Exxon Valdez Oil Spill
Restoration Project Annual Report

Genetic Discrimination of Prince William Sound Herring Populations

Restoration Project 95165
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: This project was originally conceived as a component of Restoration Science Study R58 in 1992; however the project was first initiated as Restoration Project 94165. FY94 was the first field season for this project; however due to a return failure of herring in Prince William Sound during FY94, the project was postponed until FY95. This is the first annual report. Preliminary project methods and results were presented in a poster session at the January 1996 *Exxon Valdez* Oil Spill workshop in Anchorage. FY96 and FY97 are the remaining field seasons for this project. This project will be completed with a Final Report in FY98.

Abstract: The fishery in Prince William Sound for Pacific herring (*Clupea pallasi*) has been in catastrophic decline since 1992. The Alaska Department of Fish and Game recovery effort includes incorporating a knowledge of genetic structure of herring populations into harvest management. In this project we are delineating the structure of Prince William Sound populations and related North Pacific populations using both nuclear and mitochondrial DNA analyses. We focused on sample design and collection as well as development of microsatellite (nuclear) and mitochondrial DNA markers in FY95. Tests for temporal and spatial diversity within years and temporal stability across years will be conducted as the project matures.

Key Words: *Clupea pallasi*, *Exxon Valdez* oil spill, genetic population structure, microsatellite loci, mitochondrial DNA, nuclear DNA, Pacific herring, polymerase chain reaction, Prince William Sound, restoration management.

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INTRODUCTION

Pacific herring (*Clupea pallasi*) are a major resource in Prince William Sound (PWS) from both a commercial and ecological perspective. The timing of the *Exxon Valdez* oil spill (EVOS) overlapped the annual spring migration to nearshore staging areas of herring spawners. Over 40% of the herring spawning, staging, and egg deposition areas and over 90% of the documented summer rearing and feeding areas were lightly to heavily oiled prior to the spawning events. As a result, herring encountered oil during each of their four life stages in 1989 and, to a lesser extent, in 1990. Adult herring traversed oil sheens and mousse while traveling northward and eastward. Eggs were deposited on oiled shorelines and were exposed to sheen through tidal action while incubating. Larvae that hatched contained lipophilic petroleum hydrocarbons in their yolk sacs and encountered sheen near the surface while in their most sensitive state. Post-larval or juvenile herring swam through and remained near lightly to heavily oiled shorelines, regularly encountering sheen, mousse and dissolved oil components through the summer while feeding in shallow nearshore bays and passes.

The ex-vessel value of the herring fisheries in 1992 was $12.0 million (Donaldson et al. 1993). In 1993, the ex-vessel value dropped to $2.0 million; the total observed spawning population was less than one-third of preseason predictions; and the average sizes of herring in each age class were some of the smallest on record. Only limited commercial herring fishing occurred. Preliminary pathology results suggested viral hemorrhagic septicemia (VHS) as a potential source of mortality and stress, however this has not been shown conclusively (Meyers et al. 1994). In 1994 the spawning population remained below preseason predictions. No recovery was evident in 1995, and based on this, the 1996 commercial fishing season was cancelled. Aerial surveys since 1993 indicate that the population has remained below threshold harvest levels. Herring abundance is so low that no commercial harvest has been permitted since 1993.

Pacific herring provide important forage for many species including some species severely injured by the *Exxon Valdez* oil spill. Predator species include humpbacked whales, seals, sea lions, gulls, sea ducks, shorebirds, halibut, salmon, rockfishes, and other fishes. In addition, several thousand pounds of herring and herring spawn-on-kelp were harvested annually for subsistence purposes and form an important part of the local native culture of the villages of Chenega and Tatitlek.

Alaska Department of Fish and Game is mobilizing a recovery effort that includes pathology, genetics, early life history, and oceanographic investigations. The Department drafted a stock model (Brown and Wilcock 1994) to provide a basis for restoration management. However, the stock model is based upon several assumptions about the population structure of Prince William Sound spawning groups. Genetic homogeneity of herring stocks within PWS and no recruitment to those stocks from outside of the Sound are two of the assumptions this project was designed to evaluate.

Incorporating genetically-derived population structure is crucial to the success of any fisheries or restoration program. Consistent exploitation of mixed populations has to lead to the demise of the least productive stocks (Schweigert 1993). Unfortunately, defining the population structure of herring has been particularly difficult. There is evidence that herring home (Wheeler and Winters 1984), but straying may also be substantial. Morphological and meristic differentiation of herring from discrete geographic regions has been used as evidence for the existence of genetically distinct populations, but much of this variation may be environmentally mediated and has not been confirmed with genetic data (Safford and Booke 1992; King 1985; Burkey 1986).

Allozyme electrophoresis has proven to be a useful tool for delineating the population structure of herring over broad geographic regions (Grant 1984; Grant and Utter 1984) and between spawning populations within the same area that are temporally isolated (Kornfield et al. 1982). Allozymes define two distinct races of Pacific herring (Asian/Bering Sea and eastern North Pacific), with further subdivision between Gulf of Alaska and more southerly North Pacific stocks (Grant and Utter 1984). Also, allozyme markers describe genetic divergence among local spawning populations of Pacific herring in the vicinity of northern Japan (Kobayashi et al. 1990) and among genetically distinct fjord populations in Norway (Jorstad et al. 1994).

Additional techniques to study the structure of natural populations have become available in recent years as a result of advances in molecular biology. Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) provided some evidence of genetic differentiation within Atlantic and Pacific herring (Kornfield and Bogdanowicz 1987; Schweigert and Withler 1990; Dahle and Eriksen 1990); however the utility of these techniques to detect fine genetic structure in Pacific herring from the Gulf of Alaska has not been fully assessed. Nuclear DNA microsatellite markers are a new class of markers with the potential of being useful for investigation of fine population structure (e.g., Bentzen et al. 1991; Bentzen et al. 1994; Wright and Bentzen 1994). Nuclear and mitochondrial loci evolve in response to different pressures and reflect differing patterns of relationships among populations. In this study we pursue a combination of both mitochondrial and microsatellite approaches to more accurately define the stock structure of herring from the EVOS-affected area. These data may also be used to estimate the population composition of non-spawning aggregations contributing to fall fisheries in Prince William Sound.

The goal of this project is to improve the accuracy of current stock assessment methods, thus improving resource management. Improved accuracy of stock distribution information will allow fishery managers to make fine adjustments of fishing quotas to harvest the maximum available surpluses with the lowest possible risk of overharvest, damage to the resource, or economic loss to the fishing industry.

OBJECTIVES

Our overall objective is to provide a genetic basis for the stock model used by Alaska Department of Fish and Game to manage and restore the depleted herring resource in Prince William Sound. We propose to test for genetic heterogeneity among spawning aggregations of Pacific herring within Prince William Sound, adjacent to Prince William Sound in the Gulf of Alaska and in the Bering Sea, and between year classes within and adjacent to the Sound.

The working objectives of this study are to:

1. Survey population samples using both mitochondrial and nuclear DNA approaches. Techniques will include RFLP analysis of mtDNA and microsatellite analysis of nuclear loci.

2. Evaluate the null hypothesis that a single panmictic population of herring exists in Prince William Sound. The study will include at least four putative population samples from both spatial and temporal isolates within the Sound.

3. Evaluate the structure of Prince William Sound herring populations within the context of the structure of adjacent spawning aggregates (up to four), including comparisons from across the known genetic barrier of the Alaska Peninsula.

4. Test for inter-annual stability of allele frequencies in Prince William Sound and related North Pacific populations.

SCHEDULE CHANGES

Project Initiation

This project was originally anticipated to be two and one-half years in duration (Projects 94165 and 95165) with field collections from two spawning seasons, FY94 and FY95; laboratory analysis in FY95; and reporting completed in FY96. However, the start date, and thus the completion date, of this project have been elusive. The Trustee Council first made funds available during FY94 (Project 94165), but the 1994 field season was truncated due to the surprise run failure. Inadequate samples were obtained to meet most project objectives, and the project start was deferred one year. No Trustee Council funds were spent on the project in FY94. Presently, we are mid-way through the contract lab analyses (Project 95165) and field season for FY96 (Project 96165). Based upon sampling difficulties due to the run failure, we now believe that reporting of this project will not be complete until FY98.

We are currently working under contract (FY95 funds) with the University of Washington Marine Molecular Biotechnology Laboratory and Dalhousie University Marine Gene Probe Laboratory to develop both mtDNA and microsatellite markers for use in examining Pacific herring

population structure. These laboratories have developed mtDNA and microsatellite markers and are in the process of analyzing samples collected during the 1995 spawning returns.

METHODS AND PRELIMINARY RESULTS

Field Collections

Field collections of spawning Pacific herring targeted seven representative sites within and adjacent to Prince William Sound in 1995. The collection sites within Prince William Sound were chosen to maximize the potential genetic differentiation among temporally and spatially isolated spawning aggregations. We targeted Rocky Bay, a southcentral spawning isolate on Montague Island; St. Matthews Bay, a southeast isolate; Fish Bay, a northeast isolate; and Port Chalmers on Montague Island (Figure 1). Our efforts to sample both early- and late-spawning stocks within these four sites were unsuccessful in 1995 because of the timing of the spawning returns and inclement weather conditions which hindered collection efforts. Single collections were made at these spawning sites. Early-spawning isolates were collected from St. Matthews Bay and Fish Bay, and late-spawning isolates were collected from Rocky Bay and Port Chalmers. Sampling outside of Prince William Sound included Kodiak Island, populations thought to share an ancestral tie with Prince William Sound populations (J. Wilcock, Alaska Department of Fish and Game, personal communication) and Bering Sea populations known to be genetically isolated from Gulf of Alaska stocks (Grant and Utter 1984; Figure 2).

Tissue extracts from muscle, liver, eye, and heart were collected and preserved in liquid nitrogen for transport to -80° C freezers for archiving until analysis. With the exception of the Kodiak Island collection, all tissues were dissected from freshly caught fish and frozen within two hours of capture. The remote location of capture of the Kodiak Island herring precluded immediate processing of samples. These fish were dissected and preserved in liquid nitrogen after being held at ambient air temperature (near 0°C) for several hours. Following storage at -80°C, approximately 0.5g of frozen muscle tissue was subsampled into cryovials containing 100% ethanol for transport to the contract laboratories for DNA analysis.

Genetic Analysis

The Alaska Department of Fish and Game solicited assistance from outside laboratories for the genetic analyses following standard State of Alaska procurement procedures for Project 95165. A request for proposal was issued for the molecular analyses, and contracts granted to two university laboratories. We chose the current contract laboratories for their joint proposal which incorporated both mtDNA and microsatellite analyses (Appendix A). We are currently working under contract with Dr. Paul Bentzen at the University of Washington for mtDNA and Dr. Jonathan Wright at Dalhousie University for microsatellite marker development under Trustee Council approved Project 95165 funding. The Principal Investigators in these laboratories have published extensively in the area of population genetics, applying both mtDNA and

microsatellite methods to questions of population structure (e.g. Roff and Bentzen 1989; Bentzen et al. 1991; Bentzen et al. 1993a; Bentzen et al.1993b; Bentzen and Wright 1993; Bentzen et al. 1994; Wright 1993; Wright and Bentzen 1994; O'Reilly and Wright 1995; Morris et al. 1996).

To begin analyses, each university laboratory was provided with approximately 0.5g of muscle tissue from each fish. Samples were delivered to the laboratories in early October, 1995. Because of the timing of the awarding of these contracts, final results are not expected until after the contract closure date of September 30, 1996.

Mitochondrial DNA Marker Development Progress

mtDNA Extraction Methods.--Initial efforts of Dr. Paul Bentzen focused on refining DNA extraction methods. Adequate quantity and quality of DNA was achieved with both classic phenol/chloroform and rapid extraction methods (Hoelzel and Green 1994). While rapid extraction methods (lysis) worked well for most population collections, individuals from the Kodiak Island samples required the phenol/chloroform method to extract adequate quantity and quality of DNA. The Kodiak Island fish were obtained from a remote site in less than ideal conditions and were dead for a longer period of time prior to sampling and freezing as compared to the other population collections. However, it appears that tissue quality in this collection will yield sufficient mtDNA for analyses.

mtDNA Amplification and Restriction Enzyme Surveys.--The ND1 gene of mtDNA was amplified using the polymerase chain reaction (PCR). The sequence for the ND1 primer used was:
Forward: 5' ACC CCG CCT GTT TAC CAA AAA CAT 3'
Reverse: 5' GGT ATG AGC CCG ATA GCT TA 3'
The PCR conditions used were:
94°C (120 sec)
7 cycles (94°C (60 sec) + 53°C (45 sec) + 72°C (60 sec))
20 cycles (94°C (30 sec) + 53°C (45 sec) + 72°C (60 sec))

The initial restriction digests surveyed four individuals from each of the seven populations using twelve enzymes: *HinfI, TaqI, BanII, HaeIII, RsaI, CfoI, AluI, MboI, MspI, NciI, Sau3AI,* and *Alw44I.* Polymorphisms were observed in eight of the twelve enzymes. Following additional screening of up to twelve individuals from each of the four PWS collections, the suite of restriction enzymes was narrowed to seven (*RsaI, HinfI, HaeIII, BanII, CfoI, MboI, Taq1)* based on frequencies of polymorphic haplotypes observed in both PWS and outgroup populations.

A χ^2 analysis of differences in haplotype number among the four PWS populations was used to test for significant differences among populations at each restriction enzyme (Table 2). The P-values indicate the probability of genetic homogeneity among these populations. The recommended suite of restriction enzymes was further narrowed to four, *RsaI, HinfI, BanII,* and *CfoI* . While the P-value of *HaeIII* was lower than that of *CfoI*, *HaeIII* polymorphisms were

difficult to score because of the presence of multiple bands in the 100bp range. *HaeIII* was chosen over *CfoI* because the haplotypes are less ambiguous for scoring, and it is believed that *CfoI* will be as informative as *HaeIII* in the larger survey including outgroup populations (J. Olsen, University of Washington, personal communication).

Processing of Herring Population Samples.--DNA has been extracted from all individuals from each of the four collections from PWS and from all individuals from Togiak Bay and Norton Sound collections. DNA from the remaining Kodiak Island samples will be extracted using phenol/chloroform methods. To date, extracted DNA has been successfully amplified for the ND1 mitochondrial gene, and *RsaI* and *HinfI* digestions are complete. This indicates that tissue preparation and storage has been adequate to proceed with the larger mtDNA survey of all samples from each population specified in the contract.

