

Program and Abstracts

1st Annual Meeting in Conservation Genetics

January 28-30, 2015

WSL, Birmensdorf, Switzerland

A photograph of a light-colored frog with dark spots, sitting on a pile of smooth, light-colored rocks. The frog is looking towards the left of the frame.

1st Annual Meeting in Conservation Genetics
WSL Birmensdorf, Zürich, Switzerland, January 28–30, 2015

Information on exciting and emerging conservation-genetic research
Platform for exchange among scientists, students, conservation managers and practitioners
Establishing a network for researchers in conservation genetics in Central Europe

Organizing committee:
Rolf Holderegger
Gernot Segelbacher
Axel Hochkirch
Janine Bolliger
Felix Gugerli
Alex Widmer
Frank Zachos
Niko Balkenhol

Keynotes: Mike Bruford
Lukas Keller

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Photo credit: Christoph Büchler

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Conference Program

Wednesday, January 28, 2015 (Engler- and Flury-Saal)

- 11.00 - 13.30 Registration at WSL main entrance
- 13.30 - 14.00 Rolf Holderegger, Gernot Segelbacher, Axel Hochkirch: Welcome address and opening remarks
- 14.00 - 15.00 **Keynote; Michael Bruford: Conservation genomics and the emerging policy landscape**
- 15.00 - 15.20 Gernot Segelbacher: Genetic diversity as an overlooked component in biodiversity conservation
- 15.20 - 15.50 **Coffee break**
- 15.50 - 16.10 Joscha Beninde: Refugia of the Anthropocene: multiple evolutionary lineages of wall lizards hybridize in German cities
- 16.10 - 16.30 Laura Bertola: Insights into the distribution of genetic diversity in the lion and implications for conservation
- 16.30 - 16.50 Cornelia Ebert: Implications based on non-invasive genetic population estimation: a case example of wild boar management in a protected area
- 16.50 - 17.10 Martin Fischer: Are microsatellite markers suitable for estimating genome-wide genetic diversity in a conservation context?
- 17.10 - 17.30 Božo Frajman: Does hybridisation with widespread *Knautia arvensis* threaten the long-term persistence of the Eastern Alpine rare local endemic *K. carinthiaca*?
- 17.30 - open **Welcome party (apéro riche; main entrance of WSL)**

Thursday, January 29, 2015 (Engler- and Flury-Saal)

- 09.00 - 09.20 Christine Grossen: Hybridization between domestic and wild animals: the case of a gene important for immunity
- 09.20 - 09.40 Jan Habel: Population signatures of large-scale, long-term disjunction and small-scale, short-term habitat fragmentation in an Afrotropical forest bird
- 09.40 - 10.00 Stefanie Hartmann: Can a genetic study save the endangered Pale-headed Brushfinch in Ecuador?
- 10.00 - 10.20 Sean Hoban: Improving the effectiveness and efficiency of conservation seed collections
- 10.20 - 10.50 **Coffee break**
- 10.50 - 11.10 Annette Kohlen: Monitoring and research of the European wildcat (*Felis silvestris silvestris*) in Baden-Wuerttemberg, Germany
- 11.10 - 11.30 Beatrice Nussberger: Quantifying introgression between European wildcats and domestic cats
- 11.30 - 11.50 Elvira Mächler: Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species

- 11.50 - 12.10 Sebastian Menke: Impact of land-use shifts on the gut-bacterial community of the Namibian black-backed jackal (*Canis mesomelas*)
- 12.10 - 12.30 Gilberto Pasinelli: Genetic diversity and differentiation in middle and great spotted woodpeckers
- 12.30 - 14.00 **Lunch at WSL cafeteria**
- 14.00 - 14.20 Andrea Pluess: Decreasing habitat suitability due to climate change: potential for "genetic rescue" in *Fagus sylvatica*
- 14.20 - 14.40 Tobias Reiners: Evaluation of reintroduction success using non-invasive genetic monitoring
- 14.40 - 17.30 **Extended poster session including coffee break at 15.30**

Friday, January 30, 2015 (Engler- and Flury-Saal)

- 09.00 - 10.00 **Keynote; Lukas Keller: The conservation genetics of reintroductions**
- 10.00 - 10.30 **Coffee break**
- 10.30 - 10.50 Estelle Rochat: Impact of the urbanization process on connectivity and genetic diversity - a spatially explicit simulation approach
- 10.50 - 11.10 Benedikt Schmidt: Using environmental DNA to detect aquatic species
- 11.10 - 11.30 Andrea Vaupel: Genetic exchange between populations of water frogs is predictable by landscape matrices and spatial arrangement of populations
- 11.30 - 10.50 Cristiano Vernesi: Ancient and recent demographic decline in the alpine yellow-bellied toad (*Bombina variegata*) populations: genetic variation pattern suggests that conservation actions can lead to recovery
- 11.50 - 12.00 Axel Hochkirch, Rolf Holderegger, Niko Balkenhol: Prize for the best contribution regarding conservation management, announcement of next meeting and concluding remarks

Abstracts

Keynotes

Keynote 1

Conservation genomics and the emerging policy landscape

Bruford, Michael W.

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As conservation genetics transitions into conservation genomics, a whole new series of scientific opportunities and challenges open up. Yet, despite the establishment of the Convention on Biological Diversity's Aichi Target 13, the link between increasingly complex use of genetics in conservation risks leaving policy makers behind. In this presentation, I will describe the recent political and policy-led developments in genetics for biodiversity conservation and attempt to evaluate how genomic data may be brought to bear in this context. I will describe recent initiatives at the European and IUCN level and discuss how genomic research and application in conservation prioritisation is currently developing and may develop in the future. Using our own work, I will also attempt to dissect the kinds of data that biodiversity policy makers are likely to need for the 2020 deadline and how this may (or may not) be supplied by the scientific community.

Keynote 2

The conservation genetics of reintroductions

Keller, Lukas

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Reintroduction is an important tool in conservation management, and many species have been successfully reintroduced. From a conservation genetic point of view, reintroductions often have substantial consequences in the form of genetic drift and increased inbreeding. In my talk, I will address the potential genetic consequences of reintroductions, and I will illustrate them with examples from a famous European reintroduction of the early 20th century, the Alpine ibex. I will conclude by discussing possible management actions that can be taken to reduce the undesirable genetic consequences of reintroductions.

Talks

Talk 1

Genetic diversity as an overlooked component in biodiversity conservation

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Genetic diversity is one of the key elements of biodiversity, where the Convention of Biological Diversity identified three key elements named genes, species and ecosystems. I will here review how national biodiversity strategies still neglect genetic diversity in their plans and discuss some possible solutions for this discrepancy. I will give an overview on recent activities in the field, introducing also the EU initiative ConGRESS (<http://www.congressgenetics.eu>) and the lessons learned from this project trying to link active conservationists and academic conservation geneticists. I will also present case studies, which demonstrate potential pitfalls and chances in the science-policy interface.

Talk 2

Refugia of the Anthropocene: multiple evolutionary lineages of wall lizards hybridize in German cities

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The common wall lizard (*Podarcis muralis*) is distributed mostly along the rivers Rhine, Neckar and Mosel in Germany and is often bound to anthropogenic habitats such as dry stonewalls or railway lines. In Germany, there are two native evolutionary lineages but, moreover, seven non-native and invasive evolutionary lineages have established here. Introduced mainly accidentally by railway traffic or intentionally by hobbyists, many introductions have occurred within city realms. We sampled four cities in Germany known to have a high density of the common wall lizard, two cities with only native lineages present and two cities inhabited by native and invasive lineages alike. We sequenced a mitochondrial gene (cytochrome B) to determine the evolutionary origin of individuals and analysed 17 microsatellite loci to define population membership. By analysing over 800 individuals in total (ca. 200 per city), we wanted to identify major differences between lineages concerning barriers to dispersal and pathways of gene transfer within cities. Combining sequences with a landscape genetic approach, we detected highly structured populations and located potential contact zones of still isolated populations. We detected a high degree of hybridization between lizards of different evolutionary origin. The degree of hybridization between native and invasive lineages poses a serious threat to the native lineage, and we hope to develop strategies to ensure its persistence, e.g., by developing strategic barriers to stop movement and subsequent hybridization.

