
Assessment of an established population of atypical grass snakes *Natrix natrix* in the Aire Valley, UK

DARRYN J. NASH

*Institute of Integrative and Comparative Biology, University of Leeds, LS2 9JT, UK.
darrynnash@hotmail.com*

ABSTRACT - During the past 20 years, an atypical population of grass snakes has been observed within and around the Esholt Sewage Treatment facility, West Yorkshire, UK. Based solely on phenotypic appearance, the snakes were suspected of originating from southern or eastern Europe. This study sought to ascertain the origin of the snakes through genetic means. An estimate of the population size was calculated using a capture/mark/recapture (CMR) programme. This study also investigated the possibility of discriminating between races using morphometric measurements. Phylogenetic analysis indicated that the Esholt population had originated from eastern Romania and was genetically distinct from native snakes. The CMR programme calculated a population estimate of approximately 46 snakes (95% CI = 28-106), although the actual value was expected to be closer to the latter. Morphometric analysis indicated that snakes could be discriminated by SVL to head width ratios as well as markings. There are no physical barriers enclosing the Esholt Sewage Treatment facility and striped grass snakes have been reported from the surrounding areas. Future monitoring is recommended in order to ascertain population trends and range expansion.

SINCE 1990 atypical specimens of the grass snake *Natrix natrix helvetica* (L) have been observed within and around the Esholt Sewage Treatment facility, West Yorkshire, UK (53°51'07.43, 1°43'13.12) (Sunderland, 2003). The Esholt population differs from typical British grass snakes by the presence of two dorsolateral stripes running the length of the body and a relatively indistinct collar (Fig. 1). Grass snakes with this patterning are found throughout southern and eastern Europe, ranging from the east Adriatic coast through to the Crimea (Arnold & Ovenden, 2002).

It is unclear how the striped grass snakes arrived at Esholt; however, the discovery of a striped neonate (pers. obs.) indicates that the population is breeding. The current study sought to ascertain the origin of the snakes through genetic means. Samples of four mitochondrial DNA (mtDNA) genes, collected using buccal swabs (following Beebee, 2008) were extracted, amplified and sequenced. Sequences were then inserted into the existing natricine phylogeny (Guicking et al., 2006), the results of which enabled inferences to be made regarding the origin of the snakes.

An estimate of the population size (N_e) was calculated using a capture/mark/recapture (CMR) programme. In this case, rather than applying a mark, snakes were re-identified using the unique natural markings on the anterior ventral scales. Population estimates were generated using a Peterson-Schnabel maximum likelihood census model. In addition to the census size, the effective population size N_e (i.e. the genetic population size) was also determined.

It is expected that future monitoring will be carried out by volunteers, who may not have access to a genetics laboratory. Hence a comparative morphometric study was carried out to investigate the possibility of discriminating between races using multivariate statistics (following Thorpe [1984]). A range of measurements, including lengths, widths, weights, scale counts and markings were taken from the Esholt population. These measurements were compared with grass snakes from southern Europe (Rome) and Britain (Dorset and Norfolk). A principal component analysis (PCA) using snout to vent length (SVL), head width, head depth and head length was carried out. All data were log transformed to account for the influence of



Figure 1. An adult female striped grass snake (700 mm SVL) found within the Esholt Sewage Treatment facility in 2010. Photograph by Darryn Nash.

allometry and analyzed using a combination of analysis of variance (ANOVA) and non-parametric equivalents.

RESULTS

Phylogenetic trees, based on the mtDNA genes Cytochrome b and NADH dehydrogenase subunit 4, indicated that the Esholt population was genetically distinct from native snakes (Fig. 2). The genetic sequences, obtained from the Esholt population, closely resembled those Guicking and colleagues (2006) described from the Tulcea region of Eastern Romania; in the case of Cytochrome b, the two sequences were an exact match. This particular genotype is likely to occur throughout Romania; although snakes occupying the east of the country are genetically distinct from those in the west.

The CMR programme calculated an N_c of 46

snakes (95% CI = 28-106), although this figure is considered to be conservative. Low recapture rates are typical for highly transient animals (Madsen, 1984). Based on a census estimate of 46, a N_c of 25 was calculated, assuming a constant sex ratio and excluding animals of less than 300 mm (assumed sexually immature). The principal reason for this disparity in population estimates was a skew in the sex ratio with a greater number of females recorded.

