology, Volume 8, pp. 407-454. W.S. Hoar, D.J. Randall, and J.R. Brett, editors. Academic Press, New York.

Allendorf, F.W., R.F. Leary, and K.L. Knudsen. 1983. Structural and regulatory variation of phosphoglucomutase in rainbow trout. In: Isozymes: Current Topics in Biological and Medical Research, Vol. 9, pp. 123-142. Alan R. Liss Publ. Co., N.Y.

Allendorf, F.W. 1983. Isolation, gene flow, and genetic differentiation among populations. In: Genetics and Conservation, edited by C. Schonewald-Cox, et al. Benjamin/Cummings. pp. 51-65.

Allendorf, F.W., and G. Thorgaard. 1984. Tetraploidy and the evolution of salmonid fishes. In: The Evolutionary Genetics of Fishes, B.J. Turner, ed., Plenum Press, pp. 1-53.

Allendorf, F.W., and R.F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. In: Conservation Biology: The Science of Scarcity and Diversity. M. Soule', editor. Sinauer Assoc. pp. 57-76.

Allendorf, F.W., N. Ryman, and F.M. Utter. 1987. Genetics and fishery management: Past, present, and future. In: Population Genetics and Fisheries Management, edited by N. Ryman and F.M. Utter. University of Washington Press, pp. 1-19.

Allendorf, F.W., and N. Ryman. 1987. Genetic management of hatchery stocks. In: Population

Genetics and Fisheries Management, edited by N. Ryman and F.M. Utter. University of Washington Press, pp. 141-159.
Thorgaard, G.H., and F. W. Allendorf. 1988. Developmental genetics of fishes. In: Developmental Genetics of Animals and Plants, edited by G. M. Malacinski. Macmillan Publishing Company, pp. 363-391.
Powers, D.A., F.W. Allendorf, and T. Chen. 1990. Application of molecular techniques to the study of marine recruitment problems. In: Large Marine Ecosystems: Patterns, Processes, and Yields, ed. by K. Sherman, L.M. Alexander, and B.D. Gold. Amer. Assoc. Advancement Sci., pp. 104-121.
Allendorf, F.W., and M.M. Ferguson. 1990. Genetics. In: Methods for Fish Biology, edited by C.B. Schreck and P.B. Moyle. Amer. Fish. Soc., Bethesda, Maryland, pp. 35-63.
Ferguson, M.M., and F.W. Allendorf. 1991. Evolution of the fish genome. In: Biochemistry and Molecular Biology of Fishes, edited by P.W. Hochachka and T.P. Mommsen. Elsevier. pp. 25-42.
Allendorf, F.W. 1994. Genetics and demography of grizzly bear populations. In: Principles of Conservation Biology by G.K. Meffe and C.R. Carroll. Sinauer Associates. pp. 155-156.
Allendorf, F.W., and R.S. Waples. 1996. Conservation and genetics of salmonid fishes. In: Conservation Genetics: Case Histories from Nature, edited by J.C. Avise and J.L. Hamrick. Chapman & Hall. pp. 238-280.
Allendorf, F. W., and N. Ryman. 2002. The role of genetics in population viability analysis. In: Population Viability Analysis. S. R. Beissinger and D. R. McCullough, editors. University of Chicago Press, Chicago, Illinois. In press.

ARTICLES:

Heckman, J.R., F.W. Allendorf, and J.E. Wright, Jr. 1971. Trout leukocytes: growth in oxygenated cultures. Science 173:246-247.
Allendorf, F.W., and F.M. Utter. 1973. Gene duplication in the family Salmonidae: disomic inheritance of two loci reported to be tetrasomic in rainbow trout. Genetics 74:647-654.
Utter, F.M., F.W. Allendorf, H.O. Hodgins, and A.G. Johnson. 1973. Genetic basis of tetrazolium oxidase phenotypes in rainbow trout. Genetics 73:159.
Utter, F.M., F.W. Allendorf, and H.O. Hodgins. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst. Zool. 22:257-270.
Allendorf, F.W., F.M. Utter, and B.P. May. 1975. Gene duplication in the family Salmonidae: II. Detection and determination of the genetic control of populations. In Isozymes IV: Genetics and Evolution, pp. 415-432. C. L. Markert, editor. Academic Press, New York.
May, B.P., F.M. Utter, and F.W. Allendorf. 1975. Biochemical genetic variation in pink and chum salmon: inheritance of intraspecies variation and apparent absence of interspecies introgression following massive hybridization of hatchery stocks. J. of Heredity 66:227-232.
Allendorf, F.W., and F.M. Utter. 1976. Gene duplication in the family Salmonidae: III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout. Hereditas82:19-24.
Allendorf, F.W., N. Ryman, A. Stenneck, and G. Stahl. 1976. Genetic variation in Scandinavian populations of brown trout: evidence of distinct sympatric populations. Hereditas 83:73-82.
Utter, F.M., F.W. Allendorf, and B.P. May. 1976. The use of protein variation in the

management of salmonid populations. Trans. 1st North American Wildlife and Natural Resources Conference. pp. 373-384.
Utter, F.M., F.W. Allendorf, and B.P. May. 1976. Genetic delineation of salmonid populations. In: Salmonid Genetics: Status and Role in Aquaculture. T. Nosho and W. Hershberger, editors. University of Washington Sea Grant Publication.
Allendorf, F.W., N.J. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (*Salmo trutta*): Detection and interpretation from population data. Hereditas 86:179-190.
Allendorf, F.W. 1977. Genetic variation in populations of fish. In: Fish Genetics-Fundamentals and Implications to Fish Management, pp. 35-39. C.M. Fetterholf, editor. Great Lakes Fishery Commission, Ann Arbor, Mich.
Utter, F.M., and F.W. Allendorf. 1977. Determination of the breeding structure of steelhead trout (*Salmo gairdneri*) populations through gene frequency analysis. Calif. Coop. Fish. Res. Unit Special Report 77-l:44-54.
Allendorf, F.W. 1977. Electromorphs or alleles? Genetics 87:821-822.
Allendorf, F.W. 1978. Electrophoretic distinction of rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*). Letter to the editor. J. Fisheries Res. Bd. Canada 35:483.
Allendorf, F.W. 1978. Protein polymorphism and the rate of loss of duplicate gene expression. Nature 272:76-78.
Utter, F.M., F.W. Allendorf, and B. May. 1979. Genetic basis of creatine kinase isozymes in skeletal muscle of salmonid fishes. Biochem. Genet. 17:1079-1091.
Ryman, N., F.W. Allendorf, and G. Stahl. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). Genetics 92:247-262.
O'Malley, D.M., F.W. Allendorf, and G.M. Blake. 1979. Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. Biochem. Genet. 17:233-250.
Clarke, B., and F.W. Allendorf. 1979. Frequency-dependent selection due to kinetic differences between allozymes. Nature 279:732-734.
Allendorf, F.W., F.B. Christiansen, T. Dobson, W.F. Eanes, and O. Frydenberg. 1979. Electrophoretic variation in large mammals. I. The polar bear, *Thalarctos maritimus*. Hereditas 1:19-22.
Allendorf, F.W. 1979. Rapid loss of duplicate gene expression by natural selection. Heredity 43:247-259.
Allendorf, F.W. 1979. Protein polymorphism and the rate of loss of duplicate gene expression, a reply. Nature 279:456.
Allendorf, F.W., D.M. Espeland, D.T. Scow, and S. Phelps. 1980. Coexistence of native and introduced rainbow trout in the Kootenai River drainage. Proc. Montana Acad. Sciences 39:28-36.
Allendorf, F.W., and S. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. Trans. Amer. Fish. Soc. 109: 537-543.
Allendorf, F.W., and S.R. Phelps. 1981. Use of allelic frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Sciences 38: 1507-1514.
Krueger, C.C., A. Gharrett, T.R. Dehring, and F.W. Allendorf. 1981. Genetic aspects of fisheries rehabilitation programs. Canadian Journal of Fisheries and Aquatic Sciences 38: 1877-1881.
Allendorf, F.W., and S.R. Phelps. 1982. Isozymes and the preservation of genetic variation in salmonid fishes. Ecological Bulletin (Stockholm) 34: 37-52.
Allendorf, F.W., K.L. Knudsen, and G.M. Blake. 1982. Frequencies of null alleles at enzyme loci