Talk 3

Insights into the distribution of genetic diversity in the lion and implications for conservation

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Phylogeographic studies have shown that the genetic diversity of the African lion, is greater than implied by taxonomy, which only recognizes a single subspecies. Principally, the populations in West and Central Africa, are genetically distinct from their East and Southern African counterparts. The Asiatic subspecies of the lion, confined to a single population in India, has a nested position within populations from the northern part of the range, i.e., north of the Central African rain forest. Therefore, it would be more in line with the evolutionary history of the lion, to recognize a northern subspecies, including the Asiatic lion, and a southern subspecies. This revision would have immediate implications for conservation, notably for populations in West and Central Africa. These populations are generally small, isolated and declining, a trend which was also observed in other species in the region. Because West and Central Africa harbour unique genetic lineages, conservation of populations in these regions is of highest importance. Insight into the distribution of genetic diversity can further contribute to conservation by making recommendations for translocations, reintroductions and reinforcement projects. Several projects are already being executed, but it may become an even more important strategy if we want to safeguard the long-term survival of small populations in isolated protected areas.

Implications based on non-invasive genetic population estimation: a case example of wild boar management in a protected area

Ebert, Cornelia M. (1); Sandrini, Julian (2); Hohmann, Ulf (2); Thiele, Bernhard (1)

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Non-invasive genetic approaches for population estimation have gained importance in wildlife conservation and management. Wild boar as a species is not rare or endangered, but elusive and very difficult to count. Wild boar management in protected areas where hunting is banned can be a particular challenge. We applied non-invasive genetic population estimation to assess the management status of a wild boar population in a 3700 ha forested area of which 1000 ha are protected and without hunting. Over the whole area, 4190 wild boar faeces were detected along transects during four days, corresponding to 7.2 samples per km of transect. The distribution of faeces over the area indicated that wild boars concentrated in the protected part of the area. A subsample of 351 faeces was genotyped using six microsatellites and one sex marker. The 269 successfully analysed samples were identified to belong to 181 individuals of which 76 were male and 104 were female (the sex marker failed to amplify in one case). The wild boar spring density in the area was estimated as 11.4 (9.0 – 12.5) animals/ 100 ha with a female density of 6.6 (5.1 – 7.3). When applying a reproduction rate of 250%, this would result in an estimated increase of 28.5 (22.0 – 31.3) wild boar / 100 ha for the study year. The population estimates were used for an evaluation of wild boar management measures in the area.

Talk 5

Are microsatellite markers suitable for estimating genome-wide genetic diversity in a conservation context?

Fischer, Martin C. (1); Rellstab, Christian (2); Leuzinger, Marianne (1); Roumet, Marie (1); Gugerli, Felix (2); Holderegger, Rolf (1,2); Widmer, Alex (1)

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Nuclear microsatellite markers are widely used for estimating genetic diversity and population genetic differentiation, as well as for delineating evolutionary significant units. However, it has rarely been tested whether microsatellite variation is a good proxy for genome-wide genetic variation and differentiation. The fact that often only a limited number of highly polymorphic microsatellite markers are used may introduce specific bias into estimates of genetic diversity. We assessed and quantified such potential bias by comparing microsatellite variation with genome-wide single nucleotide polymorphisms (SNPs). We genotyped 180 *Arabidopsis halleri* individuals from nine populations using 19 microsatellite markers. Twelve of these were initially developed for the close relative *A. thaliana* and seven for *A. halleri*. We then assessed genomic variation at about 2 million SNPs in the same individuals and populations with a pooled whole-genome re-sequencing approach (Pool-Seq). Our analyses revealed that expected microsatellite heterozygosity ($SSR-H_e$) was not significantly correlated with genome-wide SNP diversity estimates ($SNP-H_e$ and $\Theta_{Watterson}$). Instead, the mean number of microsatellite alleles and rarefaction-based allelic richness were strongly correlated with $\Theta_{Watterson}$ and appeared to be a better proxy for genome-wide diversity. Estimates of genetic differentiation among populations (F_{ST}) based on both marker types were correlated, but microsatellite-based estimates were significantly larger than those estimated from SNPs. Based on our results, we recommend that researchers use genome-wide SNP polymorphism data in studies that aim to accurately quantify genetic diversity and population genetic differentiation.

Talk 6

Does hybridisation with widespread *Knautia arvensis* threaten the long-term persistence of the Eastern Alpine rare local endemic *K. carinthiaca*?

Frajman, Božo (1); Čertner, Martin (2); Kolář, Filip (2); Schönswetter, Peter (1)

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Hybridisation can increase biodiversity through hybrid speciation but it can also have negative effects, as introgression might drive species to extinction. The latter is particularly problematic in narrow endemics with small population sizes. *Knautia carinthiaca* is probably the geographically most restricted *Knautia* species, being endemic to the Görttschitz valley in the Austrian federal state of Kärnten (Carinthia). Its typical habitats are limestone rock faces, open thermophilous forests and their margins as well as complexes of dry grasslands and *Pinus sylvestris* forests. Peripheral habitats are extensively mown or grazed semi-dry grasslands, where *K. carinthiaca* comes into contact with widespread *K. arvensis*, a species of meadows and pastures. Using amplified fragment length polymorphisms (AFLPs), we explored hybridisation between both taxa. Several hybrid individuals were discovered, mostly having intermediate genome size and morphology. To compare the ecological niches of both taxa and their hybrids, we have employed Landolt indicator values of accompanying vascular plant species as well as rock and vegetation cover in close proximity of investigated individuals. Most hybrids were located at the transition area between both taxa, showing that the expansion of *K. arvensis* due to human disturbance could endanger the genetic integrity and persistence of *K. carinthiaca* in the future.

Talk 7

Hybridization between domestic and wild animals: the case of a gene important for immunity

Grossen, Christine (1); Keller, Lukas (1); Biebach, Iris (1); The International Goat Genome Consortium (2); Croll, Daniel (3)

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The major histocompatibility complex (MHC), a crucial component of the defence against pathogens, contains the most polymorphic functional genes in vertebrate genomes. The extraordinary genetic variation is generally considered to be ancient. We investigated whether a previously neglected mechanism, introgression from related species, provides an additional source of MHC variation. We show that introgression from domestic goat dramatically increased genetic variation at the MHC of Alpine ibex, a species that had nearly gone extinct during the 18th century, but has been restored to large numbers since. We show that Alpine ibex share one of only two alleles at a generally highly polymorphic MHC locus with domestic goats and that the chromosomal region containing the goat-type allele has a signature of recent introgression. Our finding contradicts the long-standing view that ancient trans-species polymorphism is the sole source of the extraordinary genetic variability at the MHC. Instead, we show that in Alpine ibex introgression generated genetic diversity at a MHC locus. Our study supports the view that loci favouring genetic polymorphism may be susceptible to adaptive introgression from related species and will encourage future research to identify unexpected signatures of introgression.

Talk 8

Population signatures of large-scale, long-term disjunction and small-scale, short-term habitat fragmentation in an Afromontane forest bird

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The Eastern Afromontane biodiversity hotspot is one of the 35 centres of endemism and consists of archipelago-like mountain massifs which are covered by cloud forests on their tops. These forest habitats provide a system of long-term disjunction and thus excellent preconditions for evolutionary processes. They differ in habitat structures with highly anthropogenically fragmented or naturally fragmented forest patches but also still large and interconnected forests. Various studies on bird species were conducted recently, focusing on long-term large scale disjunction as well as short-term small scale fragmentation. Here I bundle various studies focusing on avian biogeography on different temporal and spatial scales, all conducted in the high elevation cloud forests of this biodiversity hotspot during the last years. The contributions cover (i) effects of recent habitat fragmentation on bird communities, (ii) the complex phylogeography of East African White-eyes, (iii) the evolution of bird calls in White-eyes comparing two congeners - here lowland versus highland representatives - and (iv) finally fragmentation genetics in mountain bird species, like the Mountain White-eyes, comparing potential genetic effects from different habitat histories and habitat structures.

Can a genetic study save the endangered Pale-headed Brushfinch in Ecuador?

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The Pale-headed Brushfinch (*Atlapetes pallidiceps*) was believed to be extinct for more than twenty years, when in 1998 less than 20 pairs were discovered in the Yunguilla Valley, Ecuador. With the establishment of a specific reserve, the population first recovered to about 200 individuals in 2008 but is stagnating since. In order to investigate the genetic consequences of the severe bottleneck, we assessed genetic diversity at 11 neutral microsatellites and five Toll-like Receptor (TLR) immune system loci and compared it with museum samples of the same species and concurrent samples of two co-occurring brushfinch species. Bottleneck tests confirmed genetic drift as the main force shaping genetic diversity in this species and indicated a 99% reduction in population size dating back several hundred years. Surprisingly, we found a depletion of up to 91% diversity in the immune system genes but not in microsatellites. We additionally showed that high TLR diversity is linked to decreased survival probabilities in *A. pallidiceps*. Low TLR diversity is thus probably an adaptation to the specific selection regime within its currently very restricted distribution (approx. 200 ha), but could severely restrict the adaptive potential of the species in the long run. Management should therefore aim at creating a second reserve with a translocated population to minimize extinction risk. Our study illustrates the importance of investigating both neutral and adaptive markers for recommending specific management plans in endangered species.

