

Congratulations Young Investigator Awards Winners!

As a sponsor for the SNP Workshop II, Applied Biosystems provided three Young Investigator Awards; one \$4,000 TaqMan® Assays Award to support SNP research, and two \$1,000 travel reimbursement awards to attend and contribute to this meeting. Students and post-docs were eligible to participate in the Young Investigator Awards Program for these awards. The Awards Program recognized researchers who utilized polymorphisms to study genetic relationships among populations and species. Awards were given based on the merits of an application package that included an abstract, curriculum vitae, and a letter of recommendation.

TaqMan® Assays Award Winner



M. Renee Bellinger,

Marine Fisheries Genetics Program, Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Oregon State University, 2030 Marine Science Drive, Newport, OR 37365, USA

SAGE & SNPs: Spicing up the Chinook Salmon Genetic Baseline and Advancing Our Understanding of the Genetic Basis of Migration Timing (page 53)

Applied Biosystems Travel Awards Winners



Rachel E. Simmons,

Department of Animal Science,
University of California,
Davis, CA, USA

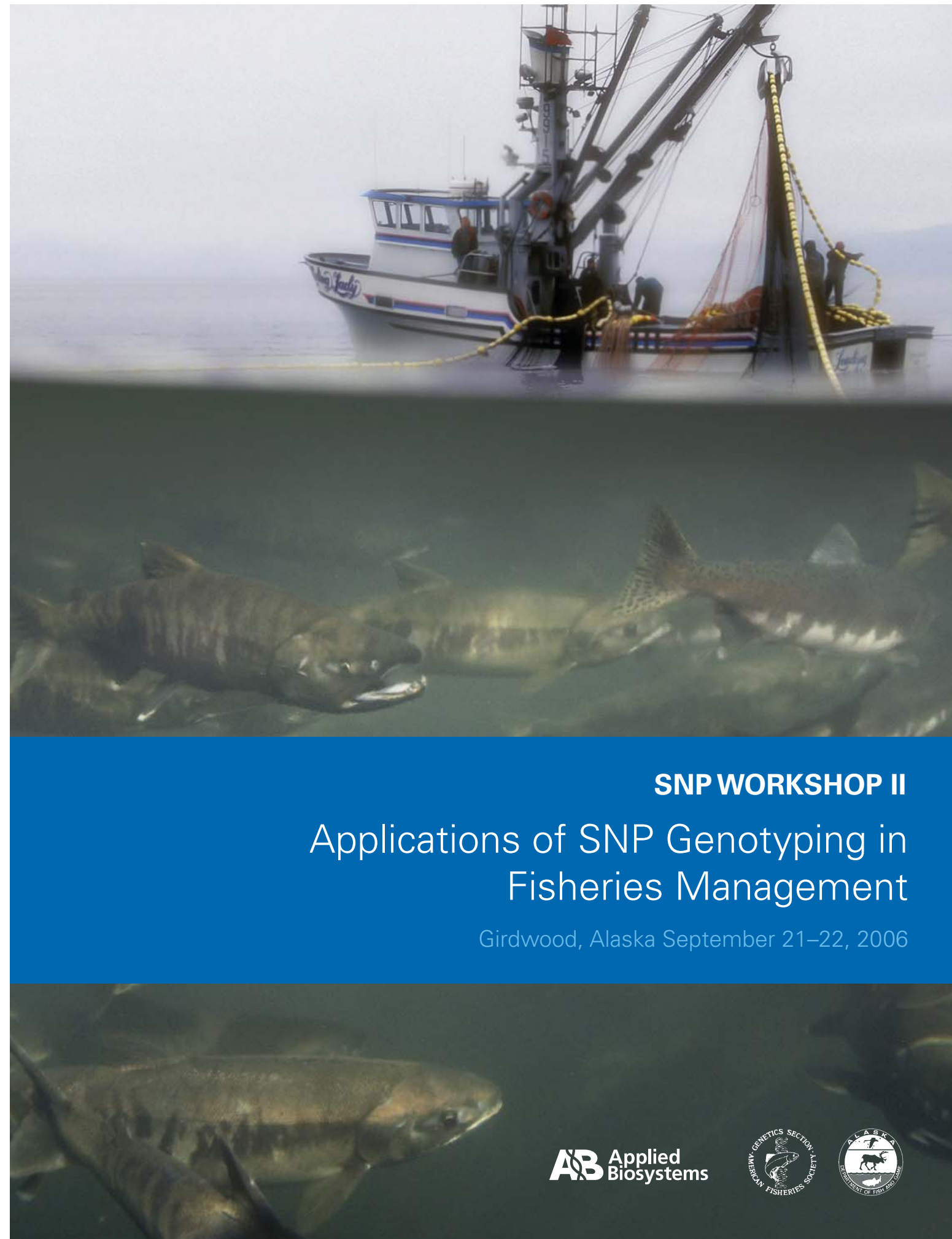
Haplotype Inference as a Method
of Producing More Powerful SNP
Markers (page 21)



Suzanne E. Roden,

Southwest Fisheries Science Center,
8604 La Jolla Shores Drive,
La Jolla, CA 92037, USA

Single Nucleotide Polymorphism
(SNP) Marker Discovery in *Chelonia*
Mydas (page 53)



SNP WORKSHOP II

Applications of SNP Genotyping in Fisheries Management

Girdwood, Alaska September 21–22, 2006



Applied Biosystems is proud to sponsor the second annual Salmon Genotyping Workshop. This two-day event provides an opportunity for laboratories to discuss SNP discovery, applications, data analysis, and other factors that can lead to the building of cooperative research. Applied Biosystems is the leader in genotyping research tools, and we will continue to provide a comprehensive line of products and services that constitute the most complete set of tools for genomic fine mapping and screening applications.