in natural populations of ponderosa and red pine. Genetics 100: 497-504.
Phelps, S.R., and F.W. Allendorf. 1982. Genetic comparison of upper Missouri River cutthroat trout to other *Salmo clarki lewisi* populations. Proc. Mont. Acad. Sci. 41: 14-22.
Allendorf, F.W., K.L. Knudsen, and S.R. Phelps. 1982. Identification of a gene regulating the tissue expression of a phosphoglucomutase locus in rainbow trout. Genetics 102: 259-268.
Simonsen, V., F.W. Allendorf, W.F. Eanes, and F.O. Kapel. 1982. Electrophoretic variation in large mammals: III. The ringed seal, the harp seal, and the hooded seal. Hereditas 97:87-90.
Phelps, S.R., and F.W. Allendorf. 1983. Genetic identity of pallid and shovelnose sturgeon (*Scaphirhynchus albus* and *S. platorynchus*). Copeia 1983(3):696-700.
Allendorf, F.W. 1983. Linkage disequilibrium generated by selection against null alleles at duplicate
loci. American Naturalist 121:588-592.
Daugherty, C.H., F.W. Allendorf, W.W. Dunlap, and K.L. Knudsen. 1983. Systematic implications of geographic patterns of genetic variation in the genus *Dicamptodon*. Copeia 1983(3):679-691.
Woods, J.H., G.M. Blake, and F.W. Allendorf. 1983. Amount and distribution of isozyme variation in ponderosa pine from eastern Montana. Silvae Genetica 32:151-157.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. Nature 101:71-72.
Thorgaard, G.H., F.W. Allendorf, and K.L. Knudsen. 1983. Gene-centromere mapping in rainbow trout: High interference over long map distances. Genetics 103:771-783.
Allendorf, F.W., K.L. Knudsen, and R.F. Leary. 1983. Adaptive significance of differences in the tissue-specific expression of a phosphoglucomutase gene in rainbow trout. Proc. Nat. Acad. Sci. USA 80:1397-1400.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1983. Consistently high meristic counts in natural hybrids between brook trout and bull trout. Syst. Zool. 32: 369-376.
Allendorf, F.W., G. Stahl, and N. Ryman. 1984. Silencing of duplicate genes: Evidence for a common null allele polymorphism for lactate dehydrogenase in brown trout (*Salmo trutta*). Molecular Biology and Evolution 1:238-248.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1984. Major morphological effects of a regulatory gene: Pgm1-t in rainbow trout. Molecular Biology and Evolution 1:183-194.
Leary, R.F., F.W. Allendorf, S.R. Phelps, and K.L. Knudsen. 1984. Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. Proc. MontanaAcad. Sci. 43:1-18.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1984. Superior developmental stability of enzyme heterozygotes in salmonid fishes. American Naturalist 124:540-551.
Allendorf, F.W., and R.F. Leary. 1984. Heterozygosity in gynogenetic diploids and triploids estimated by gene-centromere recombination rates. Aquaculture 43:413-420.
Woods, J.H., G.M. Blake, and F.W. Allendorf. 1984. Using isozyme analysis to aid in selecting ponderosa pine for coal mine spoil reclamation. Northwest Science 58:262-268.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1985. Developmental instability as an indicator of reduced genetic variation in hatchery trout. Trans. Amer. Fish. Soc. 114:230-235.
Leary, R.F., F.W. Allendorf, K.L. Knudsen, and G.H. Thorgaard. 1985. Heterozygosity and developmental stability in gynogenetic diploid and triploid rainbow trout. Heredity 54:219-225.

Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1985. Inheritance of meristic variation and the evolution of developmental stability in rainbow trout. Evolution 39:308-314.
Danzmann, R.G., M.M. Ferguson, and F.W. Allendorf. 1985. Allelic differences in initial expression of paternal alleles at an isocitrate dehydrogenase locus in rainbow trout. Developmental Genetics 5:117-127.
Ferguson, M.M., R.G. Danzmann, and F.W. Allendorf. 1985. Developmental divergence among hatchery strains of rainbow trout (*Salmo gairdneri*). I. Pure strains. Can. J. Genet. and Cytol. 27:289-297.
Ferguson, M.M., R.G. Danzmann, and F.W. Allendorf. 1985. Developmental divergence among hatchery strains of rainbow trout (*Salmo gairdneri*). II. Hybrids. Can. J. Genet. and Cytol. 27:298-307.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1985. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution 39:1318-1326.
Gyllensten, U., R.F. Leary, F.W. Allendorf, and A.C. Wilson. 1985. Introgression between two cutthroat trout subspecies with substantial karyotypic, nuclear, and mitochondrial genomic divergence. Genetics 111:905-915.
Ferguson, M.M., R.G. Danzmann, and F.W. Allendorf. 1985. Absence of developmental incompatibility in hybrids between rainbow trout and two subspecies of cutthroat trout. Biochemical Genetics 23:557-570.
Danzmann, R.G., M.M. Ferguson, and F.W. Allendorf. 1985. Does enzyme heterozygosity influence
developmental rate in rainbow trout? Heredity 56:417-425.
Hedrick, P.W., P.F. Brussard, F.W. Allendorf, J. Beardmore, and S. Orzak. 1986. Protein variation, fitness, and captive propagation. Zoo Biology 5: 91-100.
Allendorf, F.W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5: 181-190.
Danzmann, R.G., M.M. Ferguson, and F.W. Allendorf. 1986. Heterozygosity and developmental rate in a strain of rainbow trout. Evolution 40:86-93.
Scheerer, P.D., G.H. Thorgaard, F.W. Allendorf, and K.L. Knudsen. 1986. Androgenetic rainbow trout produced from inbred and outbred sperm sources show similar survival. Aquaculture 57:289-298.
Allendorf, F.W., J.E. Seeb, K.L. Knudsen, G.H. Thorgaard, and R.F. Leary. 1986. Gene-centromere mapping of 25 loci in rainbow trout. J. Heredity 77:307-312.
Allendorf, F.W., and C. Servheen. 1986. Conservation genetics of grizzly bears. Trends in Ecology and Evolution 1:88-89.
Danzmann, R.G., M.M. Ferguson, and F.W. Allendorf. 1987. Heterozygosity and oxygen consumption rate as predictors of growth and developmental rate in rainbow trout. Physiol. Zool. 60:211-220.
Marnell, L.F., R.J. Behnke, and F.W. Allendorf. 1987. Genetic identification of the cutthroat trout (*Salmo clarki*) in Glacier National Park, Montana. Can. J. Fish. Aquat. Sci. 44:1830-1839.
Leary, R.F., F.W. Allendorf, S.R. Phelps, and K.L. Knudsen. 1987. Genetic divergence and identification of seven subspecies of cutthroat trout and rainbow trout. Trans. Amer. Fish. Soc. 116:580-587.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1987. Differences in inbreeding coefficients do not explain the association between heterozygosity at isozyme loci and developmental stability in