The PCA identified two components that were significant (i.e. had eigenvalues of greater than 1); however, because of the high degree of overlap observed in Component 2 only Component 1 was considered suitable for the purpose of discrimination. Component 1 was based on the SVL to head width relationship. The value for the Esholt population ranged from 35 to 63,

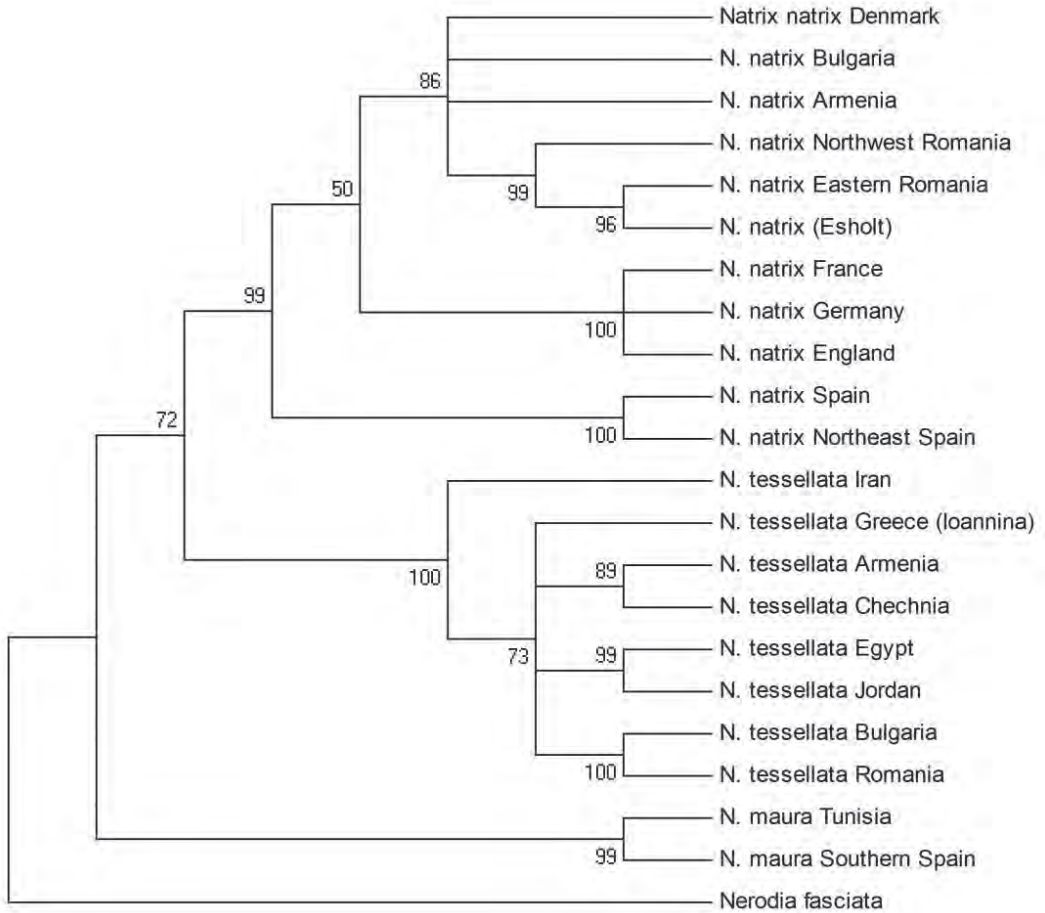


Figure 2. A phylogenetic tree based on the mitochondrion DNA gene Cytochrome b. Sequences taken from the Esholt snakes were inserted into a *Natrix* phylogeny originally produced by Guicking et al. (2006). The sequence generated from the Esholt snakes was identical to that of the Tulcea region of Eastern Romania.

considerably higher than the observed values for the native snakes (16 to 39). A combination of this morphometric relationship and markings (presence of dorsolateral striping and the relative intensity of the collar) enable discrimination of native and alien snakes with a high degree of confidence.

Despite the fact that both the southern European and Esholt populations were significantly larger (SVL) than native snakes ($F = 7.716$, d.f. = 2, $p = 0.01$) (Fig. 3), there were no significant differences in gape size (head length x width x depth) ($F = 1.11$, d.f. = 2, $p = 0.334$). As gape size is a principal determinant of prey choice in snakes, it can be assumed that similar sized prey items would be selected. On two occasions, Esholt snakes

regurgitated common frogs *Rana temporaria* during handling.

DISCUSSION

Although the Esholt population is genetically distinct from native snakes, both belong to the subspecies *helvetica*. It is unlikely that any reproductive barriers exist that would prevent either the transmission of genetic material or the production of viable offspring (R. Thorpe, pers. comm.). However, interbreeding would result in the loss of each of the unique genotypes. This could lead to the dilution of locally adapted genes, thereby lowering the population fitness (outbreeding depression [see Tallmon et al., 2004]).