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SNP Workshop II: Applications of SNP Genotyping in Fisheries Management

Alyeska Resort, Girdwood, AK
September 21–22, 2006

Welcome to the second SNP workshop hosted by the Genetics Section of the American Fisheries Society and the Alaska Department of Fish and Game. We again thank Applied Biosystems for generously providing meeting rooms, Young Investigator awards, hosted receptions, and other financial support to help make this workshop a success.

The focus of much of this workshop will be applications of SNP markers for the study of population structure and admixture analysis of Pacific salmon. However, participants from a variety of related fields will have time to develop discussions on SNP discovery and other applications. Guest speakers were selected to provide a thought-provoking introduction and will present experience papers from studies of lizards, gophers, wolves, fish, and whales. Breakout discussions will return attention to discovery, genotyping, and data management—the components necessary for developing the large data bases required for identifying population structure and population origins of migrating salmon.

Several breakthroughs in discovery in the past year enabled the case studies presented in this workshop. These case studies on Pacific and Atlantic salmon, and a special session on statistical and analytical issues, will demonstrate that we are rapidly approaching the elusive goal of using high-throughput SNP genotyping as the method of choice for both population and individual assignment.

Welcome to the participants from nearly 30 laboratories from eight countries! I hope that you have a productive workshop and a pleasant visit to Alaska.

James E. Seeb
Alaska Department of Fish and Game,
333 Raspberry Road,
Anchorage, Alaska 99518 USA

PROGRAM COMMITTEE

Jim Seeb (chair)
Phil Morin
Shawn Narum (student award chair)
Linda Park
Lisa Seeb
Christian Smith
Steven Kalinowski

Program

SNP Workshop II: Applications of SNP Genotyping
in Fisheries Management

Alyeska Resort, Girdwood, AK
September 21–22, 2006

Opening Reception

Wednesday, September 20
7:00 PM – 9:00 PM
Grand Staircase and Fireside Lobby



Day 1

SNP Workshop II: Applications of SNP Genotyping in Fisheries Management
Thursday, September 21
Prince Court

7:30 AM Continental breakfast

Session I: Overviews—Jim Seeb, moderator

8:30 AM **Jim Seeb**, Alaska Department of Fish and Game
Welcome and Introduction

9:00 AM **Phil Morin**, National Marine Fisheries Service, La Jolla, CA, USA
SNP Discovery and Genotyping in Non-model Organisms: Examples from Cetaceans

9:40 AM **Melissa Gray**, University of California, Los Angeles, CA, USA
The utility of dog-derived SNPs for reconstructing demographic history and gene mapping in wild canids

10:20 AM Break

10:40 AM **Anti Vasemägi**, Department of Biology, University of Turku, Finland
Genome scans, outliers, SNPs and microsatellites: lessons from Atlantic salmon

11:20 AM **Natalia Belfiore**, University of California, Berkeley CA, USA
Discovering and using linked and unlinked SNPs for population genetic inference

12:00 PM Lunch and Poster Session

1:30 PM Breakout groups:
I. TaqMan® Assay help (Tony Dodge, Applied Biosystems)
II. Databases and Data Management (Christian Smith, ADFG)

Session II: Statistical and analytical issues—Steven Kalinowski, moderator

2:30 PM **Steven Kalinowski**, Montana State University, Bozeman, MT, USA
Maximum likelihood approaches for estimating reproductive success and fitness in populations with unsampled parents

3:00 PM Break

3:20 PM **Rachel Simmons**, Neil Clipperton and Bernie May, University of California, Davis, CA, USA
Haplotype inference as a method of producing more powerful SNP markers

3:40 PM **Bill Templin** and Anton Antonovich, ADFG Anchorage, AK, USA
Selecting the best subset of SNP loci for distinguishing populations of Yukon River Chinook Salmon

4:00 PM **Christian Smith** and Lisa Seeb, ADFG Anchorage, AK, USA
Number of alleles as a predictor of the relative assignment power of SNP and STR baselines for chum salmon

4:20 PM **Shawn Narum**, Columbia River Inter-Tribal Fish Comm., Hagerman ID, USA
Beyond Bonferroni: less conservative analyses for conservation genetics

7:00 PM Hosted dinner

Day 2

SNP Workshop II: Applications of SNP Genotyping in Fisheries Management

Friday, September 22

Prince Court

7:30 AM Continental breakfast

8:30 AM Announcements

Session III: Case Studies, Linda Park, NMFS, Seattle, WA, moderator

8:40 AM **Lisa Seeb**, Nick Decovich, and Christian Smith, ADFG Anchorage, AK, USA

SNPs reveal high levels of genetic diversity in Chinook salmon from the Copper River, Alaska

9:00 AM **Jamie Coughlan** et al., Dept. Zoology, Ecology & Plant Science, University College Cork, Ireland