Improving the effectiveness and efficiency of conservation seed collections

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Collecting seed from natural plant populations is a key tool for conservation, ecological restoration or assisted migration, and crop breeding. In all cases, it may be advisable to capture as much phenotypic and genetic diversity as possible. Many collections rely on simple, broadly-applied rules-of-thumb for minimum sample sizes, regardless of species' natural history, but these guidelines may not be optimal for genetic representation. Here we use simulated and empirical data to quantitatively evaluate the degree to which collection effectiveness is influenced by spatial arrangement, sampling intensity, and species' reproductive biology. We demonstrate that collections with spatially limited or biased sampling arrangements, or collections from species with high selfing or limited dispersal, will need to collect substantially more samples than are commonly recommended, or else will fail to reach the targeted genetic diversity. As one example of our results, a high selfing, low dispersal species may need sample sizes five times larger than current guidelines. Overall, we clearly evidence that collection guidelines tailored to particular taxa will help facilitate optimal sampling. This should help ensure ex situ conservation collections that maximize diversity while minimizing expenditures on collections.

Talk 11

Monitoring and research of the European wildcat (*Felis silvestris silvestris*) in Baden-Württemberg, Germany

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In the early 20th century, the European wildcat (*Felis silvestris silvestris*) has undergone a severe decline in many European countries. In the federal state of Baden-Württemberg, Germany, the European wildcat was supposed to be extinct since 1912. In the years 2006 and 2007, the presence of the European Wildcat was rediscovered in the Upper Rhine valley. Since then, the lure-stick method has been used to map the current distribution in Baden-Württemberg. Additionally, the collection of wildcat carcasses and other hints of wildcat presence revealed 1156 genetic samples, 39% were assigned to wildcats. The largest known population of wildcats in Baden-Württemberg occurs in a highly fragmented landscape, which is dominated by urban and agricultural areas. In this region, a telemetry study has started in 2010 investigating the effects of landscape fragmentation on habitat use and spatio-temporal behaviour of wildcats. Twenty-one wildcats (11 females; 10 males) were captured and tagged with GPS-collars. With 20 microsatellites the fine-scale genetic structure of this wildcat population has been investigated. By genotyping additional wildcats from France and Switzerland just a weak genetic differentiation even across the River Rhine was found. With the upcoming results, management recommendations are being developed to improve wildcat conservation in Baden-Wuerttemberg.

Talk 12

Quantifying introgression between European wildcats and domestic cats

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Introgression, the flow of genes between taxa through hybridization, can cause extinction. Hence, introgression is a major concern in conservation, and assessing the impact of introgression is a crucial task for conservation genetics. However, quantifying rates of introgression is not always easy, particularly when the hybridizing species are closely related and hence lineage sorting of genes is incomplete. This is the case for the European wildcat, which has been hybridizing with the domestic cat for potentially over 2500 years. We used next-generation sequencing methods to develop SNP markers that allow reliable recognition of individual levels of introgression. Combined with a Bayesian modeling approach that does not make use of a pre-defined set of pure ancestors, these SNP markers allowed us to detect introgression between domestic cats and European wildcats with very high accuracy. We applied these markers to a large sample set of free-ranging wildcats from France, Switzerland and Germany. We found that roughly 15% of wildcats showed signs of introgression, corresponding to a migration rate of 0.02 migrants per generation. Migration rate from wildcat into domestic cat was lower. In future, research on conservation genetics of European wildcats should concentrate on the fitness consequences of introgression with domestic cats.

Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species

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Accurate knowledge of the distribution of rare, indicator, or invasive species is required for conservation and management decisions. However, species monitoring done with conventional methods may have limitations, such as being laborious in terms of cost and time, and often requires invasive sampling of specimens. Environmental DNA (eDNA) has been identified as a molecular tool that could overcome these limitations, particularly in aquatic systems. Detection of rare and invasive amphibians and fish in lake and river systems has been effective, but few studies have targeted macroinvertebrates in aquatic systems. We expanded eDNA techniques to a broad taxonomic array of macroinvertebrate species in river and lake systems. We were able to detect five of six species (*Ancylus fluviatilis*, *Asellus aquaticus*, *Baetis buceratus*, *Crangonyx pseudogracilis*, and *Gammarus pulex*) with an eDNA method in parallel to the conventional kicknet-sampling method commonly applied in aquatic habitats. Our eDNA method showed medium to very high consistency with the data from kicknet-sampling and was able to detect both indicator and nonnative macroinvertebrates. We demonstrate that an eDNA surveillance method based on standard PCR can deliver biomonitoring data across a wide taxonomic range of macroinvertebrate species (Gastropoda, Isopoda, Ephemeroptera, and Amphipoda) in riverine habitats and may offer the possibility to deliver data on a more refined time scale than conventional methods when focusing on single or few target species. Such information based on non-destructive sampling may allow rapid management decisions and actions.

Impact of land-use shifts on the gut-bacterial community of the Namibian black-backed jackal (*Canis mesomelas*)

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Next-generation sequencing approaches are increasingly applied in conservation genetics to improve our understanding of how wildlife species are impacted by an ever-growing human encroachment into former undisturbed natural habitats. Thereby, studies investigating gut-bacterial communities of wildlife and livestock hosts have the potential to identify fitness-relevant changes associated with shifts in land use. Understanding how human encroachment impacts host-bacteria relationships with negative consequences for host health is challenging, because several abiotic and biotic factors are simultaneously involved. Here we present results from a 16S rRNA gene gut-microbiome study, where we investigated the variation in bacterial communities of black-backed jackals (*Canis mesomelas*) of the central Namibian cattle ranching area in dependence of intrinsic (e.g. sex, age and body mass index) as well as extrinsic (e.g. farm type, sampling location) factors. In addition, we also sequenced bacterial communities of the respective cattle community to investigate if cattle-derived bacterial sequences were present in microbiomes of sympatric black-backed jackals. Our results revealed that although black-backed jackals exhibit inter-individual microbial variation, differences in microbial diversities were not explained by any of our intrinsic and extrinsic factors. However, black-backed jackals that lived sympatric with cattle harboured low proportions of cattle-derived bacterial sequences, which was not the case in black-backed jackals inhabiting cattle-free areas. In on-going studies, we aim to investigate whether the incorporation of gut-bacteria from livestock may pose a threat to wildlife health.

Genetic diversity and differentiation in middle and great spotted woodpeckers

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Many plant and animal species persist in fragmented habitats as a consequence of ongoing habitat loss. However, connectivity among small local populations in habitat fragments is often unknown. The middle spotted woodpecker *Dendrocopos medius* is an extreme habitat specialist bound to old deciduous forests with rough-barked trees and standing dead wood and shows a strongly fragmented distribution. Due to the presumed limited dispersal capacities of woodpeckers in general, it has long been speculated that the remaining populations of the species are at best weakly connected, possibly giving rise to genetic problems in the often small local populations. To address this, blood samples from the middle spotted woodpecker were collected in the six largest Swiss populations and in one German population from 2009 to 2011. For comparative purposes, we also collected, in the same forests, samples from the great spotted woodpecker *D. major*, an abundant and widely distributed habitat generalist. Genetic diversity and differentiation were examined with microsatellite markers. Overall, genetic diversity in the middle spotted woodpecker was lower than in the great spotted woodpecker. Local populations were more strongly differentiated in the middle spotted woodpecker than in the great spotted woodpecker. The local populations were assigned to three genetic groups in the middle spotted woodpecker and to two groups in the great spotted woodpecker. A significant isolation-by-distance pattern was found in the middle spotted woodpecker, but not in the great spotted woodpecker.

