

American Genetic Association Annual Symposium

Conservation Genomics

University of Hawaii • Hilo, HI • 26-28 July 2010

Welcome to 2010 AGA Symposium

President's Welcome

Genetics and conservation biology have a long and complex history of interaction, and the AGA has contributed to this interaction for many years. As we consider both genetics and conservation biology at the dawn of the new decade, two things seem virtually certain. First, genomes of a large number of species will become easily and cheaply available. Second, many more species will come under threat of extinction, both at home and globally. The question before us, and the goal of this year's AGA symposium is to ask if, and when a discipline of genome-enabled conservation biology will become a reality. To examine this question, we bring together genomicists (who may not think about conservation), conservation biologists (who may currently know little about genomics), and those rare individuals who currently work at the interface of both disciplines. Our goal is to brainstorm about how the genomic data that we anticipate becoming available in the next few years can be best used to conserve biodiversity on the planet.

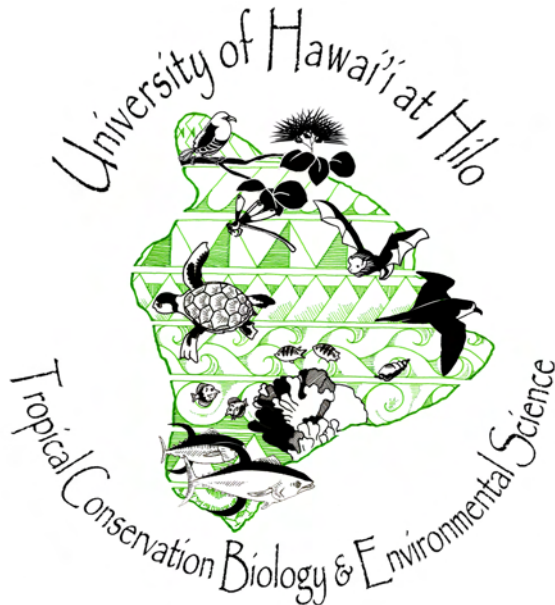
Conference organized by Brad Shaffer and Oliver Ryder

Additional support for this meeting comes from:

The College of Biological Sciences, University of California Davis

The Tropical Conservation Biology & Environmental Science Program, UH Hilo

The US Geological Survey



American Genetic Association 2010 Conservation Genomics

Program

Sunday July 25, 2010

- 12:00 pm – 5:00 pm Arrival, UH Hilo Dormitories or Hotel
- 5:00 pm – 7:00 pm **Opening Reception (beer/wine & pupus) and Registration**
at Dolphin Bay Hotel, 333 Iliahi St. Hilo (see map at end of program)

Monday July 26, 2010

- 7:45 am – 8:30 am Registration, complementary coffee and pastries
UCB 127, University of Hawaii Hilo (see map at end of program)
- 8:45 am – 9:15 am Don Straney, Chancellor, UHH and Brad Shaffer, President, AGA
Introduction and Welcome to the Symposium

Session I: Overview of Conservation Genomics (UCB 100)

Session Chair: Brad Shaffer

- 9:15 am – 9:55 am Mark W. Schwartz, *University of California, Davis*
Are there key conservation questions that genomics can answer?
- 9:55 am – 10:35 am David Haussler, *University of California, Santa Cruz*
Comparative Genomics and the Genome 10K Project
- 10:35 am – 10:55 am Short break (*more coffee*)
- 11:00 am – 11:40 am Stephen J. O'Brien, *Laboratory of Genomic Diversity NCI-NIH*
A Moving Landscape for Conservation in Mammals
- 11:40 am – 12:20 pm Kay Marano Briggs, *US Geological Survey*
**Perspectives: conservation genomics from a federal science agency's
point of view**
- 12:25 pm – 2:00 pm Lunch on your own (maps and restaurant listings enclosed)

Session II: Phylogenetics, Systematics and Genomics (UCB 100)

Session Chair: David Hillis

- 2:00 pm – 2:30 pm Robert Fisher, *US Geological Survey*
The use of molecular data for decision making in southern California.
- 2:30 pm – 3:00 pm Patrick M. O'Grady, *University of California, Berkeley*
**Evolution of Hawaiian *Drosophila*: Biogeography, Host Plant Adaptation
and Conservation**

3:00 pm – 3:30 pm Robert C. Fleischer, *Smithsonian Conservation Biology Institute*
Adaptive radiation, diversification and convergent evolution of endemic birds on the Hawaiian conveyor belt

3:30 pm – 4:00 pm David M. Hillis, *University of Texas at Austin*
Visualizing a forest of trees

4:00 pm – 6:00 pm **Poster Session, UCB 127**

Dinner on your own in Hilo (maps and restaurant listings enclosed)

Tuesday July 27, 2010

7:45 am – 8:30 am Complementary coffee and pastries, UCB 127, University of Hawaii Hilo

Session III: Hybridization, Genomics and Conservation (UCB 100)

Session Chair: Brad Shaffer

8:30 am – 9:00 am Victoria L. Sork, *University of California Los Angeles*
Landscape genomics of California oaks in the face of climate change

9:00 am – 9:30 am H. Bradley Shaffer, *University of California, Davis*
A genomic perspective on hybridization between endangered and introduced species

9:30 am – 10:00 am F.W. Allendorf, *University of Montana*
The use of genomics to detect and understand the spread of introgression from introduced rainbow trout into native westslope cutthroat trout

10:00 am – 10:30 am Benjamin M. Fitzpatrick, *University of Tennessee*
Natural hybridization and the species problem in conservation: how can genomics help?

10:35 am – 10:55 am Short break (*more coffee*)

Wilhelmine Key Lecture (UCB 100)

11:00 am – 12:00 pm Oliver A. Ryder, *San Diego Zoo's Institute for Conservation Research*
Revisiting the Evolutionary Significant Unit (ESU) Concept in the Era of Genomics

12:00 pm – 1:30 pm Lunch on your own (maps and restaurant listings enclosed)

Session IV: Disease, Functional Genomics and Metagenomics (UCB 100)

Session Chair: Scott Edwards

1:30 pm – 2:00 pm Stephen R. Palumbi, *Stanford University*
Genomic signatures of natural selection and adaptation to climate change in the sea

2:00 pm – 2:30 pm S. Randal Voss, *University of Kentucky*
Axolotl Story: Enabling an Endangered Species for Human Health and Disease Research

- 2:30 pm – 3:00 pm Louis Bernatchez, *Université Laval*
Towards genome-enabled conservation in salmonid fishes
- 3:00 pm – 3:30 pm Scott Edwards, *Harvard University*
Evolutionary genomics of disease resistance in an invasive species, the House Finch
- 3:30 – 4:00 pm William Cresko, *University of Oregon*
Sequenced RAD tags: a new tool for evolutionary and conservation genomics
- 4:00 pm – 5:00 pm **Poster Session, UCB 127**
- 5:30 pm – 8:30 pm **Luau at Onekahakaha Beach Park**
Luau included in registration. Transportation by private car (carpooling encouraged). Map enclosed.

Wednesday July 28, 2010

- 7:45 am – 8:30 am Complementary coffee and pastries, UCB 127, University of Hawaii Hilo.

Session V: Hawaii as a Model for Conservation Genomics Research (UCB 100)

Session Chair: Don Price

- 8:30 am – 8:55 am Rosemary G. Gillespie, *University of California, Berkeley*
Ecological & Evolutionary Factors Dictating the Response of Hawaiian Lineages to Global Change
- 8:55 am – 9:20 am Brian W. Bowen, *University of Hawai'i at Manoa*
The origins of tropical marine biodiversity: a phylogeographic perspective
- 9:20 am – 9:45 am Rob Toonen, *University of Hawai'i at Mānoa*
Where have all the larvae gone? Patterns of connectivity in the Hawaiian Archipelago.
- 9:45 am – 10:10 am Jennifer Schultz, *University of Hawai'i at Manoa*
Applying conservation genomics to the critically endangered Hawaiian monk seal
- 10:10 am – 10:30 am Short break (*more coffee*)
- 10:30 am – 10:55 am David Foote, *US Geological Survey*
***Drosophila* as monitors of change in Hawaiian ecosystems**
- 10:55 am – 11:20 am Donald Price, *University of Hawai'i at Hilo*
Local adaptation to changing environments: Examples from Hawaiian *Drosophila* population genetics, gene expression and behavior-physiology studies.

- 11:20 am – 11:45 am Sheila Conant, *University of Hawai'i at Manoa*
A brief overview of recent applications of molecular genetics to conservation of Hawaiian birds.
- 11:45 am – 12:10 pm Elizabeth Stacy, *University of Hawai'i at Hilo*
A next-generation sequencing approach to DNA barcoding in plants
- 12:10 pm – 12:35 pm Brian A. Perry, *University of Hawai'i at Hilo*
Conservation genetics of Hawaiian fungi: implications of fungal endophyte diversity for plant conservation on the Hawaiian Islands
- 12:40 pm – evening Lunch and afternoon on your own (maps and restaurant listings enclosed)

Thursday July 28, 2010

Field Trip to Volcanoes National Park

David Foote, *US Geological Survey, Pacific Island Ecosystems Research Center*

An Introduction to Hawaiian Montane Tropical Rainforests

Participants will meet at the Cooper Community Center in Volcano Village at 8:00 AM (45 minute drive, straight up Hwy. 11), and drive into O'laa Forest. We will explore a mature *Metrosideros-Cibotium* wet forest, including its unique bird and insect life. Then, depending on the weather, continue on to montane mesic forests to appreciate the effects of Hawaii's steep environmental gradients.

Lunch will be at the Kilauea Lodge, one of Volcano's oldest establishments (cost ca. \$15.00 per person).

After lunch we will visit Kilauea Caldera and the active volcanic vent, and end the day at Nahuku Lava Tube about 3:00 PM.

What to bring: shoes that can get muddy, a rain jacket, sweater for cool weather, binoculars (if you have them), camera, and sun screen. Snack food and water is also good to have (available at the local store, as well).

Cost: Free. A map and further instructions will be provided at the meeting.

Car-pooling from Hilo is encouraged.

Abstracts of Symposium Speakers

(listed alphabetically by speaker)

The use of genomics to detect and understand the spread of introgression from introduced rainbow trout into native westslope cutthroat trout

ALLENDORF, F.W., AND G. LUIKART.

Division of Biological Sciences and Flathead Lake Biological Station, University of Montana, Missoula, MT 59802, USA

Native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) are threatened by genomic extinction because of widespread introgressive hybridization with introduced rainbow trout (*Oncorhynchus mykiss*). Paradoxically, this introgression can spread even when the hybrids have reduced fitness because the production of hybrids is unidirectional, a sort of genomic ratchet. That is, all of the progeny of a hybrid will be hybrids. Thus, the frequency of hybrids within a local population can increase even when hybrids have greatly reduced fitness. We are employing genomic methods to better understand the dynamics of hybridization in this system. We have used molecular markers to identify the parents of progeny produced in a stream containing a hybrid swarm over a five year period. The results are striking in two ways. First, there is a strong negative relationship between the proportion of admixture from rainbow trout and fitness in both individual females and males. Second, first-generation hybrids had much greater fitness than later generation hybrids with 50% admixture. This indicates strong heterosis, apparently caused by sheltering of deleterious recessive alleles. Development of SNPs to study this system is problematic because of the tetraploid ancestry of salmonid fishes which has led us into “homeologue hell”.

Towards genome-enabled conservation in salmonid fishes

LOUIS BERNATCHEZ

IBIS (Institut de Biologie Intégrative et des Systèmes), Université Laval, Québec, Canada.

Since its first formulation by Ryder (1986), the concept of “evolutionary significant unit” (ESU) has experienced several definitions diverging mainly on the importance granted to neutral versus adaptive genetic diversity which depict distinct temporal spheres and must be assessed and interpreted differently. The former represents the particular genetic background shaped on evolutionary time scale by neutral evolutionary forces and the latter mirrors variation of adaptive significance resulting from the ongoing action of natural selection. While a wide range of methods have been available and efficiently used to measure neutral variability, characterizing adaptive genetic variation is in its infancy. As this task generally requires to first identifying a small number of adaptive loci scattered throughout the genome, genomic approaches offers the potential to accelerate substantially the discovery of such “adaptive holy grails”. I will present how the use of genomics methods, including microarray analysis of gene expression, QTL genome scans, next-generation sequencing and SNP genotyping helped in finding evidence of adaptive divergence among populations of salmonid fishes. None of these new approaches are without drawbacks, so I will also discuss the challenges and limitations of integrating their use in the context of conservation, their cost not being the least...