Samples will be assayed on agarose gels, scanned digitally, and analyzed using a Molecular Dynamics FlourImager and associated software. In cases where samples fail to amplify, reamplification will be attempted at least once in the presence of appropriate positive controls. If samples fail to amplify a second time they may be eliminated from the survey.

mtDNA Data Analysis.--Haploype frequencies will be tabulated in spreadsheet format. The basis of polymorphisms will be interpreted (i.e. whether length or restriction based), and the pattern of site gains and losses for the latter type of polymorphism inferred. Spatial heterogeneity in haplotype frequencies and the partitioning of genetic variation will be assessed using χ^2 pseudo-probability tests (Zaykin and Pudovkin 1993) and AMOVA (Excoffier et al. 1992). Relative divergences among haplotypes and their significance for population structure will also be assessed with the help of programs available in the REAP software package (McElroy et al. 1992).

Plans for Completion.--The contractor reports that mtDNA assay of all individuals from each of the seven population collections (N=690) will be completed by the August, 1996. Data analysis will be completed by September 30, 1996. Dr. Bentzen will draft the final 95165 contract report which will include results from both the mtDNA and microsatellite assays in the form of a manuscript suitable for submission to a primary fisheries journal. ADF&G anticipates receiving a draft of Project 95165 findings by late fall 1996.

Nuclear DNA Marker Development Progress

Cloning of Microsatellites from Pacific Herring.--Microsatellite analyses were conducted by Dr. Jonathan Wright. Approximately 60 ug of Pacific herring DNA from five individuals was digested overnight with *RsaI, PalI,* and *HincII.* The products of digestion were separated on a 1% agarose gel. The 300-700 base pair (bp) size fraction was recovered from the gel and cloned into dephosphorylated pUC18 digested with *SmaI.* The ligation products were extracted with phenol/chloroform and used to transform Max Efficiency DH5a (GIBCO BRL, Gaithersburg,

MD, USA) cells. The cells were plated on selective LB medium at various densities. The high success of the test transformation permitted the immediate lifting of the colonies.

After lifting approximately 10,000 colonies to Hybond-N membranes, the colonies were fixed according to the supplier's recommendations (Amersham Life Science, Inc., Arlington Heights, Il). The membranes were prehybridized for two hours. A $GT_{(15)}$ oligonucleotide probe, labeled with gamma-^{32}P using T4 polynucleotide kinase, was added to the hybridization mixture and the reaction allowed to proceed overnight at 62°C. The membranes were washed twice at room temperature in 2XSSC/0.2% SDS and exposed to X-ray film (X-OMAT-R; Kodak) for 6 hours at -80°C. Over 1,000 clones hybridized to the oligonucleotide probe.

DNA Sequencing of Microsatellites.--One hundred and eight individual clones were sequenced in the forward and reverse direction. Over 70% contained microsatellite or other repeat-like sequences. However, many of the microsatellite sequences were associated with unsuitable flanking sequences. For example, some microsatellite sequences were embedded within a larger repetitive array; located adjacent to the cloning site; and/or adjacent to a GC-rich sequence that exhibited compression on the sequencing gel, thereby making reading of the sequence difficult. Moreover, many of the microsatellites were deemed unsuitable as PCR-based genetic markers based on their large size which prohibits resolving of single repeat unit differences in microsatellite alleles. As such, microsatellites containing arrays of 15-40 bp repeat units were selected for further characterization. Efforts were also focused on producing polymorphic microsatellite markers that generated PCR products of <150 bp repeat units. Previous studies have shown that smaller loci tend to amplify more consistently and facilitate studies where available tissues may be limited, e.g., when amplifying loci from DNA extracted from scales and/or poorly preserved ancient tissue (>50 years old).

Polymorphic Microsatellites.--Six microsatellite loci were chosen for further study based on their size and suitable flanking sequences for primer design. Primer annealing for PCR amplification of all six loci was 58°C. The primers were designed to have a common annealing temperature to allow multiplexing of some of the loci. Owing to the large range of allele sizes at each locus so far observed, it is uncertain whether multiplex systems for these loci can be established. The heterozygosity and number of alleles associated with six loci are shown in Table 3. Cha63 has been dropped because is has proven difficult to interpret allele size following PCR amplification of this locus.

Preliminary evidence suggests that several of these loci may be duplicated in the herring genome; more than two distinct alleles for a single locus have been detected in some fish. Although this could be a product of the loci examined, this is unlikely because this has been observed for several different loci. However, it should be noted this is a preliminary observation. If duplicated loci prove to be products of the loci which are ambiguous, rather than a biological phenomenon, there is a large pool of potential loci available from the ligation reaction already conducted (see above).

There is now a suite of polymorphic nuclear markers to investigate genetic differentiation of Prince William Sound and outgroup herring populations. Our contractors will continue to further optimize PCR amplification of these microsatellite loci to reduce artifact bands and thus improve the ease of scoring allele sizes for increased accuracy.

Processing of Herring Population Samples.--DNA has been extracted from 50 individuals from each of the four population collections from PWS and the three outgroup populations. To date, five microsatellite loci have been successfully amplified in randomly selected samples from each population. This indicates that tissue preparation and storage has been adequate to proceed with the larger microsatellite survey of 50 samples from each population specified in the contract.

Plans for Completion.--Dr. Wright has initiated microsatellite assays of the first 50 individuals from each of the seven population collections provided with the two primer sets that have been optimized for PCR. While these assays are conducted PCR conditions will continue to be optimized for the remaining microsatellite loci. It is anticipated that microsatellite assay of 50 individuals from each of the seven populations will be completed by June, 1996, followed by scoring of gels and entry of allele sizes into databases by the end of July, 1996. Data analysis will be completed by August, 1996, and the results submitted to Dr. Paul Bentzen, University of Washington, for drafting of the final 95165 contract report.

PCR Amplification of Homologous Loci in Other Fish Species.--Although not a condition of this contract, Dr. Wright is testing the extent to which the Pacific herring microsatellite primers amplify homologous loci in related fishes. He has found that Pacific herring (Family: Clupidae) microsatellite primers amplify polymorphic loci in Atlantic herring (Family: Clupidae) but not in anchovy (Family: Engraulidae) and sardines (Family: Clupidae). These data will be included in a manuscript (in preparation for Can. J. Fish. Aquat. Sci.) that will be of great interest to researchers studying the population genetics of Atlantic herring.

DISCUSSION

Preliminary results to date look promising. The overall sample quality appears adequate for DNA extraction and amplification methods for both the mtDNA and microsatellite analyses, and sufficient levels of polymorphism for population surveys have been identified. Although this project is not on schedule as originally anticipated, we have made substantial progress. FY95 objectives will be fully realized within existing budgets because of strict criteria in the contracts. Final results will be used to evaluate the assumptions of the stock model for Pacific herring in Prince William Sound. These results will be of particular interest to researchers studying population structure of marine fishes.

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Table 1. Description of Pacific herring samples collected or proposed. Location number corresponds to Figures 1 and 2.

Location Number	N	Dates Sampled[1]	Lat. N.	Long. W.	Location
1	100	4/95	60°42'	146°20'	St. Matthews Bay, early
	100	4/96			St. Matt. Bay, early
	100	4/96			St. Matt. Bay, late
2	100	4/95	60°49'	146°25'	Fish Bay, early
	100	4/96			Fish Bay, early
	100	4/96			Fish Bay, late
3	100	4/95	60°21'	147°07'	Rocky Bay, late
	100	4/96			Rocky Bay, late
	100	4/96			Rocky Bay, early
4	100	4/95	60°15'	147°13'	Port Chalmers, late
	100	4/96			Port Chalmers, late
	100	4/96			Port Chalmers, early
5	100	4/96			Kayak Island
6	100	3/96	57°00'	135°30'	Sitka Sound
7	90	5/95	58°06'	153°04'	Kodiak Island
	100	5/96			Kodiak Island
8	100	5/96			Port Moller
9	100	5/91	58°50'	160°24'	Togiak Bay
	100	5/96			Togiak Bay
10	100	5/91	63°54'	160°50'	Norton Sound
	100	5/96			Norton Sound

[1] 1996 dates are proposed sample collections

Table 2. Results of a χ^2 test of independence among four PWS herring populations for seven restriction enzymes. Each restriction enzyme was used to digest the ND1 gene of mtDNA.

Restriction Enzyme	χ^2	df	P-value	95% CI	
				Low	High
RsaI	11.96	6	0.055	0.042	0.070
HinfI	7.52	6	0.099	0.081	0.118
HaeIII	13.40	12	0.312	0.284	0.341
BanII	9.58	9	0.397	0.367	0.428
CfoI	9.48	12	0.787	0.761	0.812
MboI	3.96	6	0.952	0.938	0.964
TaqI	2.01	3	1.000	*	*

Table 3. Sequence information and preliminary estimates of variability from microsatellite loci developed for Pacific herring.

Locus	Sequence Information (5'-3')	Number of Alleles	Heterozygosity	Sample Size (n)
Cha17	GAGACTTACTCTCATCGTCC GCACAGTAGATTGGTTCCAC	13	0.8936	17
Cha20	GTGCTAATAGCGGCTGCTG TTGTGGCTTTGCTAAGTGAG	6	ND[1]	4
Cha34	CCTATGCTATCCTAACGATGG GGACGGAGACACCCAGAACA	8	0.8182	22
Cha38	CTCTGGACCATGTAACGG GCTATGACCATGATTACGG	3	ND[1]	4
Cha63	TGCCTGCTGAAGACTTCC CCCCTAAATGTGTTCTTTTAGC	10	ND[1]	10
Cha70	GTTCCATTTCTTAAACCTGC ACACTTTATCTTGCCCACC	6	.09087	20

[1] No Data

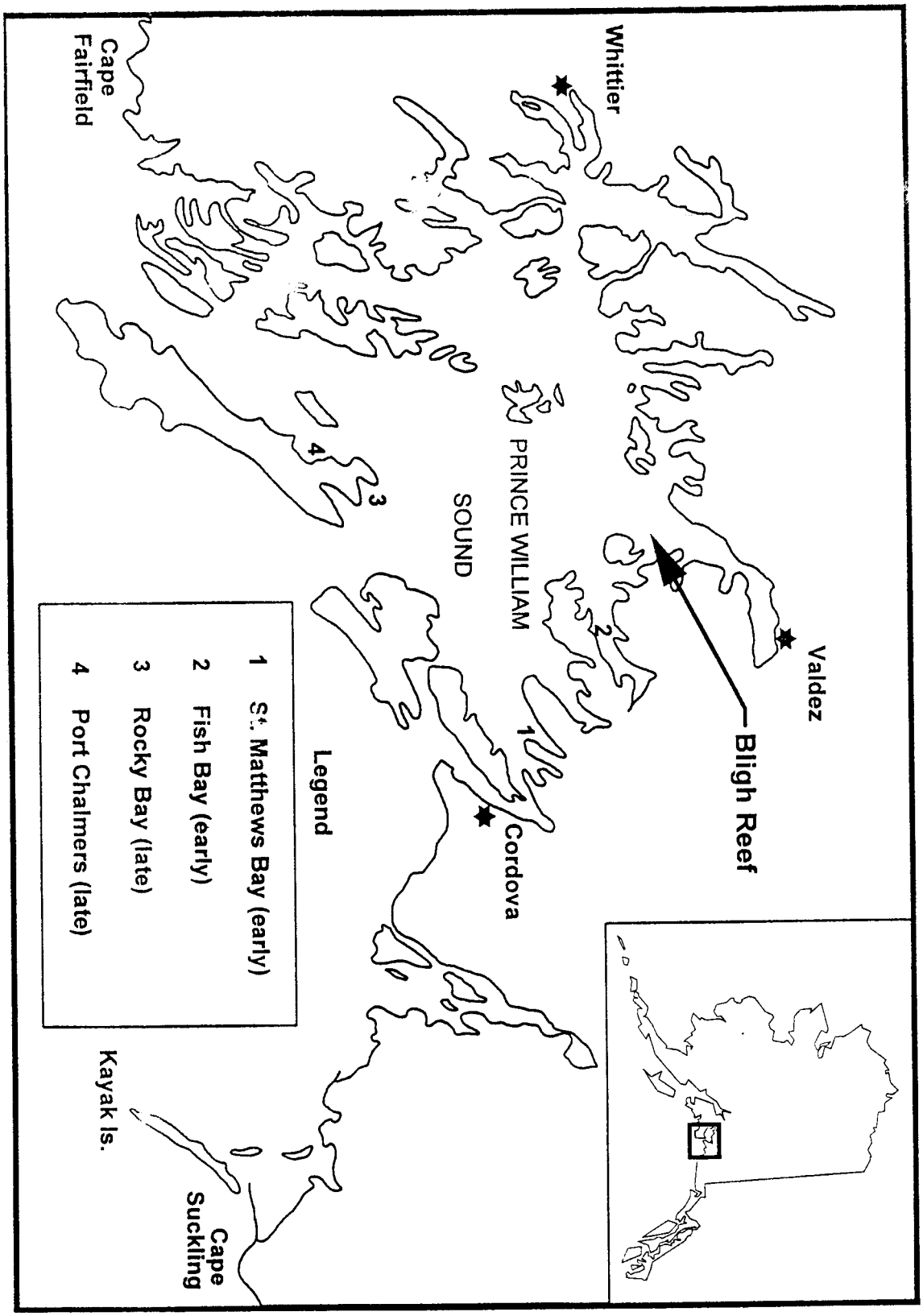

Figure 1. Prince William Sound 1995 sample collection site.