rainbow trout. Evolution 41:1413-1415.
Ferguson, M.M., R.G. Danzmann, and F. W. Allendorf. 1987. Adaptive significance of developmental rate in rainbow trout: an experimental test. Biol. J. Linnean Soc. 33:205-216.
Leary, R.F., F.W. Allendorf, S.R. Phelps, and K.L. Knudsen. 1988. Population genetic structure of westslope cutthroat trout: Genetic variation within and among populations. Proc. Montana Acad.Sci. 48:57-70.
Danzmann, R. G., M. M. Ferguson, and F. W. Allendorf. 1988. Heterozygosity and components of fitness in a strain of rainbow trout. Biol. J. Linnean Soc. 33:285-304.
Ferguson, M.M., K. L. Knudsen, R. G. Danzmann, and F. W. Allendorf. 1988. Developmental rate and viability of rainbow trout with a null allele at a lactate dehydrogenase locus. Biochem. Genet.26:177-189.
Ferguson, M.M., R.G. Danzmann, and F.W. Allendorf. 1988. Developmental success of hybrids between two taxa of salmonid fishes with moderate structural gene divergence. Can. J. Zool. 66:1389-1395.
Allendorf, F.W., and R.F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology 2:170-184.
Allendorf, F. W. 1988. Conservation biology of fishes. Conservation Biology 2:145-148.
Lesica, P., R.F. Leary, F.W. Allendorf, and D.E. Bilderback. 1988. Lack of genic diversity within and among populations of an endangered plant, *Howellia aquatilis*. Conservation Biology 2:275-282.
Harris, R.B., and F. W. Allendorf. 1989. Genetically effective population size of large mammals: Assessment of estimators. Conservation Biology 3:181-191.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1989. Genetic differences among rainbow trout spawned on different days within a single spawning season. Progressive Fish-Culturist 51:10-19.
Leary, R.F., and F.W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. Trends Ecol. Evol. 4:214-217.
Shaklee, J.B., F.W. Allendorf, D.C. Morizot, and G.S. Whitt. 1989. Genetic nomenclature for protein-coding loci in fish: proposed guidelines. Trans. Amer. Fish. Soc. 118:218-227.
Danzmann, R. G., M.M. Ferguson, and F.W. Allendorf. 1989. Genetic variability and components of fitness in hatchery strains of rainbow trout. J. Fish Biol. 35 (Supplement A):313-320.
Ferguson, M.M., R.G. Danzmann, F.W. Allendorf, and K.L. Knudsen. 1990. Metabolic effects of the expression of a phosphoglucomutase locus in the liver of rainbow trout. Canad. J. Zool. 68:1499-1504.
Shaklee, J.B., F.W. Allendorf, D.C. Morizot, and G.S. Whitt. 1990. Gene nomenclature system for protein-coding loci in fish. Trans. Amer. Fish. Soc. 119:2-15.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1991. Effects of rearing density on meristics and developmental stability of rainbow trout. Copeia 1991(1):44-49.
Forbes, S.H., and F.W. Allendorf. 1991. Associations between mitochondrial and nuclear genotypes in cutthroat trout hybrid swarms. Evolution 45:1332-1349.
Forbes, S.H., and F.W. Allendorf. 1991. Mitochondrial genotypes have no detectable phenotypic effects in cutthroat trout hybrid swarms. Evolution 45:1350-1359.
Allendorf, F.W. 1991. Ecological and genetic effects of fish introductions: Synthesis and recommendations. Canadian J. Fish. and Aquatic Sciences 48 (Suppl. 1):178-181.
Scheerer, P.D., G.H. Thorgaard, and F.W. Allendorf. 1991. Genetic analysis of androgenetic

rainbow trout. J. Exp. Zool. 260:382-390.
Campton, D.E., F.W. Allendorf, R.J. Behnke, and F.M. Utter. 1991. Reproductive success of hatchery and wild steelhead: A reanalysis. Trans. Amer. Fish. Soc. 120:816-822.
Allendorf, F.W., R.B. Harris, and L.H. Metzgar. 1991. Estimation of effective population size of grizzly bears by computer simulation. Proceedings Fourth International Congress of Systematics andEvolutionary Biology, pp. 650-654. Dioscorides Press, Portland, Oregon.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1992. Genetic, environmental, and developmental causes of meristic variation in rainbow trout. Acta Zoologica Fennica 191:77-93.
Lesica, P., and F.W. Allendorf. 1992. Are small populations of plants worth protecting? Conservation Biology 6:135-139.
Allendorf, F.W. 1993. Delay of adaptation to captive breeding by equalizing family size. Conservation Biology 7:416-419.
Ryman, N., F. Utter, F.W. Allendorf, C. Busack, and J. Shaklee. 1993. Genetic concerns about hatchery populations - A comment on conclusions of Nyman and Ring. J. Fish Biology 42:471-480.
Leary, R.F., F.W. Allendorf, and S.H. Forbes. 1993. Conservation genetics of bull trout in the Columbia and Klamath River drainages. Conservation Biology 7:856-865.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1993. Null allele heterozygotes at two lactate dehydrogenase loci in rainbow trout are associated with decreased developmental stability. Genetica 89:3-13.
Allendorf, F. W., W. A. Gellman, and G. H. Thorgaard. 1994. Sex linkage of two enzyme loci in rainbow trout. Heredity 72:498-507.
Forbes, S.H., K.L. Knudsen, T. W. North, and F.W. Allendorf. 1994. One of two growth hormone genes in coho salmon is sex-linked. Proc. Nat. Acad. Sci. USA. 91:1628-1631.
Allendorf, F.W. 1994. Comparative utility of genetic markers in the management of Pacific salmon: proteins, nuclear DNA, and mitochondrial DNA. NOAA Technical Memorandum NMFS-NWFSC-17, pp. 127-133.
Forbes, S.H., K.L. Knudsen, and F.W. Allendorf. 1994. Genetic variation in coho salmon detected by PCR amplification of growth hormone gene introns. NOAA Technical Memorandum NMFS-NWFSC-17, pp. 29-33.
Utter, F.M., and F. W. Allendorf. 1994. Phylogenetic relationships among species of *Oncorhynchus*: A consensus view. Conservation Biology 8:864-867.
Leary, R.F., F.W. Allendorf, and G.K. Sage. 1995. Hybridization and introgression between introduced and native fish. Amer. Fish. Soc. Symposium 15:91-101.
Allendorf, F.W. 1995. Genetics: Defining the units of conservation. Amer. Fish. Soc. Symposium 17:247-248.
Lesica, P.L., and F.W. Allendorf. 1995. When are peripheral populations valuable for conservation? Conservation Biology 9:753-760.
Allendorf, F.W., and N. Kanda. 1995. Genetics and the conservation of salmonid fishes in western North America and Japan. Fish Genetics and Breeding Science (Japan) 21:79-102.
Forbes, S.H., J.T. Hogg, F.C. Buchanan, A.M. Crawford, and F.W. Allendorf. 1995. Microsatellite evolution in congeneric mammals: Domestic and bighorn sheep. Molec. Biol. Evol. 12:1106-1113.
Luikart, G., and F.W. Allendorf. 1996. Mitochondrial-DNA variation and genetic-population structure in Rocky Mountain bighorn sheep (*Ovis canadensis*). J. Mammal. 77:109-123.