The origins of tropical marine biodiversity: a phylogeographic perspective

BRIAN W. BOWEN, LUIZ A. ROCHA, JEFF A. EBLE, ROBERT J. TOONEN

Hawaii Institute of Marine Biology, University of Hawaii, 46-007 Liliipuna Road, Kaneohe, Hawaii

Two biogeographic theories are proposed to explain the biodiversity hotspots in the Indo-Pacific coral triangle and Caribbean Sea. The Center of Origin theory maintains that species originate in the highly competitive environment of the coral triangle, and radiate out to peripheral areas. The Center of Accumulation theory maintains that the coral triangle is a region of overlap between Indian Ocean and Pacific Ocean faunas, and that species originate in peripheral areas. Recent phylogeographic surveys of Indo-Pacific reef fauna provide evidence for both patterns. Most of the coral reef fauna in the Central

Pacific has origins at or near the coral triangle. In contrast, several recent cases have demonstrated species originating in the peripheral Central Pacific and colonizing in towards the coral triangle. We propose that both processes are operating in concert. Successful species forged in the diverse ecosystems of the coral triangle can radiate out to depauperate peripheral habitats, where they undergo ecological release and are able to develop novel functions. These peripheral species can subsequently expand their range back into the center of biodiversity. Under this process of biodiversity feedback, both hotspots and peripheral areas contribute to the production and maintenance of tropical marine biodiversity.

Perspectives: conservation genomics from a federal science agency's point of view

KAY MARANO BRIGGS

US Geological Survey, 12201 Sunrise valley Drive, MS 301, Reston, Virginia, USA 20192

The US Geological Survey (USGS) is a multi-disciplinary science organization with expertise in biology, geography, geology, geospatial information and water resources. USGS focuses on information needs of the Department of Interior's land and wildlife management agencies providing the science and technologies to support conservation of our Nation's biological resources. Now as never before, Federal agencies whose mission includes conservation management, are faced with sweeping changes on the landscape due in part to factors including global change, energy development, and increased water demand. Dealing with these changes and potential problems requires development of new tools, technologies and creative approaches to enhance wildlife conservation. Genomics provides information about the interaction between landscape features and microevolutionary processes enabling scientists to look at numerous species, determining where they differentiate, how they move across the landscape and in what timeframe. Genomics can benefit conservation in many ways for example, by providing information on a population's adaptability and vulnerability, by identifying sources and sinks of invasive species introductions, and by detecting exposure to contaminants through differences in gene expression prior to visible signs of distress.

A brief overview of recent applications of molecular genetics to conservation of Hawaiian birds

SHEILA CONANT

Department of Zoology, University of Hawaii at Manoa, Honolulu Hawaii 96822

The Hawaiian Islands have more endangered and rare birds than any other single geographic area in the world. A combination of habitat change by humans and the effects of numerous invasive species they have introduced to the Islands are the primary causes. Disease and predation are probably the most significant current causes of decline and population limitation. Even with habitat management and predator control, many species' populations are not increasing, and some are decreasing at alarming rates. Molecular genetics has contributed to Hawaiian bird conservation in several important ways. These include descriptions of demographic history and establishment of original ranges of species with greatly reduced distributions and genetic diversity, (e.g., Nēnē, Laysan Teal, Hawaiian Petrel), within-species genetic population structure, identification of ESUs and estimation of effective population sizes (e.g., Laysan Finch, `Ālala, Nēnē), assessment of genetic diversity and sexing of individuals in captive breeding programs (e.g., `Ālala, Nēnē), and, perhaps most important, the beginnings of an understanding of disease resistance and the origin and characterization of disease organisms themselves and their vectors.

Sequenced RAD tags: a new tool for evolutionary and conservation genomics

SUSAN BASSHAM¹, PAUL HOHENLOHE¹, ERIC JOHNSON² & WILLIAM CRESKO¹

Center for Ecology and Evolutionary Biology¹ & Institute of Molecular Biology², Department of Biology, University of Oregon, Eugene, OR., USA, 97403

A central problem in ecological and evolutionary genetics is to identify the genomic basis of phenotypic variation in natural populations. Traditional genetic markers allow the estimation of some population genetic parameters, such as average heterozygosity or neutral population structure. However, genome-scale questions, such as identifying specific loci that are under selection or responsible for phenotypic variation, have been more difficult to address. A significant impediment has been the great expense of

developing the density of genetic markers required for these types of research projects in each new study organism. We developed Illumina sequenced Restriction site Associated DNA (RAD) markers to overcome this hurdle, allowing researchers to simultaneously identify and type tens of thousands of single nucleotide polymorphisms (SNPs) in nearly any organism. The rapid evolution of threespine stickleback fish provides a test case. Using sequenced RAD tags, we have found parallel patterns of divergence across the genomes of multiple stickleback populations that have adapted to similar environmental conditions, and have identified candidate genes underlying phenotypic variation in single populations. We will use these results as a starting point to discuss the general usefulness of sequenced RAD tags for various ecological, evolutionary and conservation genomic studies in non-model organisms.

Evolutionary genomics of disease resistance in an invasive species, the House Finch

CAMILLE BONEAUD¹, NIGEL DELANEY¹, SUSAN BALENGER², GEOFFREY HILL², & SCOTT EDWARDS¹

¹*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138 USA &*

²*Department of Biological Sciences, Auburn University, Auburn AL 36849, USA*

In 1994, field biologists discovered that House Finches in the eastern US were succumbing to a strain of the bacterial pathogen *Mycoplasma gallisepticum* (MG), formerly known only from poultry. Since then, House Finches have been observed to undergo phenotypic and genetic evolution as a result this epizootic. Here we describe our ongoing experiments to understand the changes in gene expression experienced by House Finches as a result of experimental infection by MG using microarrays. We find substantial differences in patterns of gene expression response between birds sampled from populations with prior exposure to the pathogen (in Alabama) as compared to birds without any prior pathogen exposure (in Arizona). In addition, we describe evolutionary dynamics across the entire 1-Mb genome MG parasite as a result of the switch to a novel songbird host. A substantial number of genes in the House Finch MG strains have been lost or pseudogenized in comparison to the chicken MG reference and the CRISPR anti-phage system has degenerated, perhaps in order to allow acquisition of virulence genes via plasmid capture. In addition to its well-characterized ecology and demography, the House Finch-*Mycoplasma* system is becoming a classic for understanding of the genomics of host-parasite coevolution.

The use of molecular data for decision making in southern California

ROBERT FISHER, AMY VANDERGAST, DUSTIN WOOD, AND JONATHAN RICHMOND

USGS Western Ecological Research Center, 4165 Spruance road, Suite 200, San Diego, Ca 92101

The USGS has been applying molecular tools to conservation planning issues in southern California for the last decade. Briefly we will review some of these projects and discuss further opportunities for applied conservation genetics. Historically the tools utilized have been single or multi-locus gene trees to determine sets of relationships between populations within declining species. Microsatellite loci have and are being utilized to understand differences between populations of critically endangered species to inform captive breeding and reintroduction strategies. More recently multi-species landscape tools are being developed and applied to create useful geospatial layers for conservation planning. These layers help identify regions where species show similar patterns in genetic diversity, divergence) or genetic similarity), all of which are useful for identifying gaps in landscape conservation plans. These “evolutionary hotspots” of diversity and divergence might represent places with the highest potential for adaptive evolution in light of selective drivers such as climate and habitat change. Genetic similarity or connectivity hotspots help identify landscape reserves that might be serving as redundant genetic populations within fragmented landscapes. The recent urgent push to permit alternative energy projects (i.e. wind, solar) over large landscapes of the southwest could and should be informed by these types of approaches in site placement and development.

Natural hybridization and the species problem in conservation: how can genomics help?

BENJAMIN M. FITZPATRICK

Ecology and Evolutionary Biology, University of Tennessee, Knoxville TN 37996

Taxonomic species are the subjects of our field guides and conservation laws. But not all organisms are easily placed in distinct taxonomic bins. This is exactly what evolutionary geneticists would predict based on the theory that the discontinuities defining species arise gradually via continuous processes. But this state of affairs is a source of confusion and frustration for natural resource managers trying to apply conservation laws that assume taxonomic species are genetically well-defined, discrete, even static entities. A genomic perspective on population and quantitative genetics can help us understand the biology of "problematic cases" and the potential value of hybrids and intergrades. Here I discuss a few examples of conservation issues created by natural hybridization, and explore how genome-scale population genetics can help challenge assumptions and clarify ethical and conceptual problems. I conclude that population genomics can help inform a more nuanced approach to conservation, but the ideal of conservation based on best available science can be realized only if the education of policy makers and government biologists integrates evolutionary and genomic biology.

Adaptive radiation, diversification and convergent evolution of endemic birds on the Hawaiian conveyor belt

ROBERT C. FLEISCHER^{1,2}, HEATHER LERNER¹, ANDREANNA WELCH¹, DAWN REDING¹, SARAH SONSTHAGEN¹, HELEN F. JAMES²

¹*Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute*

²*Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution*

The Hawaiian Islands form by movement of the Pacific Plate across a mostly stationary hot-spot, such that islands occur in a linear chain by date of emergence. Thus the main Hawaiian Islands span an age range from the present erupting volcanoes on Hawaii to the oldest large island of Kauai at about five million years. Hawaii is also extremely isolated, thus few lineages have colonized, but many of those that have made it there have diverged from their continental ancestors and radiated. We examine the patterns and tempos of radiation in several endemic avian lineages, and also phylogeography and population genetics of some non-radiating taxa. Analyses of phylogenies reveal some interesting cases of convergent evolution, both between Hawaiian and continental lineages, and within the Hawaiian radiations. We also show the utility of novel methods of DNA sequence analysis that enable extremely large datasets for resolving phylogenies, especially of rapidly radiating taxa, and that provide more accurate estimates of rates of DNA sequence evolution.

***Drosophila* as monitors of change in Hawaiian ecosystems**

DAVID FOOTE

U.S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, Hawaii 96718

Beginning in 1971 as part of the International Biological Program, the relative frequencies of populations of 14 species of picture-wing *Drosophila* were measured in the 'Ola'a Forest at Hawaii Volcanoes National Park. The fly populations were surveyed using baits placed on vegetation and all recent surveys have been conducted in a non-destructive way where individuals are identified by unique wing and thorax markings in the field. Surveys have been repeated at approximately 10-year intervals and have documented an ongoing reduction in species diversity, even within areas of the park where feral pigs and alien plants are actively excluded to promote forest restoration. In 2006, 12 species of picture-wing *Drosophila* (including 3 from 'Ola'a Forest) were listed by the U.S. Fish and Wildlife Service under the Endangered Species Act and critical habitat was designated in 2008. Recent surveys of host plants in the park indicate that Hawaiian *Drosophila* face competition for breeding substrate as well as increased predation from generalist alien insectivores. Conservation efforts to promote recovery of these endangered species will be discussed.

Ecological & Evolutionary Factors Dictating the Response of Hawaiian Lineages to Global Change

ROSEMARY G GILLESPIE, PETER CROUCHER, KARI ROESCH GOODMAN, GEORGE RODERICK
Dept. Environmental Science, 137 Mulford Hall, University of California, Berkeley, CA 94720-3114

The Hawaiian Islands are considered 'nature's test tubes' for understanding biodiversity dynamics — simple systems reproduced over a chronological framework. Likewise, they are a microcosm, with processes accentuated and accelerated, thus causing the biota to be very sensitive to climate (or other environmental) change. These properties of the islands provide an ideal system for elucidating the multifarious and complex responses of biodiversity to global change. Our research has used the Hawaiian Island system to explore community dynamics over evolutionary and ecological time scales to understand the properties that have shaped it in the past, and now, to predict future trajectories. Here, I first review current patterns of biodiversity, disturbance, and invasion across the Hawaiian Islands, focusing on arthropods. I then consider the relevance of recent advances in population genetic approaches, together with the accessibility of functional genomics to studies of non-model organisms, to assess the future of biodiversity in the archipelago. Using some preliminary examples, I will examine the interplay between isolation and selection, including the time frame within which this has occurred. I will then suggest ways in which we might use this information to infer combined ecological and adaptational responses to future environmental change.