A preliminary investigation of single nucleotide polymorphisms in Atlantic salmon and their potential use in mixed stock fishery analysis

9:20 AM **Chris Habicht** et al., ADFG Anchorage, AK, USA

SNPs provide in-season stock estimates for managing sockeye salmon in Bristol Bay: Insights on stock-specific migration pathways and schooling off-shore

9:40 AM **James Rhydderch** et al., University of Washington, Seattle, WA, USA

Small scale differentiation in space and time: Comparison of microsatellites and SNPs in measuring genetic divergence in sockeye salmon (*Oncorhynchus nerka*)

10:00 AM Break

10:20 AM **Shunpei Sato** et al., Gene Conservation Section, Salmon Research Division, National Salmon

Resources Center, FRA, Sapporo 062-0922, Japan

Origins of juvenile chum salmon inhabiting the North Pacific Ocean during the winter:
Rapid estimates by SNP markers

10:40 AM **Daria Zelenina** et al., Russian Federal Institute of Fisheries and Oceanography, Moscow, Russia

Population genetics of Russian sockeye salmon assessed by single nucleotide polymorphism

Session IV: Laboratory overviews and methods, Shawn Narum, moderator

11:00 AM **Toinette Hartshorne**, Applied Biosystems, Foster City, CA, USA

Troubleshooting TaqMan® SNP Genotyping Assays

12:00 PM Lunch and SNP discovery discussion (Phil Morin, NMFS)

1:30 PM **Shawn Narum** et al., Columbia River Inter-Tribal Fish Comm., Hagerman ID, USA

Development and evaluation of Chinook salmon SNP markers for genetic stock identification

1:50 PM **Geir Dahle**, Institute of Marine Research, Norway

Overview of research at the Institute of Marine Research, Bergen, Norway

2:10 PM **Svein-Erik Fevolden** and JI Westgaard, University of Tromso, Tromso, Norway

Activities at the Population Genetic Laboratory at the Norwegian College of Fishery Science

2:30 PM **Daria Zelenina** and Nikolai Mogue, Russian Federal Institute of Fisheries and Oceanography, Moscow, Russia
SNP-specific primers as a simple tool for population genotyping

2:50 PM Break

3:10 PM **Sewall Young**, Washington Department of Fish and Wildlife, Olympia, WA, USA

Washington Department of Fish and Wildlife Molecular Genetics Laboratory single nucleotide polymorphism activities: Multiplex SNP assay development, GAPS collaboration, and plans for local mixture analyses

3:30 PM Discussion and Wrap-up, Jim Seeb, moderator

Notes:

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SNP Discovery and Genotyping in Non-model Organisms: Examples from Cetaceans

Morin, Phillip A, Nadia Rubio-Cisneros, Nicci Aitken, Brittany Hancock, Andrew E. Dizon, Barbara L. Taylor and Sarah L. Mesnick

Southwest Fisheries Science Center, 8604 La Jolla Shores Dr., La Jolla, CA 92037, USA (PAM, NR-C, AED, SLM) (phillip.morin@noaa.gov) and University of Canberra, Bruce ACT 2601, Australia (NA)

Molecular analyses of natural populations provide valuable insights into social structures, movement patterns, population structure and evolution. Every molecular marker, however, has technological and analytical limitations. These limitations can render them inadequate for some questions and can constrain our ability to provide adequate information for management. The bigger the molecular tool box available to conservation geneticists, the more versatile and powerful our ability to answer important questions for conservation and management. We have used two approaches to develop a novel set of genetic markers, single nucleotide polymorphisms (SNP's), to study population genetic structure of sperm whales and bowhead whales. For sperm whales, we identified 39 novel SNPs in 23 sequences, and developed genotyping assays for 18 independent SNPs. These loci have been used in a pilot study to investigate population structure of sperm whales in the Eastern Pacific. Our second approach for SNP discovery makes use of existing sequences from a bowhead whale microsatellite enriched DNA library to identify anonymous non-repetitive sequences for primer design and SNP screening. To date we have identified 64 putative SNPs in 21 loci (8991bp of sequence), and designed assays for 20 independent loci. We have modified a novel SNP genotyping system called Amplifluor to use highly multiplexed pre-amplification followed by individual assay genotyping using universal fluorescent primers and real-time or end-point fluorescent detection.

Notes:

The Utility of Dog-Derived SNPs for Reconstructing Demographic History and Gene Mapping in Wild Canids

Melissa Gray¹, Nate Sutter³, Carlos Bustamante², Elaine Ostrander³ and Robert Wayne¹

1) Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, USA; 2) Biological Statistics and Computational Biology 101A Biotechnology Building Ithaca, NY, USA; 3) National Human Genome Research Institute, National Institutes of Health Building, Bethesda, MD 20892-8000, USA

The availability of a complete and a nearly complete genome sequence of the dog have permitted new insights into its evolutionary history and the population genetics of dog-like carnivores. Previous analysis of SNPs from five genomic regions has demonstrated that dog breeds vary in levels of linkage disequilibrium reflecting differences in demographic history. We demonstrate the first-time use of these SNP panels to reveal differences in linkage disequilibrium among wild canid populations. Our results show striking levels of linkage disequilibrium in gray wolf populations that accord with demographic history. We also show that SNPs developed for the dog may be useful for population genetic studies of distant relatives of the dog. Lastly, we demonstrate the utility of the dog-derived 60,000 SNP genotyping chip for studies in wild canids.

