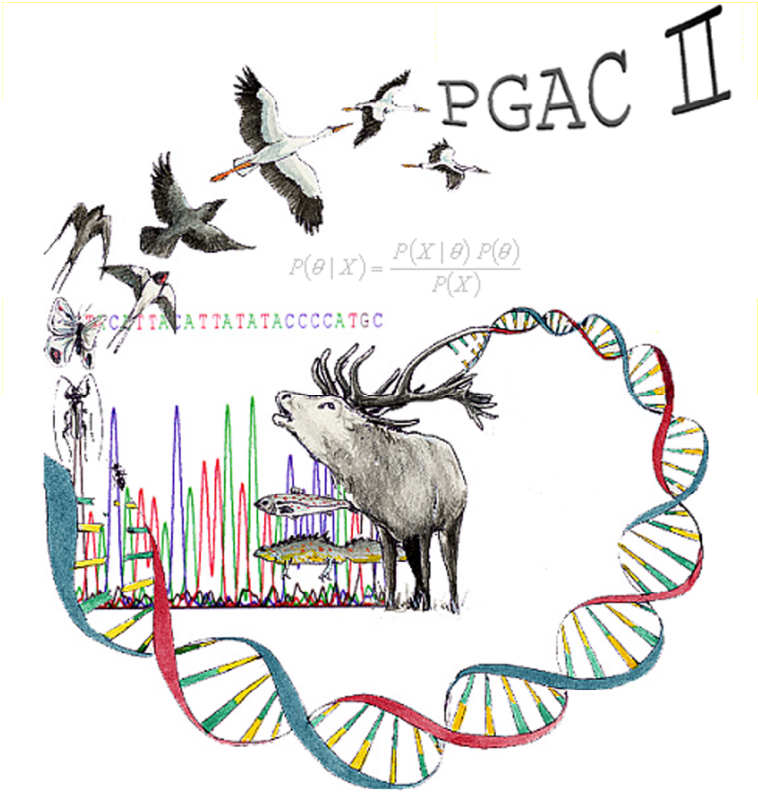
2ND INTERNATIONAL WORKSHOP

POPULATION GENETICS FOR ANIMAL CONSERVATION (PGAC II)



CENTRO DI ECOLOGIA ALPINA
VIOTE DEL MONTE BONDONE, TRENTO, ITALY
6-8 SEPTEMBER 2007

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MEETING PROGRAM

Wednesday, September 5

19.30	Arrival and welcome dinner at Centro di Ecologia Alpina (CEA)

Thursday, September 6

7.30	Breakfast at hotel
8.30	Transfer to CEA in minibuses; Registration
9.00	Welcome address

Session 1: Recent theoretical and methodological developments: Invited presentations

9.30-10.15	**Eric Anderson,** NOAA FISHERIES SERVICE, SANTA CRUZ, USA On the statistical power of single nucleotide polymorphisms for conservation-genetic applications
10.15-11.00	**Armando Caballero,** UNIVERSIDAD DE VIGO, SPAIN Impact of homoplasy of AFLP markers on the estimation of genetic diversity and the detection of selective loci
11.00-11.30	Coffee break
11.30-12.15	**Rasmus Nielsen,** UNIVERSITY OF COPENHAGEN, DENMARK Estimating demographic parameters using composite likelihood methods
12.15-13.00	**Peter Beerli,** FLORIDA STATE UNIVERSITY, TALLAHASSEE, USA Bayesian or Maximum Likelihood in gene flow inference?
13.00-14.15	Lunch at CEA
14.15-15.00	**Oscar Gaggiotti,** UNIVERSITÉ J. FOURIER, GRENOBLE, FRANCE Identifying the environmental factors that influence population processes using genetic and environmental data.

Session 2: Experimental approaches to testing the theory: Invited presentations

15.00-15.45	**Craig Primmer,** UNIVERSITY OF TURKU, FINLAND Hitch-hiking mapping for identifying functionally important genetic variation: examples in Atlantic salmon, *Salmo salar*
15.45-16.15	Coffee break
16.15-17.00	**Ilkka Hanski,** UNIVERSITY OF HELSINKI, FINLAND TITLE metapopulations?
17.00-17.45	**A. Rus Hoelzel,** UNIVERSITY OF DURHAM, UK Evolution of population structure in social cetaceans [not original session, but Pemberton won't have arrived and Acevedo-Ahitehouse is also cetaceans, so one per day better: also he HAS been studying these pops a long time...]
17.45-18.45	**Poster session** (10 posters with authors present)
18.45-19.30	Free time (to look at posters, form discussion groups, etc.)
19.30	Dinner at CEA
from 21.00	**Practical software sessions:** 1. AULA MAGNA: WHICH? 2. MULTIPOLARE BUILDING: WHICH?
from 22.00	Transfer to hotels in minibuses

Friday, September 7

7.30	Breakfast at hotel
8.30	Transfer to CEA in minibuses

Session 2: Experimental approaches to testing the theory: Invited presentations

9.00-9.45	**Josephine Pemberton,** UNIVERSITY OF EDINBURGH, UK TITLE NOT ON 6th!!! [I CANNOT DECIDE WHERE BEST TO FIT HER IN SINCE SHE IN IN SESSION 2, BUT HERE DOES NOT SEEM FAIR BECAUSE WE WILL ARRIVE VERY LATE ON THE 6TH]

Session 3: Case studies: Invited presentations

9.45-10.30	**Rob DeSalle,** AMERICAN MUSEUM OF NATURAL HISTORY, NEW YORK, USA The Role of DNA barcoding in conservation genetics
10.30-11.00	Coffee break
11.00-11.45	**Eske Willerslev,** UNIVERSITY OF COPENHAGEN, DENMARK Ancient DNA - reconstructing the past
11.45-12.30	**Pierre Taberlet,** J. FOURIER UNIVERSITY, GRENOBLE, FRANCE Non-invasive genetic sampling
12.30-13.30	Lunch at CEA
13.30-14.15	**Karina Acevedo-Whitehouse,** INSTITUTE OF ZOOLOGY, LONDON, UK Conservation genetics of marine mammals - an immunogenetic approach
14.15-15.00	**Giorgio Bertorelle,** UNIVERSITY OF FERRARA, ITALY The relative role of selection, demography and management on the genetic variation in the chamois
15.00-15.30	Coffee break

Sessions 1-3: Participant presentations

15.30-15.45	**Thomas Broquet,** UNIVERSITY OF LAUSANNE, SWITZERLAND Inferring landscape effects on dispersal from genetic data: how far can we go?
15.45-16.00	**Simone Sommer,** LEIBNIZ-INSTITUTE FOR ZOO- AND WILDLIFE RESEARCH, BERLIN, GERMANY Genes and health: the role of immune gene variability (MHC) in conservation
16.00-16.15	**Catherine Walton,** UNIVERSITY OF MANCHESTER, UK Cross-species patterns of genetic diversity in forest-dependent taxa in Southeast Asia: implications for conservation of forest biodiversity
16.15-16.30	**Gernot Segelbacher,** UNIVERSITY OF FREIBURG, GERMANY Genetic diversity in grouse - what does it mean for conservation?
16.30-16.45	**Luis Manuel Enriquez-Paredes,** AUTONOMOUS UNIVERSITY OF BAJA CALIFORNIA, MEXICO Reassessing pre-whaling stocks: genetics matches logbook records
16.45-17.00	**Jawad Abdelkrim,** UNIVERSITY OF CANTERBURY, NEW ZEALAND Genetic tools and the management of invasive species: how can we improve eradication strategies?
17.00-18.00	**Poster session** (with authors present)
18.00-19.30	Free time (to look at posters, form discussion groups, etc.)
19.30	Dinner at CEA
21.00	**Practical software sessions:**