Comparative Genomics and the Genome 10K Project

DAVID HAUSSLER

*Director, Center for Biomolecular Science and Engineering, UC Santa Cruz
Scientific Co-Director, California Institute for Quantitative Biosciences
Professor of Biomolecular Engineering, UC Santa Cruz*

The Genome 10K Project plans to sequence the genomes of at least 10,000 vertebrate species, providing an unprecedented view of vertebrate diversity and evolution. I will discuss what we have learned about the structure and evolution of the vertebrate genome from the few dozens of genomes sequenced to date, and what we hope to learn from this much more comprehensive project. The greatest challenges will be the informatics challenges.

Visualizing a forest of trees

DAVID M. HILLIS

University of Texas at Austin

Researchers often must consider hundreds or thousands of phylogenetic trees that are consistent with a given set of data. Multiple trees may result from analysis of multiple genes, from Bayesian sampling of trees, or from consideration of statistically indistinguishable trees. Faced with a forest of results, phylogeneticists have often simply examined consensus signal among the different trees. This approach can be problematic, especially if different processes or gene histories underlie the different trees obtained. I will discuss an approach (Tree Set Visualization) for visualizing, exploring, and reporting on phylogenetic analyses that result in large numbers of solutions. This method uses Multidimensional Scaling of tree-to-tree distances to visualize relationships among many tree solutions and cluster the results in two-dimensional space. Groups of trees ("tree islands") can be identified and analyzed; these tree islands often represent different fundamental solutions or histories. Consensus analyses can be restricted to subsets of trees that are statistically combinable. Tree Set Visualization is also useful for visualizing progress in Markov-Chain-Monte-Carlo searches and as an educational and presentation tool for explaining analyses and presenting phylogenetic results. As an example of the method, I will explore the differences obtained from multiple studies and multiple genes that have been collected to address the question of amphibian phylogeny.

A Moving Landscape for Conservation in Mammals

STEPHEN J O'BRIEN

Laboratory of Genomic Diversity NCI-NIH

The dwindling wildlife species of our planet have become a cause celebre for conservation groups, governments and concerned citizens throughout the world. The application of powerful new genetic technologies to surviving population so threatened mammals has revolutionized our ability to recognize hidden perils that afflict them. The lecture will connect some recent applications of conservation genetics and natural history to uncover long-forgotten adaptive adventures that left their footprints in the genomes of modern species. Illustrative examples will describe the role of genetics for *in situ* tiger and cheetah conservation management and lessons learned from Florida panther restoration in a 25 year follow up analysis. How these can help to reverse extinction events and also to unlock medical secrets will be the lessons learned from this lecture.

Evolution of Hawaiian *Drosophila*: Biogeography, Host Plant Adaptation and Conservation

PATRICK M O'GRADY

University of California, 137 Mulford Hall, Berkeley, CA 94720

Hawaiian *Drosophila* represents one of the best-understood examples of adaptive radiation in nature, with ~1000 species diversifying from a single common ancestor in the past 25 million years. Over 80% of species are ecological specialists and utilize a single type of rotting host plant. The evolution of host use is placed within a phylogenetic context to examine adaptation to substrate type (e.g., stems, leaves, flowers, flux), host plant family, and microbial community present within a given rotting plant resource. Various biogeographic patterns observed in Hawaiian *Drosophila* and other insects are also examined, including the progression rule and back colonization events. Ecological and distributional information are directly relevant to the success of individual species and may be combined with population genetic estimates (effective population size, migration rate) to construct a management plan for endangered and threatened species.

Genomic signatures of natural selection and adaptation to climate change in the sea

STEPHEN R. PALUMBI¹, MELISSA PESPENI¹, TOM OLIVER¹, DAVID GARFIELD²

¹*Stanford University, Department of Biology, Hopkins Marine Station, Pacific Grove, CA 93950*

²*Duke University, Department of Biology, Durham, NC*

A key question about climate change is the extent to which natural selection and adaptation may help populations evolve new environmental tolerance during rapid shifts in temperature and ocean acidity. Data on divergence patterns are beginning to come from genome-wide comparisons, and can show how selection accelerates divergence across the genome. We compared the genomes of two sea urchins, the shallow water *Strongylocentrotus purpuratus* with the deep sea *Allocentrotus fragilis* for patterns of rapid protein evolution. Genes involved in biomineralization, which is expected to be more difficult in the future due to ocean acidification, are evolving much more quickly in the deep water *Allocentrotus*, perhaps because deep waters are already acidic. Similar data on the population divergence of *S. purpuratus* along the US west coast shows that biomineralization genes are more likely to be diverged in allele frequencies. In this case, selection on genes involved in skeletal formation seems higher than selection on genes in other metabolic pathways. If high genetic divergence reflects increased balanced polymorphism due to spatial differences in natural selection, then evolution in the face of future acidification may be rapid.

Conservation genetics of Hawaiian fungi: implications of fungal endophyte diversity for plant conservation on the Hawaiian Islands

BRIAN A. PERRY and MALI'O KODIS

Department of Biology, University of Hawai'i at Hilo, 200 W. Kawili St., Hilo, HI 96720

Although associated with all plants that have been investigated, the taxonomic, genetic and functional diversity of endophytic fungi remains undocumented for many regions, including the Hawaiian Islands. Fungal endophytes have been shown to confer such benefits as increased draught tolerance, resistance to pathogens, and anti-herbivory properties. Additionally, fungal endophyte composition has been shown to play a large role in altering vegetation dynamics and plant community composition. Given the potential roles of fungal endophytes, it is clear that a detailed understanding the taxonomic and genetic diversity of these symbionts should be addressed as conservation and management plans for host species are developed. To assess such diversity in a native Hawaiian taxon, we examined foliar fungal endophytic community structure in elevational phenotypes of wild and cultivated *Metrosideros polymorpha* ('O'hia) using environmental PCR. Preliminary results indicate that low and high elevation populations on Hawai'i harbor disparate endophytic communities. Additionally, seeds collected from high and low elevation populations and grown together in a mid-elevation garden harbor similar endophytic communities to their parent populations, suggesting strong host selection and/or vertical symbiont transmission. These results underscore the need for more detailed investigations of endophytic diversity harbored by Hawai'i's numerous threatened and endangered plant species.

Local adaptation to changing environments: Examples from Hawaiian *Drosophila* population genetics, gene expression and behavior-physiology studies

DONALD PRICE, JON ELTON, ANNE VEILLET

Tropical Conservation Biology and Environmental Science, University of Hawaii at Hilo

Hawaiian *Drosophila* are ideal indicator species of environmental change because of the large number of single-island endemics existing in restricted ranges on all main islands. In addition, several of these species are now classified as endangered and others are becoming rarer. Hawaiian species have adapted to changes in the Hawaiian environment over long time periods and large spatial scales due to island formation and global climatic changes and over shorter time and smaller spatial scales due to geographical differences in climate, lava flows, and elevation gradients. These endemic species are now confronted with rapidly changing environments due to human activity causing further fragmentation, introducing invasive species and changing local climates. Our temperature tolerance studies indicate that Hawaiian picture-winged *Drosophila* species have very restricted tolerances compared to more temperate species. In addition, *D. sproati* populations that are somewhat genetically isolated along an elevation gradient differ in their temperature tolerance. A study of more than 14,000 genes indicates that these populations have significant differences in gene expression related to temperature tolerance. Candidate genes are being identified to determine the genes responsible for local adaptation and stress tolerance. This research highlights the importance of using neutral genetic, gene expression and adaptive trait analyses for understanding local adaptation.

Revisiting the Evolutionary Significant Unit (ESU) Concept in the Era of Genomics

OLIVER A. RYDER

San Diego Zoo's Institute for Conservation Research, 15600 San Pasqual Valley Road, Escondido, CA, 92027-7000 USA

The importance of identifying appropriate units for conservation management has generated considerable discussion and development of the concept of the evolutionary significant unit (ESU). Dramatic advances in molecular genetics – and now genomics – technologies have resulted in the growth and progression of the field of conservation genetics. It is now possible to ascribe aspects of population history, the complexity of speciation events, historical demography, and signatures of migration and dispersal from genetic studies, provided sufficient samples of suitable quality are available. Recently, conjecture has again been raised that delimiting populations genetically and restricting gene flow to avoid risk of

outbreeding depression in managed populations of wild species and those in intensive management, for example in fragmented landscapes, under habitat conservation plans and in zoos, may be detrimental to population persistence. Yet, examples of incomplete lineage sorting and/or hybridization are increasingly being documented among recognized species, as tools of genetic analysis with sufficient power to resolve such occurrences are being utilized. This presentation will outline the historical development of the ESU concept and highlight considerations that will need to be incorporated into ongoing efforts to preserve the genetic diversity of small populations, including threatened and endangered species.

Applying conservation genomics to the critically endangered Hawaiian monk seal

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The Hawaiian monk seal (*Monachus schauinslandi*) was hunted to near-extinction in the 19th century. Though the species made a partial recovery by the 1960s, it is now declining at a rate of 4% per year as a result of low juvenile survival. Two decades of monitoring has provided a wealth of data and samples, including genetic specimens from nearly 85% of the existing population. Parentage analyses would provide insight into mating systems, prevalence of inbreeding, and population connectivity; however, extremely low genetic diversity has limited progress. Of 163 microsatellite loci isolated from the species' genome, only 17 were found to be polymorphic. We mapped 98 monomorphic and 12 polymorphic loci to 35 chromosomes in the dog genome, and found no evidence of clustering. Genome-wide depletion of genetic diversity is likely a result of the 19th century bottleneck and previous population size restriction. Can emerging genomic tools be used to isolate additional variable loci to provide essential population assessment data?

Are there key conservation questions that genomics can answer?

SCHWARTZ M.W.

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At a time when conservation, struggling to avert the earth's 6th major extinction wave, has shifted attention from species protection to ecosystem function, one may wonder whether, when and how the emerging field of genomics has meaning for conservation. I argue that there are critical conservation issues that will greatly benefit from genomic approaches. Conservation is fundamentally focused on three endeavors: discovering where repositories of high biodiversity reside and protecting them; building resilient reserves for maintaining representative biodiversity; and maintaining functioning ecosystems. Genomic studies can inform each of these focal questions. A prime example is that much of conservation relies on reintroductions of species. Most vertebrate reintroductions fail for unknown reasons. Could maladapted genomes be the explanation? Secondly, many species are expected to shift their distributions considerably under the next century of climate change. Managing biodiversity through this change benefits from an understanding of the functional responses of organisms to their environments and understanding plasticity. Linking genomic questions to conservation management problems has the potential to increase management efficiency as well as developing a better understanding of key genomic processes in controlling the responses of species to environmental change.

A genomic perspective on hybridization between endangered and introduced species

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In most conservation and management contexts, we protect species or subspecies, but not hybrids or intergrades. When non-native species are introduced into novel environments, they sometimes hybridize with native, endangered taxa, creating a threat to the endangered species and an extremely difficult management problem. For the last decade, we have studied a massive hybridization event between the native, endangered California tiger salamander, *Ambystoma californiense* and a non-native invasive

congener, the barred tiger salamander, *A. tigrinum mavortium*. Using a panel of 68 unlinked genetic markers evenly spaced across the genome, we have found that most non-native gene copies are spatially restricted to a large area in central California near the barred tiger salamander release sites from the 1950s. However, three markers, or about 5% of the genome, are “superinvasive”, spreading much more rapidly within breeding populations and across the California landscape. These superinvasive markers appear to have strongly epistatic interactions, such that animals that are homozygous non-native for all three markers grow much more quickly than any other genotype. The presence of hybrids raises important ethical and management challenges for the salamander that apply equally to other cases of hybridization with endangered taxa.

Landscape genomics of California oaks in the face of climate change

VICTORIA L SORK

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Climate change over the last hundred years has jeopardized species and ecosystems throughout the world by altering local climate conditions and shifting current climate zones to new locations. This rapid climate change is particular of concern for tree species that define the ecosystem they occupy and shape local biodiversity. In this talk, I will introduce a landscape genomic approach to understanding the environmental association of adaptive variation with climate variables. Using valley oak, *Quercus lobata* Née, we are deploying cutting-edge next-generation sequencing tools to assess how the underlying genetic structure of current populations might influence this species' ability to survive climate change. Specifically, we identify candidate genes associated with functional traits for response to climate change, and test their association with geographical and environmental gradients. Our pilot study results will provide an initial assessment of the extent to which genetic variation underlying geographic variation in functional traits will help identify the potential of tree populations to respond to climate change.