E-mail: mgray9@ucla.edu

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Genome Scans, Outliers, SNPs and Microsatellites: Lessons from Atlantic Salmon

Anti Vasemägi¹, Heikki Ryyanen², Jan Nilsson³ and Craig Primmer¹

1) Department of Biology, University of Turku, Finland; 2) Department of Biological and Environmental Sciences, University of Helsinki, Finland; 3) Department of Aquaculture, Swedish University of Agricultural Sciences, Sweden

The analysis of multi-locus data to infer the signatures of selective sweeps is an active area of research both in model and non-model organisms. These methods hold the promise of identification genes and genomic regions that contribute to the adaptive phenotypic variation in natural populations but currently very little is known about the properties of various approaches. In particular, the power of identification hitchhiking effects and the effect of violation of test assumptions has been evaluated only in limited cases. I first discuss the relative merits of currently available genome scan approaches using SNP and microsatellite markers. I then address the question of whether the violation of test assumptions in model-based approaches can lead to identification of elevated levels of false positive outliers and evaluate the potential strategies that are expected to enhance the efficiency of genome scans. Finally, I provide examples of genome scans conducted in Atlantic salmon that utilize SNP, indel and microsatellite polymorphisms and discuss what evolutionary insights can be gained by conducting similar studies in other salmonid fishes.

Discovering and Using Linked and Unlinked SNPs for Population Genetic Inference

Natalia M. Belfiore, Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA

Several methods are currently being used to identify single nucleotide polymorphisms (SNPs) in non model organisms. Each method has advantages and disadvantages. I have developed multiple independent markers from two species of vertebrates, a mammal and a squamate, from screening genomic libraries. Each marker has between one and 100 individual SNPs. These markers have proven useful in numerous applications, both within the species for which they were developed, and across related species. I have used these markers as a series of linked SNPs in several population genetic and phylogenetic applications. I have also used the population data from the linked SNPs to test assumptions about ascertainment bias in SNP discovery approaches, and to evaluate the ascertainment panels used in the discovery phase. Finally, I have used the linked SNPs to determine which individual SNPs are most informative in SNP genotyping studies.

Genomic libraries were created by shearing and blunt end cloning genomic DNA from a mammal, *Thomomys bottae* (Bottae's pocket gopher), and a reptile, *Sceloporus undulatus* (Eastern fence lizard). Hybridization with genomic DNA probes permitted avoidance of presumed high copy number regions in the clone sequencing step. At least 200 clones were sequenced for each species. Most of these loci were determined to be noncoding regions, although some showed a high probability of including protein domains or being homologous to annotated genes in other taxa. Primers were designed for approximately 75 loci in each species, and primer design strategies were optimized to increase the likelihood of obtaining good quality sequence from each locus. Approximately 20 loci were screened in an ascertainment panel to assess the levels of informative variation within and across populations.

The ascertainment panel for the gopher library was selected to include 3 individuals each of 3 populations of interest, plus one individual each from distant populations. Variation within and across populations in both mammals and lizards was unexpectedly high, compared to SNP frequency estimations from published genome sequencing projects, and summaries of SNP frequencies in nonmodel organisms published to date. Patterns of relatedness among individuals in the ascertainment panel reflected the ascertainment strategy of the discovery process, as expected. Assessments of ascertainment strategies have demonstrated that biogeographically stratified panels do not differ from randomly selected panels, if the randomly selected panels sample from across the entire range or the entire region of interest of the taxon.

In principle, using a series of linked SNPs (sequence data) from multiple unlinked loci greatly increases the power to infer historical processes in populations because the data are genealogical instead of allelic. However, there are several practical tradeoffs to consider when choosing whether to sequence or genotype SNPs, and how many SNPs to assess per locus. The foremost considerations that favor SNP genotyping over sequencing in nonmodel organisms are cost of the assays, the interaction between cost per locus and number of loci, and source of the DNA sample. Sequencing by commonly used methods is expensive and cannot be multiplexed; scoring of genotypes, by various methods, is usually cheaper per individual if a large number of individuals are to be assayed;

(Continued on next page)

Notes:

genotyping can be multiplexed by some methods; SNP genotyping can theoretically be performed on highly fragmented or degraded DNA samples, whereas sequencing of longer fragments generally requires high quality DNA. New sequencing methods may bring the cost down considerably which would merit reconsideration of these practical tradeoffs.

The second category of tradeoffs to consider is the availability of analytical methods for each type of data. Analysis methods for multilocus sequence data are few and controversial; population genetic analysis methods that consider sequence data are even rarer. This gap, however, is computational, not theoretical, and it is likely that current approaches to resolving population genetic questions will continue to be adapted to use multilocus sequence data. Nonetheless, nearly all population genetic analysis programs will accept codominant allelic or genotype data, making the immediate use of data from SNP genotyping attractive.