Mills, L.S., and F.W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509-1518.
Hedrick, P., R. Lacy, F.W. Allendorf, and M. Soulé. 1996. Directions in conservation biology: Comments on Caughley. Conservation Biology 10:1312-1320.
Allendorf, F.W., and R. G. Danzmann. 1997. Secondary tetrasomic segregation of *MDH-B* and preferential pairing of homeologues in male rainbow trout. Genetics 145:1083-1092.
Allendorf, F.W., D. Bayles, D.L. Bottom, K.P. Currens, C.A. Frissell, D. Hankin, J. A. Lichatowich, W. Nehlsen, P.C. Trotter, and T.H. Williams. 1997. Prioritizing Pacific salmon conservation. Conservation Biology 11:140-152.
Leary, R.F., and F.W. Allendorf. 1997. Genetic confirmation of sympatric bull trout and Dolly Varden in western Washington. Trans. Amer. Fish. Soc. 126:715-720.
Kanda, N., R.F. Leary, and F.W. Allendorf. 1997. Population genetic structure of bull trout in the Upper Flathead River drainage. Pages 299-308, Proceedings, Friends of the Bull Trout Conference. Trout Unlimited, Calgary, Alberta.
Allendorf, F.W. 1997. The conservation biologist as Zen student. Conservation Biology 11:1045-1046.
Luikart, G., F.W. Allendorf, J-M. Cornuet, and W.B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. J. Heredity 89:238-247.
Allendorf, F.W., and B. Byers. 1998. Salmon in the net of Indra: A Buddhist view of nature and communities. Worldviews: Environment, Culture, Religion 2:37-52.
Luikart, G., W.B. Sherwin, B.M. Steele, and F.W. Allendorf. 1998. Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology 7:963-974.
Lesica, P., and F.W. Allendorf. 1999. Ecological genetics and the restoration of plant communities: mix or match? Restoration Ecology 7:42-50.
Hodges, M., and F.W. Allendorf. 1998. Population genetics and the pattern of larval dispersal of the endemic Hawaiian freshwater amphidromous gastropod *Neritina granosa*. Pacific Science 52:237-249.
Spruell, P., B.A. Greene, C. Habicht, K.L. Knudsen, K.R. Lindner, J.B. Olsen, K.L. Pilgrim, G.K. Sage, J.E. Seeb, and F.W. Allendorf. 1999. Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon (*Oncorhynchus gorbuscha*). Journal of Heredity 90:289-296.
Luikart, G., J-M. Cornuet, and F.W. Allendorf. 1999. Temporal changes in allele frequencies provide useful estimates of population bottleneck size. Conservation Biology 13:523-530.
Hughes, J.M., P.B. Mather, A.L. Sheldon and F.W. Allendorf. 1999. Genetic structure of stonefly (*Yoraperla brevis*) populations: the extent of gene flow among adjacent montane streams. Freshwater Biology 41:63-72.
Spruell, P., B.E. Rieman, K.L. Knudsen, F.M. Utter, and F.W. Allendorf. 1999. Genetic population structure within streams: Microsatellite analysis of bull trout populations. Ecology Freshwater Fishes 8: 114-121.
Funk, W.C., D.A. Tallmon, and F.W. Allendorf. 1999. Small effective population size in the long-toed salamander. Molecular Ecology 8:1633-1640.

Tallmon, D.A., W.C. Funk, and F.W. Allendorf. 2000. Genetic differentiation of long-toed salamander (*Ambystoma macrodactylum*) populations. Copeia 00:27-55.
Allendorf, F.W., and L.W. Seeb. 2000. Concordance of genetic divergence among sockeye salmon populations for allozymes, nuclear DNA, and mtDNA markers. Evolution 54:640-651.
Spruell, P., M.L.Bartron, N. Kanda, F.W. Allendorf. In press. Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. Copeia.
Lindner, K.R., J. E. Seeb, C. Habicht, E. Kretschmer, D. J. Reedy, P. Spruell, and F. W. Allendorf. 2000. Gene-centromere mapping of 312 marker loci in pink salmon by half-tetrad analysis. Genome 43:538-549.
Gemmell, N. J. , and F. W. Allendorf. 2001. Mitochondrial mutations may decrease population viability. Trends Ecol. Evol. 16:115-117.
Boyd, D.K., S.H. Forbes, D.H. Pletscher, and F.W. Allendorf. In press. Identification of Rocky Mountain gray wolves. Wildlife Society Bulletin.
Kanda, N., and F.W. Allendorf. In press. Genetic population structure of bull trout from the Flathead River basin using microsatellites and mitochondrial DNA markers. Trans. Amer. Fish. Soc.
Spruell, P., A.R. Hemmingsen, P.J. Howell, N. Kanda, and F.W. Allendorf. In press. Conservation genetics of bull trout: Geographic distribution of variation at microsatellite loci.

Conservation Genetics.
Thelen, G.C., and F. W. Allendorf. In press. Heterozygosity-fitness correlations in rainbow trout: allozyme loci or associative overdominance? Evolution.
Allendorf, F.W., P. Spruell, and F.M. Utter. In press. Whirling disease and wild trout:Darwinian fisheries management. Fisheries.
Rieman, B., and F.W. Allendorf. In press. Effective population size and genetic conservation criteria
for bull trout. N.A. Jour. Fish. Management.
Kanda, N., R.F. Leary, and F.W. Allendorf. In press. Evidence of introgressive hybridization between
bull trout and brook trout. Trans. Amer. Fish. Soc.
Weller, S.G., A.K. Sakai, F.W. Allendorf, and twelve other authors. Submitted invited paper. The population biology of invasive species. Annu. Rev. Eco. Syst.
Allendorf, F.W., R.F. Leary, P. Spruell, and J.K. Wenburg. Submitted invited paper. The problems with hybrids: Setting conservation guidelines. Trends Ecol. Evol.

OTHER KEY PERSONNEL

Dr. Paul Spruell will be responsible for direct oversight of all the activities in the laboratory and will assist with paper preparation. Eleanor Steinberg received an NSF Postdoctoral Research Fellowship in Biological Informatics to assist with data analysis. Kate Lindner and Kathy Knudsen will be responsible for all laboratory procedures (extraction of DNA, PCR analysis, and gel electrophoresis).