A next-generation sequencing approach to DNA barcoding in plants

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Species delineation by DNA barcoding is challenging in plants, especially in young groups in which hybridization is common. The most difficult challenges to the barcoding initiative are presented by young taxa, tropical taxa, and groups with strong phylogeographic structure due to limited dispersal. The evolutionary age gradient of the Hawaiian Islands anchored by the young Hawaii Island renders the archipelago an ideal location for testing the utility of multi-gene DNA barcoding for such species. We are evaluating the DNA barcoding method for two indigenous Hawaiian plant groups that are characterized by high numbers of endangered species, limited dispersal, and high hybridization, especially on Hawaii Island. Using 454 Titanium pyrosequencing technology, we sequenced pooled, non-normalized cDNA from 8-species. The sequences will be assembled, aligned and screened for phylogenetically informative regions as well as combinations of SNPs that align with taxonomic boundaries independently defined by morphological characters. We aim to identify molecular markers that will be useful to managers for rapid identification of species and hybrids in these young, taxonomically difficult Hawaiian lineages.

Where have all the larvae gone? Patterns of connectivity in the Hawaiian Archipelago

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The Papahānaumokuākea Marine National Monument, encompassing all of the Northwestern Hawaiian Islands, is the largest Marine Protected Area under US jurisdiction (>350,000 km²), but studies of connectivity between reef habitats across this broad region are currently lacking. To this end, we are surveying a broad range of fish and invertebrate species to understand connectivity patterns throughout

the Hawaiian Archipelago. We now have data for more than a dozen species, and have determined shared areas of restricted exchange across many species within the Hawaiian Archipelago. These data indicate that geographic proximity is a poor predictor of dispersal capability and that adjacent sites can be highly isolated whereas comparatively distant locations are well connected. Overall, our data show four concordant barriers that were previously unknown and suggest that there are five regions to consider for conservation and management of marine species in the Hawaiian Archipelago.

Axolotl Story: Enabling an Endangered Species for Human Health and Disease Research

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The Mexican axolotl (*Ambystoma mexicanum*), a critically endangered species in nature, is re-emerging as a model organism in biomedical research. The axolotl is best noted for its unrivaled ability to regenerate complex body parts. Here I review our studies of a second characteristic that axolotls do best - paedomorphic development. Unlike related tiger salamanders that undergo a metamorphosis during life, axolotls fail to undergo anatomical metamorphosis during the larval period and retain juvenile features as adults. The origin of axolotl paedomorphosis has long been associated with a major effect QTL that presumably functions in the brain to activate the hypothalamus-pituitary-thyroid hormone axis at the time metamorphosis is initiated. Using genetic and functional genomic approaches, including a cross to a related species that is also endangered in nature, the QTL is shown to harbor factors that explain responsiveness to thyroid hormone signaling. The results suggest that an axolotl's failure to undergo anatomical metamorphosis late in the larval period is indirectly associated with a mechanism(s) that acts earlier in development to broadly program transcription. The axolotl and related species provide important new models to identify mechanisms of early brain development that proximally and ultimately affect the expression of adult phenotypes.

Poster Presentations

(listed alphabetically by presenter)

Microsatellite mining using next generation sequencing

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Microsatellites are the genetic markers of choice for many population genetic studies, but must be isolated *de novo* using recombinant approaches where prior genetic data is lacking. Here we utilized high-throughput genomic sequencing technology (Roche/454 GS FLX) to produce millions of base pairs of short fragment reads which were screened using appropriate bioinformatics toolsets to identify primers that amplify polymorphic microsatellite loci. Using this approach we isolated 13 polymorphic microsatellites for the blue duck (*Hymenolaimus malacorhynchus*), a species for which limited genetic data were available. Our genomic approach eliminates recombinant genetic steps, reducing significantly the time and cost requirements of marker development compared to traditional approaches.

Resolving management units of the spinner dolphin (*Stenella longirostris*) using population genetics to address ecotourism concerns in Hawai'i

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Concern has grown over the last decade regarding the growing ecotourism industry focused on spinner dolphins (*Stenella longirostris*) in Hawai'i. This activity involves human interaction with free-ranging dolphins in sheltered (wave protected) daytime resting habitat, potentially disrupting diel behaviors. This resting area is essential habitat, as spinner dolphins do not consistently occur at islands within Hawai'i that lack these refuges. To aid in the development of effective regulations for this industry, we conducted a range-wide genetic survey of the Hawaiian spinner dolphin using mitochondrial DNA (mtDNA) control region sequences and 10 microsatellite loci ($N=505$). F -statistics, Bayesian cluster analyses, and assignment tests revealed population genetic differences between most islands/atolls (overall mtDNA $\Phi_{ST}=0.086$, $P < 0.001$, overall microsatellite $F_{ST}=0.015$, $P < 0.001$), indicating that each island/atoll should be managed separately. A comparison of genetic structure with available data on habitat and social structure revealed a trend toward higher gene flow between populations inhabiting smaller islands/atolls and having higher group stability. Genetic comparisons to a South Pacific location ($N=16$) indicated that Hawaiian populations are genetically depauperate and isolated from other Pacific locations (mtDNA $0.216 < F_{ST} < 0.643$, $P < 0.001$; microsatellite $0.058 < F_{ST} < 0.090$, $P < 0.001$), providing evidence that populations in Hawai'i may be vulnerable due to genetic isolation and small population sizes. We conclude that limiting resources such as resting habitat may have a critical influence on spinner dolphin distribution, dispersal patterns, population isolation, and social structure in the Hawaiian Islands. If ecotourism activities are found to be disruptive to resting behavior of Hawaiian spinner dolphins, management actions must incorporate the recognition that these dolphins occur in small, isolated populations.

Frequency and heritability of recombinant mitochondrial DNA

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Mitochondrial DNA (mtDNA) is commonly used in population and evolutionary studies due to its rapid rate of mutation, maternal inheritance, and lack of recombination. However, recent work has shown evidence of paternal inheritance, heteroplasmy, and recombination in the mtDNA of various animal species including humans. mtDNA is used in a wide range of studies spanning taxonomy, phylogeography, population structure through to the relationships among individuals, and recombination in the genome could raise uncertainty regarding population histories, particularly in near time. Therefore, further investigation is needed to better understand the frequency of mtDNA recombination and determine potential ways to account for its occurrence in evolutionary studies. Here, the Chinook salmon (*Oncorhynchus tshawytscha*) genome will be used to study mtDNA recombination. The genome will be searched for heteroplasmic sites as an indicator of paternal mtDNA and possible recombination, and the frequency of these recombinant molecules will be further examined using high-throughput DNA sequencing. As tissue samples are available from female salmon, their oocytes, and first-generation offspring, heritability of any recombinant molecules can also be studied. This information will provide insight into how strong an influence mtDNA recombination may have on interpretation of the evolutionary history of animal populations based on mtDNA-based studies.

Evidence of genetic isolation by habitat fragmentation in Indo-pacific humpback dolphins (*Sousa chinensis*) from Central Queensland, Australia

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In this study we provide evidence of genetic differentiation by habitat fragmentation in humpback dolphin, *Sousa chinensis*. 73 biopsy samples were collected from 4 neighbour populations in Queensland: Keppel Bay (KB), Port Curtis (PC), Northern Great Sandy Strait (NGSS) and Southern Great Sandy Strait (SGSS). KB-PC and NGSS-SGSS pairs are separated by ca 30 km, while PC and NGSS are 300 km apart. Habitat fragmentation by sedimentation was identified as the main cause of the highly structured society recorded in the GSS. Data from 27 microsatellite loci were used to compare the degree of genetic differentiation among populations. High levels of genetic differentiation was found among populations ($F_{ST} = 0.08$; $R_{ST} = 0.19$). Pairwise comparison tests showed significant differentiation among NGSS and SGSS but not among KB-PC. Population assignment test confirmed those results. Analysis of genetic similarity vs. geographical distance was not significant ($r = 0.82$, $P = 0.08$), with gene flow among PC-NGSS higher than among NGSS-SGSS regardless of distance. Comparison of allelic richness showed that the genetic variability in SGSS was significantly lower than in other populations. We conclude that habitat fragmentation may have limited the dispersal capabilities of humpback dolphins in the GSS, thereby reducing gene flow among the two populations.

Genomic differences among Brazilian chicken populations

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Brazilian chicken populations are the result of miscegenation of European ancestors and the climatic conditions found in the region. However, modern aviculture processes have put the variability of these birds at risk incurring the loss of an important genetic resource for future zootechnical exploitation. The

characterization of local populations at genomic level is a relevant step into decision-making for animal conservancy. Molecular markers have made possible a precise identification of genomic differences composing natural populations under the threat of extinction. In this study, genomic DNA markers were used to characterize chicken population groups naturally occurring in Brazil. Polymorphism of used marker loci revealed high discrimination power. Heterozygosity among chicken populations were at least 0.836. Excessive homozygosity detected in many *loci*, as a result of low subdivision and differentiation within populations, was also observed in established sample groups. The existence of great variability within the assessed chicken groups was also evidenced by partial genome comparisons. New Brazilian chicken lines are indicated by the analysis of molecular variance. It is clear from this study that conservation strategies should take into consideration the population subdivision and address actions towards sustainability by integrating our findings to other ecological tools.

American eel population genetics assessed by molecular and experimental approaches

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Since the 80's American eel population has declined in abundance over its entire distribution range. However, nowhere has it been more pronounced than in the St. Lawrence R. watershed where recruitment shrank more than 100 times in the past 30 years. Population enhancement through translocation may help, but the genetic consequences are poorly documented. In this context, we revisited the panmixia hypothesis and tested the hypothesis of genetic basis for quantitative traits (growth, sex ratio, gene expression) differences between eels of distinct origins. A total of 2600 eels from Florida to Newfoundland were collected and genotyped at 20 microsatellites, which confirmed the occurrence of a single, temporally stable, gene pool for the species. However, a 34-month common rearing experiment revealed pronounced differences in growth between glass eels originating from the St. Lawrence R. vs. the Canadian Maritimes. Similarly, a microarray experiment showed differential pattern of expression at hundreds of genes representing various biological functions between glass eels and elvers from these same locations. We propose a hypothesis for this apparent paradox (panmixia vs. local quantitative genetic differences) and discuss the relevance of these results for eel management and conservation.

Phylogeography, diversity and colonization history of the Hawaiian happy-face spider

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The charismatic Hawaiian happy face spider (*Theridion grallator*) exhibits a 'massive' color polymorphism, with individuals bearing yellow, red, white and black abdominal marks that create more than 20 described color morphs that are inherited in a largely Mendelian manner. The spider is found in mesic, native forest on four of the Hawaiian Islands (Oahu, Maui, Molokai, and Hawaii) and the morphs are shared between populations and islands, with many morphs being found in each population. The system is a model for balancing selection and is of particular interest because the morphs appear to have been reinvented, following extreme founder events in each island/population. Here, we critically examine the phylogeographic structure, colonization history and genetic diversity of *T. grallator* using mitochondrial (CO1, 16SrRNA, tRNA^{Leu}(CUN) and ND1) and nuclear (EF1- α) sequence data from an extensive collection of samples (>400) across all islands and volcanoes collected over the last 15 years. Phylogenetic trees and networks are used to reconstruct relationships among populations, AMOVA and spatial autocorrelation analyses are used to explore genetic diversity and Bayesian approaches are employed to infer colonization routes, times and demographic history in this unique species which is threatened by habitat loss and climate change.