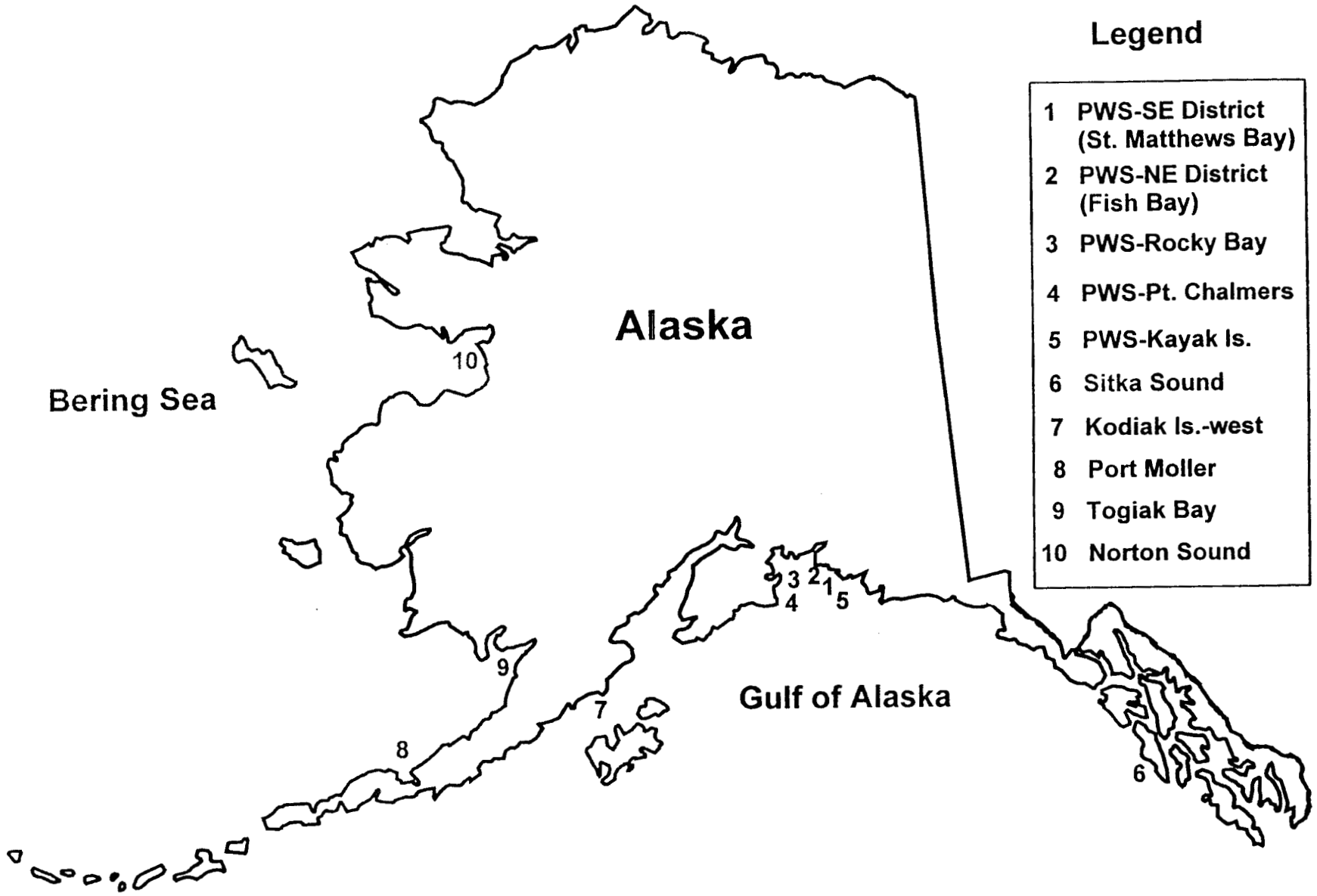

Figure 2. Collected and proposed sampling sites for Prince William Sound, Gulf of Alaska, and Bering Sea.

Appendix A. Proposals.

1) Development of mitochondrial DNA markers and use to screen Prince William Sound herring populations for genetic differentiation, by Paul Bentzen.

2) Development of microsatellite markers for genetic discrimination of Prince William Sound herring populations,
by Jonathan M. Wright.

Marine Molecular Biotechnology Laboratory
University of Washington
3707 Brooklyn Ave NE,
Seattle WA
98105-6715

August 10, 1995

Dr. James Seeb
Alaska Department of Fish and Game
Commercial Fisheries Management & Development Division
Genetics Laboratory - "Herring Project RFQ"
333 Raspberry Road
Anchorage, AK 99518-1599

Dear Jim,

Enclosed is a proposal created in response to your RFQ for work on developing genetic markers for Pacific herring , and their use in a population survey PWS herring. Please note that my proposal is only for the mtDNA portion of your RFQ, but should be viewed as a paired proposal to be taken in conjunction with Jonathan Wright's proposal to do the microsatellite part of the study.

Call or e-mail me if you have questions.

Sincerely,

Paul Bentzen

PROPOSAL: DEVELOPMENT OF MITOCHONDRIAL DNA MARKERS AND USE TO SCREEN PRINCE WILLIAM SOUND HERRING POPULATIONS FOR GENETIC DIFFERENTIATION

TO: Alaska Department of Fish and Game
Commercial Fisheries Management & Development Division
Genetics Laboratory - "Herring Project RFQ"
333 Raspberry Road
Anchorage, AK 99518-1599

FROM: Paul Bentzen
Marine Molecular Biotechnology Laboratory
School of Fisheries, University of Washington
3707 Brooklyn Ave NE,
Seattle WA 98105-6715

Phone: 206-685-9994
Fax: 206-543-1417
E-mail: pbentzen@fish.washington.edu

FEDERAL TAX IDENTIFICATION NUMBER 91-6001537

DATE: August 10, 1995

We propose to develop mitochondrial DNA (mtDNA) markers for herring (*Clupea pallasi*) and to use them to survey polymorphism in 700 herring. This proposal is made in conjunction with a collaborative proposal by Jonathan Wright to perform the microsatellite portion of this study.

WORK PLAN

Rationale for work plan: In a study such as this, where time and resource limitations preclude the "ideal" study design, researchers are forced to choose between maximizing the number of samples screened or the number of loci/polymorphic sites surveyed. The optimum choice likely differs for mtDNA and unlinked nuclear loci, such as microsatellites. In the former case, past experience of the PI and analyses by Smouse and Kobak (1994) suggest that declining returns (in terms of information useful for resolving populations) quickly set in as more restriction enzymes/polymorphic sites are added to a survey, with a large fraction of total potential resolving power available from as few as four restriction enzymes As such, it is likely better to optimize the number of samples to maximize statistical power, rather than invest effort in running many different restriction enzymes, which may lead to a proliferation of rare and relatively uniformative haplotypes. Accordingly, we propose to attempt to screen all 700 samples referred to in the RFQ, but to limit ourselves to as few as four restriction enzymes.

In a related proposal to perform microsatellite analyses (J. Wright, PI) we have adopted a different strategy. Although the large numbers of alleles typically exhibited by microsatellites make large sample sizes very desirable, this advantage must be balanced against potential gains obtained from explorative surveys of multiple unlinked loci. For instance, in Atlantic cod, we have found that one microsatellite locus of six surveyed greatly outstrips the other five in the differences it exhibits among populations on some spatial scales (P. Bentzen, unpublished data). Hence in the case of the microsatellite survey we have opted to run only 350 samples in order to maximize the number of loci we will be able to examine within time and budget constraints.

1. Primer optimization and preliminary screen. We will test and optimize the use of primer sets that will include but may not be limited to pairs that amplify ND1, ND5/6 and the D-loop. We will test at least at least 15 restriction endonucleases on two amplified fragments (amplicons) from at least 28 herring (comprising four from each putative population) for polymorphism. Our goal at this stage will be to identify particular amplicon/enzyme combinations which detect polymorphisms present at moderate-high frequency.

We have already obtained successful amplifications of ND1, ND5/6 and the D-loop for several individuals of Clupea pallasi. Preliminary data suggest that a length polymorphism exists in the herring D-loop. If confirmed by further work, we will assess the utility of this length polymorphism for population studies.

2. Main population survey. We will screen all 700 herring with a minimum of four amplicon/enzyme combinations identified as most suitable in stage 1. Samples will be assayed on agarose gels of appropriate concentration, scanned digitally and analyzed using a Molecular Dynamics FluorImager and associated software. In cases where samples fail to amplify, reamplification will be attempted at least once in the presence of appropriate positive controls. If samples fail to amplify a second time they may be eliminated from the survey. We will consult with ADF&G if the failure rate for amplifications exceeds 10%, since such a result might indicate a systematic problem with sample collection/storage procedures.

We have had very few problems amplifying DNA from properly collected and preserved fin clips, but have experienced problems on occasion with samples that have been mishandled. See sample collection protocol below.

3. Data analysis. Haplotype frequencies will be tabulated in spread-sheet format. The basis of polymorphisms will be interpreted (i.e., whether length or restriction site based) and the pattern of site gains and losses for the latter type of polymorphism inferred. Spatial heterogeneity in haplotype frequencies and the partioning of genetic variation will be assessed using χ^2 pseudo-probability tests (Zaykin and Pudovkin 1993) and AMOVA (Excoffier et al. 1992). Relative divergences among haplotypes and their significance for population structure will also be assessed with the help of programs available in the REAP software package (McElroy et al. 1992), although past experience with marine

fishes and mtDNA suggests this approach will contribute little to the assessment of herring population structure.

Data and analyses will be prepared in the form of a manuscript suitable for submission to a primary fisheries journal.

MILESTONES AND DELIVERABLES

Note that these are expressed in terms of the number of months elapsed from the establishment of an active budget account for the contract at UW, or the receipt of samples from ADF&G, whichever occurs later.

- 1 month. Completion of step 1 in the work plan.
- 8 months. Completion of step 2.
- summer 1996. Data analysis and manuscript preparation.
- 13 months. Draft manuscript made available to ADF&G.

- Brief progress reports will be made at quarterly intervals, or when requested by ADF&G.

FURTHER TERMS

1. The herring samples are to consist of fin clips (minimum 1 cm^2) removed from freshly collected herring stored individually in 100% EtOH in leak-proof vials. If freshly collected herring are not available, then 200-400 mg frozen muscle or heart tissue may be preserved in EtOH, preferably along with a non-freezer-burnt fin clip. Frozen tissues should come from specimens of suitable quality for allozyme analysis. Samples from different locations should be physically segregated.

2. Herring samples are to be shipped at ADF&G's expense to the MMBL.

3. The work shall be regarded as a collaborative project between MMBL and ADF&G, with the understanding that scientific publications that ensue directly from the project will bear joint authorship of involved personnel at both institutions. Lead authorship rights and responsibilities will rest with P. Bentzen, except in the case where both parties later agree to alter the arrangement, or Bentzen fails to provide a draft manuscript within 13 months of the inception of the contract, as defined above.

4. Hard (and if requested by ADF&G digital) copies of all gel images, along with the haplotype data in tabular spread-sheet format, will be provided to ADF&G on or before the completion of the 12 month contract period.

BUDGET

SALARIES	SUBTOTAL	TOTAL
Graduate student, Premasters Research Assistant		
50%, $1,046/mo, 7 months	7,322	
P. Bentzen, Assistant Prof.		
100%, 1 month	5,318	
		12,640
BENEFITS		
Graduate student		
7322 x 8%	586	
P. Bentzen		
5318 x 22%	1,170	
		1,756
SUPPLIES & SERVICES		
Bench fee (includes some consumables)		
$600/mo, 7 mo	4,200	
Additional consumables	585	
		4,785
Total Direct Costs		19,181
Overhead at 48.5%		9,303
TOTAL		28,484

PERSONNEL

Dr. Paul Bentzen is an Assistant Professor and runs the Marine Molecular Biotechnology Laboratory (MMBL) at the University of Washington. He has extensive expertise in molecular genetics and the population genetics of fishes. His experience with the population genetics of marine and anadromous fishes includes mtDNA studies of American shad, haddock and rainbow smelt, and mini- and microsatellite studies of Atlantic salmon, and microsatellite studies of Atlantic cod (see attached vitae).

P. Bentzen will direct the mtDNA part of this study, and assume leadership in analysis and report writing for both this and the associated microsatellite proposal submitted by J. Wright.

Mr. Jeffrey Olsen is a graduate student within the School of Fisheries who will assume responsibility for the laboratory phase of the mtDNA work. Mr. Olsen has a year of training in molecular genetics techniques and population genetics, as well as a strong background of familiarity with the PWS environment stemming from over a decade of employment with the Prince William Sound Aquaculture Corporation.

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Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

McElroy, D. P., P. Moran, E. Bermingham, and I. Kornfield. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. 83:157-158.

Smouse, P.E., and C.J. Kobak. 1994. Statistical implications of molecular data for mixed stock fishery analysis. American Fisheries Society 124th Annual Meeting, Halifax, Nova Scotia, August 1994.

Zaykin, D.V., and A.I. Pudovkin. 1993. Two programs to estimate significance of $\chi 2$ values using pseudo-probability tests. J. Hered. 84:152.

CURRICULUM VITAE *August 1995*

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Address: School of Fisheries HF-10
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Seattle, WA, USA, 98195

Telephone: (206) 685-9994 (office) (206) 527-4693 (home)
Fax: (206) 543-1417
E-mail: PBENTZEN@fish.washington.edu

Education:

NATO Advanced Studies Institute:
Molecular Techniques in Taxonomy
University of East Anglia, July 1990

McGill University
Department of Biology
Ph.D., Molecular/Evolutionary Genetics, 1989

Dalhousie University 1983-84
(began Ph.D. studies there before switching to McGill)

University of British Columbia
Department of Zoology
M.Sc., Fish Ecology, 1982

McGill University
Department of Biology
B.Sc. Marine Biology (First Class Honours),1978

Employment:

Assistant Professor
School of Fisheries, University of Washington
1993--present

Research Associate
Marine Gene Probe Laboratory/Ocean Production Enhancement Network, Dalhousie University
1991-- 1993

FCAR, NSERC Postdoctoral Fellow
Dept. of Biology, Dalhousie University
1989 1991

Current Research:

Evolutionary/population genetics of fishes.
Application of recombinant DNA technology and DNA fingerprinting to fisheries and aquaculture.
Molecular/genomic/evolutionary characteristics of variable number tandem repeat (VNTR) loci.

Refereed Publications:

McConnell, S.K., P. O'Reilly, L. Hamilton, J.M. Wright, **P.Bentzen.** 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Can. J. Fish. Aquatic Sci. (in press).

McConnell, Hamilton, D. Morris, D. Cook, D. Paquet, **P.Bentzen**, J.M. Wright1995. Isolation of microsatellite loci in Atlantic salmon and their application to the population genetics of Canadian East coast stocks. Proceeding 5th Int. Symp. on Genetics in Aquaculture. (in press).

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Taylor, E.B. and **P. Bentzen.** 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. Evolution 47:813-832.

Zwanenburg, K.C.T., **P. Bentzen** and J.M Wright. 1992. Mitochondrial DNA differentiation in Western North Atlantic populations of haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 49:2527-2537.