1. AULA MAGNA: WHICH?
2. MULTIPOLARE BUILDING: WHICH?

from 22.00	Transfer to hotels in minibuses

Saturday, September 8

7.30	Breakfast at hotel
8.30	Transfer to CEA in minibuses

Session 3: Participant presentations

9.00-9.15	**Agus Nuryanto,** JENDERAL SOEDIRMAN UNIVERSITY, PURWOKERTO, INDONESIA Strong genetic population structure and limited larval dispersal distance in the giant clams *Tridacna crocea* and *Tridacna maxima* across the Indo-Malay Archipelago
9.15-9.30	**Kathryn Elmer,** UNIVERSITY OF KONSTANZ, GERMANY Applying new approaches to assess intra- and inter-specific genetic diversity of an upper Amazonian frog
9.30-9.45	**Fulvio Maffucci,** ANTON DOHRN ZOOLOGICAL STATION, NAPLES, ITALY Juvenile loggerhead turtles foraging in the Mediterranean Sea: regional variability in the contribution from the Atlantic rookeries
9.45-10.00	**Gabriele Gentile,** UNIVERSITY OF TOR VERGATA, ROME, ITALY The diversification of land iguanas from the Galápagos Islands: a new form of iguana traces the origin of the modern Galápagos land iguanas
10.00-10.15	**Bradley Fedy,** UNIVERSITY OF MONTANA, MISSOULA, USA An apparent paradox: high inbreeding in small populations does not result in strong population differentiation or low genetic diversity.
10.15-10.30	**Sasha Vignieri,** UNIVERSITY OF CALIFORNIA, SAN DIEGO, USA Spatial genetic structure precipitates rapid bottleneck and founder effects in the patchily distributed Pacific jumping mouse
10.30-11.00	Coffee break
11.00-11.15	**Aleksandra Gondek,** POLISH ACADEMY OF SCIENCES, KRAKÓW, POLAND MHC versus microsatellite diversity in spotted suslik *Spermophilus suslicus* populations from two different parts of its range.
11.15-11.30	**Iris Biebach,** UNIVERSITY OF ZÜRICH, SWITZERLAND Genetic footprint of the reintroduction history of Alpine ibex
11.30-11.45	**Paola Lara-Ruiz,** FEDERAL UNIVERSITY OF MINAS GERAIS, BRAZIL Extreme genetic divergence between remnant wild populations of the Atlantic Forest endemic sloth (*Bradypus torquatus*)
11.45-12.00	**José Godoy,** DOÑANA BIOLOGICAL STATION, SEVILLE, SPAIN Iberian lynx conservation genetics
12.00-12.15	**Luca Fumagalli,** UNIVERSITY OF LAUSANNE, SWITZERLAND Loss of genetic diversity in historical European wolf (*Canis lupus*) populations
12.15-12.30	**Uma Ramakrishnan,** NATIONAL CENTRE FOR BIOLOGICAL SCIENCES, INDIA Indian tigers retain more than their share of genetic variation
12.30-14.00	Lunch at CEA

Session 4: Conservation genetics and conservation policy: Invited presentations

14.00-14.45	~~**Jon Ballou,** UNIVERSITY OF CALIFORNIA, LOS ANGELES, USA~~ ~~TITLE~~
14.45-15.30	**Luigi Boitani,** UNIVERSITY OF ROMA "LA SAPIENZA", ITALY Detecting and managing hybridization: implications for conservation policy.
15.30-16.00	Coffee break

16.00-16.45	**Michael Bruford**, CARDIFF SCHOOL OF BIOSCIENCES, UK From data to policy: setting the agenda for research, management and legislation in conservation genetics in the UK
16.45-17.30	**Craig Moritz**, UNIVERSITY OF CALIFORNIA, BERKELEY, USA TITLE
17.30-18.30	Round table and conclusions
from 18.30	Workshop outing and social dinner

Sunday, September 9

8.00	Breakfast at hotel and departure

POSTERS

Poster will be displayed in the Multipolare Building.

Poster sessions (with authors present):

Thursday, September 6th: 17.45-18.45

Friday September 7th: 17.15-18.15

1 **Jacqueline Bishop**, UNIVERSITY OF STELLENBOSCH, SOUTH AFRICA
The negative effects of fluctuating population size on *Ne*: past exploitation and the implications for current sustainable harvesting of the Nile crocodile

2 **Bradley Fedi,** UNIVERSITY OF MONTANA, MISSOULA, USA
Gene flow, dispersal patterns, and metapopulation dynamics of alpine Vancouver Island white-tailed ptarmigan inferred from microsatellite DNA markers and direct observations.

3 **Luisa Garofalo**, UNIVERSITY TOR VERGATA, ROME, ITALY
High genetic diversity identifies a nesting population of Caretta caretta in continental Italy as a relevant Management Unit

4 **Marc Kochzius,** UNIVERSITY OF BREMEN, GERMANY
Genetic connectivity of coral reef animals across the Indo-Malay Archipelago: implications for the conservation of marine ornamentals

5 **Riccardo Negrini,** CATHOLIC UNIVERSITY OF SACRO CUORE
Post-glacial history of Arctic charr (*Salvelinus alpinus*) populations inferred by AFLP neutral markers and mtDNA analysis

6 **Alisa Semina**, INSTITUTE OF MARINE BIOLOGY, VLADIVOSTOK, RUSSIA
Conservation genetics of chum salmon *Oncorhynchus keta*

7 **Juha-Pekka Vähä**, UNIVERSITY OF TURKU, FINLAND
Life-history and habitat features influence the within-river genetic structure of Atlantic salmon

8 **Ulo Väli,** UPPSALA UNIVERSITY, SWEDEN
Comparing and combining genome-widely developed single nucleotide markers and microsatellites for identification of hybrids between Greater and Lesser Spotted Eagles

ABSTRACTS

Acevedo-Whitehouse K
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Conservation genetics of marine mammals - an immunogenetic approach

The increasing molecular genetic tools available to conservation biologists have made it possible to determine population structure, identify populations where intrinsic genetic factors are likely to affect probability of survival and reproduction, and define management units within species. Such knowledge has helped understand extinction risks in threatened populations. However, one aspect of conservation genetics which is often overlooked or oversimplified is immunogenetics, the genetic regulation of resistance to infection/disease. The increasing emergence and re-emergence of infectious diseases as a serious threat to marine mammals, particularly to small or endangered populations such as the Vaquita and the Mediterranean monk seal, where sudden demographic declines could be catastrophic, warrant a more complete understanding of how resistance to infectious disease is regulated. Unfortunately, most marine mammal immunogenetics studies have focused on a single genetic region, the major histocompatibility complex (MHC), involved with presentation of antigens to immune cells. Research on model animal species has highlighted the importance of many other genes in regulating immune responses to different pathogens and that operate at different times following infection. MHC and non-MHC genetic regions have recently been highlighted as important for resisting a range of diseases, including cancer, bacterial and helminth infections in some marine mammal species and studies suggest that even genome-wide heterozygosity can play a role at conferring resistance to disease. Such findings urge caution when drawing general conclusions about epidemic risks in threatened and endangered populations based on studying only one aspect of the immunogenetic system.

Abdelkrim J (1, 2, 3) Pascal M (1) Samadi S (2)
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(2) Molecular Ecology Lab, Biological Sciences, University of Canterbury, Private bag 4800. Christchurch, New Zealand.
(3) Management of Invasive Species Group, INRA Station SCRIBE, Campus de Beaulieu, F-35042 Rennes, France

Genetic tools and the management of invasive species: how can we improve eradication strategies?

Biological invasions are known as one of the most important threats to wildlife conservation and the cause of numerous extinctions. Different strategies have been developed to limit their impact and to restore disturbed ecosystems. Eradication campaigns for pest species have become a real tool for conservation and enable important recoveries of endangered species. Nevertheless, such operations can have high costs, both ecologically and economically, if unsuccessful. In order to optimize the probability of success of such interventions, genetic tools can be used at different times during the

eradication process in order to provide helpful indications to wildlife managers. We will see how realistic it is to use modern population genetic tools in order to answers questions such as:
-What is the extent of migration between the target populations and the neighbouring potential sources of colonisation?
-Is it worth eradicating and if yes, what is the pertinent eradication unit to consider?
-If an eradication has not been successful, is it because of a few survivors or because a new colonisation occurred after the eradication attempt?
We will see how such questions can be answered using modern genetic tools such as microsatellite markers and recent progress in statistical analyses. Eradication campaigns of Norway rats (*Rattus norvegicus*) and ship rats (*R. rattus*) conducted on French islands and in the Caribbean will be used to illustrate these applications.