Differential gene expression between floral morphs identified by whole transcriptome sequencing

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Plant-pollinator interactions of the Rocky Mountain wildflower species *Aquilegia coerulea* are strongly influenced by floral color; importantly, which pollinators visit a flower largely determines outcrossing rate (and thus fitness). Year-to-year variation in relative pollinator abundance produces a pattern of fluctuating selection that has been suggested to maintain blue- and whiteflowered individuals in polymorphic populations of *A. coerulea*. Replicated whole transcriptome sequencing of floral tissue from blue- and white-flowered individuals identified significant expression differences between morphs. Three pools of blue sepal mRNA and three pools of white sepal mRNA (five individual plants represented in each pool) were sequenced on an Illumina GA II. The greatest gene expression difference --about ten-fold higher in blue floral tissue-- was seen for flavonoid 3'5'-hydroxylase (F3'5'H), a critical enzyme during production of the blue pigment delphinidin. A striking number of genes implicated in plant stress response were also differentially expressed between blue and white flowers, suggesting a possible negative pleiotropic consequence of producing delphinidin. These data point to cis-regulation of gene expression controlling the production of delphinidin in flowers of *A. coerulea*, and also highlight the importance of considering the impact of such changes on plant physiology as well as plant reproduction.

Molecular markers for genome analysis in western Atlantic spiny lobsters

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The spiny lobsters *Panulirus argus*, *P. laevicauda*, and *P. echinatus* are among of the most important fishery resources found along the Western Central Atlantic shelf. These species support major fisheries in northeastern Brazilian waters and in the Caribbean Sea. Conservation studies are needed to avoid reduction in diversity and elimination of distinct stocks within these resources. Therefore, polymorphic markers can be of great utility for lobster conservation studies due to their high levels of intraspecific variability. A set of nineteen polymorphic microsatellites and 2 domains of the mtDNA have been isolated and characterized for use in conservation studies of western Atlantic spiny lobsters. Several microsatellite loci were highly polymorphic, 23 alleles, and exhibited high levels of expected (0.950) and observed heterozygosity (0.938) from samples caught off Brazilian and the Caribbean coasts. Only one locus exhibited significant departure from Hardy-Weinberg equilibrium conditions. The hypervariable domains in the mitochondrial genome showed very high average nucleotide and haplotype diversities within these lobsters. Results provide a clear indication of the highly informative nature of these markers. The potential of using the developed markers for genomic analysis aiming at the conservation of these species, currently subjected to excessive fishing efforts, is discussed.

Diversity of class II genes of the major histocompatibility complex (*Mhc*) in Hawaiian honeycreepers

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Genes of the *Mhc* encode proteins responsible for eliciting adaptive immunity in vertebrates and may play a role in tolerance to disease in honeycreepers. We conducted sequencing studies involving the *Mhc* class II peptide-binding region in `amakihi (*Hemignathus virens*) and i`iwi (*Vestiaria coccinea*). To evaluate gene copy number and allelic segregation, 263 clones were sequenced from a captive family of `amakihi. Results suggest that `amakihi possess a minimum of 10 *Mhc* class II genes, with 35 unique alleles identified among family members. To evaluate diversity between species and among subpopulations of `amakihi, 158 clones were sequenced from eight `amakihi and 154 clones from eight i`iwi. 98 distinct alleles were isolated from `amakihi and 66 distinct alleles from i`iwi. The number of alleles/individual averaged 13.5 in `amakihi, and 10.6 in i`iwi. Within `amakihi, subpopulations from low and high elevations appear distinct, with only one shared allele in common. Sequences were used to design 25 probes for a microarray-based population level analysis of *Mhc* diversity in approximately 1,000 individuals of three native species. Analysis revealed three alleles specific to `amakihi, one allele specific to i`iwi, and one allele with elevational differences within `amakihi.

Conservation genomics of Arizona's large carnivores

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New technologies and bioinformatic platforms are accelerating the number of taxa with genome sequences. Depending on the phylogenetic distance, a sequenced genome may result in many "genome-enabled" taxa. Conservation geneticists can benefit from genome-enabling to better manage functional genetic variation. In endangered species, genomics will aid in examining accrued detrimental variation and lost adaptive variation associated with declining population size. We are currently studying two "genome-enabled" taxa: the puma (*Puma concolor*) and the Mexican wolf (*Canis lupus baileyi*). In pumas, we are using 454 pyrosequencing of expressed genes to identify single nucleotide polymorphisms (SNPs). We will use the SNP genotypes to address several conservation and ecological concerns. Initially, we will characterize selection on the puma correlated with its radiation into the Sonoran desert. Second, we will identify the origin and migration pattern of puma populations to improve corridor design. In the Mexican wolf study, we are examining the genetic effects of its unique history (extirpated in 1980, reintroduced in 1998) using a 22k SNP chip developed for the domestic dog. We will quantify the ancestry of Mexican wolves from different captive lineages, dogs, and coyotes, in addition to identifying genes fixed by inbreeding which has plagued the population.

Genetic consequences of introducing allopatric lineages of Bluestriped Snapper (*Lutjanus kasmira*) to Hawaii

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A half century ago the State of Hawaii began a remarkable, if unintentional, experiment on the population genetics of introduced species, by releasing 2431 Bluestriped Snappers (*Lutjanus kasmira*) from the Marquesas Islands in 1958 and 728 conspecifics from the Society Islands in 1961. By 1992 *L. kasmira* had spread across the entire archipelago, including locations 2000 km from the release site. Genetic surveys of the source populations reveal diagnostic differences in the mtDNA control region ($d = 3.8\%$;

$\phi_{ST} = 0.734$, $P < 0.001$) and significant allele frequency differences at nuclear DNA loci ($F_{ST} = 0.49$; $P < 0.001$). These findings, which indicate that source populations have been isolated for approximately half a million years, set the stage for a survey of the Hawaiian Archipelago ($N = 385$) to determine the success of these introductions in terms of genetic diversity and breeding behavior. Both Marquesas and Society mtDNA lineages were detected at each survey site across the Hawaiian Archipelago, at about the same proportion or slightly less than the original 3.4:1 introduction ratio. Nuclear allele frequencies and parentage tests demonstrate that the two source populations are freely interbreeding. The introduction of 2431 Marquesan founders produced only a slight reduction in mtDNA diversity (17%), while the 728 Society founders produced a greater reduction in haplotype diversity (41%). We find no evidence of genetic bottlenecks between islands of the Hawaiian Archipelago, as expected under a stepping-stone model of colonization, from the initial introduction site. This species rapidly colonized across 2000 km without loss of genetic diversity, illustrating the consequences of introducing highly-dispersive marine species.

Conservation biology meets genomics: resurrecting Galapagos tortoise species via high-resolution marker-assisted breeding

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Molecular genetic techniques offer conservation biologists critical information upon which to design effective management strategies. Galápagos tortoises are a flagship taxon in this respect, because captive breeding programs have been largely facilitated using genetic tools. We are embarking on an ambitious project aimed at developing and screening an information-rich panel of SNP loci capable of identifying extant hybrids carrying genes of the rarest species in the world (*Geochelone abingdoni*, represented by a single living individual, Lonesome George), as well those with genes from a recently extinct species (*G. elephantopus*, 150 years ago). Molecular identification of admixed individuals of high conservation value will be coupled with a marker-assisted captive breeding program aimed at resurrecting the evolutionary heritage of lineages that will otherwise be lost. A series of recent discoveries that have generated much excitement in the scientific community underscore the present project. We aim to develop a novel, general-purpose framework for SNP discovery in non-model organisms that can be applied in the absence of a reference genome. This is currently perceived as a major hurdle by molecular ecologists and researchers working in related fields, and so our work will contribute an important technical advance.

Genetic monitoring of New Zealand endemic subspecies: the endangered Hector's dolphin and critically endangered Maui's dolphin

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The Hector's (*Cephalorhynchus hectori hectori*) and Maui's (*C. h. maui*) dolphins inhabit coastal waters of New Zealand's South Island and North Island, respectively. Concern arises from their low abundances (Hector's $N \sim 7,000$; Maui's $N < 100$), slow rates of reproduction and susceptibility to fisheries-related mortality. Maui's dolphins are characterized by a single unique mtDNA control region haplotype, and are reproductively isolated from Hector's dolphins (9-locus microsatellite $F_{ST} = 0.172$, $p < 0.001$). Our work includes genotype capture-recapture estimates of abundance and population trends, and single-sample linkage disequilibrium estimates of effective population size (N_e). To narrow the confidence intervals of preliminary N_e estimates using 15-locus microsatellite genotypes, we are increasing the number of loci analyzed by implementing a Restriction-site Associated DNA (RAD) tag procedure to identify thousands of SNPs. The genome-wide information reflected in the SNP genotypes will also facilitate an assessment of inbreeding, which may be evident in smaller isolated populations. A better understanding of the gene flow among local Hector's dolphin populations is also being achieved by comparing sex-specific patterns based on whole mitogenome sequences and a Y-chromosome intron in the *AmeI-Y* gene. The results of this work will be used to encourage conservation strategies to maximize the survival and evolutionary potential of these subspecies.

Positive selection on mitochondrial DNA in the eastern oyster, *Crassostrea virginica*

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Recent meta-analyses of metazoan mitochondrial (mt)DNA data suggest that invertebrates have an effective population size (N_e) sufficiently large that natural selection, and not mutation-drift equilibrium, determines mtDNA diversity. However, non-neutral patterns of mtDNA variation, particularly in oysters, often are explained by mutation-drift equilibrium under exceptional demography. Like many marine species with high fecundity and high early mortality, oysters often have N_e many orders of magnitude below census size (N). A prominent hypothesis explaining these low N_e/N ratios is “sweepstakes” reproduction generating huge variance in family size. We resolve this apparent contradiction by testing for evidence of natural selection at mitochondrial and nuclear loci in the eastern oyster, *Crassostrea virginica*. Based on a battery of tests for selection, neutrality was rejected for mitochondrial DNA variation but mostly neutral patterns were found at seven unlinked nuclear loci, implicating natural selection as the force responsible for non-neutral mtDNA variation in *C. virginica*. Nuclear coalescent N_e was 1.8×10^5 , several orders of magnitude smaller than historical census size but large enough to make periodic selective sweeps of mitochondrial variation likely. We conclude that natural selection can dictate levels of mitochondrial variation despite sweepstakes reproduction or other processes dramatically reducing the N_e/N ratio.

Comparative genomics in conservation biology from an assembly-free perspective

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Genomic data for any taxa can now be obtained on even modest research budgets, but translation of these data into useful and meaningful results for conservation remains a major challenge. Traditional analyses rely upon the construction of a physical map of the target genome, which remains a costly and time-consuming endeavor. We propose that conservation biologists can best exploit short-read genomic sequence data by adopting an assembly-free perspective. Genetic differences between samples are discovered using the presence/absence and abundance of short sequence fragments. In our experience a fragment length of 15 to 25bp is optimal for most applications. *De novo* assembly of candidate markers can then be performed in a much more targeted fashion. Evolutionary significant units could be identified with assembly-free methods that estimate whole-genomic genetic variation; identify sequences associated with phenotypic variation among taxa; identify markers that differentiate populations or cryptic species; estimate demographic history with population genetics models; and reconstruct phylogenetic trees. An assembly-free perspective provides an avenue for rapid discovery of important genetic variation in the context of many questions in conservation biology without prior knowledge of the target genome.

Genetic and genomic patterns of differentiation in *Sida fallax* (Malvaceae) in Hawaii

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Remote island archipelagoes such as the Hawaiian Islands are often associated with the process of explosive adaptive radiation. Dramatic phenotypic differences between species are often accompanied by little molecular genetic differentiation, however. These patterns suggest a role for genomic regulation in the differentiation and adaptation process. To investigate these linkages, I am examining differentiation in the Hawaiian indigenous species *'ilima* (*Sida fallax*). The common and widespread nature of the species in the main Hawaiian Islands along with its tremendous morphological and habitat divergence makes it particularly suited for a model species of adaptive evolution in an island setting. Coastal *'ilima* has a prostrate growth form and small, hair-covered grayish leaves. In contrast, upland *'ilima* grows upright and has large, green leaves. Each of the forms is found across the islands. The populations are usually isolated from one another and where they do occur in close geographic proximity intermediates are

typically not seen. I am using AFLP markers to characterize differentiation between islands and coastal and upland ecotypes with a goal of identifying quantitative trait loci (QTL) associated with phenotypic patterns of differentiation. I am particularly interested in whether the same QTL underlie divergence across islands.

