

Aim of the visit:

Exchange ideas and expertise to use the newly developed domestic cat SNP chip in the conservation genomics of Britain's rarest mammal, the wildcat. Discuss a potential Europe-wide wildcat genomics working group, and possibilities of applying for European funding for it, such as LIFE+.

Description of the proposed work:

Genomic technology has been widely used to facilitate human biomedicine and subsequently animal breeding. Industry has had the necessary funds to push forward genetic marker development, such as single nucleotide polymorphisms (SNPs). Due to a lack of comparable funding, the conservation genetics community has had little opportunities to benefit from these developments. However, the increasing availability of genomic resources in domesticated animals makes possible to carry out genomic studies in a few wild species, such as wolf (via dogs) or wild boar (via pigs). Last year, a large scale SNP resource for domestic cats based on Illumina SNP chip technology became available. An obvious conservation genomic application is to use this chip for wildcat genotyping.

Wildcats are threatened large carnivores with a Europe-wide distribution. Their densities have been low due to eradication campaigns in the early last century. During the last decade, especially in Western Europe, the wildcat has increased in numbers. However, its status in Scotland, where the wildcat is Britain's last and only large carnivore, is unclear. There, it is not only threatened by low population densities but also by hybridisation with domestic cats, which threatens its genetic integrity. Conservation genetic studies have so far been inconclusive in assessing the current genetic status. Therefore, the powerful domestic cat SNP chip should be utilised to investigate Scottish wildcat populations. The only possibility to solve the Scottish wildcat puzzle is a comparative study with a range of well-studied mainland European wildcats. My research team, the conservation genetics group of the Senckenberg Institute, has collected several hundred wildcat samples from their native range. Moreover, we currently develop methods to genotype SNP sets from non-invasively collected genetic material. Combined, the expertise of the proposed host (i.e., running the domestic cat SNP chip on Scottish wildcats), and our background in wildcat genetics and sample collection, will secure the survival of Britain's last large carnivore.

Travel Report:

A series of meetings were carried out in Edinburgh, Scotland, to discuss collaboration on conservation genomics of beaver and wildcat (discussions on beaver added to the agenda *a posteriori*). Added value through discussions about beaver conservation in Scotland was due to upcoming relevance of coordinated and illegal beaver reintroductions in Scotland. While there is a test area in the west of Scotland where national authorities currently test the reintroduction of beaver from a Scandinavian lineage (Norway), there have been illegal releases in the east, sourced from unknown European population(s). As a result, national Scottish authorities have called to the Royal Zoological Society of Scotland (RZSS, the host institution) to study the new Scottish beavers with respect to population origin, genetic diversity, and family structure. Traditional approaches of conservation genetics employing microsatellites have been mostly unsuccessful in studying these questions to great depth due to extreme genetic impoverishment in most of the European beaver

relict populations. The RZSS has developed new tools by so-called RAD-tag sequencing to develop SNP genetic markers. The development stage has been passed successfully and in a next step an inventory of subpopulations and genetic diversity in European beavers will be created. A set of 384 SNPs have been chosen to genotype a wide panel of beaver samples. Current work on beaver in the Senckenberg Institute (the grant recipient institute) complements this effort. Several discussions about sampling design and analysis of the data set were fairly successful. We arranged the exchange of beaver samples from our Senckenberg samples stock to be genotyped on the 384 SNP panel. Further, we discussed how data analyses and writing will be shared among co-authors of a proposed paper on European beaver genetic structure. RZSS will carry out genotyping and general statistics, as well as drive the writing of a manuscript. Senckenberg plans to carry out mtDNA sequencing (“haplotyping”) of samples that have not been haplotyped before and will contribute to population genetics analyses as well as more detailed assessments of the data in a local German beaver population context.

A meeting with European wildcat specialists with a wider group could not be carried out due to issues with the schedules of the planned participants. However, detailed discussions about the current status of the wildcat conservation genomics work led to a good understanding of how the project can continue. Currently, original Scottish wildcat samples from museum skins (assessed by Andrew Kitchener, a world authority on wildcat pelage and morphology) have been genotyped on an Illumina 60k cat chip (obtained from the Morris Foundation, USA), alongside with reference samples from German wildcat populations (supplied by Senckenberg), contemporary Scottish wildcats, and a reference set of domestic cats. Examination of the genotype data indicates that there are thousands of SNP markers that segregate between wildcats and domestic cats. Based on these results it was discussed how to continue the study to find diagnostic (or nearly so) SNP markers to differentiate between wildcats, domestic cats, and their hybrids. A small panel of SNPs (in the order of 24-96 SNPs) of the most promising SNPs will be genotyped in single-plex assays for a wide array of wildcat and domestic cat samples (amounting to hundreds of samples) to confirm their diagnostic nature. We discussed that a sanctuary for Scottish wildcats (once they have been identified by the aforementioned SNP panel) will be installed on Ardnamurchan, a peninsula in the West of the Scottish highlands. This peninsula can be locked from land by building a relatively short stretch of fences (< 10 km), so that no domestic cats or hybrids can enter the area, i.e., wildcats living there can breed pure. On two days of excursion we investigated habitat structure and suitability on that island personally. In conclusion, although not very large, the habitat seems highly suitable for wildcats. Although only few larger patches of dense forest exist on the peninsula there are belts of highly structured and rich forest all along most of the shore (except for the more western parts). This continuous, well suitable wildcat habitat connects most of the areas there, and we expect this area to be a very suitable place for installing a park to host Scottish wildcats and provide sufficiently large territories for around 20 wildcats, or more. In the near future it will be possible to bring leading European wildcat specialists to a round table to present these plans, and to start collaboration in a Europe-wide framework. Should the European reference samples of wildcat (some of them already provided by Senckenberg) appear to be genetically closely related to the indigenous Scottish wildcats (based on SNP data from museums) we will even discuss supplementing the potentially hybridised Scottish wildcat population with rather pure wildcats from mainland Europe.