# ADDER GENETIC RESEARCH SUMMARY

## Background Motivation & Knowledge Gaps

The adder (Vipera berus) is the UK's only venomous snake species. Although widespread across Great Britain, the adder exhibits a patchy distribution and significant variation in regional abundance. In recent decades, evidence of widespread population declines and county level extinctions have resulted in increased conservation focus[1–7]. The adder is listed as a priority species for conservation under section 41 of the Natural Environment and Rural Communities Act (2006) and has been listed as Vulnerable in a recent National Red List Assessment of British herpetofauna[8]. Species Distribution modelling has estimated a 39% decrease in occupancy (at a 1km resolution) in England between 1990 and 20116. Furthermore, adders are estimated to occupy just 29% of suitable habitat in England and 7% of 1km squares nationally. Most recently, analysis of data gathered as part of a UK-wide citizen science monitoring programme 'Make the Adder Count' has revealed a significant decline in mean peak count among small adder populations (< 10 individuals) between 2005 and 2016 (n=117 sites)[7]. The study concluded that, if current rates of decline continue unabated, small adder populations will become extinct by 2032. This is of considerable concern given that over 90% of populations assessed in this study had a mean peak count less than 10 individuals. Results mirror an earlier study in which a third of adder populations occurred on small (less than 6HA), isolated sites[5]. The disproportionate number of population declines to stable or increasing populations indicates a declining national trend.

Historic declines are attributed to extensive habitat loss and fragmentation driven by a decline in traditional practises, agricultural intensification, and urbanization. However, public pressure and unsympathetic habitat management are increasingly reported as negative factors. Nevertheless, the effects of habitat fragmentation on genetic diversity remains poorly understood. Anthropogenic land use change has left many populations isolated with little prospect for genetic exchange. This

leaves populations susceptible to genetic drift and inbreeding depression, leading to a decrease in fitness and an increased risk of extinction. For example, inbreeding depression has resulted in a decrease in relative clutch size and a higher incidence of stillbirths and congenital deformities in a small adder population in southern Sweden[9]. Furthermore, low genetic diversity may result in reduced adaptive potential, decreasing a population's resilience to stochastic events such as disease or extreme weather (e.g. droughts). Genetic analysis of four adder populations from Gwynedd (northwest wales), Staffordshire (west midlands) and Anglesey (an island off the north west coast of Wales) has revealed very low levels of genome-wide heterozygosity[10]. Population simulations indicate a recent genetic bottleneck likely caused by anthropogenically-driven fragmentation of a previously large and interconnected population.

## Aims & Significance

Adder records collated to date reveal large areas of data deficiency in Alston/South Tynesdale, Teesdale and Stainmore. RSPB Geltsdale represents a significant adder population in the Northwest of the North Pennines, comprised of reproductively distinct populations with potential for genetic exchange. Thus, the adder population at RSPB Geltsdale represents an important source of genetic data against which to compare the genetic diversity of smaller, isolated populations.

The Adders Up Project is a three-year project funded by the National Lottery Heritage Fund that aims to build a more complete picture of the distribution and conservation status of the adder in the North Pennines. In the first year of the project, the North Pennines National Landscape are partnering with The University of Newcastle, under the supervision of Dr Simon Maddock, to conduct a genetic study of adder populations across the North Pennines Landscape. The aim of this research is to determine how genetically isolated adder populations are and the degree of genetic diversity within populations. Our goal is to identify adder populations at greatest risk of extinction and priority areas for improving habitat connectivity to facilitate genetic exchange. Furthermore, phylogenetic analysis will indicate potential source populations for translocation.

## Sampling Protocol

Field work will take place between July and October. Permission will be sought to obtain adder samples from a representative sample of sites distributed across the North Pennines. Adder sampling will be carried out be specially trained and experienced personnel – Lucy Struthers and Samuel Betts, under the guidance of Dr Simon Maddock. A minimum of 4 adder samples are required per population to allow for poor sequencing. A maximum of 200 samples can be processed, equivalent to 50 populations.

Adder sampling involves visual searches (using standardard survey techniques). Adders are safely captured by hand, wearing HexArmour Venom Defender gauntlets and transferred to a snake bag while field equipment is unpacked. The adder is then placed into an appropriately sized restraining tube while 3-4 transverse clippings are taken from the ventral (belly) scales (approximately 2 x 5mm) using a sterile pair of high-precision iridectomy scissors. The process is harmless to the snake and the scales will regrow after a couple of shed cycles.

Scale clipping is preferred to cloacal and buccal swabbing as this method is less stressful and poses no risk to gravid females. Gravid females will be sampled however, care will be taken to ensure that handling time is minimised. Neonates (new born), juveniles (less than 1 year old) and small immature (more than 1 year but not yet sexually mature) adders will not be sampled as their small size makes taking scale clipping challenging and increases risk of injury to the animal. Adders that exhibit excessive signs of stress, for example excessive biting or defensive posturing, will be released without sampling. Snakes than can not be 'tubed within 2 minutes' will be released. If multiple snakes are caught simultaneously, we will use an insulated bag to hold the snakes and process one at a time. Adders that have been sampled have been seen to return to their basking position within 20 minutes. This includes a gravid female from Cuthberts Moor that was seen basking in the same location on a subsequent visit. This indicates that the sampling process is unlikely to be of significant detriment.

Each specimen will be photographed for individual identification; weighed and measured before being released in exact location of capture. The whole process should take no longer than 10 minutes from capture to release. Environmental variables are also recorded for each capture or sighting. Several visits may be required to obtain the required sample size. Larger may have more than one reproductively distinct population (deme). Therefore, additional samples may be collected where distinct populations are identified.

Scale clippings are immediately placed in labelled ethanol microtubes, which are then stored ina freezer until processing. Recently shed skins can also serve as a source of DNA and should be stored in containers to minimise contamination and handling, with silica gel to remove moisture and preserve the specimen. Unused material will be sent to Amphibian and Reptile Conservation's Gene Bank.

## Sample Processing and Analysis

DNA extraction and purification will be performed my L. Struthers and S. Betts using using a commercially available kit (DNeasy Blood & Tissue Kit, QIAGEN) following the manufacturer's protocol. Sample processing will be carried out at the University of Newcastle, under the supervision of Dr Simon Maddock.

Library preparation, whole-genome sequencing and mapping will be carried out by an external commercial facility, Novogene UK Ltd., Cambridge, using an Illumina NovaSeq platform, producing paired-end 150bp sequencing reads, aiming for 8x genome coverage (accounting for sequencing errors).

Genetic analysis will be carried out by L.Struthers, following the methods of Pozzi et al. (2023)[10], under supervision of Dr Simon Maddock. Population structure will be analysed via principle component analysis to determine the how connected or isolated populations are. A measure of genetic admixture

(gene flow), genome-wide heterozygosity and nucleotide diversity will also be determined for each population. Phylogeny will be investigated using a distance-based neighbour joining approach.

This research, including adder sampling protocol has been approved by the Animal Welfare and Ethical Review Body. No license is required for the disturbance or handling of adders.

## References


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