Refereed Publications: (cont'd)

Bentzen, P., A.S. Harris, and J.M. Wright. 1991. Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of salmonids and tilapia. In: T. Burke, G. Dolf, A.J. Jeffreys, and R. Wolff (eds.), DNA Fingerprinting: Approaches and Applications. Birkhauser Verlag, Basel/Switzerland. pp.243-262.

Franck, J.P.C., A.S. Harris, **P.Bentzen,** E.M. Denovan-Wright, and J.M. Wright. 1991. Organization and evolution of satellite, minisatellite and microsatellite DNAs in teleost fishes. Chapter 3 in: N. Maclean (ed.), Oxford Surveys on Eukaryotic Genes. Volume 7, 1991. Oxford University Press. pp.51-82.

Bentzen, P., G.G. Brown, and W.C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure and life history variation in American shad (*Alosa sapidissima*). Can. J. Fish. Aquat. Sci. 46:1446-1454.

Roff, D.A., and **P. Bentzen**. 1989. The statistical analysis of mitochondrial DNA polymorphisms: Chi-square and the problem of small samples. Mol. Biol. Evol. 6:539-545.

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Bentzen, P., S. McConnell, D. Denti, L. Hamilton and J.M. Wright. Genetic differentiation of Atlantic salmon (*Salmo salar*) populations revealed by mini- and microsatellite markers. In preparation for Molecular Biology and Evolution.

Bentzen, P., K.C.T. Zwanenburg, A. Shedlock, D. Power, L. Bryden and J.M. Wright. Lack of genetic divergence between western North Atlantic redfishes (*Sebastes spp.*) revealed by mitochondrial DNA analysis. In preparation for Can. J. Fish. Aquat. Sci.

Bentzen, P., M. Sargent, L. Bryden, K.C.T. Zwanenburg and J.M. Wright. Tandem repeats and heteroplasmy in the mitochondrial DNA of North Atlantic redfishes (*Sebastes spp.*). In preparation for Molecular Biology and Evolution.

Other publications:

Bentzen, P., D.E. Ruzzante, C.T. Taggart, and D. Cook. 1995. Genetic variation among NW Atlantic cod and Northern cod (*Gadus morhua* L.) populations based on nuclear DNA microsatellite analysis. 1995 Newfoundland Regional Groundfish Assessment Meeting, Working Paper WP/12.

Bentzen, P., J.M. Wright, I. Kornfield and F. Roberts. 1992. Single-locus DNA fingerprinting of Atlantic salmon from Maine and Newfoundland. Proceedings of Atlantic salmon workshop, Rockland Maine, 1992.

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Bentzen, P., D. Cook, D. Denti, A.S. Harris, J. Hofman and J.M. Wright. 1990. One tube DNA extraction procedure for molecular fingerprinting. Fingerprint News 2(4):17-21.

Abstracts and Presentations:

Bentzen, P. and J.M. Wright. Emerging technologies and the search for new "markers". Invited presentation at A.F.S. Ann. Mtng., August 1994, Halifax, Canada.

Bentzen, P., C.T. Taggart, S. McConnell and J.M. Wright. Microsatellite markers and genetic differentiation in fishes: an early perspective. Invited presentation at AFS sponsered conference on Evolution and the Aquatic Ecosystem; Defining Unique Units in Population Conservation. May 1994, Monterrey, CA.

McConnell, S., **P. Bentzen,** D. Morris, D. Cook, L. Hamilton, D. Paquet, J.M. Wright. Isolation of microsatellite loci in Atlantic salmon and their application to the aquaculture and population genetics of Canadian east coast stocks. 5th Intn. Symp. on Genetics in Aquaculture, June 1994, Halifax, Canada.

Bentzen, P., J.M. Wright, F. Roberts and I. Kornfield. A survey of polymorphism in variable number tandem repeat loci in Atlantic salmon (*Salmo salar*) populations in eastern North America. Genetics & Evolution of Aquatic Organisms, Bangor, Wales, Sept. 1992.

Wright, J.M. and **P. Bentzen**. DNA fingerprinting of Nova Scotia and Maine Atlantic salmon populations. Atlantic Salmon Workshop, U.S. Fish and Wildlife Service, Rockland, Maine, March 1992.

Bentzen, P. and J.M. Wright. Cloning and characterization of variable number tandem repeat loci in salmonid fishes. International Symposium on Biochemical Genetics and Taxonomy of Fish., Belfast, U.K., July 1991.

Taylor, E.B., and **P. Bentzen**. Morphological and molecular genetic studies of diversity in northeastern North American populations of smelt (*Osmerus*) with contrasting life histories. International Symposium on Biochemical Genetics and Taxonomy of Fish., Belfast, U.K., July 1991.

Zwanenburg, K.C.T., **P. Bentzen**, and J.M. Wright. Biochemical systematics of redfishes (*Sebastes*). Int. Symposium on Biochem. Genet. Fish, Belfast, U.K., July 1991.

Abstracts and Presentations: (cont'd)

Bentzen, P., A.S. Harris, and J.M. Wright. Hypervariable genetic markers cloned from the DNA of fishes. Canadian Conference For Fisheries Research, Guelph, Ontario, January 1991.

Bentzen, P. Mitochondrial divergence in clupeid fishes. NATO Advanced Studies Institute, "Molecular Techniques in Taxonomy", Norwich, U.K., July 1990.

Zwanenburg, K.C.T., **P. Bentzen**, and J.M. Wright. Mitochondrial DNA variation in northwest Atlantic populations of haddock. NATO ASI "Molecular Techniques in Taxonomy", Norwich, U.K., July 1990.

Harris, A., **P. Bentzen**, and J.M. Wright. Cloning of hypervariable minisatellite loci from tilapia. CFBS symposium, Halifax, June 1990.

Harris, A.S., **P. Bentzen** and J.M. Wright. Cloning of hypervariable loci from fish DNA. DNA mini-symposium, Canadian Society of Forensic Science, Ottawa, 1990.

Harris, A., **P. Bentzen**, and J.M. Wright. DNA fingerprinting in aquacultural genetics. 2nd DNA Fingerprinting Workshop, University of Nottingham, December 1989.

Bentzen, P. Mitochondrial polymorphism in American shad and its implications for population structuring. West Vancouver Lab. of D.F.O., June 1988.

Bentzen, P. Geographic variation in the frequencies of mitochondrial genotypes of American shad. Ann. Meeting of the Soc. for the Study of Evolution, Asilomar, CA, June 1988.

Bentzen, P. and G.G. Brown. Biogeographic and molecular aspects of heteroplasmy in shad mitochondrial DNA. Genetics Soc. of America Ann. Meeting, San Francisco, June 1987.

Bentzen, P., and G.G. Brown. Heteroplasmy in shad mitochondrial DNA. Canadian Society of Zoologists Annual Meeting, Montreal, May 1987.

Invited Lectures:

Microsatellite polymorphism and the population genetics of Atlantic cod University of Washington, Zoology Dept. , May 1995.

Microsatellite polymorphism and the population genetics of Atlantic cod and Atlantic salmon. UC Davis, Bodega Bay Marine Laboratory, Bodega Bay, CA, December 1994.

Application of variable number tandem repeat loci to population genetic studies of marine fishes. Rutgers University, June 1993.

Invited Lectures: (cont'd)

Development and use of variable number tandem repeat markers for population and aquacultural genetics of salmonids. National Oceanic and Atmospheric Administration workshop, "Application of DNA Technology to the Management of Pacific Salmon", Seattle, March 1993.

Micro- and minisatellite DNA and new approaches to the population genetics of marine organisms. University of Washington, Seattle, January 1993.

Looking for molecular evolution in the fast lane. U.B.C., Vancouver, B.C., June 1992.

Molecular population genetics of marine fishes: some case studies. University of Maine, December 1991.

Molecular approaches to the population biology of marine organisms. Dept. of Oceanography, Dalhousie U., Feb. 1991.

Two approaches to evolutionary genetics: VNTRs and mitochondrial DNA. Dept. of Zoology, U. Guelph, October 1990.

Two approaches to the evolutionary genetics of fishes. Dept. of Biology, Memorial University, March 1990.

Molecular approaches to stock discrimination. Inst. Maurice Lamontagne, D.F.O., April 1989.

Mitochondrial polymorphism in shad (*Alosa*). Dept. of Biology, Dalhousie University, August 1988.

The application of mitochondrial DNA analysis to stock discrimination. Theme session on methodological frontiers in fishery science, Canadian Conference for Fisheries Research, Ottawa, January 1988.

The application of mitochondrial DNA analysis to biogeographic and stock discrimination studies. Bedford Institute of Oceanography, Dartmouth, Nova Scotia, March 1987.

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August 4, 1995

Dr. Jim Seeb
Alaska Department of Fish and Game
Commercial Fisheries Management &
Development Division
Genetics Laboratory - "Herring Project RFQ"
333 Raspberry Road
Anchorage, AK 99518-1599
U.S.A.

Dear Dr. Seeb,

Please find enclosed a proposal entitled, "The development of microsatellite markers for genetic discrimination of Prince William Sound herring populations" for consideration in the RFQ contract competition. Also enclosed is a copy of my curriculum vitae that details my expertise in this area of research.

Although the results of our microsatellite project can stand alone as a potentially valuable contribution to Prince William Sound herring fisheries management, this work would be enhanced by comparative analyses with mitochondrial DNA studies proposed by Dr. Paul Bentzen, University of Washington, in this same competition. As such, we have coordinated our application with Dr. Bentzen with regard to sample analysis and overall funding of the project.

Yours sincerely,

Jonathan Wright, Ph.D.
Associate Professor

JMW/cy

HD#11:SEEB.LET

PROPOSAL: DEVELOPMENT OF MICROSATELLITE MARKERS FOR GENETIC DISCRIMINATION OF PRINCE WILLIAM SOUND HERRING POPULATIONS.

TO: Alaska Department of Fish and Game
Commercial Fisheries Management & Development Division
Genetics Laboratory - "Herring Project RFQ"
333 Raspberry Road
Anchorage, AK 99518-1599

FROM: Jonathan M. Wright
Marine Gene Probe Laboratory
Department of Biology
Dalhousie University
Halifax, NS B3H 4J1

Phone: 902-494-6468
Fax: 902-494-3736
E-mail: JMWRIGHT@AC.DAL.CA

DATE: August 9, 1995

INTRODUCTION

During the past decade, there has been an explosion in the development of molecular biological techniques for the population discrimination of marine species. The objectives of much of this work has been: population differentiation; assessment of temporal and spatial diversity within years and temporal stability across years; assessment of the impact of escaped stocks on indigenous populations; evaluation of stocking programs, etc. (reviewed in Park and Moran, 1994; Ward and Grewe, 1994; Wright, 1993). What remains to be seen is which DNA-based marker system will provide fisheries managers with cheap, reliable and informative genetic data to make rational decisions.

At the forefront of DNA-based technologies has been the use of mitochondrial DNA (mtDNA) polymorphism, a molecular marker that in most instances is inherited maternally. While mtDNA has proved particularly useful in the discrimination of some pelagic fish populations (e.g., Zwanenburg et al, 1992), several species of fish show insufficient variability in this molecule for population differentiation. As such, much interest is now directed towards the application of so-called "DNA fingerprinting" technology to fisheries research because it detects highly polymorphic nuclear loci that provide substantially more information than can often be derived from mtDNA (reviewed in Wright, 1993).

Initial DNA fingerprinting studies applied to fisheries and

aquaculture genetics used the multilocus approach developed by Jeffreys et al (1995a,b)(see e.g., Harris et al, 1991; Bentzen et al, 1991, Wright, 1993). Because of problems with its reproducibility and the lack of information about the heterozygous/homozygous state at a given locus (which reduces potential information of value in population genetic analyses), many researchers have abandoned mutlilocus fingerprinting in favour of single-locus DNA fingerprinting using cloned VNTR or minisatellite loci (e.g., Bentzen et al, 1991; Taggart and Ferguson, 1991; Bentzen and Wright, 1993; Harris and Wright, 1995). Although the information derived from single-locus fingerprints is superior to multilocus fingerprints, this technology suffers from similar problems of multilocus fingerprinting, i.e., it requires high quality DNA, employs the Southern blot/hybridization method, often requires binning of alleles, thereby underestimating genetic diversity in a given population. Moreover, it necessitates development of species-, or at best genus-specific, single-locus probes. As a result, single-locus fingerprinting is time consuming and expensive.

The currently favoured DNA fingerprinting approach in a myriad of genetic studies is based on single-locus microsatellite fingerprinting. Microsatellites are short stretches of DNA composed of di-, tri-, or tetranucleotide arrays predominantly embedded in unique DNA (Tautz, 1989; Weber and May, 1989). Since the tandem array of a microsatellite is highly susceptible to mutation due to slipped-strand mispairing during DNA replication (Levinson and Gutman, 1987), this results in polymorphism at the microsatellite locus (reviewed in Wright, 1993; Wright, 1994; Wright and Bentzen, 1994; O'Reilly and Wright, 1995).

Microsatellites have recently received considerable interest from fisheries geneticists because of their unique attributes among DNA-based marker systems (reviewed in Wright and Bentzen, 1994; O'Reilly and Wright, 1995). These include: (1) various levels of polymorphism at different loci allowing for selection of markers appropriate to the degree of genetic differentiation present in populations; (2) codominant Mendelian inheritance of alleles; (3) amplification of microsatellite alleles by the polymerase chain reaction (PCR) such that only a minute amount of tissue is required for assay, e.g., drop of blood, scale, or otolith. (Samples can be dried or preserved in alcohol. We have even assayed dried scales over 80 years old for retrospective studies of population structure); (4) cross-species priming, e.g., PCR-primers to Atlantic cod microsatellites amplify polymorphic loci in other gadids such as haddock (Brooker et al 1994); (5) potential for multiplexing. (We have recently developed 4 microsatellite loci from Atlantic salmon that can be amplified in one tube and alleles at each locus identified in a single lane on a sequencing gel (O'Reilly and Wright, 1995)); (6) potential for automation.

In this project, we propose to develop a suite of polymorphic microsatellite loci for Pacific herring and evaluate their use in

genetic discrimination of populations in Prince William Sound, Alaska.

PRIOR EXPERIENCE IN THE DEVELOPMENT OF MICROSATELLITE MARKERS

In the Marine Gene Probe Laboratory (MGPL), we have developed considerable facility in the cloning, sequencing, optimization of PCR-amplification and application of highly polymorphic microsatellite markers to population, conservation and breeding genetics of a variety of species (Table 1).