Decreasing habitat suitability due to climate change: potential for "genetic rescue" in *Fagus sylvatica*

Pluess, Andrea R. (1,2); Frank, Aline (1); Heiri, Caroline (1); Lalagüe, Hadrien (3,4); Vendramin, Giovanni G. (5); Oddou-Muratorio, Sylvie (3)

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Local habitat suitability is expected to change largely under climate change. The persistence of long-lived species such as trees is thereby especially challenged. Accordingly, forest ecosystems face changes in species composition and structure likely with a high impact on ecosystem functions. However, species occurring over a multitude of environmental conditions might be under differential selection pressures resulting in populations with potential pre-adaptation to climate change, especially at their physiological distribution limits. Determination of divergent adaptation is a first step towards an informed conservation strategy for climate-resistant forests. Here we investigate 78 natural *Fagus sylvatica* stands that represent the whole climatic envelope of this species within Switzerland. Neutral genetic diversity based on 12 SSR-markers resulted in a small degree of relatedness up to 150m inter-pair distance. Yet, there was no large scale spatial genetic structure, indicating genetic mixture at a regional scale. An environmental association analysis based on 88 environmental factors, 144 SNPs out of 52 candidate genes and the latent factor mixed model (LFMM) as well as a logistic regression approach revealed that 16 SNPs out of 10 genes responded to one or several environmental factors. The associations often reflected specific gene functions. The lack of large-scale neutral genetic patterns despite divergence of candidate genes suggests that gene flow allows the spread of advantageous alleles in adaptive genes. Moreover, practitioners might enhance the occurrence of climate-change pre-adapted genotypes through management. Thereby, "genetic rescue" and adaptation processes are likely at the scale studied.

Evaluation of reintroduction success using non-invasive genetic monitoring

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Reintroductions have led to a successful return of several mammal species in Germany. One crucial but notoriously difficult task is the assessment of reintroduction success and the long-term trend of the reintroduced population. This is particularly demanding in cases when still existing relict populations are restocked with animals from distant origins. Here we present how non-invasive genetic monitoring methods are applied in four ongoing or recently completed reintroduction projects on common hamsters, beavers, lynx and wildcats to evaluate the success of reintroduction or restocking action. In three cases hair or blood samples from reintroduced animals could be obtained, allowing for direct genotype comparisons between released animals and field samples from the reintroduction area. Using clustering methods and parentage analysis, we could proof population admixture and/or successful reproduction after release, supporting evidence for reintroduction success. Unfortunately, in most cases genetics was only included years after reintroduction took place, resulting in a lack of scientific design, reference samples of reintroduced animals, or sufficient funds for the analyses. Anyhow, we clearly show that genetic monitoring provides important insights into both the short- and long-term fate of reintroduced populations and hence shall be routinely incorporated in future reintroduction programmes. Especially in the field of animal reintroductions, the above studies showed that non-invasive genetic monitoring is highly important in practical conservation management.

Impact of the urbanization process on connectivity and genetic diversity - a spatially explicit simulation approach

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Urbanization leads to fragmentation, degradation or loss of natural environments and reduces connectivity between remaining habitat patches. This affects dispersal and establishment of species and their genes and might have adverse effects on biodiversity in urban areas. Here we assess functional connectivity between green spaces for one plant (*Plantago major*) and one insect (*Pieris rapae*) model species in the Geneva urban area. We also estimate the impact of future urbanization projects planned until 2030 in Geneva (constructions and road projects) on the connectivity network. We show that the connectivity between small green spaces in the urban centres and larger ones in peripheral areas is fragile and unstable. Potential barriers to dispersal for the study species are mainly buildings, roads, but also large areas of forests, crops and vineyards. Furthermore and based on spatially explicit simulations of gene flow, we test how the distribution of genetic variation among populations along rural-urban gradients is affected by urban landscape elements. We compare these results to empirically observed population responses of the butterfly species *P. rapae* in the region of Marseille, France. For different dispersal scenarios and taking into account the effect of future intensification of urbanization, results show a decrease in genetic diversity in areas characterized by medium to high urban densities. In order to conserve and promote genetically stable and diverse populations, it is therefore important to maintain or increase landscape connectivity, especially between green spaces in dense urban centres and those in more natural peripheral areas.

Using environmental DNA to detect aquatic species

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The use of environmental DNA to detect species in ponds and streams is a relatively new addition to the toolbox of ecologists. The method is relatively simple and could change the way in which surveys are carried out. A small sample of water is taken, and then PCR is conducted to detect DNA of the focal species. While the molecular parts of the methods are comparatively well developed, little work has been carried out to put the eDNA method into an inferential framework. That is, how can eDNA be used in surveys and monitoring programs? Like any other methodology, the eDNA approach needs to be tested and compared to other detection techniques. Here we present a case study in which we compared the detection of an endangered amphibian, the Crested newt, using eDNA and traditional torch light surveys. We showed that both methods provided similar detection probabilities. Species detection using eDNA depends on abundance, with larger populations being more easily detected. We conclude that eDNA is a valuable, complementary method. Whether it should be used in a survey or monitoring programs depends on the questions, the habitats and the resources that are available.

Genetic exchange between populations of water frogs is predictable by landscape matrices and spatial arrangement of populations

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For conservation management it is important to know which landscape elements are barriers or corridors to genetic exchange between populations. Moreover, effective planning relies on the knowledge about how these elements affect genetic structure in combination with population size or density. Amphibians are of high conservation concern worldwide. In Switzerland, amphibian habitats are strongly fragmented by a dense network of traffic infrastructure. We studied genetic exchange between 53 populations of water frogs (*Pelophylax* sp.) distributed along two highway segments in the Swiss lowlands. We drew transects between all pairs of populations and calculated several measures of landscape composition and configuration. Due to a nearly complete sampling of all populations in the study areas, we were able to include measures of spatial arrangement of populations in our analysis. We calculated linear models using landscape matrices, population topology and population characteristics (e.g. population size, sex ratio) as predictor variables for gene flow (F_{ST}). The models with the highest predictive accuracy included predictors that negatively affected genetic exchange (e.g. traffic infrastructure) as well as predictors that positively affected genetic exchange (e.g. number of populations within the maximum dispersal distance) between populations. Our study demonstrates that gene flow can be well predicted by the interplay of the different types of predictor variables. We found that traffic infrastructures are barriers to gene flow between populations and that measures of spatial arrangement are important predictors of gene flow.

Ancient and recent demographic decline in the alpine yellow-bellied toad (*Bombina variegata*) populations: genetic variation pattern suggests that conservation actions can lead to recovery

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Amphibians are worldwide experiencing population declines due to anthropogenic and natural factors. Evidences of reduction in population size and even local extinctions have been reported for the yellow-bellied toad, *Bombina variegata*, along all its distributional range. We typed 200 individuals, collected in an area of Northern Italy, at mtDNA cytochrome b and 11 nuclear microsatellites. We investigated fine-scale population structure and tested for genetic signatures of historical and recent population decline using several approaches, including likelihood methods and approximated Bayesian computation. Five major groups of genetically divergent populations were found, largely corresponding to the geographic structure of the sampled area but with a clear exception of strong genetic isolation in a highly touristic area. The estimated inbreeding within populations was high, and the effective sizes in the last few generations, as estimated from the random association among markers, never exceeded few dozens of individuals. Several analyses converged in suggesting that genetic variation was shaped in all groups by a 7- to 45-fold demographic decline, which occurred between few hundreds to few thousand years ago. Weaker evidence is also supporting a genetic impact of the more recent demographic decline related to human activities, but with no clear association with specific environmental conditions. The alpine *B. variegata* populations, with highest priority given to two genetically isolated populations, should be monitored and protected to stop their recent decline and to prevent local extinctions. However, their current genetic variation pattern, being mostly shaped in earlier times, allows a reasonable optimism that complete recovery can be reached.

Posters

Poster 1

Genetic and fitness consequences of using commercial seeds in ecological restoration

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Commercially produced wildflower seed mixtures are often used in grassland restoration. However, the genetic and fitness consequences of sowing seed mixtures have not yet been entirely evaluated. We studied the genetic diversity and fitness of naturally occurring and sown populations of the grassland plant *Lychnis flos-cuculi* in a Swiss agricultural landscape. In addition, we carried out an experiment in the study area and in an experimental garden to examine the effects of local adaptation on fitness. Finally, in a climate chamber experiment we studied the effects of soil moisture and nutrient content on the fitness of plants from sown and natural populations of *L. flos-cuculi* as well as from several seed suppliers. Sown populations were characterized by significantly higher inbreeding coefficients and different genetic composition compared to natural populations of *L. flos-cuculi*. However, higher inbreeding did not influence the fitness of sown populations. There was also no clear evidence that plants from natural populations were better adapted to the local environment than plants from sown populations or from seed suppliers. Nevertheless, experimental results revealed that plants of natural origin invested more into generative reproduction and tended to flower earlier than plants originating from sown populations or from seed suppliers. Our results show that the use of commercial seed mixtures can have both genetic and fitness consequences, which may potentially affect restoration success.