Eric C. Anderson

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On the statistical power of single nucleotide polymorphisms for conservation-genetic applications

In the last decade, the discovery and use of hundreds of thousands of single nucleotide polymorphisms (SNPs) has revolutionized genetic studies of humans and model organisms. SNPs have many desirable properties: they are abundant, allele calls do not require difficult cross-laboratory standardization, and they are amenable to high-throughput genotyping with low genotyping error rates and minimal human interaction. While dense genetic maps of SNPs are not yet available for most non-model organisms of conservation concern, a moderate, and increasing, number of SNPs have been developed for such organisms. I will discuss the statistical power of such SNP data sets for two population-genetic analyses commonly employed in animal conservation: genetic stock identification (a generalized version of population allocation) and parentage inference. Simulation results suggest that upwards of 200 neutrally-evolving SNPs would be required to match the power of 13 microsatellite loci (with an average of 25 alleles each) for genetic stock identification. On the other hand, with only 100 SNPs it is possible to accurately infer parentage from amongst very many candidate parents. These analyses will be illustrated with examples and empirical genetic data used in the management of Pacific salmon fisheries.

Beerli P (1,2)

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Bayesian or Maximum Likelihood in gene flow inference?

Recent approaches in population genetics often employ models that are too complicated to solve analytically. Approximations, most prominently the Metropolis-Hastings (MH) algorithm, allow us to infer parameters of these models, but often we do not have a good measure whether the results are

correct or not. For coalescence-based population genetics maximum likelihood (ML) inference and Bayesian inference (BI) are using the MH algorithm to estimate the same parameters in a somewhat different way. Both inference methods will deliver similar results with good data, but when the data is not very informative results can differ. Often the arbitrary choice of prior distributions for BI is considered to be problematic; but with data that does not allow to estimate all parameters of a complex model, BI makes it rather easy to detect this insufficiency because the posterior distribution is mimicking the prior distribution. In contrast, current ML applications sometimes may fail to deliver a correct distribution because multiple peaks in the probability landscape of the high dimensional functions, and the results do indicate this failure.

Bertorelle G (1), Crestanello B (1,2), Mona S (1), Dronnet F (1), Pecchioli E (2), Vernesi C (2), Hauffe HC (2), Martinkova N (3), D'Amelio S (4), Rossi (5)

(1) Department of Evolution and Biology, University of Ferrara, via Borsari 46, Ferrara, Italy; ggb@unife.it
(2) CEA, Centro di Ecologia Alpina, Viote di Monte Bondone, Trento, Italy
(3) Institute of Population Biology, Academy of Sciences, Studenec, Czech Republic
(4) Dipartimento di Scienze di Sanità Pubblica, University of Rome "La Sapienza", Rome, Italy
(5) Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia, University of Torino, Italy

The relative role of selection, demography and management on the genetic variation in the chamois

Genetic variation at two mitochondrial DNA markers (control region and cytochrome b) and 11 nuclear microsatellites was analysed in 259 chamois (Genus Rupicapra) from 16 different sampling sites located in Italy, Spain, Slovakia, and Czech Republic. A partially overlapped sample of 182 alpine chamois (Rupicapra rupicapra) was also sequenced at the exon 2 of a DRB class II MHC locus in 10 populations in the Eastern Alps. The impact of selection, demography, and management was identified using different statistical methods, including a general approach based on the nonparametric combination of the p-values obtained in several non-independent tests.

Biebach I, Keller L

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Genetic footprint of the reintroduction history of Alpine ibex

Many vertebrate species that were extirpated in the last two centuries have been successfully reintroduced to parts of their former range. However, such reintroductions were usually associated with low number of founders, which may have long-term genetic consequences. Here we investigate these genetic consequences, loss of genetic diversity and how it is influenced by reintroduction parameters, in the reintroduced Alpine ibex. All Alpine ibex populations descend from one common ancestral population. The reintroductions, often with multiple bottlenecks are well documented for many populations. Genetic diversity was explored both with neutral markers and with a gene under balancing selection, the major histocompatibility

complex (MHC). The observed genetic structure of 37 neutral microsatellite loci clearly reflects the reintroduction history. This suggests that genetic drift was the main factor determining current genetic structure in Alpine ibex. Genetic diversity was strongly affected by source diversity: genetic variation was highest when founder individuals came from different source populations. However, contrary to our original expectations, number of founder individuals did not influence genetic diversity. Genes under balancing selection are expected to maintain a higher diversity compared to neutral markers following a bottleneck. However, in Alpine ibex the DRB exon II of the MHC turned out to have only two alleles. While one allele is ibex specific the other one is a domestic goat allele, suggesting rare hybridization events. These results are important for understanding the genetic effects in small ungulate populations and give valuable information for future reintroductions.

Bishop JM (1, 2), Leslie AJ (1), Bourquin S (1), O'Ryan C (2)

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(2) Evolutionary Genetics Group, Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa

The negative effects of fluctuating population size on *Ne*: past exploitation and the implications for current sustainable harvesting of the Nile crocodile

Overexploitation of wildlife resources during the last few centuries has had immense effects on ecosystems, increasing the rate of both population decline and species extinction. The Okavango River supports a population of Nile crocodiles that was repeatedly exploited by hide hunters in the 20th century; records indicate that ~50 000 adults were removed, resulting in repeated cycles of population collapse. In this study we investigated whether life history traits inherent to Nile crocodiles, namely being a long-lived species with overlapping generations, have buffered the negative genetic effects expected of repeated population collapse. We found that while Okavango crocodiles are characterised by moderate levels of genetic diversity at microsatellite loci (*Hobs*=0.51), the population has undergone a five-fold decrease in effective size (*Ne*=480 to 90 over 4-6 generations) despite its clear demographic recovery. In light of CITES quotas that permit the ongoing removal of both egg clutches and breeding females to stock crocodile farms, we explored the prospect of maintaining extant levels of genetic diversity by means of simulation. Results indicate that, even with a stable effective size, genetic diversity will be lost; ~26% of existing allelic diversity will be lost after 200 years/~15 generations, suggesting that the possibility of genetic recovery of this population is further diminished by its continued exploitation. We conclude that while the presence of a pool of juvenile individuals may buffer the effects of population decline in long-lived species, these very traits may mask the reality of diminishing effective size and impede management for long-term persistence.

Boitani L, Ciucci P

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Detecting and managing hybridization: implications for conservation policy.

Hybridization occurs naturally across many taxa and in a variety of geographic and ecological contexts and genetic markers are used to detect and study hybridization processes at individual, population and species levels. Conservation concerns are raised when rare or endangered species (or ESU) are threatened by hybridization with common species, particularly with domestic species. Notable examples are found in canid (wolves, dogs, coyotes, etc) populations that show cases of hybridization and introgression in different areas of the world. In this review, we focus on the conservation and management implications of hybridization. The first major challenge is to detect hybridization in time before it produces extensive introgression and to assess its actual and potential impact on large temporal and spatial scales; technical difficulties and uncertainties still exist when hybridization involves closely related 'species' (e.g., domestic and wild cat, dog and wolf, pig and wild boar). The second major problem is how to manage a population where hybridization occurs: action depends on the on the spatial and temporal extent of the problem, but most proposed techniques are logistically difficult, most often 'politically incorrect', and surely socially unacceptable. Hybrid animals are often elusive to laws as they are not listed for conservation or control, their damages are not compensated, their selective control is often impossible. However, hybrids animals often provide the same ecological functions (e.g., predation) of their wild ancestors and may be protected for their services. We conclude with a plea for closer integration of genetics, ecology and ethology in guiding management of hybrid animals.

Broquet T, Jaquiéry J, Hirzel AH, Yearsley J, Perrin N

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Inferring landscape effects on dispersal from genetic data: how far can we go?