Finally, I propose a combined approach to using linked vs unlinked SNPs for population studies. There are multiple practical reasons to obtain sequences from a subset of individuals for each population or taxon to be studied, not the least of which is to be certain there is not hidden polymorphism in priming or probe hybridization sites. If enough variation is present in a given locus that multiple SNPs are under consideration for genotyping, there are several methods available that will operationally consider each SNP, and assign rankings to individual and combinations of SNPs for each locus with respect to their information content. At this level, it is extremely efficient to select the most informative SNPs if one is to genotype only a subset of SNPs per locus. This approach can be taken to the next level. With high probabilities, a subset of SNPs in a locus can be used to analytically reconstruct whole haplotypes.

In this way, studies of nonmodel organisms will be able to take advantage of the tools and methods being developed and vetted in the field of human population genetics. As analytical tools become increasingly available for population genetic analysis of multilocus sequence data, these reconstructed haplotypes will prove increasingly useful. In a practical sense, we can sequence a moderate number of individuals from each population or taxon under study, at each locus. Using this population information, we can query the data to ask how many and which SNPs should be ideally genotyped to best infer haplotypes for each individual at each locus. Then, we can genotype those SNPs in the rest of the individuals in each population, including individuals for which only forensic quality DNA is available. Finally, we can analyze all SNPs as allelic or genotype data, linked or unlinked, with currently available tools; we can infer haplotypes and use the growing number of methods available that consider multilocus sequence data for population genetic analysis.

Notes:

Maximum Likelihood Approaches for Estimating Reproductive Success and Fitness in Populations with Unsourced Parents

Steven T Kalinowski

Department of Ecology, Montana State University, Bozeman, MT 59717, USA

A common method for estimating the fitness of salmon stocks in the wild is to genotype adults as they ascend rivers to spawn and smolts as they head to the ocean. Parentage tests can then be used to identify the offspring of the adults and thereby estimate the relative fitness of each stock. This experimental design has been used to show that wild-reared individuals have as much higher reproductive success than hatchery-reared fish. The approach, however, is prone to two difficulties. First, if too few loci are genotyped, parentage assignment can be ambiguous. Second, sampling all parents can be difficult, and this also complicates parentage assignment. In my presentation, I will show how maximum likelihood methods can be used to address both problems.

Notes:

Number of Alleles as a Predictor of the Relative Assignment Power of SNP and STR Baselines for Chum Salmon

Christian Smith and Lisa Seeb

Gene Conservation Laboratory, Alaska Department of Fish and Game, Anchorage, AK 99518, USA

Simulation studies have indicated that number of independent alleles is a good predictor of the power of genetic markers for estimating genetic divergence and for mixed-stock analyses. Empirical data comparing STR markers to one another and to allozyme markers have further supported this hypothesis.

Extant STR baselines for chum salmon contain hundreds of alleles and it has been suggested that several hundred SNP markers will be required before SNP baselines will have equivalent assignment power to these STR baselines

Empirical data for several species, however, has indicated that individual SNP markers may have more power than STRs exhibiting many alleles. Further, the process of SNP ascertainment makes such loci more likely than random to be included in a set of markers used in any application. In the present study we plan to compare 16 STRs exhibiting 394 independent alleles to 60 SNP markers containing 60 independent alleles to analyze closely related populations of chum salmon. We will compare the power of these two baselines for mixed-stock analyses, and further compare subsets of the STR marker sets that contain ~60 alleles to the SNP baseline. We predict that the discrepancies between published simulation results and our data will be caused due to a failure of those simulation studies to account for ascertainment processes commonly used in developing SNP baselines.

Beyond Bonferroni: Less Conservative Analyses for Conservation Genetics

Shawn R. Narum

Columbia River Inter-Tribal Fish Commission, 3059-F National Fish Hatchery Road, Hagerman, ID 83332, USA

Studies in conservation genetics often attempt to determine genetic differentiation between two or more temporally or geographically distinct sample collections. Pairwise p-values from Fisher's exact tests or contingency Chi-square tests are commonly reported with a Bonferroni correction for multiple tests. While the Bonferroni correction controls the experiment-wise α , this correction is very conservative and results in greatly diminished power to detect differentiation among pairs of sample collections. An alternative is to control the false discovery rate (FDR) that provides increased power, but this method only maintains experiment-wise α when none of the pairwise comparisons are significant. Recent modifications to the FDR method provide a moderate approach to determining significance level. Simulations reveal that critical values of multiple comparison tests with both the Bonferroni method and a modified FDR method approach a minimum asymptote very near zero as the number of tests gets large, but the Bonferroni method approaches zero much more rapidly than the modified FDR method. I compared pairwise significance from three published studies using three critical values corresponding to Bonferroni, FDR, and modified FDR methods. Results suggest that the modified FDR method may provide the most biologically important critical value for evaluating significance of population differentiation in conservation genetics. Ultimately, more thorough reporting of statistical significance is needed to allow interpretation of biological significance of genetic differentiation among populations.