TABLE 1. DEVELOPMENT OF POLYMORPHIC MICROSATELLITES IN MGPL.

Fish	Mammals
Atlantic salmon	American mink
Rainbow trout	Sperm whale
Atlantic cod	Harbour seal
Nile tilapia	
Shiranus tilapia	Invertebrates
Thai carp	Sea scallop
Common carp	
Sea bass	

In the past 18 months, my group in MGPL has published 15 articles in the primary literature describing the development and application of microsatellite markers to population, behavioral and conservation genetics for a variety of species (see attached curriculum vitae). Moreover, we have a large inter-disciplinary group in the MGPL focused on the application of genetic engineering technology to fisheries and aquaculture, consisting of 25 research personnel housed in a "state-of-the-art" facility of approximately 6,000 square feet.

EXPERIMENTAL STRATEGY

Objectives:

1. To develop polymorphic microsatellite markers for Pacific herring.

2. To assess the level of polymorphism at 3-6 microsatellite loci in selected populations. We will assay 350 samples, approximately 50 per population.

3. To compare the utility of microsatellite markers with mtDNA markers for population differentiation of Pacific herring (in collaboration with Dr. Paul Bentzen, University of Washington).

Developing a set of microsatellite markers for a new species such a Pacific herring is a relatively straightforward procedure for a highly skilled team such as ours. However, it is fairly time-consuming (about 6-8 months) and involves an unpredictable risk element (see below). However, we propose to follow the procedure that has proved successful in the development of polymorphic microsatellites previously isolated in MGPL from other marine species (see Table 1).

Procedure:

1. Create a Pacific herring library of size-selected restriction fragments using genomic DNA (or DNA extracted from tissue) supplied by ADFG.

2. Screen this library with a radiolabelled $(GT)_{15}$ oligonucleotide to detect and then isolate clones potentially bearing microsatellite sequences.

3. Sequence the clones by the dideoxy- chain terminating method to determine suitable candidate microsatellite loci for assay by the polymerase chain reaction (PCR).

4. Evaluate unique flanking regions to microsatellite arrays for appropriate sequence composition and sufficient length to synthesize oligonucleotides for PCR primers.

5. Conduct a preliminary optimization of PCR conditions to amplify candidate microsatellite loci.

6. Assess the level of polymorphism at candidate microsatellite loci using DNA or tissue from presumably genetically unrelated individuals (supplied by ADFG).

7. Evaluate the level of polymorphism and allele frequency distribution at candidate microsatellite loci in 350 individuals, approximately 50 fish from the seven populations of Pacific herring (supplied by ADFG).

For experimental details see Wright and Bentzen, 1994; Brooker et al, 1994; McConnell et al, 1995a,b; Morris et al, 1995; Colbourne et al, 1995; O'Reilly and Wright, 1995.

The element of risk previously mentioned occurs at stages 6 and 7. At stage 6, we have found that there is a very large difference between species (populations) in the proportion of microsatellite which are polymorphic, and this cannot be predicted in advance. In our experience, 30% of microsatellite on average of the PCR-primer sets used, detected polymorphic microsatellite loci in the sample tested. From one species to another this ranged from 8-80%.

For this reason, MGPL cannot guarantee the exact number of informative microsatellite generated in this contract. We do guarantee that a minimum of 20 candidate PCR-primer sets complementary to sequences flanking microsatellites will be tested. On average, we have found that this should yield 4 to 6 informative microsatellite markers, normally sufficient for population differentiation of marine species. The complete sequence information of microsatellites, PCR conditions for amplification of loci and results of tests on 350 individual Pacific herring will be provided to ADFG.

At stage 7, we cannot guarantee the quality of DNA (or tissue sample) for PCR-amplification of microsatellite loci as samples were not collected using quality-controlled conditions employed by MGPL. Depending on the preservation quality of a sample, either all samples can be amplified at all microsatellite loci, only some but not all loci can be amplified, or owing to very poor quality of the DNA, none of the microsatellite loci can be amplified by PCR.

We will attempt to PCR-amplify all 350 samples at candidate microsatellite loci. Those samples that fail to amplify will be repeated once using appropriate control samples; lack of PCR-amplification at microsatellite loci in these samples will be deemed due to poor quality of DNA owing to inappropriate sampling and storage of tissue by ADFG

From previous experience we have found that a preliminary survey of the one proposed here for Prince William Sound herring populations will provide sufficient data to indicate the utility of the microsatellite markers developed in this project for population differentiation. Although we do not guarantee it as a deliverable in this contract, if time and resources permit (i.e., if few problems arise in the development of polymorphic markers during the early stages of the project), we will assay additional samples beyond the 350 fish stated in the contract. This will enhance the robustness of statistical analysis.

Reporting of results:

At least quarterly, we will provide ADFG with a concise summary of progress detailing number of clones isolated and sequenced, primers synthesized and tested, levels of polymorphism, etc. A full report will be supplied to ADFG upon completion of the contract. If problems arise in finding sufficient polymorphic microsatellites, we shall review with ADFG the feasibility of continuance of the project with respect to survey of populations after 6 months of the contract.

Publication rights:

Although the information derived from this contract will belong to ADFG, the MGPL requests right to publish information on primer sequences and preliminary population surveys of Pacific herring with appropriate co-authorship given to contributing staff of ADFG.

Networking:

Dr. Paul Bentzen, University of Washington, will coordinate duplicate sample analysis and conduct a comparative analysis of the two marker systems used. Dr. Bentzen was a Research Associate in MGPL from 1989 to 1993 prior to taking up his position at the University of Washington. Bentzen and I have co-authored 10 publications in the primary literature since 1991 and continue to collaborate on a number of projects. Clearly, our two laboratories have a good track record of collaboration and publication

An optional component in the budget is for a senior scientist or technician from ADFG to come to MGPL to learn the assay of microsatellite markers for use in the Anchorage Genetics Laboratory. The cost will be based on standard bench/user/professional fees at MGPL. We have successfully trained numerous personnel from different countries in the microsatellite assay and data analysis.

BUDGET (US$):

1.	**Salaries:**	
	-Mary Dillon (8 months + 6.6% benefits).	17,067
	-Dr. Mick O'Connell (3 months + 6.6 % benefits, partial salary).	2,500
2.	**Professional fees:**	
	-Dr. Jonathan Wright (PI: 20 hrs/month).	4,000
3.	**Bench fees:**	
	-11 months @ $250/month.	2,750
4.	**Consumables:**	
	-11 months @ $300/month for general supplies.	3,300
	-oligonucleotide synthesis by GIBCO/BRL $12.5 setup + $1.5/base (normally 20-mer).	2,000
5.	**Equipment:**	
	-2000 volts power supply (built by Dalhousie Electronics Shop).	800
6.	**Dalhousie University overhead:**	
	-38.6% on salaries and benefits ($23,567).	9,097
	TOTAL	41,514
7.	**Optional training period:**	
	One researcher from ADFG to train in MGPL for 4 weeks at standard MGPL fees.	550

RESEARCH PERSONNEL, EXPERIENCE AND CONTRIBUTION TO PROJECT:

Dr. Jonathan Wright is an Associate Professor in the Department of Biology and a Principal Investigator in the Marine Gene Probe Laboratory at Dalhousie University. He has extensive expertise in molecular genetics, especially the development of microsatellites markers for fisheries and aquaculture genetics. Wright will provide coordination of the project, responsibility for report

writing and networking with Dr. Bentzen at the University of Washington. CV attached.

Dr. Mick O'Connell is a postdoctoral fellow in MGPL who has developed polymorphic microsatellite markers for rainbow trout and American mink. He will devote approximately 50% time in the first 3 months of the project, but will continue supervision in the day-to-day experimental procedures throughout the project.

Mary Dillon will complete her Ph.D. thesis in November, 1995. She has experience in cloning, sequencing, PCR and population genetic studies of the sperm whale. Ms. Dillon will contribute 100% time for 8 months in the development and assay of Pacific herring microsatellites.

Pam Gaines, MGPL administrator, will provide administration assistance in the project.

REFERENCES CITED

Bentzen, P., Harris, A.S., and **Wright, J.M.** 1991. Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of salmonids and tilapia. In: *DNA fingerprinting: approaches and applications*. (T. Burke, A.J. Jeffreys, R. Wolff and G. Dolf, eds.). Birkhauser Verlag, Switzerland. pp. 243-262.

Bentzen, P, and **Wright, J.M.** 1993. Nucleotide sequence and evolutionary conservation of a minisatellite VNTR cloned from Atlantic salmon (*Salmo salar*). Genome 36: 271-277.

Brooker, A., Cook, D., **Bentzen, P., Wright, J.M.**, and Doyle, R.W. 1994. The genomic organization of microsatellites differs between mammals and cold-water teleost fishes. Can. J. Fish. Aquatic. Sci. 51: 1959-1966.

Harris, A.S., Beiger, S., Doyle, R.W., and **Wright, J.M.** 1991. DNA fingerprinting of tilapia, *Oreochromis niloticus*, and its application to aquaculture genetics. Aquaculture 92: 157-163.

Jeffreys, A.J., Wilson, V., and Thein, S.L. 1985a. Hypervariable "minisatellite" regions in human DNA. Nature 314: 67-79.

Jeffreys, A.J., Wilson, V., and Thein, S.L. 1985b. Individual-specific 'fingerprints' of human DNA. Nature 316: 76-79.

Levinson, G., and Gutman, G.A. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol. Biol. Evol. 4: 203-221.

McConnell, S.K., O'Reilly, Hamilton, L., **Wright, J.M.**, and **Bentzen, P.** 1995a. Polymorphic microsatellite loci from Atlantic salmon

(*Salmo salar*): genetic differentiation of North American and European populations. Can. J. Fish. Aquat. Sci. In press.

McConnell, S.K., Hamilton, L., Morris, D.B., Cook, D., Paquet, **Bentzen, P.**, and **Wright, J.M.** 1995b. Isolation of microsatellite loci in Atlantic salmon and their population genetics of Canadian east coast stocks. Proceedings of the 5th International Symposium on Genetics in Aquaculture. Aquaculture. In press.

Morris, D.B., Richard, D.R., and **Wright, J.M.** 1995. Polymorphic microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Can. J. Fish. Aquat. Sci. In press.

O'Reilly, P., and **Wright, J.M.** 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture genetics. Proceedings of the International Symposium on *Molecular Biology in Fish, Fisheries and Aquaculture*. J. Fish Biology. special edition. In press.

Park, L.K., and Moran, P. 1994. Developments in molecular genetic techniques in fisheries. Rev. Fish Biol Fish. 4: 272-299.

Taggart, J.B., and Ferguson, A. 1990. Hypervariable minisatellite DNA single locus probes for Atlantic salmon, *Salmo salar*. J. Fish Biology 37: 991-993.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. 17: 6463-6471.

Ward, R.D., and Grewe, P.M. 1994. Appraisal of molecular genetic techniques in fisheries. Rev. Fish Biol. Fish. 4: 300-325.

Weber, J.L., and May, P.E. 1989. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. American J. Human Genetics. 44: 38 -396.

Wright, J.M. 1993. DNA fingerprinting of fishes. In: *The Biochemistry and Molecular Biology of Fishes*. (T. Mommsen and P.W. Hochachka, eds.). Elsevier Press, Amsterdam. pp. 57-91.

Wright, J.M. 1994. Mutation at VNTRs; are minisatellites the evolutionary progeny of microsatellites? Genome 37: 345-347.

Wright, J.M., and **Bentzen, P.** 1994. Microsatellites: genetic markers for the future. Rev. Fish Biol. Fish. 4:384-388.

Zwanenburg, K.C.T., **Bentzen, P.**, and **Wright, J.M.** 1992. Mitochondrial DNA differentiation in western north Atlantic populations of haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 49: 2527-2537.

CURRICULUM VITAE
JONATHAN MARK WRIGHT

PERSONAL DATA

Born: March 8, 1955; Stoke-on-Trent, UK.
Canadian/British citizen
Married, two children

EDUCATION

1978 B.Sc., Biology (Honours), Mount Allison University, Sackville, N.B., Canada. Thesis: Studies on the differential sensitivity of the peptidoglycan of Caryophanon latum to egg white lysozyme (Supervisor: W.C. Trentini).

1985 Ph.D., Molecular Biology, Faculty of Medicine, Memorial University of Newfoundland, St. John's Newfoundland, Canada. Thesis: Negative transcriptional control of the ornithine decarboxylase gene (speC) by cyclic AMP in Escherichia coli (Supervisor: Stephen M. Boyle).

1984-1986 Alberta Heritage Foundation for Medical Research Postdoctoral Fellow, Department of Medical Biochemistry, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada. Research: The cloning and characterization of chromosomal protein genes from rainbow trout, their regulation and DNA-protein interactions (Supervisor: Gordon H. Dixon).

ACADEMIC AND RESEARCH APPOINTMENTS

1986-1991 Assistant Professor, Department of Biology, Dalhousie University. Halifax, N.S. Canada.

1991- Associate Professor with tenure, Department of Biology, Dalhousie University.

1991- Honourary Research Associate, Department of Plant Science, Nova Scotia Agricultural College, Truro, N.S. Canada.

1994- Consultant to the Dept. of Pathologia, Faculdade de Medicina, UNESP, Botucatu -Sao Paulo, Brazil.

MEMBERSHIP IN PROFESSIONAL ASSOCIATIONS

Genetics Society of Canada
Canadian Society for Biochemistry and Molecular Biology
Nova Scotia Institute of Science

AWARDS

1978 Atlantic Universities Undergraduate Biology Conference. First Prize. Mount Allison University.

1979-1994 Faculty of Graduate Studies Scholarship, Memorial University of Newfoundland.

1981 Faculty of Medicine, Graduate Student Research Award, Memorial University of Newfoundland.

1984-1986 Alberta Heritage Foundation for Medical Research post-doctoral fellowship. Department of Medical Biochemistry, The University of Calgary.

1987 NSERC International Scientific Exchange Award (with Dr. S. Boyle).

1988 American Society for Biochemistry and Molecular Biology Travel Award.

TEACHING (Current Classes)

Biology 1000R. Introductory lectures in genetics (7 lectures).
Biology 2030B. Genetics (13 lectures).
Biology 3014A. Nucleic Acids Biochemistry and Molecular Biology (13 lectures).
Biology 4012A. Advanced Laboratory in Biochemical Techniques (32 hours of laboratory).
Biology 5705C. Modules in Molecular Genetics (8 lectures for graduate students).
Biology 5825A. Special Topics in Molecular Biology (open tutorials for graduate students).