Dispersal plays a key role in determining the demographic and genetic future of fragmented populations because it governs the isolation of local demes. This is especially true when population sizes are small because demographic stochasticity and the loss of diversity induced by genetic drift will be important factors determining the extinction risk of isolated populations. Therefore, dispersal behaviour of threatened species needs to be understood in order to develop efficient conservation policies. More specifically, inferring the effect of environmental features on dispersal is a highly relevant but problematic issue. Such inferences have recently been attempted using genetic-based methods (broadly grouped under the term 'landscape genetics'). These methods lack a clear statistical framework, and their power and accuracy are unknown. We used stochastic simulations of a fragmented population to investigate several of these statistical methods. Our results show that the performance of these methods is highly dependent upon both the absolute and relative effects of each landscape feature (i.e. the strength of a dispersal barrier). The resolution of the landscape (i.e. the number of distinct landscape

features and their spatial scale) also appears to be important. The highest success occurs when a small number of broad classes are considered. Finally, these methods appeared to be fairly robust when not all individuals within a population are sampled. These methods give insight into the biology of a species in simple situations, but their applicability in complex scenarios needs to be improved.

Bruford MW

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From data to policy: setting the agenda for research, management and legislation in conservation genetics in the UK

Despite the fact that conservation genetics is a maturing discipline and that the links between genetic diversity and biodiversity resilience are well established, the relationship between science and policy remains obscure in many cases. In contrast to the US, where the Endangered Species Act makes explicit reference to genetically-informed units for conservation, the situation in Europe and elsewhere is less developed, with research data being translated into policy on a largely ad hoc basis. In the UK, the government's Joint Nature Conservation Committee's Biodiversity Research Advisory Group (BRAG) recently formed a Conservation Genetics Subgroup (www.jncc.gov.uk/page-3912) to address the question the role of genetic data on the focus and shape of UK biodiversity conservation policy over the foreseeable future. Although some of the issues covered by this group are specific to the UK, many others are general issues and identify priorities which are relevant in most if not all countries. Using examples from the literature and our own work, I will seek to highlight some of the issues facing conservation geneticists attempting to feed their results into policy in a UK and European context that were identified as a result of the BRAG exercise.

Caballero A, Quesada H, Rolán-Alvarez E

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Impact of homoplasy of AFLP markers on the estimation of genetic diversity and the detection of selective loci

AFLP markers are becoming one of the most popular tools for the genetic analysis in the fields of evolutionary genetics and ecology and conservation of genetic resources. The technique combines a high information content and fidelity, with the possibility of carrying out genome-wide scans. However, a possible problem with this technique is the lack of homology of bands with the same electrophoretic mobility, what is known as homoplasy for the length of fragments. This implies that fragments of the same size may correspond to more than one locus and, therefore, the inferences obtained from the bands can be difficult to interpret. We carried out a theoretical analysis aimed at quantifying the impact of AFLP homoplasy on the estimation of within and between population genetic diversity and the detection of selective loci in a model of a structured finite population with migration among subpopulations.

The results indicate that the biases produced by homoplasy are an overestimation of the frequency of the most common allele, an underestimation of the degree of differentiation between subpopulations, and an overestimation or underestimation of the heterozygosis, depending on the allelic frequency of the markers. The homoplasy also reduces the efficiency of the current methods to detect selective genes from the comparison between genetic differentiation and heterozygosis. However, the biases are reduced quickly by reducing the number of fragments analysed per primer combination, such that with 100 fragments or less per combination the biases become negligible.

DeSalle R

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The Role of DNA Barcoding in Conservation Genetics

Recent excitement over the potential for DNA barcoding in modern biology has included statements that the conservation biology community would benefit from this approach. This talk will examine this premise from two perspectives. First, the nuances of barcoding from the perspective of species identification and species discovery will be discussed. Next case studies of the application of barcoding approaches to conservation problems will be presented and their efficacy with respect to conservation efforts will be discussed.

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Applying new approaches to assess intra- and inter-specific genetic diversity of an upper Amazonian frog

Describing population genetic diversity is fundamentally based on how the sample population is delineated, the estimators used to summarize diversity, and the assumptions inherent in those methods. In this study we assess the patterns of genetic diversity of a neotropical leaflitter frog, *Eleutherodactylus ockendeni*, in the megadiverse upper Amazon of Ecuador without *a priori* population delineation. We mapped the location of each collected frog across a 5.4 by 1 km primary and secondary forested landscape, genotyped 185 individuals using species-specific DNA microsatellites, and sequenced a fragment of mitochondrial cytochrome b for a subset of individuals (n = 51). The microsatellites were characterised by high allelic diversity and homozygote excess across all loci, suggesting the sample is not a panmictic population. We conclude that the lack of panmixia is not attributable to null alleles, inbreeding, or a Wahlund effect caused by discrete population clusters. Using distance-based estimators such as multivariate spatial autocorrelation, kinship coefficients, and relatedness coefficients, we assess

inter-individual genetic distances and identify a continuous isolation by distance population structure. MtDNA corroborates the observation of high genetic diversity at fine scales with multiple haplotypes within the sample locality. In a tangential project at higher spatial scales, microsatellite markers and mtDNA sequences indicate the presence of previously undescribed but sympatric cryptic species of *E. ockendeni*. Our findings suggest that the renowned Amazonian biodiversity of *Eleutherodactylus* may be characterized by high intraspecific and inter-specific genetic diversity even at small geographic scales.

Enríquez-Paredes LM (1), De la Rosa-Vélez J(1), Taylor BL (2), Gendron D (3)

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Reassessing pre-whaling stocks: genetics matches logbook records

Although whaling is known to have reduced most large cetacean populations, historical abundance estimates, based on catch records, remain controversial. In 2003, Roman & Palumbi (Science 301:508-510) used genetic data to estimate historical abundances that can differ by an order of magnitude from demographic back-calculation estimates, which are based on whaling data. For example, back-calculated estimate for fin whales in the North Atlantic is 40,000 compared to their estimate of 360,000; a figure that challenges the practice of using the back-calculated estimates in current management. We present an alternate molecular approach to estimate historical population size which, by incorporating two demographic-history footprints derived from phylogeny reconstruction (number of haplotypes for each lineage and an intraspecific/intrapopulation mutation rate), matches back-calculated estimates closely. We first calculated genetic diversity as the mean nucleotide diversity times the number of haplotypes occurring in a population. We then calculated the mutation rate for each population as the mean number of substitutions since the time of the most recent common ancestor (TMRCA) for all haplotypes, which was recovered from the phylogenetic tree topology. This method estimates 41,960 fins (95% C.I. 27,891 – 58,722) for the North Atlantic prior to commercial whaling. To test the method we also estimated historical population size in two isolated populations of fin whales that were not extensively hunted and, as expected, these results were close to current abundances. If validated, our estimates could set a more confident baseline to evaluate recovery levels for whale stocks and, consequently, to improve management schemes.

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An apparent paradox: high inbreeding in small populations does not result in strong population differentiation or low genetic diversity.

Decreased gene flow between small, subdivided populations often leads to increased inbreeding and decreased genetic diversity. Unlike most previous studies in population genetics of subdivided populations, this study examines a species which has evolved in naturally patchy and successionally stable habitats. We used microsatellite markers to examine the population genetics of an alpine grouse species, white-tailed ptarmigan (*Lagopus leucura*) on Vancouver Island, British Columbia, Canada. All populations demonstrated high levels of diversity (mean HE = 0.78) combined with high FIS values (significant heterozygote deficiencies) across all 10 loci. Analysis of population subdivision revealed only weak genetic structure among populations. An apparent paradox exists in that high inbreeding within populations did not result in strong genetic differentiation among populations or decreased genetic diversity. Two scenarios could potentially explain these unique patterns. First, sampling may have captured a snapshot of a group of populations progressing towards severe isolation; suggesting that population isolation may have existed long enough to increase inbreeding, but not long enough for drift to result in strong differentiation and decreased diversity. The second scenario evokes a pattern of infrequent dispersal between populations sufficient to maintain high levels of diversity, combined with low densities and limited mate choice resulting in the relatively quick accumulation of homozygosity within populations.