Poster 2

Molecular examination of commercial fishes of the family Batoidae

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Batoidae is a superorder of cartilaginous fish commonly known as rays and skates. Rays are vital for the health of coral reefs; however they are subjected to overfishing in the Red Sea and worldwide. Rays take a long time to reach sexual maturity, and when they finally can reproduce, they have very few offspring every 1-3 years. Some Batoidae are extremely vulnerable to even local fisheries. They are believed to have small, genetically independent, island-associated stocks. With little exchange between members of neighbouring stocks, a fishery could deplete a single stock quite rapidly with little chance of recovery. The main aim of this study was to study the genetic diversity of Batoidae in the Egyptians coasts of the Gulf of Suez and Red Sea. The study also assessed the conservation status of ray species along Egypt and will stress on the vulnerability of their capture and the status of their commercial fisheries.

Genetic diversity of sea cucumber of the Red Sea

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The use of DNA Barcoding technique as a tool for sea cucumber identification was tested in this study in order to assess its accuracy and potential of the technique in identifying Seacucumber individuals. A total of 18 different (species) of Seacucumber were collected from the Egyptian coast of the Red Sea and Gulf of Aqaba and examined for both morphological and molecular characteristics. One new species of Seacucumber were identified from the Egyptian coast of the Red Sea (*Actinopyga* sp. nov). A cryptic species complex was also identified for the *Holothuria atra* population in the Red Sea using molecular analysis of the mitochondrial COI gene. In this study, another experiment was conducted in order to identify Seacucumber species from cooked or dried materials using the molecular techniques as well as testing the possibility of using DNA barcoding in order to identify badly or long time preserved museum samples.

Understanding landscape effects on functional connectivity: is matrix resistance all that matters?

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Understanding the causes and consequences of functional connectivity is crucial for many fundamental research questions in ecology, evolution, and conservation. For populations, a particularly important aspect of connectivity is the exchange of migrants and genes, which occurs if organisms successfully move from one location to another and subsequently reproduce at the new location. Landscape genetic research on such dispersal-mediated connectivity has proliferated in recent years, but it often focuses exclusively on the effects of matrix quality among sampling locations. Here we highlight that dispersal is affected by both local environmental conditions and landscape resistance found among locations, making a simultaneous consideration of these environmental effects pivotal for developing an in-depth understanding of dispersal-mediated connectivity. Specifically, understanding environmental effects on population connectivity requires an assessment of how individuals are influenced at each of the three phases of dispersal (emigration, transience, immigration). We show how the different environmental effects on individual dispersal behaviour can be analysed by simultaneously incorporating both local environmental conditions and landscape resistance in population- and individual-based studies. Importantly, landscape genetic approaches for understanding environmental effects on functional connectivity should ideally be combined with other research techniques, such as mark-recapture or telemetry. Such analyses will promote a better link between individual dispersal behaviour and population-level processes and help to clarify the relative importance of local vs. matrix effects for population connectivity.

Local adaptation of *Empetrum hermaphroditum* to habitats with different snow cover

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The evergreen, wind pollinated dwarf shrub *Empetrum hermaphroditum* is a dominant species of several subarctic heath and mountain birch forest communities. The species mostly reproduces vegetatively, but fruit production can be abundant. We studied the morphology of *Empetrum* in three different habitats across four study areas differing in climate (sub-oceanic vs. sub-continental) and latitude (Northern Sweden vs. Central Norway). The habitats were birch forest with deep snow cover (b), alpine tundra with deep snow cover in wind-sheltered depressions (d) and alpine tundra with shallow snow cover on wind-exposed, elevated ridges (s). We observed significant differences in growth of *Empetrum* among the three habitats. Ramet height, shoot length, number of lateral shoots and total biomass were lowest in the s-habitats, intermediate in the d-habitats and highest in the b-habitats. Number of leaves per mm stem and relative leaf mass were highest in s-habitats, intermediate in d-habitats and lowest in b-habitats. To study whether habitat-specific variation in growth is driven by phenotypic plasticity or genetic variation, we used AFLP markers. We analysed 30 shoots from each habitat in each study area (N=360). We expect that different habitats will be characterized by separate gene pools. Furthermore, we will analyse the local clonal structure of *Empetrum* in different habitats to quantify the proportion of vegetative vs. sexual reproduction. We expect that sexual reproduction will be higher in sites with shallow snow cover since these habitats are characterized by high proportions of bare soil for seedling recruitment.

Plant genetic diversity in tropical lowland rainforest transformation systems in Sumatra (Indonesia)

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Due to the expansion of agriculture, tropical rainforests are transformed to other types of land use throughout the globe, at a rate of approximately 14 % in Sumatra and Kalimantan (Indonesia) in the last 15 years. A common result of the transformation of natural ecosystems to managed systems is a loss of species diversity. In Jambi Province, Sumatra, tropical lowland rainforests are transformed into oil palm plantations, rubber plantations and 'jungle rubber'. The aim of this project is to analyse the intra specific genetic diversity of vascular plants in reference forests compared to the three mentioned transformation systems. In 32 plots (50m x 50m) of these four different ecosystems, 10 individuals of 10 dominant species are sampled. Based on anonymous AFLP markers the consequences of land use changes on the genetic diversity of plants caused by the different species composition in each system will be analysed. Preliminary results indicate that oil palm and other species of the transformation systems have a lower intra specific genetic diversity than the analysed species of the forest plots. Our results on intraspecific variation will contribute to a comprehensive quantitative assessment of the impacts of tropical forest conversion to other land uses on biodiversity.

The dwarf bulrush (*Typha minima*) - genetic basics for reintroductions

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Reintroductions have become increasingly important for the recovery of rare native species and restoration purposes. Genetic information about the target species, however, is often missing in reintroduction projects. The dwarf bulrush (*Typha minima*) is a formerly widespread pioneer plant species of braided alpine rivers, and several reintroduction projects have been conducted in Switzerland and Austria. We studied neutral genetic diversity of this rare and endangered plant species and evaluated fitness parameters in a greenhouse experiment. We genotyped 1512 samples of *T. minima* from 21 locations at eight rivers in Austria, France, Italy and Switzerland with 16 nuclear microsatellite markers. The amount of clonal growth (number of multilocus genotypes divided by number of ramets) in a population ranged from 1.7% to 86.2%. Samples of the same river system mainly clustered together in the same genetic group. 6480 seeds were planted in a greenhouse, and germination rate as well as shoot length, dry weight of shoots, roots and rhizomes after one year of growth were assessed. Average germination rate was 15%, with two isolated populations showing significantly lower germination rates. There were no significant differences between populations in the other measured parameters. Based on the results of the study, we recommend that plant material for reintroductions be only taken from the same river system. As clonal growth varied considerably in the studied populations, care should be taken to avoid source populations with only few genotypes.

Human-mediated translocation impacts lineage integrity of chub (*Squalius cephalus*) and its sister species cavedano (*S. squalus*) by hybridization

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European fish species distributions were shaped by glaciations and the geological history of river networks, until human activities abrogated the restrictions of biographical regions. The nearby origins of the rivers Rhine, Rhone, Danube and Po allow the examination of historical and human-influenced patterns on a small scale. We investigated these patterns in the widespread chub (*Squalius cephalus*) and its proposed southern sister species cavedano (*S. squalus*). A mitochondrial network constructed from Cyt b and CO1 sequences showed a clear separation of chub and cavedano and a single common haplotype prevalent in chubs from Rhine and Rhone. The separation into two species was also supported by shape differences quantified by geometric morphometrics as well as traditional morphological traits. Bayesian clustering of microsatellite genotypes divided the individuals into five clusters corresponding to the Rhine, upper Rhone, lower Rhone, Danube and Po rivers. However, there was evidence for mitochondrial and nuclear introgression of chub into cavedano in southern Switzerland, presumably the result of hybridization following human-mediated translocations, e.g., by live bait or stocking. The desirable preservation of distinct evolutionary lineages will thus require the prevention of such translocations.