A Preliminary Investigation of Single Nucleotide Polymorphisms in Atlantic Salmon and Their Potential Use in Mixed Stock Fishery Analysis

Jamie Coughlan¹, Phil McGinnity², Shau Neen Liu-Cordero³, Willie Davidson⁴, Thomas Moen⁵, Ken Whelan² and Tom Cross¹

1) Dept. Zoology, Ecology & Plant Science, University College Cork, Ireland; 2) Marine Institute, Newport, Co Mayo, Ireland; 3) Biotrove Inc., Woburn, MA; 4) Dept. Molecular Biology and Biochemistry, Simon Fraser University, BC, Canada; 5) AKVAFORSK, The Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway

A renewed interest in genetic stock identification (GSI) of Atlantic salmon (*Salmo salar*) has been triggered by the instigation of strict conservation measures coupled with rising concerns about increasing marine-phase mortality and potential changes in migration patterns with respect to climate change. Microsatellite DNA loci are currently the markers of choice for GSI and mixed-stock-analysis (MSA) and while these perform well, they have a number of disadvantages particularly associated with inter-laboratory calibration of allele/genotype designations and different laboratory preferences with respect to specific locus suites. Single nucleotide polymorphisms (SNPs) may offer a viable alternative to microsatellite loci and while the information revealed by one SNP locus can be reduced compared to a typical microsatellite locus, the use of automated technology and array-based screening, can mean that the analysis of many more SNP loci for a larger number of individuals, can be achieved over similar time scales. Here, we present the results of a pilot study in which 595 juvenile Atlantic salmon individuals from six distinct river systems from across the Atlantic species range and from four major tributaries of the River Moy, Ireland, were screened for variation at 51 SNP loci (mostly derived from ESTs). The samples were screened using the Biotrove open array genotyping system using ABI TaqMan assays. Up to 35 loci revealed reliably scorable and potentially informative genotype information, although there were some indications of highly significant linkage associations between a few of the loci. High levels of differentiation were observed between river samples, which increased with geographic distance. A similar pattern of genetic relationships compared to microsatellite loci was apparent from dendrograms. Heterozygosity at the different loci was highly variable and a number of loci appeared to approach fixation for alternate alleles when western and eastern Atlantic samples were compared. Levels of individual assignment (IA) were generally slightly lower but comparable to those produced by microsatellite loci and also appeared to be related to geographic distance between samples. In addition, we analysed a sample of 32 adults caught near the mouth of the River Moy and compared assignment results with those from a recent microsatellite DNA study. We estimate that up to 100 SNPs (distributed widely over the nuclear and mtDNA genome) will be needed to obtain reliable MSA and IA to levels informative for fisheries managers and the development of conservation strategies.

Small Scale Differentiation in Space and Time: Comparison of Microsatellites and SNPs in Measuring Genetic Divergence in Sockeye Salmon (*Oncorhynchus nerka*)

Rhydderch, J.G., Newton, L.R., Lin, J. and Hauser, L.

SNPs are being hailed as the new marker for population genetics, despite concerns on selective effects, ascertainment bias and low variability. In particular, the power of SNPs to detect fine scale genetic differentiation is still untested, as most comparisons involve large geographic ranges with considerable genetic differentiation. Here, we present SNP data from samples of geographically proximate populations of sockeye salmon beach and creek spawners in Little Togiak Lake, Alaska, over two years. Microsatellite analyses from 12 loci showed considerable differentiation between the two creek populations ($F_{ST}=0.038$) as well as between creek fish and the adjacent beach population ($F_{ST} = 0.020 - 0.066$). Furthermore, large temporal differentiation in one creek appeared to be due to the replacement of one population by another, suggesting metapopulation dynamics in small sockeye populations. In contrast, little spatial and temporal differentiation was observed in beach populations ($F_{ST} =0.007$). Genetic diversity was higher in beach ($H_E=0.789$) than in creek spawners ($H_E =0.730$). Data from 37 SNP loci were collected from the same populations using a novel chemistry reducing costs of initial assays and allowing calling of genotype scores on a plate reader. Comparisons of the two data sets allow an investigation of the effects of selection, locus variability and number of loci on estimators of genetic diversity and differentiation, as well as on results from recent assignment approaches.

Development and Evaluation of Chinook Salmon Single Nucleotide Polymorphism (SNP) Markers for Genetic Stock Identification

Shawn Narum¹, Michael Banks², Matt Campbell³, Carlos Garza⁴, Chuck Guthrie⁵, Kristi Miller⁶, Paul Moran⁷, Ruth Phillips⁸, Lisa Seeb⁹, Christian Smith⁹ and Sewall Young¹⁰

1) Columbia River Inter-Tribal Fish Commission; 2) Oregon State University; 3) Idaho Department of Fish & Game; 4) Southwest Fisheries Science Center, NOAA Fisheries; 5) Alaska Fisheries Science Center, NOAA Fisheries; 6) Canadian Department of Fisheries and Oceans; 7) Northwest Fisheries Science Center, NOAA Fisheries; 8) Washington State University Vancouver; 9) Alaska Department of Fish and Game; 10) Washington Department of Fish and Wildlife

This collaborative project entails the development and evaluation of single nucleotide polymorphism (SNP) genetic markers for genetic stock identification (GSI) of Chinook salmon in mixed stock fisheries. SNP markers potentially offer a more cost-effective and less error-prone alternative to existing genetic tools that may be used independently or in tandem with existing microsatellite data to improve accuracy and precision of stock assignments. The Genetic Analysis of Pacific Salmonids (GAPS) consortium has recognized the potential value of SNP markers as a tool for fishery genetics and identified two important tasks necessary before SNPs can be widely utilized for GSI purposes: SNP ascertainment and SNP evaluation throughout the species range. SNP ascertainment is necessary to increase the number of markers for Chinook salmon, since many SNPs will be needed to meet broad GSI needs and few are currently available. These SNP loci then need to be genotyped on a coast-wide scale to determine the geographic extent of polymorphism of individual loci, to estimate the number of SNPs needed for GSI, and to compare assignment power of marker types (SNPs versus microsatellites). Initial development of SNPs will provide the foundation for adding informative SNP loci for GSI purposes. Currently, 40 SNPs have been ascertained, verified among labs, and genotyped for greater than 20 coast-wide populations of Chinook salmon. Further SNP ascertainment and genotyping is ongoing.