PAUL SPRUELL: Research Scientist

BORN: August 14, 1965 Bloomington, IL USA

EDUCATION

B.S.	1987	University of Illinois Ecology, Ethology, & Evolution
M.S.	1989	Michigan State University Fisheries & Wildlife (major professor, Donald Garling)
Ph.D.	1994	Washington State University Zoology (major professor, Gary Thorgaard)

POSITIONS HELD

1994-1995	Post-Doctoral Research Associate, Washington State University (with Gary Thorgaard and John N. Thompson)
1995 - 2000	Post-Doctoral Research Associate, University of Montana (with Fred Allendorf)
2000-present	Research Assistant Professor, University of Montana

RESEARCH INTERESTS

Conservation Genetics of Fishes
Evolutionary Genetics of Salmonids
Evolution of Salmonid Life Histories
Application of Molecular Genetics to Conservation Issues

TEACHING EXPERIENCE

Introductory Biology (Honors BIOL101, UM; BIOL101, WSU)
Introductory Genetics (GenCB301, WSU)
Introductory Genetics and Evolution (Honors BIOL223, UM)
Fish Biology & Management (ZOOL412, WSU)
Ichthyology (FW471, MSU)
Conservation Genetics (BIOL495, UM)

PUBLICATIONS

Spruell P, Bartron ML, Kanda N, Allendorf FW (*In press*) Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. *Copeia*.

Neraas LP & Spruell P (*In press*) Fragmentation of riverine systems: the origins of bull trout (*Salvelinus confluentus*) collected at the base of Cabinet Gorge Dam, Montana. *Molecular Ecology*.

Allendorf FW, Spruell P & Utter FM (2001) Whirling disease and wild trout: Darwinian fisheries management. Fisheries ***26,*** 27-29.

Lindner KR, Seeb JE, Habicht C, Knudsen KL, Kretschmer E, Reedy DJ, Spruell P, Allendorf FW (2000) Gene-centromere mapping of 312 loci in pink salmon by half tetrad analysis. *Genome.* ***43,*** 538-549.

Spruell P, Rieman BE, Knudsen KL, Utter FM, Allendorf FW (1999) Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecology of Freshwater Fish. ***8***, 114-121.

Spruell P, Pilgrim KL, Greene BA, Habicht C, Knudsen KL, Lindner KR, Olsen JB, Sage GK, Seeb JE, Allendorf FW (1999) Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon. Journal of Heredity. ***90***, 289-296.

Spruell P, Thorgaard GH (1996) SINE sequences detect DNA fingerprints in salmonid fishes. Heredity ***76***, 317-324.

Spruell P, Cummings SA, Kim Y, Thorgaard GH (1994) Comparison of three anadromous rainbow trout (*Oncorhynchus mykiss*) populations using DNA fingerprinting and mixed DNA samples. Canadian Journal of Fisheries and Aquatic Sciences ***51***, (Suppl. 1) 252-257.

Thorgaard GH, Spruell P, Cummings SA, Peek AS, Brannon EL (1995) Mixed DNA fingerprint analysis differentiates sockeye salmon populations. Pages 295-303 in Nielsen JL (ed.) Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society Symposium 17, Bethesda, Maryland.

Thorgaard GH, Spruell P, Wheeler PA, Scheerer PD, Peek AS, Valentine JJ, Hilton B (1995) Incidence of albinos as a monitor for induced triploidy in rainbow trout. Aquaculture 137: 121-130.

InReview

Spruell P, Hemmingsen AR, Howell PJ, Kanda N, Allendorf FW (*In review*) Conservation genetics of bull trout: geographic distribution of variation at microsatellite loci. *Conservation Genetics*.

Kanda N, Leary RF, Spruell P & Allendorf FW (*In review*) Molecular genetic markers identifying hybridization between Colorado River cutthroat trout or greenback cutthroat trout and yellowstone cutthroat trout or rainbow trout. *Transactions American Fisheries Society*.

Weigel DE, Peterson JT, Spruell P A (*In review*) Probabilistic model to detect introgression between westslope cutthroat trout and rainbow trout based on phenotypic characteristics. *Transactions American Fisheries Society*.

ELEANOR STEINBERG: NSF Postdoctoral Fellow

EDUCATION

B.A. Zoology, University of California-Berkeley, 1989
Ph.D. Zoology, University of Washington, 1999

HONORS

Nominated to Sigma Xi	1993
NSF Graduate Fellowship in Mathematical Biology	1996-1998
NSF Postdoctoral Fellowship in Biological Informatics	2000-2002

POSITIONS

Consulting Biologist (The Nature Conservancy, Seattle, WA; U.S. Fish and Wildlife Service, Sacramento, CA; H.T. Harvey and Associates, Alviso, CA; Parametrix Incorporated, Kirkland, WA)	09/89-12/99
Visiting Scholar (University of Illinois, Chicago)	01/94-12/94
Visiting Scholar (University of California, Berkeley)	10/96-05/97
Graduate Research and Teaching Assistant (University of Washington)	01/92-06/99
Postdoctoral Research Associate (University of Montana) 01/00-present	

PUBLICATIONS

Steinberg, E.K. and C.E. Jordan. 1997. Using molecular genetics to learn about the ecology of threatened species: the allure and illusion of measuring genetic structure in natural populations. Pp. 440-460 in *Conservation Biology for the Coming Decade* (P. Fiedler and P. Kareiva, eds.). Chapman and Hall, New York.

Steinberg, E.K. and P.M. Kareiva. 1997. Challenges and opportunities for empirical evaluation of "spatial theory". Pp. 318-332 in *Spatial Ecology: The Role of Space in Population Dynamics and Interspecific Interactions* (D. Tilman and P. Kareiva, eds.). Princeton University Press, Princeton.

Hartway, C. and E.K. Steinberg. 1997. The influence of pocket gopher disturbance on the distribution and diversity of plants in western Washington prairies. *Proceedings of The Ecology and Conservation of the South Puget Sound Prairie Landscape.*

Steinberg, E.K. and D. Heller. 1997. Using DNA and rocks to understand the patchy distribution of pocket gophers in Puget Sound prairies. *Proceedings of the Ecology and Conservation of the South Puget Sound Prairie Landscape*.

Sork, V.L., D. Campbell, R. Dyer, J. Fernandez, J. Nason, R. Petit, P. Smouse, and E.K.Steinberg. 1998. Proceedings from a Workshop on Gene Flow in Fragmented, Managed, and Continuous Populations. National Center for Ecological Analysis and Synthesis, Santa

Barbara, California. Research Paper No. 3.

Steinberg, E.K. 1999. Diversification of genes, populations, and species: evolutionary genetics of real and virtual pocket gophers (*Thomomys*). Ph.D. Dissertation, University of Washington.

Steinberg, E.K. 1999. Characterization of polymorphic microsatellites from current and historic populations of North American pocket gophers (Geomyidae: *Thomomys*). Molecular Ecology 8:1075-1092.

Steinberg, E.K. and J.L. Patton. 2000. Genetic structure and the geography of speciation in subterranean rodents: opportunities and constraints on evolutionary diversification. Pp.301-331 in *The Biology of Subterranean Rodents: Evolutionary Challenges and Opportunities* (E.A Lacey, G.N. Cameron, and J.L. Patton, eds.), University of Chicago Press, Chicago.

Steinberg, E.K., K.R. Lindner, J. Gallea, J. Meng, A.Maxwell, and F.W. Allendorf. Rates and patterns of microsatellite mutations in pink salmon. Submitted to Molecular Biology and Evolution.

LITERATURE CITED

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. In *Genetics and conservation*. Eds. C. Schonewald-Cox, S. Chambers, B. MacBryde, and L. Thomas, pp. 51-65. Menlo Park, CA. Benjamin/Cummings.

Allendorf, F.W., and R. G. Danzmann. 1997. Secondary tetrasomic segregation of *MDH-B* and preferential pairing of homeologues in male rainbow trout. Genetics 145:1083-1092.