Feral cats – an invasive species in the Indian Ocean

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Cocos Keeling and Christmas Island are offshore islands halfway between Indonesia and Australia in the Indian Ocean. Both islands have a past of minimal human disturbance since exploration of both islands began as recently as 1800 by Europeans. The fauna shows a high level of endemism and it is severely threatened by invasive feral cats. Therefore, a major cat eradication program started on the islands, conducted by the Department of Environment and Conservation, Western Australia, to protect the indigenous fauna. Cat population genetics will provide important information about the demographics and population dynamics which enables us to develop more effective control and eradication strategies. We study mitochondrial and nuclear genes (microsatellites) to evaluate genetic diversity and population structure, assess gene flow from villages and identify the origin of invasive populations. Preliminary results show that island populations are genetically differentiated and cats of both islands originate from different European regions.

Effects of Copper Exposure on Pond-breeding Amphibians

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An artificial wetland was constructed on the Savannah River Site to treat process and storm water discharge from an industrial facility. Constructed wetlands can provide new habitat for local wildlife, but if poor water quality limits recruitment, these wetlands could become “population sinks.” In order to assess potential impacts to local pond-breeding amphibians, we are experimentally examining the effects of trace metal exposure on the southern toad (*Anaxyrus terrestris*), the eastern narrowmouth toad (*Gastrophryne carolinensis*), and the southern leopard frog (*Lithobates sphenoccephalus*). Copper (Cu) concentrations in the system can vary temporally and spatially, ranging from 7-37ppb and have reached up to 590 ppb in the retention pond; these concentrations may reduce larval survival, increase larval period, decrease body mass at metamorphosis, and cause a reduction in population level genetic variation. Our experiments are ongoing but data to date show that survivorship is strongly affected by Cu treatment. We are now collecting genetic data to assess whether Cu exposure may affect population genetics by causing a reduction in genetic variation and/or alteration of allele and genotype frequencies. These shifts can result from fitness differences among individuals exposed to contaminants or from reduced population size caused by decreased survivorship and reproduction.

Phylogenetic relationships in the spoon tarsus subgroup of Hawaiian *Drosophila*: conflict and concordance between gene trees

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The Hawaiian Drosophilidae is comprised of approximately 1000 species, placed in species groups and subgroups based on male secondary sexual characteristics. Members of the spoon tarsus subgroup possess a cup-shaped modification on their foretarsi. The majority of species in this subgroup are endemic to the Big Island of Hawaii, suggesting that they have diverged within the past 500,000 years. This radiation has occurred so quickly that determining the relationships within this group has proven difficult due to high gene tree conflict and low resolution with molecular markers. We use 14 genes, including 10 nuclear introns, to estimate relationships within the spoon tarsus species, as well as to test the monophyly of this subgroup. A variety of analytical approaches are used, including individual and partitioned concatenated analyses, Bayesian estimation of species trees and Bayesian concordance

analysis. We find widespread agreement between phylogenetic estimates derived from different methods, although some incongruence is present. Notable, our analyses suggest that the spoon tarsus subgroup, as currently defined, is not monophyletic. These methods and markers can be applied to other species groups of Hawaiian *Drosophila* threatened by habitat loss and invasive predators, including the listed picture wing species, to infer well-defined lineages.

Revisiting Wallace's haunt, with molecular tools

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It has been argued that conservation efforts should be targeted at evolutionary significant units, rather than at variation representative of putative local adaptation. To assess the utility of the subspecific rank as an indicator for historical significance, we used 16 bird species that are co-distributed across insular Southeast Asia (Sundaland) as a test case. We defined a match between taxonomy and molecular data as individuals of a particular subspecies forming a monophyletic clade on phylogeographic trees. Overall, the number of strongly supported Sundaic clades ranged from one to three. There was congruence between subspecific taxonomy and molecular phylogenies 63% of the time. When there was incongruence (six species), oversplitting by traditional taxonomy was twice as common as overlumping. We further probed the evolutionary population dynamics of three focal species, which possess different ecological characteristics, using data from 11 independent loci. The demographic histories of populations from different parts of Sundaland (mainland vs. Borneo) were different. This could be due to regional differences in long-term environmental changes. The species with the narrowest habitat breadth had the lowest amount of historical inter-landmass gene flow. This study advances our knowledge on avian evolution in a poorly studied but highly imperiled tropical region.

Conservation genetics of the crab *Portunus trituberculatus* in China

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Portunus trituberculatus is the most widely fished species of crab in the world, with 98% of the catch coming off the coast of China. In order to effectively manage this important fishery it is crucial to identify the limits of populations or stocks of this species. We sampled crabs from three locations off the coast of China: Bohai Sea, Yellow Sea, and East China Sea. We genetically characterized these three populations using Amplified Fragment Length Polymorphisms (AFLPs). A total of 60 individuals (20 per population) were analyzed using 10 primer combinations; and 466 loci were obtained, 360 of which were polymorphic. We conducted population genetics analyses using the software programs TFGA and PopGene V1.31, GENALEX6.3, and Structure V2.2, to determine genetic diversity and whether genetic population structure occurs. Our results indicate a high-level of genetic diversity within each population. High genetic differentiation is observed between the Bohai Sea population and the other two populations. A lower degree of genetic differentiation, yet significant, is observed between the Yellow-Sea and East China Sea populations. Our results have important implications for the management of this fishery, since they indicate each locality constitute a different stock or population.

Distribution, dispersal, and impacts of the introduced *Macrobrachium lar* within anchialine pool ecosystems – proposed investigations

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Anchialine pools are rare coastal ecosystems where groundwater and tidal marine lenses mix creating brackish conditions. Hawaii's anchialine pools support numerous endemic organisms (shrimp, mollusks, damselflies), including candidate and endangered species. Throughout Hawai'i, anchialine pools are threatened by development, groundwater withdrawal and pollution, introduced species, and sea level rise. My research will focus on the population and ecological genetics of the introduced *Macrobrachium lar* (Tahitian prawn) in the anchialine pool ecosystem. Specifically, I will focus on using population genetic

tools to study the introduction, distribution and dispersal of this species. Exclusion experiments will be used to study the ecological impact of this invasive taxon.

Assessing genomic variability in species of high conservation priority

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Many indigenous animal species have been reduced to small, isolated populations. Their insular nature may lead to inbreeding effects and a reduction in genetic diversity, compromising the ability of populations to evolve and persist long term. The focal species for my project, the New Zealand sea lion (*Phocarcos hookeri*), is the rarest sea lion in the world and is classed as 'Vulnerable' by the IUCN. Epizootic episodes in 1997, 2002 and 2003 were responsible for the deaths of approximately 40% of pups born during these breeding seasons, with increasing mortality among adult females. Neutral genetic variability may accurately represent the overall genetic diversity of many species and may be a good indicator of fitness. However, neutral markers may not always accurately reflect the diversity of loci that have important functional roles in e.g. inbreeding avoidance, growth and disease resistance. Here we use 21 neutral microsatellite markers in concert with several candidate genes in which variability is associated with disease resistance and growth/survivorship to test if neutral genetic variability is useful for predicting individual fitness, specifically disease resistance and growth/survivorship in NZ sea lions. This approach has the capacity to be applied to other species of conservation significance, and may help better enable the prediction of the future evolutionary potential of threatened species, aiding species conservation.

A genome wide set of SNPs detects population substructure and long range linkage disequilibrium in wild sheep

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The development of genomic resources for wild species is still in its infancy. However, cross-species utilization of technologies developed for their domestic counterparts has the potential to unlock the genomes of organisms that currently lack genomic resources. Here we apply the OvineSNP50 BeadChip, developed for domestic sheep, to two related wild ungulate species: the bighorn sheep (*Ovis canadensis*) and the thinhorn sheep (*Ovis dalli*). Over 90% of the markers were successfully genotyped in both species and identified 868 SNPs. Of these, 570 were detected in bighorn sheep, while 330 SNPs were identified in thinhorn sheep. The total panel of SNPs was able to discriminate between the two species, assign population of origin for bighorn sheep, and detect known relationship classes within one population of bighorn sheep. Using an informative sub-set of these SNPs we examined the extent of genome-wide linkage disequilibrium (LD) within one population of bighorn sheep, and found high levels of LD persisting over 4 Mb. In taxa where no genomic resources are available typing individuals on a platform such as the OvineSNP50 BeadChip can be more efficient and cost effective than other marker discovery efforts, such as SNP discovery through sequencing or attempts at cross-species microsatellite amplification.

Post-introduction dynamics and genetic variability in an isolated Japanese sika deer (*Cervus nippon*) population

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The aim of this study was to assess temporal changes in genetic differentiation after a bottleneck event, in this case following the introduction of the sika deer (*Cervus nippon*) onto an isolated island. The sika deer population in Nakanoshima Island (5.2 km²) in Lake Toya, Hokkaido, Japan was established by three individuals between 1957 and 1965, and has experienced three population crashes, in 1984, 2001

and 2004. We analysed seven polymorphic microsatellite markers for 505 individuals between 1984 and 2004, and estimated the changes in genetic diversity and effective population size (N_e). Even though the population was established by only three individuals, overall allelic richness (AR) per locus ($AR = 4.3$) and overall expected heterozygosity ($H_e = 0.41$) were not very low. The estimators of N_e and N_e/N ranged from 21.4 to 33.0 and from 0.06 to 0.12, respectively. A significant reduction (35%) in N_e was observed after the first population crash. The allelic composition was significantly different between 1984 and levels observed in 2001 and 2004, suggesting that these population crashes may have led to changes in genetic diversity in the islands' remaining population.

Adaptation of redband trout in desert and montane environments

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Natural populations that evolve under extreme climates are likely to diverge due to selection in local environments. To explore whether local adaptation has occurred in redband trout (*Oncorhynchus mykiss gairdneri*) occupying differing climate regimes, we used a limited genome scan approach to test for candidate markers under selection in populations occurring in desert and montane streams. An environmental approach to identifying outlier loci, Spatial Analysis Method (SAM), and linear regression of minor allele frequency with environmental variables revealed six candidate markers ($p < 0.01$). Putatively neutral markers identified high genetic differentiation among desert populations relative to montane sites, likely due to intermittent flows in desert streams. Additionally, populations exhibited a highly significant pattern of isolation-by-temperature ($p < 0.0001$) and those adapted to the same environment had similar allele frequencies across candidate markers, indicating selection for differing climates. These results implicate that many genes are involved in adaptation of redband trout to differing environments, and selection acts to reinforce localization. The potential to predict genetic adaptability of individuals and populations to changing environmental conditions may have profound implications for species that face extensive anthropogenic disturbances.

The shift in genetic structure due to seasonal intrusion in a black bear population

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Some of the Asian black bears inhabiting Japan invade residential areas, a phenomenon called mass intrusion. Although mass intrusion occurs in autumn in central and western Japan, such intrusion could result in genetic mingling. We examined the influence of intrusion on the genetic structure of black bears. Tissue samples were collected from invasive bears in central Japan in the autumn of two mass intrusion years: 2004 and 2006. We set hair-traps overlapping the area where tissues were collected in the summers from 2005 to 2008, except for 2007. The genotypes of eight microsatellite loci were determined using a PCR method. Relatedness was negatively correlated with the distance among normal bears, whereas no such relationship was observed among invasive bears. Spatial autocorrelation analysis of the normal bears in 2006 revealed a significantly positive rc value within the 0–65 km distance class. The analysis of invasive bears in 2006 revealed a significantly positive rc value between the 0–15 and 0–65 km distance classes. From these results, we concluded that the mass intrusion mingled the genetic structure in autumn. Subsequently, the normal structure would recover with seasonal movements.

Targeted next generation sequencing for conservation genetics in Ambystomatid salamanders

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Conservation genetics relies on the ability to accurately measure population genetic parameters including genetic diversity and structure, heterozygosity, inbreeding, gene flow, and selection. Accurate assessment of these parameters requires genotyping at hundreds of genome wide codominant markers. Next generation sequencing technology provides novel opportunities for gathering genome-scale sequence data in natural populations. We present the preliminary results of a first attempt to use the next generation methods to sequence, in parallel, ~9,000 barcoded PCR amplicons across the range of the tiger salamander species complex, a widely distributed North American clade containing many endangered and imperiled species. Prior to sequencing, we screened over 250 EST loci for positive PCR from a test pool of representative individuals. From this set of loci we identified 95 that amplified across all individuals and were polymorphic at one or more positions. We then used standard PCR methods to amplify all 95 loci from 94 individual samples representing most of the currently described species and subspecies of the tiger salamander complex. PCR amplicons from each individual were uniquely barcoded and pooled for 454 next generation sequencing. These techniques are likely to become extremely useful for the rapid and large-scale sequencing and genotyping of populations for conservation genetics.