Overview of Research at the Institute of Marine Research, Bergen, Norway

Geir Dahle

Institute of Marine Research, Norway

Institute of Marine Research (IMR), Norway is one of the largest European marine research institutes, employing in excess of 600 persons, including scientists, technicians, post docs and seamen. The Institute is a research institute under the Ministry of Fisheries, but it also includes master and PhD students in several projects in close collaboration with the University of Bergen. The scientific activity of the Institute of Marine Research is organized in the form of 19 interdisciplinary research groups.

Population genetic Research group Activity

- Genetic and ecological impacts of escaped salmon and cod
- Genetic problems associated with coastal cod and the development of lobster and scallop ranching
- Species identification based on genetic methods
- Further development of genetically marked cod broodstock
- Development of a pilot monitoring programme in the Hardangerfjord
- Significance of salmon louse infections for the population structure of wild salmon
- Genetic tracking of fish-farm escapees
- Genetic studies of king crabs
- Genetic aspects of establishing American lobsters
- Biobanks
- Population genetics studies of wild stocks

Molecular biology Laboratory

A modern genetics laboratory, well equipped for both protein and DNA analyses. Available equipment includes: several PCR machines, an automated gene analyzer (ABI 3130XL), set-ups for all types of electrophoresis; vertical, horizontal, starch, agarose, PAGE, IEF, pulse-gel, etc.

The fully automated gene analyzer, ABI 3130XL Genetic Analyzer, is a fluorescence based DNA analyzing system with 16 capillaries. The ABI 3130XL together with a semi-automated DNA isolation system makes it possible for the laboratory to analyze several hundred samples every day. The laboratory is thus well suited for large screening projects of wild populations as well as genotyping and surveillance of different farmed species. The gene analyzer is used for micro-satellite analyses of species such as salmon, cod, lobster and halibut, but will also be used for SNP analyses.

Notes:

Activities at the Population Genetic Laboratory at the Norwegian College of Fishery Science

SE Fevolden and JI Westgaard

Norwegian College of Fishery Science, University of Tromsø, 9037 Tromsø, Norway

Our main focus in recent years has been the population structure of Atlantic cod (*Gadus morhua*) in the NE Atlantic, in particular the differentiation between the migratory North-East Arctic Cod and the Norwegian Coastal Cod.

Other species that have been studied are:

- Capelin (*Mallotus villosus*)
- Brown trout (*Salmo trutta*)
- Arctic charr (*Salvelinus alpinus*)
- Deep sea shrimp (*Pandalus borealis*)
- Icelandic scallop (*Chlamys islandica*)

Present and future activities also include genetic studies of key organisms in the arctic/polar basin such as:

- Polar cod (*Boreogadus saida*)
- Ice cod (*Arctogadus glacialis*)
- Calanoid copepods (*Calanus finmarchicus*, *C. glacialis*, *C. hyperboreus*)

Finally, we are involved in the possible species differentiation between the Pacific *Theragra chalcogramma* and *Theragra finmarchica*, which is found in small numbers off the coast of northern Norway.

Other Young Investigator Awards Abstract Submissions

Applied Biosystems Travel Award Winner #2

Single Nucleotide Polymorphism (SNP) Marker Discovery in *Chelonia Mydas*

Suzanne E. Roden^{1,2}, *P.A. Morin*¹ and *P.H. Dutton*¹

1) NMFS, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037 USA; 2) University of San Diego, Marine Science Dept., 5998 Alcalá Park, San Diego, CA 92110, USA

Chelonia mydas was used as a model to develop a series of nuclear SNP loci. DNA extracts from 39 green turtles sampled in Caribbean, East Pacific, Central Pacific, Mediterranean, and Indo-Pacific locations were used for two methods of SNP discovery. The first approach employed amplified fragment length polymorphism (AFLP) techniques to generate random fragments of DNA. The second technique used a microsatellite library to screen sequences of DNA segments not containing complex repeats. Site specific primers were designed for 16 candidate clones from the library and used to amplify identical regions, ranging in size from approximately 250-550 bp, across a set of individuals. The resulting homologous sequences were compared for differences across green turtles to identify single point mutations. A total of 15 loci (approximately 5900 bases) were screened resulting in the discovery of 54 SNPs, or an average of one SNP every 110 bp. Minor SNP alleles ranged in frequency from 0.02 to 0.5. One SNP will be chosen from each locus to generate a set of 15 independent SNP markers. These markers will be used to assess green turtle population structure in the Pacific.