Allendorf, F. W., W. A. Gellman, and G. H. Thorgaard. 1994. Sex-linkage of two enzyme loci in *Oncorhynchus mykiss* (rainbow trout). Heredity 72:498-507.

Allendorf, F.W., and R.F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. In: Conservation Biology: The Science of Scarcity and Diversity. M. Soule', editor. Sinauer Assoc. pp. 57-76.

Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Can. J. Fish. Aquat. Sci. 38: 1507-1514.

Allendorf, F.W., and L.W. Seeb. 2000. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mtDNA markers. Evolution 54: 54:640-651.

Allendorf, F. W. and G. H. Thorgaard. 1984. Tetraploidy and the evolution of salmonid fishes. Pages 1-53 in B. J. Turner, ed. Evolutionary Genetics of Fishes. Plenum Publishing Corp., New York.

Altukhov, Y. P., E. A. Salmenkova, V. T. Omel'chenko, G. A. Rubtzova, and Y. E. Dubrova. 1987. Balancing selection as a possible factor maintaining uniformity of allele frequencies of enzyme loci in populations of Pacific Ocean pink salmon *Oncorhynchus gorbuscha* (Walbaum). Genetika SSSR 23:1884-1896.

Altukhov, Yu. 1990. Population genetics: diversity and stability. London: Harwood Academic Publishers.

Altukhov, Yu. P., S. V. Mezhzerin, E. A. Salmenkova, and V. T. Omel'chenko. 1989. Effect of selective hatchery on adaptive genetic and biological structure of pink salmon *Oncorhynchus gorbuscha* (Walb.) population. Genetika 25:1843-1853.

Altukhov, Yu. P., E. A. Salmenkova, and Yu. P. Kartavtsev. 1991. Relation between allozyme heterozygosity and viability and growth rate of pink salmon. Cytology and Genetics 25:47-51.

Amos, W., S.J. Sawcer, R.W. Feakes, and D.C. Rubinsztein. 1996. Microsatellites show mutational bias and heterozygote instability. Nature Genetics, 13, 390-1.

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

Banks, M.A., M.S. Blouin, B.A. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity 90:281-288.

Becker, J. P. Vos, M. Kuiper, F. Salamini, and M. Heun, 1995. Combined mapping of AFLP and RFLP markers in barley. Mol. Gen. Genet. 249:65-73.

Beacham, T. D., R. E. Withler, C. B. Murray, and L. W. Barner. 1988. Variation in bodysize, morphology, egg size, and biochemical genetics of pink salmon in British Columbia. Transactions of the American Fisheries Society 117:109-126.

Biggs, E., T. Baker, M. McGurk, J. E. Hose and R. Kocan. 1991. Injury to Prince William Sound Herring. State/Federal Natural Resources Damage Assessment Draft Preliminary Status Report Unpub. rep. Alaska Department of Fish and Game, Cordova, AK.

Braat, A.K, T. Zandbergen, S. Van de Water, H.J.Th. Goos, and D. Zivkovic. 1999. Characterization of zebrafish primordial germ cells: morphology and early distribution of *vasa* RNA. Developmental Dynamics, 216:153-67.

Bue, B.G., S. Sharr, and J.E. Seeb. 1998. Evidence of damage to pink salmon populations inhabitating Prince William Sound, Alaska, two generations after the *Exxon Valdez* oil spill. Trans. Amer. Fish. Soc. 127:35-43.

Cronin, M. A., and J. W. Bickham. 1998. A population genetic analysis of the potential for a crude oil spill to induce heritable mutations and impact natural populations. Ecotoxicology 7:259-278.

Dallas, J.F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mammalian Genome, 3, 452-6.

Doyle, R. W., C. Herbinger, C. T. Taggart, and S. Lochmann. 1995. Use of DNA microsatellite polymorphism to analyze genetic correlations between hatchery and natural fitness. Amer. Fish. Soc. Symposium 15:205-211.

Dubrova, Y. E., V. N. Nesterov, N. G. Krouchinsky, V. A. Ostapenko, R. Neumann, D. L. Neil, and A. J. Jeffreys. 1996. Human minisatellite mutation rate after the Chernobyl accident. Nature 380:683-86.

Dubrova, Y. E., E. A. Salmenkova, Y. P. Altukhov, Y. F. Kartavtsev, E. V. Kalkova, and V. T. Omel'chenko. 1995. Family heterozygosity and progeny body length in pink salmon *Oncorhynchus gorbuscha* (Walbaum). Heredity 75:281-289.

Ferguson, M.M. 1992. Enzyme heterozygosity and growth in rainbow trout - genetic and physiological explanations. Heredity 68:115-122.

Forbes, S. H., K. L. Knudsen, T. W. North, and F. W. Allendorf. 1994. One of two growth hormone genes in coho salmon is sex-linked. Proc. Nat. Acad. Sci. USA 91:1628-1631.

Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler, S. G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon Aquaculture, 173:117-130.

Gharrett, A. J., and W. W. Smoker. 1994. Introduction to genetics of subarctic fish and shellfish. Canadian Journal of Fisheries and Aquatic Sciences 51, Suppl. 1:1-3.

Goodier, J. L. and W. S. Davidson. 1994. *Tc*1 transposon-like sequences are widely distributed in salmonids. J. Mol. Biol. 241:26-34.

Greene, B. A., and J. E. Seeb. 1997. SINE and transposon sequences generate high-resolution DNA fingerprints, "SINE prints", that exhibit faithful Mendelian inheritance in pink salmon (*Oncorhynchus gorbuscha*). Molecular Marine Biology and Biotechnology 6:328-338.

Habicht, C., W.B. Templin, L.W. Seeb, and J.E. Seeb. 1998. Genetics of pink salmon inhabitating Prince William Sound, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 96196), Alaska Department of Fish and Game, Anchorage, Alaska.

Hunt, R.L. 1969. Overwinter survival of wild fingerling brook trout in Lawrence Creek, Wisconsin. J. Fish. Res. Board Can. 26:1473-1483.

Hurst, L. D. 1999. The evolution of genomic anatomy. Trends Ecol. Evol. 14:108-112.

Jacob, H.J. and 19 other authors. 1995. A genetic linkage map of the laboratory rat, *Rattus norvegicus*. Nature Genetics 9:63-69.