ADMINISTRATIVE RESPONSIBILITIES

National

1993-1996 Member, NSERC Strategic Grants, Food, Agriculture and Aquaculture Panel.

University

1986-present Member, Radiation Safety Committee
1988-1992 Chair, Scientific Advisory Committee, Marine Gene Probe Laboratory
1994-1995 Member, Search Committee for Dean, Faculty of Graduate Studies, Dalhousie University.

Faculty

1991-1994 Member and Chair (1993-1994), Faculty of Graduate Studies, NSERC Postgraduate Scholarship Committee
1994 Representative for the Faculty of Graduate Studies on the Interim Review Committee of the Department of Biochemistry, Dalhousie University.

Department

1986-1992 Member, Graduate Admissions Committee
1986 Member, Search Committee for faculty position in Molecular Biology
1987-1992 Chair, Departmental Radioisotope Suite User Committee
1988-present Member (and co-ordinator, 1989-1994), Biology Honours Committee
1988-present Member (ex officio 1988-present, but voting 1991-1992), Undergraduate Curriculum Committee
1991 Member, ad hoc Committee on the Biology Overhead Fund

CONFERENCE ORGANIZATION

1989-1991 Organizing committee for the Annual Meeting of the Canadian Federation of Biological Societies, Halifax, N.S. Canada
1991 Session Chair, "Gene Structure and Expression" Annual Meeting of the Canadian Federation of Biological Societies, Halifax, N.S. Canada
1995 Session Chair, "Genetics and Breeding", International Symposium on Molecular Biology in Fish, Fisheries and Aquaculture. Fisheries Society of the British Isles. Plymouth, UK. July 10-13.

RESEARCH INTERESTS

1. Nuclear and mitochondrial genome structure, gene expression and evolution (especially in teleost fishes).
2. Molecular dynamics (and mutational processes) of repetitive DNA's: SINEs, LINEs, satellite, minisatellite and microsatellite sequences.

3. Application of DNA technology to: (i) animal and plant breeding; (ii) population and behavioural genetics of aquatic species (e.g. fish and marine mammals).
4. Biotechnology.

RESEARCH SUPPORT

Years of tenure	Title proposal and funding source	Amount (Can$)
1986-1988	Dalhousie Development Fund to establish research laboratory.	21,900
1986-1987	Biology Department Overhead Fund to establish research laboratory.	10,380
1987-1990	Nuclear matrix of Physarum polycephalum. NSERC Operating Grant	78,900
1987-1988	The nuclear lamina of Physarum polycephalum. The Banting Research Foundation.	7,442
1988	Dalhousie Development Fund to establish a DNA analysis workstand in the Dept. of Biology, Dalhousie University.	2,900
1989-1992	Marine Bioadhesives. NSERC Strategic Grant (PI: Wright, with Drs. Vining, Chapman & Jeff Wright).	197,100
1989-1992	Marine Gene Probe Laboratory. Infrastructure support from the Nova Scotia Ministry of Industry, Trade and Technology, Gov't of Nova Scotia. With Drs. Doyle, Zouros, Pohajdak and Lazier.	2.8 million
1990-1993	Molecular evolutionary genetics of tilapia. NSERC Operating Grant.	96,660
1990-1994	Ocean Production Enhancement Network, part of the National Centres of Excellence Program. Amount to Marine Gene Probe Laboratory (with Drs. Doyle, Pohajdak, Zouros and Lazier) was approx. $800,000.	23 million
1991-1993	Species descrimination of redfishes using the polymerase chain reaction. Canadian Dept. of Fisheries and Oceans Subvention Grant. (PI: J. Wright, Co-applicant: P. Bentzen).	24,000
1991	Extraction, purification and initial sequence analysis of fish mitochondrial DNA. Canadian Departments of Supply and Services, and Fisheries and Oceans . Contract.	5,500
1992-1995	DNA fingerprinting technology for improvement and diversification of salmonid broodstock. NSERC Strategic Grant (co-applicants: Drs. Bentzen and Doyle).	335,091
1992	DNA fingerprinting of apples. Agriculture Canada Contract.	2,500

1992-1993	DNA fingerprinting of Maine populations of Atlantic salmon. University of Maine, subcontracted from U.S. Dept. of Interior, Fish and Wildlife Service. (PI: J. Wright, co-applicant: P. Bentzen).	32,000
1993-1996	Molecular evolutionary genetics of tilapia. NSERC Research Grant.	115,500
1994	Sex-specific markers in tilapia Dalhousie Research and Development Fund. (co-applicant: Dr. S. McConnell).	2,500
1994-1995	Cod genetics and physiology. Interim Funding for the Ocean Production Enhancement Network, Centres of Excellence, (PI: Dr. C. Taggart; co-applicants: Drs. Wright, Doyle, Zwanenburg et al.).	206,800
1994-1997	Improving reproductive performance of mink by controlling inbreeding using microsatellite DNA probes. NSERC Strategic Grant. (PI: J.M. Wright; co-applicant: Dr. H. Farid).	210,000
1994-1996	DNA markers for selection of cold tolerant alfalfa. Development program. Nova Scotia Dept. of Agriculture and Marketing. (PI: J. Nowak. Co-applicant: J.M. Wright).	14,000
1995	Detection of cold-induced alfalfa genes in red clover. Nova Scotia Department of Agriculture and Marketing. (PI: J. Nowak. Co-applicants: J.M. Wright and S. Laberge).	4,000
1995	DNA marker(s) for cold hardiness in red clover. Nova Soctia Department of Agriculture and Marketing. (PI: J. Nowak. Co-applicant: J.M. Wright).	6,000
1995	DNA fingerprinting of alfalfa. Nova Scotia. Department of Agriculture and Marketing. (PI: J. Nowak. Co-applicant: J.M. Wright).	5,000
1995-1997	Assessment of the genetic impact of stocking on Atlantic salmon populations in two Nova Scotian rivers using microsatellite markers. DFO/NSERC Subvention grant. (PI: J.M. Wright. Co-applicant: S. McConnell).	18,000
1995	DNA markers for the damsel fly, Calopteryx maculata, Acadia University. Contract.	2,485

PUBLICATIONS

REFEREED JOURNALS:

1. Wright, J.M., and Boyle, S.M. 1982. Negative control of ornithine decarboxylase and arginine decarboxylase by cyclic adenosine-3',5'-monophosphate in Escherichia coli. Molecular and General Genetics 186: 482-487.
2. Wright, J.M., Gulliver, W.P., Michalski, C.J., and Boyle, S.M. 1982. Ornithine decarboxylase activity and polyamine levels during zoospore germination and hormone-induced differentiation of Achlya ambisexualis. J. General Microbiology 128: 1509-1515.
3. Wright, J.M., and Boyle, S.M. 1984. Intergeneric homology of the speC gene encoding biosynthetic ornithine decarboxylase in Escherichia coli. J. Bacteriology 159: 1074-1076.
4. Boyle, S.M., Markham, D.G., Hafner, E.W. Wright, J.M., Tabor, H., and Tabor, C.W. 1984. Expression of the cloned genes encoding the putrescine biosynthetic enzymes (speA, speB, speC) and methionine adenosyltransferase (metK) of Escherichia coli. Gene 30: 129-136.
5. Pentecost, B.T., Wright, J.M., and Dixon, G.H. 1985. Isolation and sequence of cDNA clones coding for a member of the family of high mobility group proteins (HMG-T) in trout and analysis of HMG-T-mRNA's in trout tissues. Nucleic Acids Research 13: 4871-4888.
6. Wright, J.M., Satishchandran, C., and Boyle, S.M. 1986. Transcription of the speC (ornithine decarboxylase) gene of Escherichia coli is repressed by cyclic AMP and its receptor protein. Gene 44: 37-45.
7. Voigt, M.N., Tek, T.M., Park, L.E., Wright, J.M., and Hall, D.E. 1987. Influence of avian aflatoxicosis on the synthesis of polyamines. Poultry Science 66: 1217-1232.
8. Wright, J.M., Wiersma, P.A., and Dixon, G.H. 1987. Use of protein blotting to study the DNA-binding properties of histones H1 and H1 variants. European J. Biochemistry 168: 281-285.
9. Wright, J.M., and Dixon, G.H. 1988. Induction by torsional stress of an altered DNA conformation 5' upstream of the gene for a high mobility group protein from trout and the specific binding to flanking sequences by the gene product, HMG-T. Biochemistry 27: 576-587.
10. Wright, J.M. 1989. Nucleotide sequence, genomic organization and evolution of a major repetitive DNA family in tilapia (Oreochromis mossambicus/hornorum). Nucleic Acids Research 17: 5071-5079.
11. Denovan, E.M., and Wright, J.M. 1990. A satellite DNA family from pollock (Pollachius virens). Gene 87: 279-283.
12. Harris, A.S., Beiger, S., Doyle, R.W., and Wright, J.M. 1991. DNA fingerprinting of tilapia, Oreochromis niloticus, and its application to aquaculture genetics. Aquaculture. 92: 157-163.
13. Denovan-Wright, E.M. and Wright, J.M. 1991. Immunologically-related nucleic acid-binding proteins associated with the nuclear matrix of Physarum polycephalum. Biochimica et Biophysica Acta. 1088: 25-30.
14. Harris, A.S., Young, J. and Wright, J.M. 1991. DNA fingerprinting of harbour seals (Phoca vitulina concolor): Male mating behaviour may not be a reliable indicator of reproductive success. Can. J. Zoology 69: 1862-1866.

15. Edens Magor, K.A., and Wright, J.M. 1992. Chromosomal proteins of Physarum polycephalum with preferential affinity for the sequence, poly d(A-T). poly d(A-T). Mol. Biol. Rep. 16: 105-115.
16. Zwanenburg, K., Bentzen, P., and Wright, J.M. 1992. Mitochondrial DNA differentiation in western north Atlantic populations of haddock (Melanogrammus aeglefinus). Canadian J. Fisheries and Aquatic Sciences. 49: 2527-2537.
17. Franck, J.P.C., Wright, J.M., and McAndrew, B.J. 1992. Genetic variability in a family of satellite DNAs from tilapia (Pisces: Cichlidae). Genome. 35: 719-725.
18. Franck, J.P.C. and Wright, J.M. 1993. Conservation of a satellite DNA sequence (SATB) in the tilapiine and haplochromine genome (Pisces: Cichlidae). Genome. 36:187-194.
19. Dillon, M.C., and Wright, J.M. 1993. Nucleotide sequence of the D-loop region of the sperm whale (Physeter macrocephalus) mitochondrial genome. Mol. Biol. Evol. 10: 296-305.
20. Bentzen, P., Taylor, E.B. and Wright, J.M. 1993. A novel synthetic probe for DNA fingerprinting salmonid fishes. J. Fish Biology. 43: 313-316.
21. Bentzen, P. and Wright, J.M. 1993. Nucleotide sequence and evolutionary conservation of a minisatellite VNTR cloned from Atlantic Salmon, Salmo salar. Genome. 36: 271-277.
22. Nelke, M., Nowak, J., Wright, J.M., and McLean, N. 1993. DNA fingerprinting of red clover (Trifolium pratense L.) with Jeffreys' probes: detection of somaclonal variation and other applications. Plant Cell Reports. 13: 72-78.
23. Franck, J.P.C., Kornfield, I., and Wright, J.M. 1994. The utility of SATA satellite DNA sequences for inferring phylogenetic relationships among the three major genera of tilapiine cichlid fishes. Molecular Phylogenetics and Evolution. 3:10-16.
24. Richard, K., McCarrey, S., and Wright, J.M. 1994. DNA sequence from the SRY gene of the sperm whale (Physeter macrocephalus) for use in molecular sexing. Can. J. Zoology. 72: 873-877.
25. Wright, J.M.1994. Mutation at VNTRs: Are minisatellites the evolutionary progeny of microsatellites? Genome. 37: 345-347.
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27. Coltman, D.W., and Wright, J.M. 1994. Can SINEs: A family of tRNA-derived retroposons specific to the superfamily Canoidea. Nucleic Acids Res. 14: 2726-2730.
28. Boyle, S.M., Burroso, L., Moore, R.C., Wright, J.M., and Patel, T. 1994. Primary structure of the speC gene encoding biosynthetic ornithine decarboxylase in Escherichia coli. Gene. 151: 157-160.
29. Nielsen, J.L., Gan, C.A., Wright, J.M., Morris, D.B., and Thomas, W.K. 1994 Biogeographic distributions of mitochondrial and nuclear markers for southern steelhead. Molecular Marine Biology and Biotechnology. 3: 281-293.
30. Harris, A.S., and Wright, J.M. 1995. Nucleotide sequence and genomic organization of cichlid fish minisatellites. Genome. 38: 177-184.

31. McConnell, S.K., P. O'Reilly, L. Hamilton, J.M. Wright and Bentzen, P. 1995. Polymorphic microsatellite loci from Atlantic salmon (Salmo salar): genetic differentiation of North American and European populations. Canadian J. Fisheries and Aquatic Sciences. In press, Feb. 10, 1995.
32. Morris, D.B., Richard, K.R., and Wright, J.M. 1995. Polymorphic microsatellites from rainbow trout (Oncorhynchus mykiss) and their use for genetic study of salmonids. Canadian J. Fisheries and Aquatic Sciences. In press. July 17.
33. Denovan-Wright, E.M., Ramsey, N.B., McCormick, C.J., Lazier, C.B. and Wright, J.M. 1995. Nucleotide sequence of transferrin cDNAs and tissue-specific expression of the transferrin gene of Atlantic cod (Gadus morhua). Comparative Biochemistry and Physiology. In press.
34. Colbourne, J.K., Neff, B.D., Wright, J.M., and Gross, M.R. 1995. DNA fingerprinting of bluegill sunfish (Lepomis macrochirus) using (GT)n microsatellites and its potential for assessment of the relative success of male reporductive strategies. Can. J. Fisheries and Aquatic Sciences. Accepted pending revision, July 17.
35. Coltman, D.W., Bowen, W.D., and Wright, J.M. 1995. PCR primers for harbour seal (Phoca vitulina concolour) microsatellites amplify polymorphic loci in other pinniped species. Molecular Ecology. Accepted pending revision. June 21.