Trapped in urban refugia? ‘Cityscape’ genetics of a flightless ground beetle

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Urbanization leads to changes in abiotic conditions, increased disturbance frequency and habitat fragmentation. Populations inhabiting the remaining green patches in cities are therefore often small and, if the exchange of individuals is also affected, genetically isolated. Especially forests provide an ecosystem with a wide range of habitats for specialized species. Using ground beetles as model organisms, we studied differently sized within-city-forest-patches in three cities in northern Germany. We analysed 330 individuals of the forest dwelling, flightless ground beetle *Abax parallelepipedus* from 15 forest patches caught by means of pitfall traps. We analysed 13 polymorphic microsatellite loci. Results show that *A. parallelepipedus* was only found in forest patches older than 200 years. The overall genetic differentiation was high ($F_{ST}=0.1$) indicating reduced gene flow between the patches. Our data highlight the special importance of ancient forests within cities and point towards a careful conservation and urban planning for these sites.

German wolf recovery under genetic observations

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Wolves in Germany severely suffered from human persecution, leading to their complete extinction within the first half of the 19th century. Strict legal protection recently resulted in a successful reestablishment of wolf packs in the eastern part of the country. The rapid population growth and range expansion to the North West caused speculation about the origin of German wolves and their ability to find new habitats. Hence, understanding wolf population dynamics is mandatory to design sound conservation strategies. Here we describe the recolonization by wolves of Germany, from the initial production of a litter by a pair of wolves in 2000. Non-invasively collected samples of almost every German wolf pack and some reference samples of the Baltic population, the Carpathian population, and the Alpine population were genotyped at 14 microsatellite loci and a diagnostic mitochondrial DNA control-region sequence. Few individuals did not belong to the local wolf population as determined by assignment analyses, suggesting a certain level of immigration in the population from the east. We reconstructed the genealogy of German packs, aiming to detect immigrants, estimate kinship, relatedness between reproductive wolves, and detect turnover of breeding pairs. The inferred pedigree shows strong evidence of inbreeding. One pack in lower Saxony was founded by full sibs. Overall, however, due to ongoing immigration, the population was not inbred. Our results show the process of natural spread of wolves and can be used to predict future expansion of the population.

Molecular identification of endangered rosewood species from Madagascar

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Internationally traded valuable timber species are increasingly in the focus of conservation geneticists. Authorities need to be able to assess the legality of traded wood, which requires methods that allow verification of species identity and origin. The genus *Dalbergia*, better known as rosewood, is an example of a highly sought after tropical timber species, and several species are highly threatened as a consequence of excessive illegal logging, even in protected areas. Once logged, the different *Dalbergia* species are difficult or impossible to distinguish and identify in the absence of traits typically used for species identification, such as bark, leaves, fruits and flowers. To better characterise and distinguish these rosewood species and to identify the species most affected by logging, we are working towards a DNA-based identification system for Malagasy rosewoods using standard DNA barcoding and nuclear microsatellite analysis. First results indicate that barcoding allows identifying rosewoods from Madagascar and microsatellites help distinguishing among Malagasy species. Such a method – once fully developed and validated – may be useful as a forensic tool for conservation purposes and international trade regulation.

Genetic research at the CRC for the conservation of captive and wild populations

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Within the Centre for Research and Conservation (CRC), molecular genetic and statistical tools are used to study wild and captive populations. Delineating (genetic) differences is of prime interest in conservation. Hence, samples from the wild and musea are analysed (microsatellites, sanger sequencing or NGS) to determine both the taxonomic position (e.g. military macaws, *Ara militaris*) or to understand population dynamics (e.g. effect of reintroductions in storks, *Ciconia ciconia*). In terms of captive breeding, most of this work centres around avoidance of hybridisation. On a “lower” level, individual subpopulations are subject of genetic surveys. Genetic analysis of non-invasive stool samples of the Cross river gorilla (*Gorilla gorilla diehli*) for instance enables us to get insights in population sizes. These estimates are essential in modeling and the management of these wild populations. Within ex-situ breeding populations, we study the role of inbreeding depression and how genes affect the expression of traits (e.g. life-history traits in Congo peafowl, *Afropavo congensis*) and whether genetic variability of these traits changes throughout time. Within captive breeding, genetic goals are set and evaluated based on pedigrees, which often include gaps and assume founders to be unrelated. Here management of breeding programs can be supported by DNA analyses. As such, samples of, e.g., golden-headed lion tamarins (*Leontopithecus chrysomelas*) are currently analysed to resolve questions on founder relatedness and paternity, by integrating the molecular data with the available pedigree data. At the individual level, analyses on Major Histocompatibility Complex genes of Eurasian black vultures aim to optimize pair formation.

Public knowledge and awareness of genetic diversity

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With the International Year of Biodiversity in 2010, public knowledge of biodiversity has been improved and a small, but increasing percentage of the general public can name genetic diversity as one level of biodiversity. However, little is known on whether its meaning or definition is known by the general public. In summer 2014, a Switzerland-wide representative survey was conducted to investigate people's knowledge and awareness of genetic diversity. Around 1150 people were asked whether they have heard about the term, whether they are familiar with its meaning and what associations they have with genetic diversity. Further, they were asked whether they think that genetic diversity is threatened or not and to state the most severe threats to humans. Almost 50% of the respondents have heard about the term 'genetic diversity', whereas only 15% knew the correct definition. Genetic diversity is mainly associated with agriculture and nature conservation but also with genetic engineering. Almost 70% of the respondents think that genetic diversity is threatened or rather threatened. Our results show that there is a concern about genetic diversity, however, there is a need to increase the level of knowledge of the term and the concept of genetic diversity and to clarify misunderstandings. Environmental education programs concerning genetic diversity might foster the understanding of the different levels of biodiversity and raise awareness of the crisis of biodiversity loss.

An ordinary species in ordinary landscapes – The genetic structure of *Abax parallelepipedus* in the Biodiversity Exploratories

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Habitat history and habitat structure and their resulting effects on genetic structure are central topics in conservation genetics. Often, however, studies have concentrated on rare species or those found in extremely fragmented landscapes, so little is known about how common species react to common landscape structures. Like in much of central Europe, the current forests in Germany are embedded in complex landscapes and are the result of a long history of changing land uses. We sampled 143 populations of *Abax parallelepipedus*, a stenotopic and flightless forest ground beetle in the forest plots of the Biodiversity Exploratories and sequenced a set of 14 microsatellite loci. The plots are located in three separate regions across Germany and represent a gradient of land use types and intensities and of habitat histories. Each Exploratory contains a complex landscape structure, including towns, streets, forests, and agricultural areas. The drivers of genetic diversity were found to all be related to current day population sizes, while no effects of forest history were found. We additionally found that genetic differentiation between the plots is not driven by landscape or barriers. We conclude that *A. parallelepipedus* was able to efficiently recolonize previously cleared forest patches and that the non-extreme fragmentation levels in our landscapes together with the large population densities have prevented the development of genetic differentiation or the loss of genetic diversity. These results are a reminder that genetic processes at historical time scales must be interpreted in the context of gene flow and population sizes.

Genetic Diversity Centre (GDC)

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The Genetic Diversity Centre (GDC) is a technology and knowledge platform of the Department of Environmental System Sciences (D-USYS) at ETH Zurich. Its aim is to provide easy access to instruments, support, training, and expertise in the generation and analysis of genetic and genomic data. We have an active user community and promote the transfer of know-how among research groups. Although the GDC is formally a departmental platform, it is open to all researchers of academic institutions. Established in 2009, the GDC currently supports over 200 projects and has 410 active users from more than 60 different research groups. The GDC is offering access to professionally managed laboratory instruments including massive parallel sequencing related equipment. We provide tested protocols and support the users actively in the lab. We also offer bioinformatics support and access to a powerful computing environment. The GDC is supporting projects in the fields of molecular ecology, population biology, evolutionary biology and conservation genetics. The GDC actively assists the implementation of new methods and plays an important role in developing custom-made solutions primarily for non-model organisms. Over the years, the GDC staff and its users have built up expertise in several fields, including genome assembly and annotation, genome-wide variant calling, genetic barcoding, marker based genotyping, transcriptomics, metagenomics, metatranscriptomics, expression analysis, and experimental evolution.