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Gene flow, dispersal patterns, and metapopulation dynamics of alpine Vancouver Island white-tailed ptarmigan inferred from microsatellite DNA markers and direct observations.

The dispersal of individuals among populations affects the demographic and adaptive trajectories of animal populations and is fundamental to understanding population dynamics. White-tailed ptarmigan (*Lagopus leucura*) are a high elevation grouse species that live year-round in patchily distributed alpine areas. In this study we investigate the patterns of dispersal and identify barriers to gene flow for a threatened sub-species, the Vancouver Island white-tailed ptarmigan (*L. l. saxatilis*). Population connectivity among seven populations was examined using 10 microsatellite loci and direct movement observations using radio-telemetry. The results showed low, but significant genetic differentiation between most populations. Direct and genetic estimates of dispersal suggested limited gene flow among populations. The stepping-stone model of dispersal was the most likely explanation for patterns of dispersal in Vancouver Island white-tailed ptarmigan. Analysis of molecular data also showed a generally consistent pattern of isolation by distance. However, large areas of unsuitable low

elevation habitat might act as barriers to gene flow. We conclude that white-tailed ptarmigan dispersal and population rescue strategies function as tenuous buffers against the threat of extinction.

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Loss of genetic diversity in historical European wolf (*Canis lupus*) populations

Loss of genetic diversity in small, isolated populations reduces the ability to adapt to environmental changes. Recent technical improvements in DNA extraction and amplification protocols make it possible to measure ancient levels of genetic diversity from the historical DNA record. This gives us a unique opportunity to detect genetic loss of diversity. Formerly widespread throughout Europe, the wolf (*Canis lupus*) has declined continuously over the last few centuries due to human persecution and alteration of the ecosystem. A massive decline has occurred across the species' former range, such that today almost all populations in western Europe have been eradicated. Mitochondrial (mt) DNA studies have suggested a general loss of genetic variability in remnant populations, possibly due to random genetic drift. In order to compare contemporary vs. historical levels of genetic diversity, we analysed mtDNA sequence data from 170 wolf specimens collected in several European museums. Almost all samples are from the 19th century and cover most of the species' former range. The results show an overall loss of genetic diversity in contemporary European populations. Compared to the less perturbed populations of eastern Europe, the genetic diversity of western European populations shows a massive decline (40% reduction in H). These results provide a direct measure of the rate at which genetic diversity has been lost in this species, and allow inferences about historical processes and arguments for the preservation of biodiversity.

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Identifying the environmental factors that influence population processes using genetic and environmental data.

Statistical genetics methods are frequently used to estimate population parameters (e.g. growth and migration rates) using only the information contained in the genetic samples. The results are then interpreted by looking at the spatial patterns observed for environmental variables that are considered important for the species under study. This approach, although valid, does not make use of all the information available in an efficient and rigorous way. In my lecture I will explain how the hierarchical Bayesian approach combined with MCMC methods can help develop methods that combine all sources of information available. I will explain some methodological aspects using as examples three different statistical methods and will then present the results of two analyses of real data sets, the first one

from the subterranean rodent *Ctenomys australis* and the second one from herring populations inhabiting the North and Baltic seas. These two examples illustrate the extent to which Bayesian methods can help us to better understand complex evolutionary and ecological scenarios.

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High genetic diversity identifies a nesting population of *Caretta caretta* in continental Italy as a relevant Management Unit

The main Italian nesting ground for loggerhead turtles *Caretta caretta* in Calabria, Italy, was monitored from 2000 to 2006. Non-invasive sampling was performed on non-hatched eggs, egg remains and dead hatchlings, for a total of 2,471 specimens. We sequenced the mtDNA D-loop in one individual per nest. Out of 26 sequences, haplotype CC-A2 was the most common (65%) as in other Mediterranean rookeries, followed by CC-A20 (31%) and CC-A31 (4%). CC-A20 may indicate female-driven gene flow with Atlantic populations. However, this must be compared with the low frequency of CC-A1 and the possibility of an independent origin for the Calabrian haplotype CC-A20 (C172T as compared to CC-A2). CC-A31 was not found in Mediterranean nesting sites and only once in juveniles from South-eastern Italy. Haplotype diversity (0.50) equals that of Turkish rookeries and, therefore, represents one of the highest levels in the Mediterranean. Haplotype composition differentiates Calabria from the well-studied rookeries of Greece, Cyprus and Turkey (Φst= 0.15, 0.29 and 0.32, respectively). These data identify the Calabrian nesting population as a relevant source of diversity, characteristic of the central Mediterranean and distinct from both eastern and southern colonies. We also developed multiplex fluorescent PCR reactions to type the dinucleotide microsatellites CC7, CC141 and CCM2. These are used to identify a possible genetic contribution by multiple males to the same clutch and to identify the genotype of females returning to nest in the area on the same or subsequent years. Our data urge the recognition of the loggerhead population of Calabria as a relevant management unit for conservation purposes.

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The diversification of land iguanas from the Galápagos Islands: a new form of iguana traces the origin of the modern Galápagos land iguanas

The land iguanas (genus *Conolophus*) of the Galápagos Islands are among the most significant representatives of the fauna from that archipelago. Their evolution consists primarily of a history of extinctions and recolonizations, most of which left few traces in the fossil record and in the genes of the remnant populations. We used a 2.2kb sequence of mtDNA and nine microsatellite DNA loci to investigate patterns of genetic diversification and times of divergence between all extant populations. We also included the genetic analyses of a rare form of land iguana (that we refer to as *rosada*) from the Wolf Volcano on Isabela island. Both mtDNA and microsatellites indicated major differentiation between western and central islands. MtDNA data indicated that the current patterns and levels of genetic differentiation are not completely consistent with the age of the presently emerged islands. At the base of the evolution and diversification of the Galápagos land iguanas, stem two lineages: the first led to the lineages of the extant land iguanas, while the second led only to the *rosada* form. The estimated time of such a divergence event predated the age of the oldest extant island. Despite the remarkable diversification found in the mtDNA haplotypes, microsatellite data indicate possible incomplete genetic isolation due to occasional introgression between the *rosada* and the syntopic yellow form. An alternative scenario that proposes a recent diversification of the *rosada* from populations that already had colonized Isabela was not supported by the analysis.

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Iberian lynx conservation genetics

Continuous range contraction, fragmentation and population declines during the last century have ultimately led to the cataloguing of Iberian lynx as critically endangered [CR C2a(i)] by the IUCN. The only two remnant populations of Doñana and Sierra Morena, harbor less than 50 and 160 individuals, respectively, and are effectively isolated from each other. We have analysed contemporary genetic variation in Iberian lynx for mitochondrial and microsatellite markers. A high level of genetic differentiation is observed between the two extant Doñana and Sierra Morena populations. Mitochondrial diversity is extremely low with only two haplotypes observed which differ at only one position. Overall, nuclear microsatellite diversity levels appear to be low when compared to other felid species, and differ dramatically between populations, with Doñana showing about 30% lower diversity than Sierra Morena, both for expected heterozyosity and allelic diversity. A signal for a recent demographic bottleneck is evident in the data for both populations. Observed genetic patterns are consistent with the predominance of genetic drift in recent times affecting both populations, but more intensely Doñana, where the magnitude and rate of accumulation of inbreeding could be severely affecting population viability. Risks of inbreeding depression and loss of genetic diversity, indirect evidences for recent historical connection, and absence of evidence for adaptive divergence between populations call for the genetic management of the

species as a single unit and recommend the translocation into Doñana of wild or captive-born individuals with ancestry in Sierra Morena. Genetic patterns are also being used to guide the management of the *ex-situ* conservation program through the estimation of co-ancestry between founders.

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MHC versus microsatellite diversity in spotted suslik *Spermophilus suslicus* populations from two different parts of its range.