REFEREED CONFERENCE PROCEEDINGS AND BOOK CHAPTERS:

1. Boyle, S.M., Wright, J.M., Satishchandran, C., and Buch, J. 1986. Expression of the putrescine biosynthetic genes (speA, speB, speC) in Escherichia coli. In: The Physiology of Polyamines. (U. Bachrach and Y.H. Heimer, Eds.). CRC Press, Inc., Boca Raton, Florida. pp. 3-11.
2. Boyle, S.M., Wright, J.M., and Satishchandran, C. 1985. Analysis In vitro and In vivo of the role of cyclic AMP on speC expression in Escherichia coli. (L. Selmici, M.E. Brosnan and N. Seiler, Eds.). pp. 3-8. Proceedings of the International Congress of Polyamines, Budapest.
3. Franck, J.P.C., Bentzen, P., Harris, A.S., Denovan-Wright, E.M. and Wright, J.M. 1991. Organisation and evolution of satellite, minisatellite and microsatellite DNAs in teleost fishes. In: Oxford Surveys on Eukaryotic Genes. (N. Maclean, Ed.) Oxford University Press. Invited Monograph. pp. 51-85.
4. Bentzen, P., Harris, A.S. and Wright, J.M. 1991. Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of salmonids and tilapia. In: DNA fingerprinting: Approaches and applications. (T. Burke, A. Jeffreys, R. Wolf, and G. Dolf, Eds.). Birkhauser Verlag, Switzerland. Invited monograph. pp. 243-262.
5. Bentzen, P., Wright, J.M., Kornfield, I., and Roberts, F. 1992. Single-locus DNA fingerprinting of Atlantic Salmon from Maine and Newfoundland. Proceedings of the Northeast Atlantic Salmon Workshop. US Fish and Wildlife Service.
6. Wright, J.M. 1993. DNA fingerprinting of fishes. In: The Biochemistry and Molecular Biology of fishes. (T. Mommsen and P.W. Hochachka, eds.). Elsevier Press. Amsterdam. Invited chapter. pp. 57-91.

7. Nielsen, J.L., Gan, C., Wright, J.M., and Thomas, W.K. 1994. Phylogeographic patterns in California Steelhead using mitochondrial DNA and microsatellites. Proceedings of Calcofi conference, "Genetics of organisms of the California current" 35: 90-92.
8. Bentzen, P., Morris, D.B., and Wright, J.M. 1994. Development and use of variable number tandem repeat markers for population and aquacultural genetics of salmonids. National Oceanic and Atmospheric Administration Workshop, Proceedings. "Application of DNA technology to the management of Pacific salmon". Seattle. NOAA Tech. Memo. MFS-NWFSC-17. pp. 85-90.
9. Wright, J.M., and Bentzen, P. 1994. Microsatellites: genetic markers for the future. In: Reviews in Fish Biology and Fisheries (G.R. Carvalho and T.J. Pitcher, eds.) Chapman and Hall, London. Invited commentary. 4: 384-388.
10. McConnell, S. K., L. Hamilton, D. B. Morris, D. Cook, D. Paquet, P. Bentzen and J.M. Wright. 1995. Isolation of microsatellite loci in Atlantic salmon and their application to the population genetics of Canadian East coast stocks. Proceedings of the 5th International Symposium on Genetics in Aquaculture. Accepted Jan. 12.
11. Herbinger, C.M., R.W. Doyle, E.R. Pitmann, D. Paquet, K.A. Mesa, D.B. Morris, J.M. Wright and D. Cook. 1995. DNA fingerprint based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. Proceedings of the 5th International Symposium on Genetics in Aquaculture. In press.
12. O'Reilly, P., and Wright, J.M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture genetics. Proccedings of International Symposium on Molecular Biology in Fish, Fisheries and Aquaculture. J. Fish Biology. In press, July 13.

REPORTS (NON-REFEREED):

1. Bentzen, P., Cook, D., Denti, D., Harris, A.S., Hofman, J. and Wright, J.M. 1990. One tube DNA extraction procedure for molecular fingerprinting. Fingerprint News 2(4): 17-21.

INTERVIEWS:

1. Wright, J.M. 1990. DNA fingerprinting: applications to aquaculture and fisheries. CBC Mainstreet (Fredericton) news programme. December 17.
2. Wright, J.M . 1995. Sperm whales and DNA fingerprinting. CBC Morningside (national broadcast). January 16.
3. Wright, J.M. 1995. The "Morin Case", forensics and DNA-typing. CBC Information Morning (Halifax). January 24.
4. Wright, J.M. 1995. DNA fingerprinting. Halifax Cable 10 channel. "What's happening in Halifax" Program. February 6.
5. Wright, J.M. 1995. DNA: the ultimate determinant of identity. Atlantic Television (ATV) Network Evening News (Halifax). February 6.

ABSTRACTS AND PRESENTATIONS:

1. Trentini, W.C., Wright, J.M., and Elliot, D. 1978. Trichome ghosts of Caryophanon latum. Annual Meeting, American Society for Microbiology, Las Vegas.
2. Wright, J.M., and Trentini, W.C. 1978. Studies on the differential sensitivity of the peptidoglycan of Caryophanon latum to egg white lysozyme. Annual meeting, Canadian Society of Microbiologists, Montreal.
3. Wright, M.M., and Boyle, S.M. 1982. Negative control of ornithine decarboxylase and arginine decarboxylase by cyclic adenosine-3',5'-monophosphate in Escherichia coli. Annual Meeting, American Society for Microbiology, Dallas.
4. Wright, J.M., and Boyle, S.M. 1983. Intergeneric homology of the speC gene coding for ornithine decarboxylase of Escherichia coli. Annual Meeting Canadian Federation of Biological Societies, Ottawa.
5. Wright, J.M., and Boyle, S.M. 1983. Negative transcriptional control by cyclic AMP of the speC gene encoding ornithine decarboxylase in Escherichia coli. Queen's University Symposium of Gene Expression, Kingston.
6. Wright, J.M., and Barnsley, P. 1984. Examination of mycoplasma DNA for homology to various cloned Escherichia coli genes. Fifth International Congress of the International Organization of Mycoplasmology, Jerusalem.
7. Wright, J.M., and Boyle, S.M. 1984. Intergeneric homology of speC among the Enterobacteriaceae. Annual Meeting Canadian Federation of Biological Societies. Invited paper for the Symposium on Gene Evolution, Saskatoon.
8. Satishchandran, C., Wright, J.M., and Boyle, S.M. 1984. Quick-blot analysis of procaryotic messenger RNA. In: DNA and RNA Probes: Strategies and applications. Albany, New York.
9. Wright, J.M., and Boyle, S.M. 1984. Analysis in vitro of the effect of cAMP and CRP on speC (ornithine decarboxylase) expression in Escherichia coli. International Congress of Polyamines, Budapest.
10. Dixon, G.H., Pentecost, B.T., Lee, K.L.D., and Wright, J.M. 1985. High mobility group (HMG-) cDNA's and mRNA's. Camerino Chromatin Meeting, Camerino, Italy.
11. Pentecost, B.T., Wright, J.M., and Dixon, G.H. 1985. Isolation and sequence of cDNA clones coding for a member of high mobility group chromosomal proteins (HMG-T) in trout and analysis of HMG-T-mRNA's in trout tissue. Annual Meeting Canadian Federation of Biological Societies, Toronto.
12. Pentecost, B.T., Wright, J.M., and Dixon, G.H. 1985. cDNA and genomic clones coding for a high mobility group (HMG-T) protein in trout. Thirteenth International Congress of Biochemistry, Amsterdam.
13. Wright, J.M., Wiersma, P.A., and Dixon, G.H. 1986. DNA-binding properties of histone H1 and differentiation stage-specific H1 variants from trout, chicken and the annelid, Platynereis durnerlii. Canadian Biochemical Society Symposium, Banff.
14. Wright, J.M. and Dixon, G.H. 1986. A cruciform structure in the 5' flanking region of the gene for a high mobility group (HMG-T) protein from trout. Annual Meeting Canadian Federation of Biological Societies, Guelph.

15. Wright, J.M., Wiersma, P.A., and Dixon, G.H. 1986. Use of protein-blotting to study the DNA-binding properties of histones H1 and H5. Annual Meeting Canadian Federation of Biological Societies, Guelph.
16. Wright, J.M., and Dixon, G.H. 1986. Induction by torsional stress of a cruciform conformation 5' upstream of the gene for a high mobility group protein from trout and the specific binding to flanking sequences by the gene product, HMG-T. American Society for Cell Biology, Washington, D.C.
17. Wright, J.M., Barasso, L., and Boyle, S.M. 1988. Interaction of the cAMP receptor protein and RNA polymerase with the speC gene of Escherichia coli. Fourteenth International Congress of Biochemistry, Prague.
18. Denovan, E.M., Franck, J.P.C., and Wright, J.M. 1988. Antibodies to nuclear matrix proteins of Physarum polycephalum. Fourth International Congress of Cell Biology, Montreal.
19. Edens Magor, K., and Wright, J.M. 1988. Low mobility group (LMG) chromosomal proteins of Physarum polycephalum. Fourth International Congress of Cell Biology, Montreal.
20. Beiger, S., and Wright, J.M. 1989. DNA fingerprinting of fish and its application to aquaculture. Canadian Aquatic Biotechnology Network, Aquatech '89. Vancouver. (Invited paper)
21. Harris, A.S., Bentzen, P., and Wright, J.M. 1989. DNA fingerprinting and aquaculture genetics. Second DNA Fingerprinting Workshop. Nottingham, U.K.
22. Franck, J.P.C., McAndrew, B.J., and Wright, J.M. 1990. Evolution of a satellite DNA family in tilapia. Annual Meeting Canadian Federation of Biological Societies. Halifax.
23. Harris, A.S., Bentzen, P., and Wright, J.M. 1990. Cloning of hypervariable minisatellite loci from tilapia. Annual Meeting Canadian Federation of Biological Societies, Halifax.
24. Denovan, E.M., and Wright, J.M. 1990. A satellite DNA family from pollock (Pollachius virens). Annual Meeting Canadian Federation of Biological Societies, Halifax.
25. Lazier, C.B., McKay, M.E., Langely, S., and Wright, J.M. 1990. Studies on the sex hormone regulation of serum proteins in tilapia (Oreochromis niloticus). Annual Meeting Canadian Federation of Biological Societies, Halifax.
26. Edens Magor, K.A., and Wright, J.M. 1990. Nuclear DNA-binding proteins of Physarum polycephalum which display preferential affinity for poly d(A-T). Annual Meeting Canadian Federation of Biological Societies, Halifax.
27. Pohajdak, B., Wright, J.M., Zouros, E., Lazier, C.B., and Doyle, R.W. 1990. Opportunities in aquaculture for genetically-engineered products. Aquaculture and Veterinary Products Conference Proceedings, Stamford, CT.
28. Wright, J.M. 1990. Genetic markers for population definition of marine species. (Invited paper). Shellfish life histories and shellfishery models. International Council for the Exploration of the Sea. Universite de Moncton.
29. Zwanenburg, K.C.T., Bentzen, P., and Wright, J.M. 1990. Mitochondrial DNA variation in northwest Atlantic populations of haddock. NATO Advanced Studies Institute. Molecular techniques in taxonomy. University of East Anglia, Norwich, U.K.

30. Wright, J.M., Bentzen, P., and Harris, A.S. 1990. Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of salmonids and tilapia. First International Conference on DNA fingerprinting, Berne, Switzerland.
31. Harris, A.S., Bentzen, P. and Wright, J.M. 1990. Cloning of hypervariable loci from fish DNA. DNA mini-symposium, Can. Soc. of Forensic Sci. Ottawa.
32. Bentzen, P., Harris, A.S. and Wright, J.M. 1991. Hypervariable genetic markers cloned from the DNA of fishes. Can. Conf. Fisheries Research. Guelph, January.
33. Mosher, R., Skeat, J., Wright, J.M., and Vining, L.C. 1991. Adhesive polymers from marine sources. American Chemical Society. Atlanta, GA.
34. Wright, J.M., Franck, J.P.C., and Harris, A.S. 1991. Clustered and dispersed repetitive DNAs in the tilapiine (Cichlidae) genome. International Symposium on the Biochemical Genetics and Taxonomy of Fish. The Queen's University of Belfast, Belfast, N. Ireland.
35. Bentzen, P. and Wright, J.M. 1991. Cloning and characterization of variable number of tandem repeat loci in salmonid fishes. International Symposium on the Biochemical Genetics and Taxonomy of Fish. The Queen's University of Belfast, Belfast, N. Ireland.
36. Zwanenburg, K.C.T., Bentzen, P., and Wright, J.M. 1991. Biochemical systematics of redfishes (Sebastes). International Symposium on the Biochemical Genetics and Taxonomy of Fish. The Queen's University of Belfast, Belfast, N. Ireland.
37. Langley, S., Ramsey, B.N., Lazier, C.B. and Wright, J.M. 1991. Androgen inhibition or vitellogenesis in tilapia (Oreochromis niloticus). Annual Meeting Can. Fed. Biol. Soc. Kingston, Ontario.
38. Wright, J.M., Bentzen, P., Kornfield, I., and Roberts, F. 1992. DNA fingerprinting of Atlantic salmon populations from Nova Scotia and Maine. Northeast Atlantic Salmon Workshop, US Fish and Wildlife Service, Rockland, ME.
39. Morris, D.B., Richard, K.R., and Wright, J.M. 1992. The cloning and characterization of microsatellites from rainbow trout (Onchorhynchus mykiss). Int. Conference on the Genetics and Evolution of Aquatic Organisms. Bangor, Wales.
40. Bentzen, P., Wright, J.M., Kornfield, I., and Roberts, F. 1992. A survey of variable number of tandem repeat loci in Atlantic Salmon in eastern North America. Int. Conference on the Genetics and Evolution of Aquatic Organisms. Bangor. Wales.
41. Wright, J.M., and Harris, A.S. 1992. Towards digital DNA fingerprinting of tilapia for evolutionary and population studies. International Conference on the Genetics and Evolution of Aquatic Organisms. Bangor, Wales.
42. Bentzen, P., Morris, D.B., and Wright, J.M. 1993. Development and use of variable number tandem repeat markers for population and aquacultural genetics of salmonids. National Oceanic and Atmospheric Administration Workshop, Proceedings. "Application of DNA technology to the management of Pacific salmon". Seattle.