New SNP arrays for standardised genetic surveys of mammals from non-invasively collected samples

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Wild mammals in Europe, such as wolves, bears and wildcats, have been, and still are, extensively studied with mitochondrial DNA (mtDNA) sequences and microsatellite markers. These studies yield important ecological and genetic information and assist in the development of conservation plans. In Europe, the ranges of many of these species are currently expanding following conservation measures and extend over many different countries, with the result that the same species is studied by many different laboratories, yielding data that are not readily comparable. Microsatellite markers are platform dependent, and shifts in exact allele sizes can be expected across machines. Additionally, allele sizes are prone to the expert but subjective assessment of the person performing the scoring. These problems can be solved through calibration practices that require the exchange of samples with known profiles, which complicates studies. In our laboratory, we are currently exploring the suitability of SNP panels that, once validated, can be implemented in custom-designed Fluidigm arrays, in which 96 SNPs are analysed simultaneously. Amplification of small amplicons (ca. 100 bp) hold great promise for the implementation of this technique in samples with low DNA quality and quantity, such as non-invasively collected material (scats, hair, feathers). Additionally, the same SNP chip can hold biparentally, maternally and paternally inherited markers (nuclear, mtDNA and Y chromosome markers). Here we will show current performance and results for the above-mentioned species.

Poster 17

Noninvasive genetic monitoring of endangered large carnivores in Germany

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Since five years our laboratory serves as national reference centre for large carnivore genetics in Germany. During this time, we have generated thousands of DNA profiles from scats, hairs, bones, saliva, urine, and blood of wolves, lynxes, wildcats, and further mammals. The analyses are conducted in close cooperation with national and federal state authorities as well as NGOs, aiming to provide a link between molecular science and legal species monitoring. We show some selected results of genetic wolf, lynx, and wildcat monitoring activities, documenting that both wolves and wildcats show no or only minor signs of hybridization, inbreeding, or genetic erosion. Selected examples show the importance of genetic data when aiming to understand dispersal and recolonization patterns, locate source populations, assess gene-flow across landscape barriers, and investigate grades of spatial isolation. Besides showing scientific results, we discuss differences between applied and basic research and why bridging the gap between legal nature conservation and scientific research in Germany is not a trivial task.

Genetic consequences of species' range shifts and contractions due to climate and land-use changes

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Recent environmental changes, both climate and habitat-related, have impacted species at previously unseen rates, causing distribution shifts and declines. Increasing concern over the implications of such changes for biodiversity has led to the use of environmental niche models to predict potential future declines in species' distributions. However, these models often focus on climate while omitting land-use changes, and surprisingly little has been done to investigate the genetic consequences of species' range shifts and declines. We use environmental niche models for a set of butterfly species in the UK that experienced declines in their range over the past century. We build models for both the present and the past, using both climate and land-use data, aiming to capture the important variables for species presence, and to assess whether these variables change over time. We aim to further sample and genotype a subset of these species at several time points during the range decline, using populations from museum collections as repositories of past diversity. We plan on comparing past and present neutral diversity, and then building individual-based simulation models and using an Approximate Bayesian Computation (ABC) framework to investigate the demographic process behind the declines. Our results will help increase our understanding of how declines and shifts impact genetic diversity and whether under some conditions it can be better preserved. They may also highlight differential effects of climate or land-use changes, as the latter tend to happen more rapidly, allowing less time for adaptation.

Heterosis and inbreeding depression in alpine populations of *Arabidopsis thaliana*

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Climate warming is pushing species ranges upwards in elevation. This problem raises interest in studies focusing on adaptation at range margins and on how changes in connectivity among populations may affect their evolutionary trajectory. In this study, we investigate the processes shaping genetic diversity in alpine populations of the model species *A. thaliana* and discuss how inter-population gene flow could affect their evolutionary trajectories. To address these issues we have chosen six populations located along an altitudinal gradient in the Swiss Alps. These populations are typically small, isolated and show restricted within-population diversity. The impact of genetic drift on their evolution is thus expected to be strong and can theoretically result in an accumulation of recessive deleterious mutations. To investigate the magnitude of the drift load, we conducted a crossing experiment consisting of comparing the performance of offspring from selfings, within-population and between population-crosses. Results revealed that, within populations, selfing is often associated with fitness reduction (inbreeding depression), while inter-population outcrossing may have either positive (heterosis) or negative (outbreeding depression) effects. Inbreeding depression and heterosis indicate the existence of a significant drift load that segregates within and among populations. The occurrence of outbreeding depression shows that the combined effects of divergent selection and random genetic drift can lead to the accumulation of genetic incompatibilities between closely adjacent populations. Overall, our results confirm the important role of genetic drift in the evolution of natural alpine population of *A. thaliana*.

DNA quantity and quality from remnants of traffic-killed specimens of a highly endangered and protected longhorn beetle: a comparison of different approaches

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The sampling of living insects should be avoided, especially when the species is highly endangered and sampling of living specimens would further increase the risk of population extinction. Non-lethal sampling (wing clips or leg removals) can be an alternative to obtain DNA of individuals for population genetic studies. However, non-lethal sampling is not possible for all insect species. We examined whether remnants of traffic-killed specimens of the endangered and protected longhorn beetle *Iberodorcadion fuliginator* can be used as a resource for population genetic analyses. Using traffic-killed specimens and insect fragments collected over 15 years, we determined the most efficient DNA extraction method in relation to the preservation state of the specimen (crushed, fragment or intact), type of storage (dried, in ethanol or in airtied tubes), storage duration and weight of the sample by assessing the quantity and quality of genomic DNA. A modified CTAB method provided the highest recovery rate of genomic DNA and the largest yield and highest quality of DNA. We further used traffic-killed specimens to evaluate two DNA amplification techniques (quantitative PCR and microsatellites). Both qPCR and microsatellites revealed successful DNA amplification in all degraded specimens or beetle fragments examined. However, relative qPCR concentration and peak height of microsatellites were affected by the state of specimen, type of storage (best in dried specimens) and storage duration but not by specimen weight. Our investigation demonstrates that traffic-killed specimens of a beetle can serve as a source of high quality genomic DNA which allows to address conservation genetic issues.

Towards monitoring genetic diversity of a butterfly species in Switzerland

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The assessment and monitoring of biodiversity primarily focus on the species level and occasionally consider ecosystem diversity. However, the third level of biodiversity, i.e. genetic variation, is widely neglected. Our study evaluated the feasibility of a monitoring of neutral genetic diversity based on samples from the Biodiversity Monitoring Switzerland (BDM). The widespread but scattered butterfly species *Melanargia galathea* was chosen as study organism, and leg samples were collected across Switzerland within the BDM program (indicator «species diversity in landscapes») in summer 2013. We genotyped 424 samples of *M. galathea* from 56 locations for seven microsatellite loci newly developed. Population genetic parameters were chosen to quantify genetic diversity within individuals (observed heterozygosity), within populations (allelic richness), between populations (differentiation) and across landscapes (genetic clusters). Four genetic clusters were identified across Switzerland, differing significantly in allelic richness. The genetic clusters were separated by high mountain ranges that limit gene flow and hence caused or maintained genetic differentiation. Although large-scale patterns of observed heterozygosity and allelic richness were found across Switzerland, these measures neither significantly differed between single populations, nor did the two parameters correlate. Our study implies that genetic monitoring of selected species is feasible with limited additional effort, given that the sampling can be done within an existing monitoring scheme. However, it requires further work of operationalization, e.g. selecting adequate taxa and defining meaningful indicators. Nevertheless, this procedure could be a first step towards a monitoring of genetic diversity, which provides complementary facets of biodiversity hitherto largely ignored.

Establishing time series data to assess temporal changes in genetic diversity of crop wild relatives

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Assessment and monitoring of genetic diversity, which is part of in situ conservation strategies of crop wild relatives (CWR), require time series data. While we can today identify sites in which we will measure and monitor diversity and effectiveness of conservation actions in the future, knowledge about past trends is equally important to inform conservation strategies. Analyses of past trends have been constrained for CWR because of the patchy, scarce availability of historic data and seed material. Past germplasm collecting missions and their associated documentation might partially fill this gap. The Bioversity collecting database (BCD) contains original passport data for over 200,000 landrace and CWR samples, which were collected from over 100 countries mainly between 1975 and 1995 and then conserved in genebanks. Three features in particular make the BCD an interesting data resource: (1) the high percentage of 27% of CWR records; (2) 73% of CWR samples are geo-referenced, which allows mapping and re-visiting historical collecting sites and (3) for many records the original seed material can be traced in genebanks to which the material had been sent for storage after collecting. Many CWR species were collected from more than 20 sites within one country and a number of them also from up to five different countries. A case study of wild barley (*Hordeum vulgare* ssp. *spontaneum*) re-collected from historical sites in Jordan is presented.