Jackson, T. R., M. M. Ferguson, R. G. Danzmann, A. G. Fishback, P. E. Ihssen, M. O'Connell, and T. J. Crease. 1998. Identification of two QTL influencing upper temperature tolerance in three

rainbow trout (*Oncorhynchus mykiss*) half-sib families. Heredity 80:143-151.
Jin, L., and R. Chakraborty. 1995. Population structure, stepwise mutations, heterozygote deficiency and their implications in DNA forensics. Heredity 74:274-285.
Johnson, K. R., J. E. Wright Jr., and B. May. 1987a. Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics 116:579-591.
Johnson, O. W., F. M. Utter, and P. S. Rabinovitch. 1987b. Interspecies differences in salmonid cellular DNA identified by flow cytometry. Copeia 1987:1001-1009.
Johnson, S. L., M. A. Gates, M. Johnson, W. S. Talbot, S. Horne, K. Baik, S. Rude, J. R. Wong, and J. H. Postlethwait. 1996. Centromere-linkage analysis and consolidation of the zebrafish genetic map. Genetics 142:1277-1288.
Jones, A.G., G. Rosenqvist, A. Berglund, and J.C. Avise. 1999. Clustered microsatellite mutations in the pipefish *Syngathus typhle*. Genetics 152:1057-1063.
Karl, S. A. and J.C. Avise. 1992. Balancing selection at allozyme loci in oysters - Implications from nuclear RFLPs. Science 256:100-102.
Kartavtsev, Y. P. 1992. Allozyme heterozygosity and morphological homeostasis in pink salmon, *Oncorhynchus gorbuscha* (Walbaum) - Evidences from family analysis. Journal of Fish Biology 40: 17-24.
Katagiri, T., I. Hirono, T. Aoki, and M. Sakai. 1996. Isolation of major histocompatibility complex class I cDNA from pink salmon (*Oncorhynchus gorbuscha*). Developmental and Comparative Immunology 20:217-228.
Kido, Y., M. Aono, T. Yamaki, K. Matsumoto, S. Murata, M. Saneyoshi, and N. Okada. 1991. Shaping and reshaping of salmonid genomes by amplification of tRNA-derived retroposons during evolution. Proc. Natl. Acad. Sci. USA 88: 2326-2330.
Kocher, T. D., W. J. Lee, H. Sobolewska, D. Penman, and B. McAndrew. 1998. A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). Genetics 148:1225-1232.
Koehn, R.K., W.J. Diehl, and T. M. Scott. 1988. The differential contribution of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, *Mulina lateralis*. Genetics 118:121-130.
Lander, E.S. and 6 other authors. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.
Lander, E.S., and Schork, N.J. 1994. Genetic dissection of complex traits. Science 265:2037-2048.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1987. Differences in inbreeding coefficients do not explain the association between heterozygosity at isozyme loci and developmental stability in rainbow trout. Evolution 41:1413-1415.
Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1989. Genetic differences among rainbow trout spawned on different days within a single spawning season. Progressive Fish-Culturist 51:10-19.
Levinson, G. and G.A. Gutman. 1987. Slipped strand mispairing: a major mechanism for DNA sequence evolution. Molecular Biology and Evolution. 4:203-221.
Lewontin, R.C. 1991. 25 Years ago in genetics - electrophoresis in the development of evolutionary genetics - milestone or millstone? Genetics 128: 657-62.
Lie, O., A. Slettan, F. Lingaas, I. Olsaker, I. Hordvik, and T. Refstie. 1994. Haploid gynogenesis: A powerful strategy for linkage analysis in fish. Anim. Biotech. 5:33-45.
Lindner, K.R., J. E. Seeb, C. Habicht, K.L. Knudsen, E. Kretschmer, D. J. Reedy, P. Spruell, and

F. W. Allendorf. 2000. Gene-centromere mapping of 312 loci in pink salmon by half-tetrad analysis. Genome 43: 538-549.
Longwell, A. C. 1977. A genetic look at fish eggs and oil. Oceanus 20(4):46-58.
Lynch, M., and B. Walsh. In preparation. Genetics and Analysis of Quantitative Traits. Volume 2: Evolution and Selection of Quantitative Traits. Sinauer Assoc. Sunderland, MA. http://nitro.biosci.arizona.edu/zbook/volume_2/vol2.html
Maheswaran, M., P. K. Subudhi, S. Nandi, J. C. Xu, A. Parco, D. C. Yang, and H. Huang, 1997. Polymorphism, distribution and segregation of AFLP markers in a doubled haploid rice population. Theor. Appl. Genet. 94:39-45.
Malkin, D. 1994. Germline p53 mutations and heritable cancer. Annual Reviews in Genetics 28:443-465.
Matova, N. and L. Cooley. 2001 Comparative aspects of animal oogenesis. Developmental Biology 231, 291-320.
McGregor, A. J., S. Lane, M. A. Thomason, L. A. Zhitovsky, W. W. Smoker, and A. J. Gharrett. 1998. Migration timing, a life history trait important in the genetic structure of pink salmon. N. Pac. Anadr. Fish. Comm. Bull. 1:262-273.
Miller, K. M., and Withler R. E. 1998. The salmonid class I MHC: limited diversity in a primitive teleost. Immunological Reviews 166:279-293.
Mironov, O. G. 1969. The development of some Black Sea fishes in seawater polluted by petroleum products. Probl. Ichthyol. 9(6):1136-1139.
Moles, A., M. M. Babcock and S. D. Rice. 1987. Effects of oil exposure on pink salmon (*Oncorhynchus gorbuscha*) alevins in a simulated intertidal environment. Marine Environment Research 21:49-58.
Moran, P., J. I. Izquierdo, A. M. Pendas, and E. Garciavazquez. 1997. Fluctuating asymmetry and isozyme variation in Atlantic salmon: Relation to age of wild and hatchery fish. Transactions of the American Fisheries Society 126:194-199.
Morizot, D. C., S. A. Slaugenhaupt, K. D. Kallman, and A. Chakravarti. 1991. Genetic linkage map of fishes of the genus *Xiphophorus* (Teleostei, Poeciliidae). Genetics 127:399-410.
Mousseau, T. A., K. Ritland, and D. D. Heath. 1998. A novel method for estimating heritability using molecular markers. Heredity 80:218-224.
Naruse, K. and 19 additional authors. 2000. A detailed map of Medaka, *Oryzias latipes*: Comparative genomics and genome evolution. Genetics 154: 1773-1784.
Nelson, D.L., S.A. Ledbetter, L. Corbo, M.F. Victoria, R. Ramirez-Solis, T.D. Webster, D.H. Ledbetter, and C.T. Caskey. 1989. Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. Proc. Nat. Acad. Sci. USA 86:6686-6690.
O'Brien, S. J., J. E. Womack, L. A. Lyons, K. J. Moore, N. A. Jenkins, and N. G. Copeland. 1993. Anchored reference loci for comparative genome mapping in mammals. Nature Genetics 3:103-112.
Pamilo, P., and S. Pálsson. 1998. Associative overdominance, heterozygosity and fitness. Heredity 81:381-389.
Phillips, R. B., and A. R. Kapuscinksi. 1988. High frequency of translocation heterozygotes in odd-year populations of pink salmon (*Oncorhynchus gorbuscha*). Cytogenetics and Cell Genetics 48:178-182.
Phillips, R. B., M. P. Matsuoka, W. W. Smoker, and A. J. Gharrett. 1999. Inheritance of a