43. Nelke, M., Nowak, J., Wright, J.M., and McLean, N.L. 1993. DNA fingerprinting of red clover (Trifolium protense L.) with Jeffreys' probes: detection of somaclonal variation and other applications. Atlantic Plant Tissue Culture Association, Fredericton, N.B.
44. Dillon, M.C., and Wright, J.M. 1993. Population and phylogenetic studies of the sperm whale using mitochondrial D-loop sequences. Tenth biennial conference on the biology of marine mammals. Galveston, U.S.A.
45. Richard, K.R., Wright, J.M., and Whitehead, H. 1993. Genetic study of social organisation in sperm whales. Tenth biennial conference on the biology of marine mammals. Galveston, U.S.A.
46. Herbinger, C., Doyle, R.W., Pitman, E.R., Paquet, D., Morris, D.B., Cook, D., and Wright, J.M. 1994. Analysis of paternal and maternal effects on offspring growth performance in a rainbow trout farm using DNA fingerprints to infer parentage of the communally reared offspring. 5th International Symposium on Genetics in Aquaculture. Halifax.
47. Nelke, M., Wright, J.M., and Nowak, J. 1994. DNA changes in the red clover genome during regeneration in tissue culture. Atlantic Plant Tissue Culture Association, Kentville, N.S.
48. McConnell, S. Bentzen, P., Morris, D. Cook, D., Hamilton, L., Paquet, D. and Wright J.M. 1994. Isolation of microsatellite loci in Atlantic salmon and their application to the aquaculture and population genetics of Canadian East Coast Stocks. 5th International Symposium on Genetics in Aquaculture. Halifax.
49. Bentzen, P., Taggart, C.T., McConnell, S. and J.M. Wright. 1994. Microsatellite markers and genetic differentiation in fishes: an early perspective. Meeting of Evolution and the Aquatic Ecosystem - Defining Unique Units in Conservation. Monterey, CA.
50. Bentzen P., and J.M. Wright 1994. Emerging technologies and the search for new "markers". American Fisheries Society Ann. Meeting, Halifax.
51. Coltman, D.W., and J.M. Wright. 1994. A family of short interspersed elements (SINES) specific to the superfamily Canoidea provides a new source of genetic markers for pinniped studies. International Symposium on Marine Mammal Genetics. La Jolla, CA. Sept. 23-24.
52. Wright, J.M. 1995. The "pro's and con's" of various DNA-based technologies for population definition of aquatic species. Annual Meeting, Society of Experimental Biology, St. Andrews, Scotland. Plenary lecture, April 3-7.
53. McConnell, S.K., Wright, J.M., Cook, D., Doyle, R.W., Bentzen, P., and Taggart, C. 1995. Use of polymorphic microsatellites for stock discrimination of Atlantic salmon and Atlantic Cod. Ann. Meeting, Society of Experimental Biology, St. Andrews, Scotland. April 3-7.
54. Wright, J.M. 1995. The evolving technology of DNA fingerprinting. International Symposium on the Molecular Biology in Fish, Fisheries and Aquaculture, Fisheries Society of the British Isles, Plymouth, July 10-13. Keynote speaker.

55. O'Connell, M., Danzmann, R.G., McConnell, S.K., Wright, J.M., and Ferguson, M.M. 1995. Isolation of microsatellite DNA from rainbow trout (Oncorhyuchus mykiss) and its potential to fishery arrangement. International Symposium on the Molecular Biology in Fish, Fisheries and Aquaculture, FSBI, Plymouth, July 10-13.
56. McConnell, S.K., O'Reilly, P., Hamilton, L., Wright, J.M., and Bentzen, P. 1995. Microsatellite analysis of genetic variation in Atlantic salmon populations from Atlantic Canada. International Symposium on the Molecular Biology in Fish, Fisheries and Aquaculture, FSBI, Plymouth, July 10-13.

INVITED LECTURES (Universities, Research Institutes and High School Teacher Training)

Department of Pathology, Dalhousie University, Halifax, N.S., Canada. March 23, 1995. "The evolving technology of DNA fingerprinting".

Depto Pathologia, Faculdade de Medicina, UNESP, Campus de Botucatu, Botucatu, Sao Paulo, Brazil. Feb. 15, 1995. "Overview of DNA fingerprinting".

Nova Scotia Institute of Science, Halifax, N. S., Canada. Feb. 6, 1995. "DNA: the ultimate determinant of identity."

Nova Scotia Association of Science Teachers, Annual Meeting. Halifax, N.S., Canada. Oct. 1994. "The Genetic Revolution".

Department of Biology, St. Mary's University, Halifax, N.S. Canada. Jan. 1994. "DNA fingerprinting: approaches and applications."

Department of Wildlife and Marine Resource, State of South Carolina, Charleston, South Carolina, U.S.A. April 1994. "DNA fingerprinting of fishes: application to fisheries and aquaculture."

Canadian Department of Fisheries and Oceans, St. John's, Newfoundland Canada. 1993. "DNA fingerprinting of Atlantic salmon: approaches and applications to fisheries and aquaculture."

Faculty of Medicine, 25th Anniversary Alumni Symposium, Memorial University of Newfoundland, NFLD, Canada. 1993. "DNA fingerprinting: approaches and applications."

Nova Scotia Agricultural College, Truro, N.S., Canada. April 1992. "DNA fingerprinting".

Department of Biochemistry, Dalhousie University, Halifax, N.S. Canada. Jan. 1991. "Fingerprinting and other uses for junk DNA."

Department of Biology, Dalhousie University, Halifax, N.S. Canada. Nov. 1990. "Probe Technology."

Institute of Aquaculture, University of Stirling, Stirling, Scotland. October 1990. "Application of DNA technology to fisheries and aquaculture."

Summer Science Institute, Department of Education, Government of Nova Scotia, Dalhousie University. July 1990 and July 1989. "A series of lectures on genetic engineering presented to high school science teachers."

Nova Scotia Agricultural College, Truro, N.S. Canada. May 1990. "Genetic engineering: Fish production in the Maritimes."

Department of Pharmacology, Dalhousie University, Halifax, N.S. Canada. January 1990. "Dalhousie's Marine Gene Probe Laboratory."

Atlantic Regional Laboratory, National Research Council of Canada, Halifax, N.S., Canada. January 1990. "Fisheries genetics."

Department of Biology, Mount Allison University, Sackville, N.B., Canada. December 1989. "Molecular approaches to aquaculture."

Department of Zoology, University of Maine, Orono, ME. U.S.A. December 1989. "Molecular approaches to aquaculture."

APICS Conference, Mount St. Vincent University, Halifax, N.S. Canada. August 1989. "Genetic engineering - what next in the biological revolution?"

Department of Biology, Acadia University, Wolfville, N.S. Canada. March 1987. "Science fiction and the nuclear matrix of Physarum polycephalum."

Departments of Biochemistry and Pathobiology (Veterinary College), Virginia Tech., VA, U.S.A. May 1986. "Structure and expression of the gene for a high mobility group (HMG-T) protein from rainbow trout."

Department of Biochemistry, Dalhousie University Halifax, N.S. Canada. November 1986. "Are the genes for the high mobility group (HMG) proteins autogenously regulated?"

THESES SUPERVISED:

Edens Magor, K. 1980. Characterization of two nuclear DNA-binding proteins of Physarum polycephalum. M.Sc. thesis, Dalhousie University.

Franck, J.P.C. 1993. Organization and evolution of two satellite DNA families, SATA and SATB, from the Tilapiine and Haplochromine Genome (Pisces: Cichlidae). Ph.D. thesis, Dalhousie University.

Morris, D.B. 1993. The isolation and characterization of microsatellites from rainbow trout (Onchorhynchus mykiss). M.Sc. thesis, Dalhousie University.

Brooker, A.L. 1994. Polymorphic microsatellites: Tools for measuring genetic diversity in subpopulations of Atlantic cod (Gadus morhua). M.Sc. thesis, Dalhousie University.

Harris, A.S. 1995. Organization and evolution of minisatelite VNTR's in the tilapiine genome. Ph.D. thesis, Dalhousie University.

Richard, K. 1995. A molecular genetic analysis of kinship in free-living sperm whales. Ph.D. thesis, Dalhousie University. (Jointly supervised).

TRAINING OF HIGHLY QUALIFIED PERSONNEL

Undergraduate & Honours Students

Student	Year	Scholarship
Eileen Denovan	1987-1989	NSERC (1988); Sarah Lawson (1987,1988)
Diane Srivastava	1988	NSERC
Sophie Bieger	1988-1989	NSERC
Brenda Briel	1988	NSERC (scholar from Concordia University)
Monique Brison	1989	NSERC (scholar from Mt. St. Vincent Univ.)
Christian Bachmann	1989	NSERC
Colin Peircey	1990-1991	
Chris McCormick	1991	Exchange student from the University of Bath, UK.
Dawn Forbes	1992-1993	

Steven McCarry	1992-1993	NSERC
Kathy MacEachern	1994-1995	

Six students entered Ph.D. programs (three with NSERC 1967 scholarships; two with NSERC postgraduate scholarships; two with NSERC/Killam Scholarships); one each entered programs in medicine, veterinary medicine and education, respectively.

Graduate Students	Degree	Years	Scholarship
Kathy Edens Magor[1]	M.Sc.	1986-1989*	
Jens Franck[2]	Ph.D.	1987-1992*	
Andrew Harris[4]	Ph.D.	1987-1995*	NSERC Award
Mary Dillion	Ph.D.	1990-1995	NSERC and Killam Scholar
Dianne Morris	M.Sc.	1990-1993*	
Marek Nelke	Ph.D.	1990-1995	Co-supervised
Kenny Richard	Ph.D.	1988-1995*	NSERC and Killam Scholar, Co-supervised
Louis Bryden	M.Sc.	1991-1995	Co-supervised
Amanda Brooker[3]	M.Sc.	1991-1994*	
Patrick O'Reilly	Ph.D.	1992-1996	
David Coltman	Ph.D.	1992-1996	NSERC Award, Co-supervised
Joyce Chew	M.Sc.	1994-1996	NSERC Award, Co-supervised
Andrew Duffy	M.Sc.	1994-1996	
Merilee Temple	M.Sc.	1995-1997	Co-supervised
Lorraine Hamilton	M.Sc.	1995-1997	

Position subsequent to leaving my laboratory:
*Graduated
[1] Ph.D. graduate of Medical University of South Carolina, 1994.
[2] PDF at Chicago then Stanford University, CA, 1994.
[3] Ph.D. candidate at Australian Institute of Marine Science, 1994.
[4]Medical Student, 1992.

Postdoctoral fellows	Years	Scholarship
Dr. Stewart McConnell	1993-present	
Dr. Michael O'Connell	1995-present	
Dr. Paul Bentzen[1]	1989-1993	NSERC PDF + RA (MGPL)
Dr. Bruce Ramsey[2]	1990-1992	

Position subsequent to leaving my laboratory:
[1] Assistant Professor at Univ. of Washington as of 1993.
[2] Medical Student.

Technicians	
K. Prinoski	1989 - 1991
S. Wu	1990 - 1991
J. Skeat	1990 - 1992
D. Denti	1989 - 1993
M. Sargent	1992 - 1993

B. Edgar	1991 - 1993
T. Lagace	1991 - 1992
D. Ellies	1991 - 1992
L. Hamilton	1993 - 1995

OTHER RESEARCH CONTRIBUTIONS:

We have isolated and characterized several DNA sequences that are of value in genetic research of important aquacultural and capture fishery species. We have established links with industrial and government agencies to exploit these genetic probes for commercial applications. Many researchers world-wide have contacted us for our DNA fingerprinting probes for tilapia, salmonids and other species.

Reviewer for Canadian NSERC research grants, US Sea-Grants program (New Hampshire, Maine and Washington), US Saltonstall-Kennedy grants, Natural Enviroment Research Council of U.K. and several overseas agencies 1993-1995.

Reviewer for the journals: Gene, Genome, BioTechniques, Bio/Technology, J. Molecular Evolution, Can. J. Fisheries and Aquatic Sciences, Molecular Biology and Evolution, Molecular Ecology, J. Experimental Marine Biology and Ecology and J. Marine Science. 1993-1995.

COLLABORATIONS:

1. **Genome mapping and molecular genetics of fishes**

Dr. M. Dobson, Biochemistry, Dalhousie. Cloning and characterization of telomeres and telomere-associated sequences from tilapia.

Dr. C. Lazier, Biochemistry, Dalhousie. Hormonal regulation of gene expression in tilapia.

Drs. B. McAndrew and D. Penman, University of Stirling, Scotland. Use of gynogenetic lines of tilapia for centromere-gene mapping using polymorphic markers.

Dr. F. Alledorf, University of Montana. Use of gynogenetic lines of rainbow trout for centromere-gene mapping using polymorphic markers.

Drs. R. Danzmann and M. Ferguson, University of Guelph. Microsatellite linkage map in rainbow trout.

2. **Population and behavioural genetics**

Drs. P. Omeira and J. Ritter, DFO, Halifax. Population genetics of Atlantic salmon in Nova Scotia using microsatellite markers.

Dr. P. Bentzen, University of Washington. Population and molecular genetics of redfish, Atlantic salmon and Atlantic cod.

Dr. M. Gross, University of Toronto. Paternity assessment in bluegill sunfish using microsatellites.

Dr. Hal Whitehead, Biology, Dalhousie University. Population and behavioural genetics of sperm whales.

Dr. Don Bowen, Bedford Institute of Oceanography. Behavioural genetics of

harbour seals on Sable Island.

Dr. Marty Leonard, Biology, Dalhousie. Paternity studies in the Ipswich sparrow of Sable Island.

Drs. M. Synder and P. Taylor, Acadia University. Development of microsatellite markers for population genetics of damsel fly.

3. **Plant and animal breeding**

Dr. J. Nowak, N.S. Agricultural College and Dr. S. Laberge, Agriculture Canada. DNA fingerprinting of alfalfa, and cloning of DNA markers and cold-induced genes from red clover; molecular basis of somaclonal variation in red clover.

Dr. H. Farid, N.S. Agricultural College. Use of microsatellites to assess inbreeding and reproductive performance in American mink.

4. **Centre for Molecular Biology UNESP, Botucatu Campus, Sao Paulo, Brazil**

Dr. Marcello Franco, Faculdade de Medicina, UNESP, Botucata. I serve as a genetics advisor for the establishment of this facility.