Next Generation Sequencing and the Classroom: a conservation model

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Molecular biological enquiry has entered a new age with the development of high-throughput, massively parallel data acquisition platforms. While this technology has revolutionized genome biology, it remains largely inaccessible to the individual scientist. As part of the AGA sponsored conference held at UConn in 2009, Next Generation Sequencing in Non-Traditional Model Systems, we included a modular workshop on 454 whole genome shotgun sequencing. Workshop students were trained to prepare a library for sequencing, set up an emulsion PCR, perform bead breakage and sequencing on the 454 instrument. The goal of the workshop was to expose students to this next generation technology as well as the principles behind de novo sequence assembly and annotation. The subject of the workshop was the genome of *Limulus polyphemus* (horse shoe crab), which was sequenced and analyzed for gene content and novel repeats. This species has been isolated for over 500 million years, providing a glimpse into an "ancient" genome harboring a unique suite of mobile DNA residents. While this workshop served to introduce a broad range of students from diverse backgrounds to next generation sequencing technology, it also served as proof of principle for complex eukaryotic genome assembly in the classroom environment.

Ecological genomics in space and time: the signature of natural selection in wild populations of the water flea, *Daphnia magna*

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Elucidating the genetic basis of adaptation to different environments represents a central goal in evolutionary biology. To unravel patterns and mechanisms of adaptation to anthropogenic and natural stressors in natural populations, we use the water flea *Daphnia magna*. We capitalize on key assets of this model system - its well-documented ecology, the possibility to analyze subfossil dormant egg banks, and the short generation time allowing an experimental evolution approach. Using a genome-wide approach, we try to elucidate the genetic basis of local adaptation to key biotic (predation and parasite exposure) and anthropogenic stressors (land use, temperature rise). For the genome scan analysis we use populations of *D. magna* hatched from sediment cores with known evolutionary history as well as populations distributed along independent orthogonal gradients, known to induce micro-evolutionary

responses for fish predation, parasite infection and land use intensity. To investigate the genetic responses to temperature changes we use experimental populations pre-adapted to different temperatures and natural populations sampled from sediment cores. With this approach we identified genomic regions linked to individual environmental stressors. We confirmed the link between the target genomic region and fitness along manipulated gradients in experimental evolution trials.

Distinct phylogeographic patterns of sympatric West Atlantic exploited seahorses

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We are interested in providing a better understanding of population connectivity and the identification of evolutionary lineages in 2 sympatric seahorse species from the western Atlantic: *Hippocampus erectus* and *Hippocampus reidi*. With sequences from cytochrome *b*, the control region, and 7 polymorphic microsatellite loci, we analyzed 120 specimens from six distinct geographic populations across their overlapping ranges. Our mtDNA results indicate that *H. erectus* and *H. reidi* exhibit highly different patterns of evolution. Fst values among populations of *H. erectus* are not highly structured, grouping into two clades: the Gulf of Mexico and the Caribbean Sea. Populations of *H. reidi* display greater structure, with the population from Brazil being almost completely isolated. Percentage of sequence divergence between populations of *H. reidi* from Brazil and the Caribbean (0.7%) is very similar to the sequence divergence between *H. reidi* from Brazil and its sister species from the eastern Atlantic, *H. algiricus* (0.8%). Although the work on microsatellite loci is still in a preliminary stage, our results suggest that these two species exhibit differential patterns of population connectivity, dispersal abilities, abundance, and distribution. All of these processes determine and maintain phylogeographic patterns, the elucidation of which can help inform conservation decisions

Genotype by environment interaction affects genetic load in the Pacific oyster, *Crassostrea gigas*

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Inbreeding depression is a major concern in the management and conservation of endangered species. Inbreeding generally reduces fitness, but its magnitude and effects are highly variable because they depend on the genetic constitution of a population and on interaction with the environment. In general, harsher environments significantly increase inbreeding depression. However, most studies have examined the interaction of environment and inbreeding depression at the population level; less is understood about underlying genetic mechanisms. Using QTL methods, we performed a genome scan for deleterious (viability) loci in an inbred F₂ population of the Pacific oyster, *Crassostrea gigas*, reared in two environments; nutrient-poor (single-algal) and nutrient-rich (mixed-algal) diets. We found 14 viability loci, causing 99.8 % mortality up to the juvenile stage. There were four types of environmental interaction with viability loci: 1) selection against the affected allele increased in the harsher environment, 2) dominance increased exposing heterozygotes to more selection (harsher environment) 3) viability loci were lethal in both environments (no interaction), and 4) viability loci were only present in the harsher environment. These findings suggest that individual viability loci respond differently to the environment and have implications for small or inbred populations coping with climatic shifts and habitat destruction.

Linkage mapping in free-living bighorn sheep

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The construction of genetic linkage maps in free-living populations is a promising tool for the study of adaptive genetic variation. However, such maps are rare because it is difficult to develop wild pedigrees and corresponding sets of molecular markers that are sufficiently large. We took advantage of long-term field studies of pedigreed individuals (Ram Mountain, Alberta, Canada; National Bison Range, Montana, USA) and microsatellite loci mapped in domestic sheep (*Ovis aries*) to construct the first genetic linkage map for bighorn sheep (*Ovis canadensis*). Using genotypes from 500 pedigreed individuals, we ordered

248 loci along all 26 ovine autosomes and the X chromosome. We then combined genotypic and phenotypic information to locate chromosomal regions containing genes influencing ecologically important traits. The availability of genomic resources for bighorn sheep will allow for investigations into a variety of conservation related topics, including the genetic basis of local adaptation, inbreeding depression, genetic rescue, as well as the evolutionary consequences of selective harvesting.

Conservation Genetics of the Endangered Saint Croix ground lizard *Ameiva polops*

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The endangered lizard *Ameiva polops* is restricted to four islets located around the St. Croix Island: Protestant Cay, Ruth Cay, Green Cay and Buck Island. This lizard was once abundant in the main island, but went extinct there, apparently as a consequence of mongoose introduction. Protestant Cay and Green Cay appear to correspond to natural populations. The population of Ruth Cay was established with 10 individuals from Protestant Cay and one from Green Cay. The population of Buck Island was recently established with individuals from Green Cay. Sequences of a 950 bp fragment of the mitochondrial gene ND2 were obtained for 71 individuals, and only two haplotypes that were different by two nucleotides were observed. One of these haplotypes was found in the individuals from Protestant Cay (n = 10) and Ruth Cay (n = 27), and the alternative haplotype in the individuals from Green Cay (n = 34). These results indicate very low within-population genetic diversity and the potential for two management units. Analyses of eight microsatellites confirmed the Green Cay population is genetically different from Protestant Cay and Ruth Cay, which are genetically identical. Therefore, two distinct conservation units are identified for this lizard.

Phylogeny and Evolution of equids

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In conservation, the main goal of phylogenetics is to establish an evolutionary framework for understanding patterns of intraspecific genetic variation, necessary to identify evolutionary significant units (ESUs). However, processes such as hybridization and lineage sorting might confound phylogenetic inferences, jeopardizing species' designation. This is particularly relevant in the case of endangered species, where no conservation policies have been established for hybrids. Here, we examine the phylogenetic relationships of eight species of equids by analyzing mitochondrial and nuclear datasets, and evaluate the role of speciation processes in determining patterns of intraspecific genetic variation. In equids, discrepancies among gene trees were detected among datasets, likely due to lineage sorting or genetic introgression. Intraspecifically, the Estrogen Receptor 1 gene showed signature of genetic admixture between the common species, Burchell's zebra, and the endangered Grevy's zebra for a Rare Genomic Change. Our results emphasize the importance of understanding lineage sorting and genetic introgression as evolutionary processes limiting the resolution of phylogenetic trees. This is relevant in a conservation context, validating the incorporation of ecological and demographic/population data in addition to phylogenies for consistently identifying ESUs among equids.

Genomics in conservation of cave adapted nemobiinae crickets of Hawai'i Island

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Determining conservation needs and management strategies for cave adapted crickets on Hawai'i Island depends on knowing how many species exist, their ranges, habitats (including surface ecosystems) and the threats to each species. Based on morphology, four species of cave adapted nemobiinae crickets have been described from Hawai'i Island. Additional evidence from chromosome differences, sex determining mechanisms, and presence or absence of vision appeared to support these species

determinations. Two cave species, *C. varius* (able to respond to light, and having 12 chromosomes and an XX/X0 sex determination) and *C. uuku* (blind, 10 chromosomes and $X_1X_1X_2X_2/X_1X_2Y$ sex determination), were considered to have wide ranges on the Big Island. Two additional cave species, *C. albus* and *C. paralbus* (sight, chromosome numbers and sex determination unknown), were known only from Hawai'i Volcanoes National Park. However, mtDNA results indicate that *C. varius* and *C. uuku* may include more than one species, and may represent hybrid populations in some areas. *C. albus* was found to be more widespread than previously realized. Further evidence from genomics, in combination with morphology, chromosome numbers, sex determination and vision is necessary to decide the number cave species and their ranges, as the basis for further conservation efforts.

Molecular phylogenetics of *Caralluma s.l.* species occurring in India

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Caralluma s.l. (Asclepiadoideae, Apocynaceae) consists of a diverse assemblage of perennial species, mainly distributed in dry regions of tropical Asia, Mediterranean, and Africa. Recent revisions tend to split it into several smaller genera. There are about 14 species of *Caralluma s.l.* occurring in the foothills of Western and Eastern Ghats of India, with five being endemic and many threatened with extinction due to habitat destruction and large-scale indiscriminate collection for medicinal use. To inform conservation prioritization, phylogenetic studies are the first step to clarify species diversity and their evolutionary distinctiveness. In this study, we investigated phylogenetic relationships of *Caralluma s.l.* with focus on 17 Indian taxa (13 species and 4 varieties). We performed Maximum likelihood and Bayesian analyses on nuclear ITS and chloroplast *trnL* intron and *trnL-F* intergenic spacer sequences. The phylogenies based on either ITS or cpDNA or the combined data indicate that most Indian taxa belong to two new genera *Caralluma s.s.* and *Boucerosia*. Only two species fall under *Apteranthes* and *Caudanthera*, respectively. This information can assist conservation biologists in identifying the unit of conservation and in setting priorities to protect those distinctive species that are valuable for understanding speciation and evolution, in addition to their economic values.

Neutral versus functional genetic diversity in populations with different bottleneck histories

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In conservation genetics, genetic diversity is usually measured at neutral loci, particularly microsatellites. However, neutral loci have no adaptive value and do not provide information about how selection acts upon a population or species. Functional genetic diversity may, therefore, be of interest to conservation managers concerned about how populations will respond to changing environments. We are examining both microsatellite and functional major histocompatibility complex (MHC) variation in New Zealand birds, and are comparing contemporary populations to pre-bottlenecked museum specimens. New Zealand saddlebacks suffered severe population declines following the introduction of mammals and lost relatively more microsatellite diversity compared to New Zealand robins, which experienced more moderate bottlenecks. However, given that selective forces act on MHC diversity, patterns may be different to those observed at microsatellite loci. In other vertebrate species severe bottlenecks often lead to loss of both neutral and functional diversity, but this is not always the case. This research will be useful for understanding the genetic effects of population bottlenecks on both neutral and functional genetic diversity in species with different histories of decline. It will also help identify the utility of different markers for conservation genetics.

A historic seahorse from Hilo, *Hippocampus hilonis* (Jordan and Evermann 1902) revived

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The holotype *Hippocampus hilonis* from Hilo, Hawai'i, and five specimens of *Hippocampus kuda* of O'ahu were compared morphologically with five specimens of *H. kuda* from the western and South Pacific. Both taxa have a posteriorly-projecting coronet preceded by a ridge (keel) that is narrower and higher in *H.*

hilonis and *H.kuda* from Hawai'i. In addition, differences were noted in the number of dorsal-fin rays between Hawai'i and the rest of the Pacific seahorses. Such morphological observations were further investigated using *Cytb* DNA sequences from *H. hilonis* and 34 contemporary Hawai'i *H. kuda* samples. All sequences were identical (HK_01). A comparison of HK_01 to all 67 Indo-Pacific *H. kuda* *Cytb* haplotypes (GenBank) indicates that HK_01 is found only in Hawai'i and differs from Taiwan and Philippines haplotypes by only one or two base pairs, respectively. We conclude that the Hawaiian population of *Hippocampus kuda* is best treated as a subspecies; *H. kuda hilonis*. The unique *Cytb* haplotype found only in Hawai'i, the distinctive narrow ridge anterior to the coronet, and the probable mean difference in the number of dorsal fin rays indicate a highly isolated population in the archipelago.