Fragmentation of populations leads to depletion of genetic variation but balancing selection can, to some degree, prevent this process. Specifically, highly polymorphic MHC genes, coding for proteins crucial in vertebrate immune response, are thought to be under balancing selection and therefore retain some degree of polymorphism despite population bottlenecks. We compared genetic variation in MHC DRB genes and microsatellite loci in spotted suslik populations located in two different parts of its range. We tested genetic variation in 10 populations severely affected by habitat loss and size reductions and located at the western part of the species range. They were compared with 4 populations from the eastern part of the range, occupying habitat with higher levels of connectivity. To reveal whether MHC is under selection from parasites in fragmented populations, we also examined the prevalence and intensity of gut parasites in the isolated populations. MHC DRB in the suslik was polymorphic and highly divergent, and there is evidence of positive selection acting in the past, indicated by an excess of non-synonymous substitutions, suggesting that selection is a result of parasite pressure. In contrast to microsatellite results, an AMOVA did not reveal differences in MHC allele composition between Eastern and Western populations. This may indicate that similar selection pressures maintained MHC variation in both regions. However, in agreement with the microsatellite analysis, western populations show a much stronger population structure for MHC and considerably lower allelic richness, reflecting the impact of the recent population fragmentation. Nonetheless we have found that Spsu-DRB*03 allele is associated with susceptibility to nematode infection.

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Genetic connectivity of coral reef animals across the Indo-Malay Archipelago: implications for the conservation of marine ornamentals

Indonesia is the world's number one exporter of fish ornamentals. Therefore, baseline data for management and sustainable use of these living marine resources are needed. Data on connectivity of populations are necessary to identify populations that export larvae to areas of exploitation. Knowledge about sources and sinks of larvae is a prerequisite for sustainable use of living marine resources. However, a comprehensive multi species study on the genetic structure of many different groups of coral reef dwelling animals across in this region is missing. In the framework of the German-Indonesian research programme SPICE, a study on the genetic structure of different taxonomic groups, such as fish, giant clams, starfish, snails, anemones, corals, and ascidians was initiated. Samples were taken at more than 20 sites across the Indonesian Archipelago, as well as Malaysia, Philippines, Taiwan and Okinawa. Even though the life histories of various sampled species are very different, the genetic population structure of the clownfishes *Amphiprion ocellaris* and *A. clarkii* as well as the giant clams *Tridacna crocea* and *T. maxima* are quite similar. $\square_{st}$-values for *A. ocellaris*, *A. clarkii*, *T. maxima*, and *T. crocea* are high and significant, indicating limited gene flow between populations. Even though the analysis of the blue startfish *Linckia laevigata* revealed a very shallow genetic structure, the $\square_{st}$-value was significant. However, its ectoparasitic snail *Thyca crystallina* does not show limited gene flow within the Indo-Malay Archipelago, indicated panmixing. Such data are important for defining stocks and management units, as well as the size and spatial distribution of marine protected areas.

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Evolution of population structure in social cetaceans

Many of the delphinid cetacean species (dolphins) live in social groups of varying permanence. Two of the most stable social societies are found among killer whales and bottlenose dolphins, and these species are the focus of this presentation. The similarities include the existence of long-term individual bonds, predictable local ranging behaviour and specialisation on food resources. Both species show fission/ fusion associations to some extent and dynamics of social group formation over time, but this is the primary distinction between social groups in the two species, with killer whales showing the most coherent long-term social group affiliations. Both species show genetic differentiation among populations over a much finer geographic scale than represented by their known capacity for dispersal. For example killer whale pods have been tracked over thousands of km in a single season, but populations with summer foraging ranges in proximate areas (such as in the eastern North Pacific) are highly differentiated. Population boundaries in both species are consistent with oceanic habitat boundaries that could affect the distribution of their prey, and MHC markers reveal possible local selective environments. Coalescent models suggest ongoing gene flow at a low level, and the pattern of variation among populations is often consistent with founder events establishing local populations, especially in near-shore habitat. Overall, the application of

various genetic markers has suggested a pattern of isolation and differentiation driven largely by dependence on local habitat and resources, likely facilitated by social group affiliations.

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Extreme genetic divergence between remnant wild populations of the Atlantic Forest endemic sloth (*Bradypus torquatus*).

The maned sloth (*Bradypus torquatus*) is a poorly known endangered species endemic to the Atlantic Forest that inhabits remaining forest fragments in four Brazilian states. We analyzed 70 samples from the largest remnant wild populations to characterize their geographic structure and to produce genetic diversity estimates. The analysis of mtDNA haplotypes indicated that the remnant populations are reproductively isolated and extremely divergent. There were no shared haplotypes among populations and hierarchical grouping analysis indicated a high degree of differentiation ($\square_{ST}$ > 0.95). Phylogeographic analysis evidenced an extremely discontinuous distribution, with divergent haplotype clusters specific to different geographical regions. Phylogenetic analysis indicated that all populations may be derived from an ancestral northern population. The analyses also indicated allopatric fragmentation as the main factor shaping the observed pattern of divergence between northern and southern clades. The existence of deeply divergent clades in this species is likely to be related to climatic and vegetation changes during the Pleistocene, and justifies their designation as at least two independent evolutionary units. The analyses also indicate that populations separated by more than 100 km should be considered different management units. Thus, current devastation of primary remains of the Atlantic Forest Biome is responsible for an unrecoverable loss of genetic diversity in this species. These conclusions should direct management actions undertaken in order to preserve the evolutionary distinctiveness of each evolutionary unit, as well as to preserve the demographic distinctiveness of the different management units.

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Juvenile loggerhead turtles foraging in the Mediterranean Sea: regional variability in the contribution from the Atlantic rookeries

The loggerhead turtle, *Caretta caretta*, spends the greater part of its life in large foraging aggregations that may combine individuals from several demographically independent populations. Determining which rookeries contribute to a particular feeding ground is essential to evaluate the effects of exploitation or of management actions, especially when potential source populations differ substantially in conservation outlook. In the Mediterranean Sea, loggerheads from local rookeries are known to be joined by juveniles

from larger and more stable Atlantic populations in regional feeding habitats but little is known about the pattern of mixing of these populations, particularly in the eastern basin. Using mitochondrial DNA markers and Bayesian analysis, we investigated the demographic structure of loggerhead feeding aggregations (N=240) at five Mediterranean locations (Sardinia, mainland Italy, Tunisia, Libya and Israel) and compared these samples to previously analysed Mediterranean feeding habitats. Significant regional population structure was detected ($\square_{st}$.= 0.21, P = 0.000) with loggerheads from Mediterranean nesting populations dominating the composition of feeding aggregations along European and African continental coasts, whereas Atlantic juveniles were predominant in Mediterranean pelagic habitats. These results are consistent with the natal homing behaviour of juvenile loggerheads that preferentially recruit to coastal habitats in the vicinity of their natal rookery. This must be considered in the formulation of effective conservation plans, since long-line fishery and other offshore activities mainly impact the Atlantic stock, while coastal fisheries and recreational activities almost exclusively affect the smaller and more vulnerable local populations.

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Estimating demographic parameters using composite likelihood methods

The nature of population genetic data is rapidly changing. While most current and previous studies are based on a limited number of loci, it is now feasible to generate data from thousands of loci using SNP genotyping or whole-genome re-sequencing. Full Bayesian and maximum likelihood methods are not directly applicable to this data. Composite likelihood methods which combine individual likelihood functions in an estimation procedure can instead be used. I will discuss some examples of this approach and discuss statistical aspects of the composite likelihood estimators.

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Post-glacial history of Arctic charr (*Salvelinus alpinus*) populations inferred by AFLP neutral markers and mtDNA analysis

Arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), is a species of the Salmonidae family characterised by varying degrees of anadromy and an extremely variable morphology. *S. alpinus* has an Holarctic distribution and, being adapted to cold-water habitats, is widespread at very high latitudes and altitudes. During Pleistocene glaciations, Arctic charr populations underwent repeated changes in distribution and density due to the impact of the ice

cover extension, which led to repeated isolation and bottleneck events. Previous studies described genetic variation within the species by means of mitochondrial molecular markers or microsatellites, revealing low levels of variability in the former case, but high genetic diversity, both within and among populations, in the latter. In this study we characterised 40 Arctic charr populations (537 total specimens) from the Alps and Fennoscandia using almost 100 AFLP polymorphisms and sequences of the mitochondrial D-Loop region. Relationships among individuals were estimated by Factorial Analysis of Correspondence, while the relationships between populations were evaluated by Reynolds genetic distances and plotted by Principal Component Analysis. The Bayesian-based clustering approach implemented in the software Structure was used to investigate the hidden population genetic structure. A Median Network was constructed to evaluate the relationships among the mitochondrial haplotypes. In most cases, individuals of the same population clustered together and different populations were subdivided by the first and second principal components according to geographic origin. As expected, some populations showed a very low level of polymorphism, whose probably caused by severe bottlenecks or strong anthropic impact in the last decades.