Applied Biosystems TaqMan® Assays Award Winner

SAGE & SNPs: Spicing up the Chinook Salmon Genetic Baseline and Advancing Our Understanding of the Genetic Basis of Migration Timing

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The recently developed standardized Chinook salmon genetic baseline (GAPS consortium, Seeb et al., submitted) has enabled scientists the unprecedented ability to match ocean-harvested fish with their most-likely source population. Current efforts to combine the origin of an individual fish with its at-sea collection location holds promise to revolutionize our understanding of schooling behavior and ocean distribution of Chinook (i.e. www.projectCROOS.com).

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Real-time assessment of stock composition by harvest-location allows managers to maximize harvest of healthy stocks while avoiding harvest of weak stocks and thereby decreasing the overall impact of ocean fisheries on endangered populations. Efforts to assign ocean-harvested Chinook salmon to their most-likely population of origin rely on rapid and economical methodologies. While technology for rapid population assignment exists via the recently developed standardized microsatellite baseline (Seeb et al. submitted), sample processing is expensive and the baseline requires continual maintenance as new alleles must be monitored and standardized among all participating laboratories. A simpler and potentially cheaper methodology for population assignment is to use Single Nucleotide Polymorphisms (SNPs), which do not require standardization or continual updates because alleles are scored only as either homozygous or heterozygous. Population assignment using SNPs is currently restricted because few (<100) have been developed and accurate population assignment can require up to several hundred.

LongSAGE is one methodology whereby RNA is captured directly from a sample and 21 base-pair expressed sequence tags (SAGE tags) are identified. This methodology is ideal for gene expression experiments as it has the ability to discover novel and lowly expressed genes (Chen et al. 2002; Sun et al. 2004), unlike microarrays in which sample RNA binds to known oligonucleotides from cDNA or genomic libraries (Lu et al. 2004). Bernier et al. (manuscript in prep.) used serial analysis of gene expression (LongSAGE) to identify 221 differentially expressed sequence tags (ESTs) in fall and spring migrating Chinook salmon using ocean-harvested salmon as a control. Putative gene functions were assigned to 125 of the 221 tags, with 96 tags classified as hypothetical proteins or unknowns. Since ESTs with known functions have previously been data-mined for SNPs, I plan to continue Bernier's research by isolating "unknown" tags and assessing their usefulness in discriminating closely-related populations, particularly those separated primarily by migration timing. Furthermore, identification of these unknown genes may contribute to our understanding of the genetic mechanism that enables extreme variability of life-history in salmon. Using each "unknown" SAGE tag cDNA sequence as a "forward" primer and a random hexamer as a "reverse" primers, I will attempt to amplify these unknown regions using PCR. Next I will run the PCR product on a gel, excise all bands that amplified, and will clone the PCR product. Then I will sequence the cloned product and re-design primers to amplify the targeted region in a set of Chinook salmon that represent multiple populations and various life-histories. If sequences are variable, I will submit the gene sequences to ABI for TaqMan assay development, and screen all successful SNPs with the set of populations identified by the GAPs consortium.

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Mitochondrial D-loop Sequence Variation Among Chinook Salmon (*Oncorhynchus tshawytscha*) Populations

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Sequence analysis of the mitochondrial control region (D-loop) is often used to determine differences in population genetic structure. We analyzed genetic population structure of North American Chinook salmon (*Oncorhynchus tshawytscha*) by examining single nucleotide polymorphisms (SNPs) throughout 414bp of the 3' end of the mitochondrial DNA variable region (D-loop), the 68bp phenylalanine tRNA region and 81bp of the 5' end of the 12s ribosomal region. Sixteen unique haplotypes were detected among 230 individuals from 10 populations. Analysis of haplotype distribution and diversity among north (Alaska), central (B.C. to Oregon) and south (California) regions revealed: 1) A cline of decreasing average haplotype diversity from south to north; 2) Four highly prominent haplotypes in the central region, of which two are shared exclusively with the north, and two exclusively with the south; and 3) The northern and southern regions share no common haplotypes. Genetic studies of both rainbow trout (*O. mykiss*) and coho salmon (*O. kisutch*) suggested the existence of ice free refugia in both northern and southern regions during the Pleistocene glaciations. The cline in haplotype diversity, independence of northern and southern haplotypes, and the existence of both northern and southern haplotypes in the central region found in this study also supports the multiple refuge hypothesis for *O. tshawytscha*.

Examining the Differences Between Coastal and Offshore Populations of Bottlenose Dolphins (*Tursiops truncatus*) in the Gulf of Mexico Using Both Nuclear and Mitochondrial DNA Information

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The Marine Mammal Protection Act was established by the United States Government to protect, restore and sustain marine mammal populations within U.S. waters. To successfully manage and maintain bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico, it is imperative to gain an understanding of the degree of genetic differentiation existing between populations, especially when human impacts are not evenly distributed across all areas. In the Gulf, morphological and geographical variations are not obvious, therefore, in order to identify discrete populations or manageable stocks of bottlenose dolphins, genetic differences must be examined. In this study, skin samples from bottlenose dolphins were collected between 1994 and 2006 from both coastal and offshore areas. Over 700 samples have been received and will be analyzed for this research. Evidence for population structure will be investigated using mitochondrial DNA control region sequence data and 19 nuclear microsatellite loci. Also for this study, single nucleotide polymorphisms (SNPs) will be developed and utilized. Currently over 300 samples have been sexed and sequenced and 39 mtDNA haplotypes have been identified, including 24 offshore

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