Standardized large-scale genetic wildcat census in Germany

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European wildcats (*Felis silvestris*) are considered as an important target and umbrella species for semi-natural, rich-structured forests in Germany and other European countries. Currently, large-scale conservation projects aim at reconnecting isolated wildcat populations and provide migration corridors for forest communities. Here we introduce data from a large-scale genetic wildcat census, the “Wildkatzensprung”, with the aim of investigating the connectivity among wildcat populations across Germany. This was supported by more than 550 volunteers, who sampled 800 valerian lure sticks. These sticks acted as hair traps, which were inspected for samples weekly, during the mating season and over three consecutive years. We present our findings for the first time for the three years 2012 to 2014. Microsatellite analyses of 1670 samples resulted in 1237 wildcat detections representing 519 individual genotypes. The study provides estimates of population size and changes in population composition over the years. Combining our individual data with habitat models will help to provide a well-founded census estimate for wildcats in Germany.

Postglacial recolonisation in a cold climate specialist in Western Europe: patterns of genetic diversity in the adder (*Vipera berus*) support the central-marginal hypothesis

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Understanding the impact of postglacial recolonisation on genetic diversity is of major interest. The central-marginal hypothesis (CMH) predicts a reduction in genetic diversity from the core of the distribution, or the refugium, to peripheral populations. While the CMH has received considerable empirical support, its broad applicability is still debated and alternative hypotheses predict opposite patterns. Using microsatellite markers, we analysed the genetic diversity of the adder (*Vipera berus*) in Western Europe to reconstruct postglacial recolonisation. Approximate Bayesian Computation (ABC) analyses suggested a postglacial recolonisation from two routes: a western route from the Atlantic Coast up to Belgium and a central route from the Massif Central to the Alps. This cold-adapted species likely used two isolated glacial refugia in Southern France, in areas free of permafrost during the last glacial maximum. Adder populations further away from putative glacial refugia have lower genetic diversity and, therefore, our results support the CMH. Finally, our study also illustrates the utility of highly variable nuclear markers, such as microsatellites, and ABC to test competing recolonisation hypotheses.

Rapid species identification of predators from saliva traces on kills – SNP (Single Nucleotide Polymorphism) based approaches for wolf/dog discrimination

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Large predators, such as wolves and lynx are currently reoccupying Germany. With their return, kills of livestock and game species are increasing, reducing human tolerance for coexistence. Hence, management plans in combination with compensation fees are of continuously growing importance. In many kill instances, genetic based methods are essential for reliable predator identification. The routinely applied methods are laborious, expensive and show low success rates. Thus, we aimed to develop reliable, fast, and cheap molecular detection methods that allow for safe species discrimination from kill samples. Sampling of saliva traces from kills was carried out with sterile cotton swabs at wound sites of the carcass, with focused sampling around the throat and neck area. We tested two different SNP (Single Nucleotide Polymorphism)-based marker system approaches: (i) The SNPtype Assays (Fluidigm Corp.) and (ii) TaqMan® SNP Genotyping Assays (Life Technologies). Both assay systems offer the most cost-effective, flexible, and fastest way to determine SNP genotypes, designed for wolf/dog/fox discrimination, with detection on a real-time thermocycler or an end point reader. Here we compare both approaches and show the results of initial tests on both reference samples and real-life saliva traces obtained from kills across Germany.

Biodiversity dynamics and the effect of urban environment on the distribution of genetic variation in the Geneva cross-border area

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Population growth, urbanization and agriculture lead to degradation and loss of natural environment. This has an adverse effect on the diversity of habitats, species and genes and might cause decline in the quality of life of the resident population. In this context and in the densely populated Geneva cross-border area, we study biodiversity dynamics and the effect of the environment on the distribution of genetic variation (URBANGENE project). We use genetic information of three model species (a plant, an insect and an amphibian) in combination with environmental and landscape data to evaluate their potential to disperse and adapt to urban environments. Based on a representative population survey, we further investigate how urban residents perceive their quality of life with a local biodiversity perspective, compared with health data measured in the same areas. Using genome-wide SNP data, we assessed genome-environment associations for *Plantago major*, a synanthropic plant, in order to obtain information on its ability to disperse among habitats and to adapt to the urban environment. Butterfly (*Pieris rapae*) and amphibian model species (*Bufo bufo*) will be sampled in 2015. As regards the amphibians, we developed a WebGIS participatory platform, making it possible for Geneva residents to indicate the geographic location of amphibian habitats on an interactive map, and to transmit information on the species they observed in this area. The integration of results from this interdisciplinary project should allow for a more sustainable urban development and a better conservation and management of biodiversity in urban areas.

eDNA-based detection of crayfish plague in Germany

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Crayfish plague (*Aphanomyces astaci*) is a fatal disease among European crayfish species. Invasive species of North American origin, e.g. the signal crayfish *Pacifastacus leniusculus*, are latent carriers of the pathogen and pose a constant infection risk due to continuous release of *A. astaci* spores. With environmental DNA (eDNA) techniques, it is possible to detect *A. astaci* directly from water samples. The aim of our study was to investigate the seasonal dynamic of spore release in natural water bodies and the detectability of crayfish plague in different environmental conditions. We filtered water in varying volumes and caught crayfish in parallel for each sampling event during one year (monthly and biannual). Water samples and crayfish tissues were tested for presence of *A. astaci* DNA by species specific real-time PCR and pathogen load was measured in semi-quantitative agent levels. We show that *A. astaci* spores can be released over the entire year, which supports the evidence that no time period can be proclaimed safe from infection transmission. We further demonstrate that the pathogen can be detected in water samples of streams with differing crayfish distribution and pathogen load showing varying *A. astaci* distribution patterns. We analysed an acute outbreak event and inlet/outlet water of a fish aquaculture to show that surveillance and risk assessment is crucial to monitor disease transmission. The successful application of eDNA techniques shows the high potential of this method to improve biomonitoring in aquatic ecosystems by means of low costs, ease of sampling and reliability of results.

Estimation of population size and connectivity of a fragmented capercaillie (*Tetrao urogallus*) population in the Black Forest (Germany)

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As a result of habitat destruction and fragmentation, the endangered capercaillie (*Tetrao urogallus*) has experienced a severe population decline in the Black Forest. The current capercaillie population of about 600 individuals is highly fragmented and close to the minimum viable population size of 500 birds. Thus, the connectivity between the spatially disjoint patches is of crucial importance for the long-term survival of the local capercaillie population. A prominent conflict between capercaillie conservation and land use in the Black Forest is the construction of wind power plants. In this context, the project “Capercaillie and Wind Power” of the Forest Research Institute of Baden-Wuerttemberg (FVA) has been launched to evaluate the impact of wind power plants on capercaillie. To estimate and monitor population size and to investigate the connectivity between population patches and the functionality of the corridors connecting patches genetic analyses are conducted. Therefore, we use non-invasive genetic sampling and subsequent genotyping using 12 microsatellites to identify individuals in three consecutive years. Population size is estimated implementing a capture-recapture approach. We attempt to verify the functionality of corridors applying a parentage analysis and inferring movements of related individuals. In a pilot study the ability to identify individuals and to estimate population size was confirmed. Additionally, the elaboration of a systematic sampling approach is under progress, prior to start working on the main study questions.

Genetic structure of the red deer (*Cervus elaphus*) populations in Rhineland-Palatinate (Germany)

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Fragmentation and loss of habitats are increasing problems in modern European landscape. Reduced migration may lead to decreasing genetic diversity within populations and increases the risk of inbreeding. Red deer, *Cervus elaphus*, is adapted to large open or semi-open habitats. It is often considered a key species in conservation, hunting and forestry and, therefore, it is of particular interest for the development of effective hunting and conservation management. Genetic diversity and genetic population structure of red deer populations in Rhineland-Palatinate (Germany) was studied by microsatellite analysis. From 2009 to 2013, a total of 2300 individuals was sampled. The individuals were genotyped at 17 microsatellite loci and one sex-specific locus. Measures of genetic diversity (allelic richness, expected heterozygosity etc.) were calculated as well as measures of genetic differentiation (F_{ST} , D_{est}). Furthermore, parentage analyses were conducted. Genetic diversity differed between management areas. For one area marginal signs of inbreeding were detected. Significant genetic differentiation occurred among the areas; particularly for those, which were separated by larger distances or river systems. The assignment test conducted with the software STRUCTURE created four most appropriate genetic clusters. The location of these clusters fitted well with natural landscape elements (particularly rivers), but may also be explained by the management in separated red deer areas. Our set of microsatellite loci seems to be suitable to create the basic data for future management plans for *Cervus elaphus*.