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Strong genetic population structure and limited larval dispersal distance in the giant clams *Tridacna crocea* and *Tridacna maxima* across the Indo-Malay Archipelago

The Indo-Malay Archipelago hosts the world's greatest diversity of marine species. However, studies of the genetic population structure and gene flow of marine organisms within the Indo-Malay Archipelago are rather rare. This study aims to investigate the genetic population structure of the giant clams *T. crocea* and *T. maxima*. The analyses are based on a 456 bp and 484 bp fragment of the cytochrome oxidase I gene from 300 individuals of *T. crocea* and 211 individuals of *T. maxima*, respectively. Samples were collected from 15 localities across the Indo-Malay Archipelago. *T. crocea* and *T. maxima* show a very strong genetic population structure and isolation by distance, indicating restricted gene flow between almost all sample sites. The observed $\square_{st}$-value of 0.28 (*T. crocea*) and 0.72 (*T. maxima*) is very high compared to other studies on giant clams. According to the pronounced genetic differences among *T. crocea* populations, the sample sites can be divided into five groups from West to East: (1) Padang (Indian Ocean), (2) Java Sea, (3) Makassar Strait (including northern Borneo), (4) eastern coast of Sulawesi (including Sembilan, Komodo, and Kupang), and (5) Biak (Western Pacific). In *T. maxima*, the sample sites can be assigned to three different regions: (1) Padang and Java Sea, (2) Sulawesi (including Komodo and Kupang), and (3) Biak (West Pacific). These complex genetic population structures and patterns of connectivity, characterised by restricted gene flow between some sites

and panmixing between others can be attributed to the geological history and prevailing current regimes in the Indo-Malay Archipelago.

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Hitch-hiking mapping for identifying functionally important genetic variation: examples in Atlantic salmon, *Salmo salar*

The identification of functionally important genetic variation underlying phenotypic traits of ecological and evolutionary importance has received considerable attention in the literature recently. Such studies are also highly relevant for conservation biology, as knowledge of the genetic basis of e.g. local adaptation would assist the preservation of functionally important genetic diversity. Numerous methods are available for identifying genes potentially underlying a certain phenotypic trait. One approach which has been applied quite commonly is the hitchhiking mapping or 'genome scan' approach, whereby large numbers of markers are genotyped in sets of individuals taken from one or more populations or species. Genomic regions or 'outlier loci' that exhibit patterns of variation that deviate from the rest of the genome, are thereby flagged as candidates for being potentially affected by the forces of selection. Hitchhiking mapping is generally considered as being a rather indirect method for identifying functionally important genetic variation. However, the recent expansion of genome projects to a more phylogenetically diverse range of taxa enables genome scan approaches which assess variation of potential functional importance more directly through the analysis of e.g. EST-linked microsatellites and coding sequence SNPs. This talk will present the results of genome scans which utilise recently developed Atlantic salmon genomic resources to identify genomic regions potentially affected by natural selection in Altantic salmon populations. The relevance of the findings from a conservation perspective will also be discussed.

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Indian tigers retain more than their share of genetic variation

Around 5000 tigers remain in the wild, necessitating their conservation and management. Scientific conservation efforts thus far have focused on a better understanding tiger ecology. A recent phylogeographic study revealed six subspecies of tigers, with moderate levels of genetic variation. Given that around 50% of the worlds tigers are in the Indian subcontinent, we decided to further investigate the genetic variation in this region. We sampled 25 wild individuals from across the Indian subcontinent. Sequence data from 750bp reveal much higher levels of genetic variation within India than previously suggested. When compared to tigers outside of India, we found that about 70% of all genetic variation was within the Indian subcontinent. The additional diversity we discovered was because we

collected non-invasive samples across tiger populations in India, unlike the previous study where sampling was geographically restricted. In terms of conservation, our results emphasize the conservation status of *Panthera tigris tigris*, the Bengal tiger. This result is especially relevant given that less than 11% of the remaining global tiger habitat is currently occupied by the Bengal tiger. Our results also hint at high levels of pre-exploitation genetic diversity, which we hope to explore using historical skin samples in future research.

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Genetic diversity in grouse - what does it mean for conservation?

Population fragmentation and isolation have extremely detrimental effects both on the fitness and viability of extant populations, and also the evolutionary potential of the species. Population genetic structure is predicted to affect population persistence and long-term survival where small and isolated populations run a higher risk of extinction. Most studies that have attempted to monitor genetic diversity within and among threatened populations have used so-called neutral genetic markers to quantify variation. These markers are excellent for estimating effective population size, migration rates and other population genetic processes since, on the whole, they are not affected by selection. I am using grouse as a model system to investigate how lowered genetic variability in grouse populations is affected by both population size and isolation, and to estimate the potential impact of both of these factors. I compare populations from within a continuous distribution (e.g. boreal landscape) with contiguous populations (e.g. Alps) and recently isolated populations to infer the effect of habitat fragmentation on genetic diversity. I discuss whether heterozygosity is a useful marker to describe recent population decline, and will demonstrate how the results of non-invasive genetic studies can be used to identify conservation priorities at multiple spatial scales. I will also highlight the importance of sex-specific analyses when characterizing potential barriers to gene flow and illustrate how management strategies can be improved when spatial information is incorporated into the analyses of genetic structure.

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Conservation genetics of chum salmon *Oncorhynchus keta*

We present the results of mtDNA analyses in natural, artificial, and mixed populations of chum salmon from the rivers of the Sea of Japan and the Okhotsk Sea, and in chum salmon seasonal races of the Amur. A significant level of polymorphism among the majority of the populations studied was detected, except for the Sakhalin populations, where the absence of genetic differences might be caused by the transportation of eggs among the fish hatcheries located on those rivers. The effect of such genetic homogenizing and the reduction of genetic variability has previously been shown in chum

salmon populations of the south Primorye. The groups of chum salmon from the Sea of Japan and the Okhotsk Sea displayed the most pronounced differences. The analyses of their genetic variability demonstrated that periodic paleogeological and climatic changes in the past were the most probable factor that caused the divergence of these groups. The ocean level regressions and transgressions might have caused isolation of chum salmon in the refugia belonging hypothetically to the paleo-Shuifen and paleo-Amur river systems. Genetic differences between the seasonal races of the Amur chum salmon are insignificant. Most of the east Sakhalin populations appeared to be genetically close to the late Amur chum salmon, which corresponds to the conception that Amur and Sakhalin rivers used to compose a common river system, the paleo-Amur. The early chum salmon is close to the north Okhotsk Sea populations.

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Genes & Health: The Role of Immune Gene Variability (MHC) in Conservation

Current discussions in evolutionary ecology and conservation genetics focus on the relative importance of using selective neutral markers or markers of coding genes to identify adaptive and evolutionary relevant processes. Adaptive variation is of interest because it simultaneously reflects historical evolution and determines the population's future phenotypic response to evolutionary processes, conferring the basis for adaptation to environmental changes. In vertebrates, growing evidence suggests that genetic diversity is particularly important at the level of the major histocompatibility complex (MHC) because its gene products play an important role in immune functions. The central role of the major histocompatibility complex in terms of pathogen defense is indisputable. The remarkable MHC diversity found in most vertebrate studies to date is a result of diversifying selection acting on this gene complex. However, there are also examples of species with low MHC diversity, and potential causes and consequences have been controversially discussed. On one hand it has been hypothesized that exposure to high pathogen diversity leads to increased MHC variation, whereas low pathogen richness leads to reduced selective pressure on MHC diversity. On the other hand it is well known that fragmentation of habitats often prevents migration and gene exchange among populations which might cause a reduced genetic diversity and parasite resistance. In addition, differences in mating systems and bottleneck effects might explain the differing levels of genetic variation. These contrasting pressures and processes raise the hot-debated question: what causes low MHC-diversity observed in some species and what are the potential consequences? Here, I summarise our investigations on the importance of overall genetic variability as well as adaptive MHC-diversity in parasite resistance as well as the genetic and fitness effects of fragmentation or degradation in numerous free-ranging animal populations (primates, carnivora, rodents, marsupials) with different levels of genetic diversity.