chromosomal polymorphism in odd-year pink salmon from southeastern Alaska. Genome. 42:816-820.
Pogson, G. H., K. A. Mesa, and R. G. Boutilier. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: A comparison of allozyme and nuclear RFLP loci. Genetics 139:375-385.
Postlethwait, J.H. and 14 other authors. 1994. A genetic linkage map for the zebrafish. Science 264:699-703.
Primmer, C.R., H. Ellegren, N. Saino, and A.P. Moller. 1996. Directional evolution in germline microsatellite mutations. Nature Genetics, 13, 391-3.
Roush, W. 1997. A zebrafish genome project? Science 275:923.
Roy, N. K., J. Stabile, J. E. Seeb, C. Habicht, and I. Wirgin. 1999. High frequency of K-ras mutations in pink salmon embryos experimentally exposed to Exxon Valdez oil. Environmental Toxicology and Chemistry 18:1521-1528.
Sakamoto, T., R. G. Danzmann, K. Gharbi, P. Howard, A. Ozaki, S. K. Khoo, R. A. Woram, N. Okamoto, M. M. Ferguson, L. Holm, R. Guyomard, and B. Hoyheim. 2000. A microsatellite linkage map of Rainbow Trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rates. Genetics 155: 1331-1345.
Sakamoto, T., R. G. Danzmann, N. Okamoto, M. M. Ferguson, and P. E. Ihssen. 1999. Linkage analysis of quantitative trait loci associated with spawning time in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 173:33-43.
Schug, M.D., T.F.C. Mackay, and C.F. Aquadro. 1997. Low mutation rates of microsatellite loci in *Drosophila melanogaster*. Nature Genetics, 15, 99-102.
Seeb, J. E., C. Habicht, J. B. Olsen, P. Bentzen, J. B. Shaklee, and L. W. Seeb. 1998. Allozyme, mtDNA, and microsatellite variants describe structure of populations of pink and sockeye salmon in Alaska. N. Pac. Anadr. Fish. Comm. Bull. 1:300-318.
Seeb, J.E., C. Habicht, W.B. Templin, and L.W. Seeb. 1996. Genetics of pink salmon inhabitating Prince William Sound, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Projects 94320D and 95320D), Alaska Department of Fish and Game, Anchorage, Alaska.
Shimoda, N., E. W. Knapik, J. Ziniti, C. Sim, E. Yamada, S. Kaplan, D. Jackson, F. deSauvage, H. Jacob, M. Fishman. 1999. Zebrafish genetic map with 2000 microsatellite markers. Genomics 58:219-232.
Shum, B. P., K. Azumi, S. Zhang, S. R. Kehrer, R. L. Raison, H. W. Detrich, and P Parham. 1996. Unexpected β2-microglobulin sequence diversity in individual rainbow trout. Proc. Natl. Acad. Sci. USA 93: 2779-84.
Slatkin, M. 1995. Hitchhiking and associative overdominance at a microsatellite locus. Mol. Biol. Evol. 12:473-480.
Spruell, P. and G. H. Thorgaard, 1996. SINE sequences detect DNA fingerprints in salmonid fishes. Heredity 317-324.
Spruell, P., K. L Pilgrim, B. B. Greene, C. Habicht, K. Knudsen, K. R. Lindner, J. Olsen, G. K. Sage, J. E. Seeb, and F. W. Allendorf. 1999. Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon (*Oncorhynchus gorbuscha*). Journal of Heredity 90:289-296.
Smoker, W.W., A.J. Gharrett, and M.S. Stekoll. Manuscript. Genetic variation in timing of anadromous migration within a spawning season in a population of pink salmon. Presented at Proc. Int. Symp. on Biol. Interactions of Enhanced and Wild Salmonids, Nanaimo, B.C.

1992.
Stanley, J. G. 1983. Gene expression in haploid embryos of Atlantic salmon. J. Hered. 74:19-22.
Unwin, M. J. 1997. Fry-to-adult survival of natural and hatchery-produced chinook salmon (*Oncorhynchus tshawytscha*) from a common origin. Canadian Journal of Fisheries and Aquatic Sciences 54:1246-1254.
Utter, F. M., J. E. Seeb, and L. W. Seeb. 1993. Complementary uses of ecological and biochemical genetic data in identifying and conserving salmon populations. Fisheries Research 18:59-76.
Van der Beek, S., and Van Arendonk, J.A.M. 1993. Criteria to optimize designs for detection and estimation of linkage between marker loci from segregating populations containing several families. Theor. Appl. Genet. 86:269-280.
Wada, H., K. Naruse, A. Shimada, and A. Shima. 1995. Genetic linkage map of a fish, the Japanese medaka *Oryzias latipes*. Molec. Marine Biol. and Biotech. 4:269-274.
Waples, R. S. 1995. Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. American Fisheries Society Symposium 17:8-27.
Weber, J.L. and C. Wong. 1993. Mutation of human short tandem repeats. Human Molecular Genetics 2:1123-1128.
Wierdl, M., M. Dominska, and T.D. Petes. 1997. Microsatellite instability in yeast: dependence on the length of the microsatellite. Genetics, 146, 769-79.
Woodruff, R. C, H. Huai, and J. N. Jr. Thompson. 1996. Clusters of identical new mutations in the evolutionary landscape. Genetica 98:149-160.
Young, W. P., P. A. Wheeler, V. H. Coryell, P. Keim, and G. H. Thorgaard. 1998. A detailed linkage map of rainbow trout produced using doubled haploids. Genetics 148:1-13.
Zhivotovsky, L. A., K. I. Afanasiev, and G. A. Rubtzova. 1987. Selection for enzyme loci in the pink salmon (*Oncorhynchus gorbuscha* Walbaum). Genetika 23:1876-1883.
Zhivotovsky, L. A., A. J. Gharrett, A. J. McGregor, M. K. Glubokovsky, and M. W. Feldman. 1994. Gene differentiation in Pacific salmon (*Oncorhynchus* sp): Facts and models with reference to pink salmon (*Oncorhynchus gorbuscha*). Canadian Journal of Fisheries and Aquatic Sciences 51, Suppl. 1:223-232.
Zouros, E.W., and D. Foltz. 1986. The use of allelic isozyme variation for the study of heterosis. In: M.C. Rattazzi, J.G. Scandalios, and G.S. Whitt (eds.), Isozymes: Current Topics in Biological and Medical Research, Volume 13. Alan R. Liss, New York, New York.

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002	LONG RANGE FUNDING REQUIREMENTS					
Personnel	$132.0	$28.0						
Travel	$14.1	$0.9						
Contractual	$0.0	$0.0						
Commodities	$35.5	$2.5						
Equipment	$0.0	$0.0						
Subtotal	$181.6	$31.4	Estimated					
Indirect	$41.9	$8.9	FY 2003					
Project Total	$223.5	$40.3	$75,000.0					
Full-time Equivalents (FTE)	3.2	0.5						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

Indirect costs are based on the University of Montana rate of 43.7% of salaries and wages.

Travel costs are included to attend one scientific meeting to present results.

Personnel time includes time for report and manuscript preparation.

FY02

Prepared: July, 2001

Project Number: 02190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:			Months	Monthly		
	Name	Position Description	Budgeted	Costs	Overtime	
	F Allendorf	Project Director	0.5	10.7		
	K Lindner	Research Specialist	5.8	3.9		
		Subtotal	6.3	14.6	0.0	
					Personnel Total	

Travel Costs:		Ticket	Round	Total	Daily	
	Description	Price	Trips	Days	Per Diem	
	Travel to a national meeting to present results.	0.6	1	3	0.1	
					Travel Total	

FY02

Prepared: July, 2001

Project Number: 02190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:	
Description	
Contractual Total	
Commodities Costs:	
Description	
Materials and supplies for microsatellite, allozyme, and morphological analysis. Equipment repair and maintenance	
Commodities Total	

FY02

Prepared: July, 2001

Project Number: 02190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

New Equipment Purchases:	Number of Units	Unit Price	
Description			
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	

Existing Equipment Usage:	Number of Units	
Description		
Hitachi FMBIO 100 Fluorescent Imaging Scanner	1	

FY02

Project Number: 02190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

Prepared: July, 2001