Altered patterns of gene flow following evolution in response to climate change

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Climate change is currently a subject of major concern across the globe, specifically regarding its effects on the natural environment. Climate change can alter traits and distributions of natural populations. Recent research has shown this effect through the rapid evolution in the flowering time of two populations of *Brassica rapa* in wet and dry habitats, in which flowering time became more synchronous following a drought period. Given this convergence in flowering time it is predicted that the exchange of genetic material between the populations increased following the drought. Known microsatellites of *B. rapa* will be sequenced for individuals from each population to analyze the gene flow between the populations and compare the degree of gene flow before and after the drought. These results will be important in demonstrating the effect of climate change on the movement of genes between populations. Understanding the genetic basis of how species respond to large-scale changes such as global climate change can assist in the conservation of species in a changing world.

Phylogeography using parallel tagged next-generation sequencing: a case study of New Zealand endemic frog species *Leiopelma hochstetteri*

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Rapid improvements in next-generation sequencing (NGS) have greatly increased the quantities of genetic data that can be affordably processed and obtained in relatively short time. These technologies have already found very broad application in ecology, evolutionary and conservation biology using genomic approaches and parallel tagging (multiplexing) of individual samples. However, up to date, only a few studies have employed parallel tagged amplicon NGS in phylogeography and population genetics. One reason for this might be the cost and time related to tagging the large number of individual samples that are typically required in population genetic and phylogeographic studies. Here we aim to examine how accurate and appropriate parallel tagged pyrosequencing on pooled population samples is for estimating species diversity and reconstructing phylogeographic patterns. To do so, we have used the endemic New Zealand frog *Leiopelma hochstetteri*, in which we have previously documented strong genetic structure. We have then employed a pooling approach, in which the individual mtDNA gene amplicons have been combined by population and tagged with population specific barcodes for 454 sequencing. The accuracy and feasibility of this population (rather than individual) parallel tagged NGS for population genetic and phylogeographic analyses will be examined and discussed.

MAPS AND RESTAURANTS

Map of Hilo town. Distance from airport to Hilo Hawaiian is about 3 miles. Distance from airport to Dolphin Bay Hotel is about 4.5 miles. Distance from airport to University of Hawaii Hilo dorms is about 4 miles. Red line shows path from airport to UHH Kawili Street entrance.



Map showing the location of the Dolphin Bay Hotel for the Sunday early-evening reception and registration. The Dolphin Bay is at 333 Iliahi; directions are in the insert map. The reception will have beer and wine (free) and pupus (appetizers to you mainlanders), 5:30-7:30 Sunday, 25 July. It will be in the Dolphin Bay Garden behind the hotel (you can't miss it), rain or shine (often, it rains).

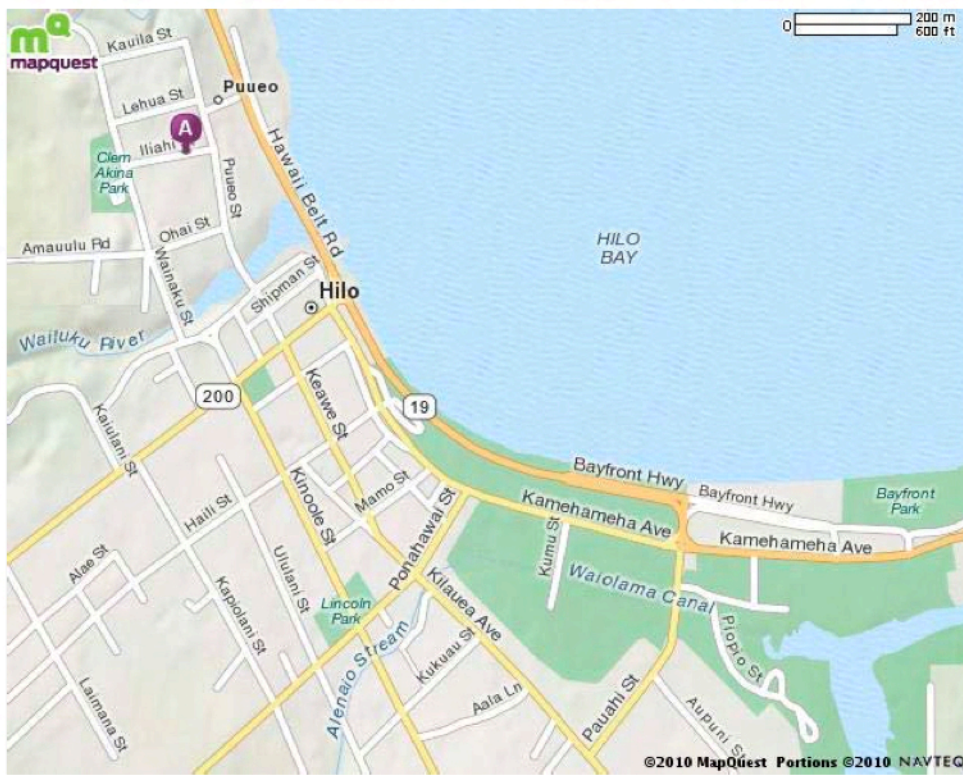


Map of Dolphin Bay

333 Iliahi St, Hilo, HI 96720 - (808) 935-1466

Notes

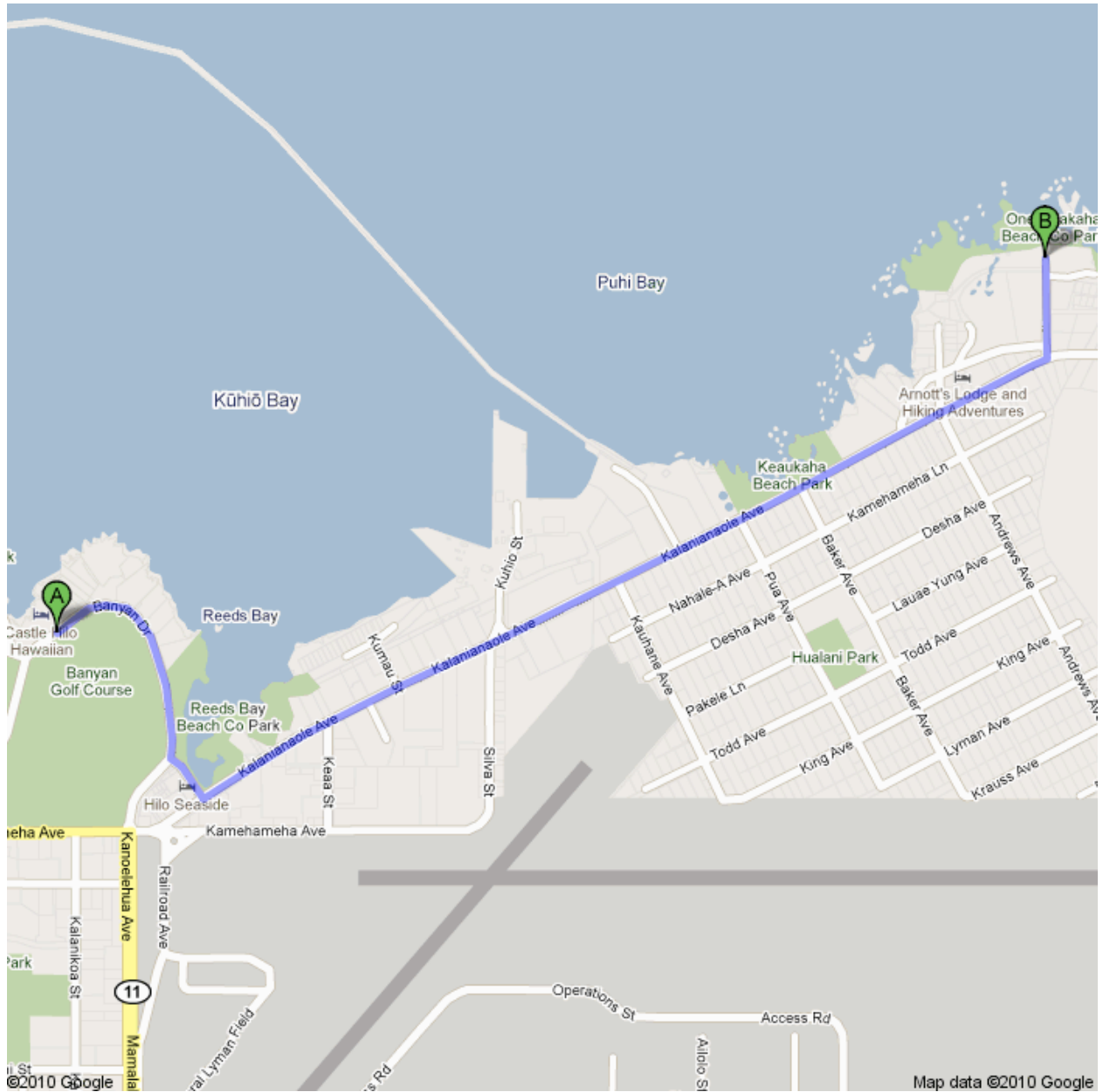
Stay on Kilauea Avenue through the downtown area. At one point the street name will change to Keawe Street and it will become one-way (your way) - keep going STRAIGHT on Keawe Street. Cross the white arched bridge, and turn LEFT at the second street which will be Iliahi Street. Dolphin Bay Hotel is on the LEFT, parking is behind the hotel.



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Map showing Onekahakaha Beach Park, site of the Luau Tuesday evening. Onekahakaha is labeled "B" on the map. Distance from UHH to Onekahakaha is about 5 miles, from Hilo Hawaiian ("A") to Onekahakaha (blue path) is about 2.4 miles. Drinks start around 5:30, dinner at 6:30. Bring your swim suit!



Some Restaurant Ideas

(many restaurants close early; call ahead for hours)

- Café 100 (cheap local food, home of the loco moco) 969 Kilauea Ave (808) 935-8683
- Café Pesto (“creative island cuisine”) 308 Kamehameha Ave (808) 969-6640
- Cronies (sports bar) 11 Waiuanuenue Ave. (808) 935-5158
- Emerald (pub food, music) 168 Keawe St. (808) 961-5400
- Garden Snack Club (Thai) 80 Kilauea Ave. 808-933-9664
- Hilo Bay Café (high end drinks, eclectic menu) 315 E Makaala St # 109 (808) 935-4939
- Hilo Burger Joint (pub food, good beer selection) 776 Kilauea Ave (808) 935-8880
- Hilo Farmers Market (food stalls on Wednesday and Saturday, best green papaya salad in Hilo)
- Hilo Lunch Shop (Japanese deli, closes at 1pm, bentos) 421 Kalanikoa St (808) 935-8273
- Hilo Hawaiian Queen’s Court (American/International, buffet excellent) 71 Banyan Drive (808) 935-9361
- Island Naturals Market and Deli (natural foods grocery store) 1221 Kilauea Ave (808) 935-5533
- Kawamoto Store (Japanese deli, good bentos, opens early) 808-935-8209
- Ken’s House of Pancakes (full menu served 24/7) 1730 Kamehameha Ave. (808) 935-8711
- Miyos (Japanese) 400 Hualani St. (808) 935-2273
- Naung Mai (Thai) 86 Kilauea Ave. (808) 934-7540
- Ocean Sushi (Japanese), 250 Keawe Street (808) 961-6625
- Pescatore (up-market Italian) 235 Keawe St. (808) 969-9090
- Puka Puka Kitchen (eclectic, local plate lunches, bentos) 270 Kamehameha Ave. 808-933-2121
- Short N Sweet (pastry, light lunches) 374 Kinooole Street (808) 935-444
- Suisan Fish Market (take-out poke rice bowl bentos) 93 Lihiwai St. 808-935-9349
- Yen’s Café (great Vietnamese) 235 Waiuanuenue Ave. 808-933-2808