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Non-invasive genetic sampling

Non-invasive sampling allows genetic studies of free-ranging animals without the need to capture or even observe them, and thus allows questions to be addressed that cannot be answered using conventional methods. Most of the time, DNA is extracted from hair or feces. In the early nineties, this sampling strategy promised to exploit fully the existing DNA-based technology for studies in ethology, conservation biology and population genetics. However, it appeared that non-invasive sampling suffers from genotyping errors. Despite this, many of the difficulties of non-invasive sampling have been overcome with improved methodology, including the implementation of appropriate pilot studies. After a brief historical presentation of the non-invasive genetic sampling, the technical difficulties will be analyzed in details. This concerns the design of the experimental protocol, the sampling in the field, the preservation of the sample, the DNA extraction, the genotyping, and the data analysis in the presence of genotyping errors. Each study is specific, and it is difficult to establish a sound experimental protocol without understanding the technical and theoretical difficulties linked to non-invasive sampling. Then a few landmark studies will be presented. Finally, new DNA-based perspectives will be examined, in the light of the most recent advances.

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Life-history and habitat features influence the within-river genetic structure of Atlantic salmon

Defining populations and identifying ecological and life-history characteristics affecting genetic structure is important for understanding species biology and hence, for managing threatened or endangered species or populations. In this study, populations of the world's largest indigenous Atlantic salmon (*Salmo salar*) stock were inferred using information from 29 microsatellite markers and model-based clustering methods, in order to identify the life-history and habitat variables best predicting the genetic diversity of these populations. This study revealed that natal homing of Atlantic salmon within the Teno River system is accurate at least to the tributary level. In general, defining populations by the nearest main tributary was found to be a reasonable approach in this large river system, whereas in the main stem of the river, the number of inferred populations was fewer than the number of distinct sampling sites. Population structure and variation in genetic diversity among populations were poorly explained by geographical distance. In contrast, age-structure, as estimated by the proportion of multi-sea-winter spawners, was the most predictive variable in explaining the variation in the genetic diversity of the populations. This observation, in agreement with theoretical predictions, emphasizes the importance of large, multi-sea-winter

females in maintaining the genetic diversity of populations. Our results show that despite this species' high capacity for migration, tributaries foster relatively closed populations with little gene flow, which is important to consider when developing management strategies for the system. To further study the genetic variation over time, we included two to five replicate samples (n=2021) spanning up to five generations from 14 sites in Teno and a neighbouring river Näätämö. Preliminary results of AMOVA imply a rather stable temporal genetic structure: 1% of genetic variation over time (~25 years), and 8.8% in space among tributary samples and 0.3 % and 0.7% in the mainstem populations, respectively. More detailed results of the evolutionary stability of population structure in the light of conservation and management will be discussed.

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Comparing and combining genome-widely developed single nucleotide markers and microsatellites for identification of hybrids between Greater and Lesser Spotted Eagles

Genetic identification of hybrid individuals is important in hybridisation studies, but choosing the appropriate set of markers may be complicated and allopatric reference samples of rare species hard to collect. In the current study we demonstrate the development of single nucleotide polymorphic markers (SNPs) for detection of hybrids between the threatened Greater and Lesser Spotted Eagles using individuals from a sympatric population, and compare the efficiency of SNPs with that of microsatellites. We amplified introns from 122 avian genes, obtained single-band PCR products from 87 introns, and sequenced 48 of them in eleven spotted eagles. Nine SNPs (eight of them detectable by restriction analysis) were selected and tested successfully in 65 sympatric and allopatric spotted eagles, and their hybrids, using different Bayesian model-based approaches. Only one out of twelve previously described avian nuclear intron markers showed significant species-specific allelic difference. Twenty microsatellites outperformed nine SNPs in species identification, but were poorer in hybrid detection, whereas the power of ten of the microsatellites remained too low for correct assignment. Combined use of nine SNPs and twenty microsatellites resulted in the most efficient and accurate identification of all individuals.

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Spatial genetic structure precipitates rapid bottleneck and founder effects in the patchily distributed Pacific jumping mouse

Exploring the nature of the loss of genetic diversity has been one of the central themes in conservation genetics. Generally, however, our understanding of this process is largely based upon theoretical definitions of a population as a completely interbreeding group of individuals. No spatial

variation is inherent in this idealized definition, yet many populations in nature display strong spatial genetic structuring. In this study, an unexpected mortality event, in the form of a 25-year flood, occurred during the course of a three year mark-recapture study of the riparian affiliated Pacific jumping mouse. Strong spatial genetic structure among individuals exists in this population and the occurrence of the flood allowed for the detailed characterization of the interaction between spatial genetic structuring and disturbance-induced mortality. Spatial survival patterns and microsatellite data revealed that extremely rapid bottleneck and founder effects were precipitated due to the interaction between spatial patterns of relatedness and the spatial nature of flood induced mortality. The results of this study demonstrate that within-population patterns among individuals can have strong impacts on genetic structure and diversity. Such dynamics should be considered in much greater detail by conservation geneticists and current and emerging technologies that can facilitate the integration of spatial data and population genetics will allow us to do so.

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Cross-species patterns of genetic diversity in forest-dependent taxa in Southeast Asia: implications for conservation of forest biodiversity

The tropics are rich in biodiversity, much of which is contained within tropical forests that are currently under huge threat. We have been studying the patterns of genetic diversity in forest-dependent species of mosquitoes and black flies in mainland Southeast Asia. Surprisingly consistent patterns across eleven species were noted: 1. Northwest Thailand/Myanmar/Yunnan has high levels of intra-specific genetic diversity; 2. Moving southwards and eastwards from this region there is reduced genetic diversity and signals of recent population expansion; 3. The presence of a suture zone on the Thai-Myanmar border. Since the species studied are dependent on forest (with shared patterns probably due to common responses to Pleistocene climatic and environmental change), it is likely that other forest-dependent taxa will have similar patterns of diversity. Our findings therefore have several important conservation implications since: areas of high genetic and species diversity should have higher conservation priority; the loss of genetically divergent lineages needs to be minimised; genetically distinct populations should form independent management units; and previously unknown, cryptic species need to be detected if they are to be conserved. We are therefore now determining if the patterns of genetic diversity found to date also occur in forest-dependent species of conservation concern. We have chosen to focus primarily on amphibians for several reasons: amphibians are some of the most vulnerable, endangered taxa; they are charismatic; they can serve as indicator species for forest biodiversity in general; and conservation efforts directed at frogs will indirectly conserve other forest-dependent biodiversity.

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Ancient DNA - Reconstructing the Past

In the past two decades, ancient DNA research has progressed from the retrieval of small fragments of mitochondrial DNA from a few late Holocene specimens, to large-scale studies of ancient populations, phenotypically important nuclear loci, and even large scale genomics of extinct species. However, the field is still regularly marred by erroneous reports, which underestimate the extent of contamination within laboratories and samples themselves. An improved understanding of these processes and the effects of damage on ancient DNA templates has started to provide a more robust basis for research. Recent methodological advances have included the characterization of Pleistocene mammal populations and discoveries of DNA preserved in ancient sediments and ice cores. Increasingly, ancient genetic information is providing a unique means to test assumptions used in geology, evolutionary and population genetics studies to reconstruct the past. With the advent and uptake of appropriate methodologies, ancient DNA is now positioned to become a powerful tool in biological research and is also evolving new and unexpected uses, such as in the search for extinct or extant life in the deep biosphere and on other planets.

ADDRESSES