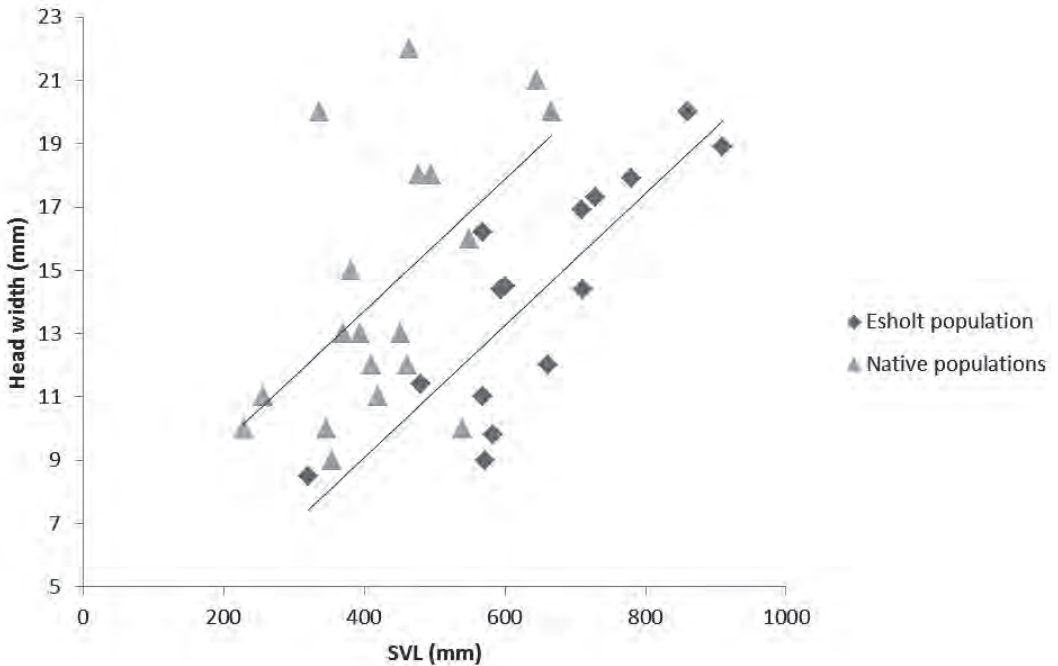


Figure 3. Head widths plotted against snout to vent lengths (SVL) of native and non-native grass snakes. Native specimens exhibited a larger head width relative to body length, when compared with individuals from the Esholt population.

It is important to note that no native grass snakes were located during the surveys and local records were ambiguous. Should the Esholt population encounter native snakes, the effects of interbreeding are likely to be localised and short lasting, with the Romanian genotype being overwhelmed by the native form.

The study population was found to occur at a density of approximately 3 ha⁻¹, comparable to that of native snakes (Beebee & Griffiths, 2000). The Esholt Sewage Treatment facility comprises approximately 140 ha of suitable habitat, which includes unmanaged grassland, hard standing, woodland, scrub and a series of drainage ditches. The River Aire and Leeds-Liverpool Canal both horizontally bisect the site and link up with parcels of habitat in the wider landscape. There are no physical barriers enclosing the Esholt Sewage Treatment facility and striped snakes have been reported in the surrounding areas.

Future monitoring is expected to concentrate on two key areas: monitoring population trends (there

is already speculation that sightings have declined) and range expansion.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to the British Herpetological Society and the Wharfedale Naturalists Society for providing financial support. I would like to thank all of those who assisted surveying in Britain and Italy, particularly Rowland Griffin, Gary Powell, David Price and Jamie Bowkett. Museum specimens were kindly provided by the Museo Civico di Zoologia, Rome. Finally, I wish to thank the following who have provided technical input throughout this project: Prof. Luca Luiselli, Dr. Massimo Capula, Prof. Trevor Beebee, Prof. Roger Thorpe, Dr. David Sewell, Dr. Daniela Guicking, Dr. John Baker and Dr. Todd Lewis.

REFERENCES

Arnold, E.N. & Ovenden, D.W. (2002). *A Field Guide to the Reptiles and Amphibians of Britain*

- and Europe, second edition*. London; Harper Collins Publishers Ltd.
- Beebee, T.J.C. (2008). Buccal swabbing as a source of DNA from squamate reptiles. *Cons. Genetics* **9**, 1087-1088.
- Beebee, T.J.C. & Griffiths, R.A. (2000). *Amphibians and Reptiles*. London: Harper Collins Ltd.
- Guicking, D., Lawson, R., Joger, U. & Wink, M. (2006). Evolution of phylogeny of the genus *Natrix* (Serpentes: Colubridae). *Biol. J. Linn. Soc.* **87**, 127-143.
- Madsen, T. (1984). Movements, home range and habitat use of radio-tracked grass snakes (*Natrix natrix*) in southern Sweden. *Copeia* **3**, 707-713.
- Sunderland, K. (2003). Continental grass snakes (*Natrix natrix natrix*) in Upper Airedale. *Yorkshire Naturalists' Union Bulletin* **40**, 1-3.
- Tallmon, D.A., Luikart, G. & Waples, R.S. (2004). The alluring simplicity and complex reality of genetic rescue. *Trend. Ecol. and Evol.* **19**, 489-496.
- Thorpe, R.S. (1984). Geographic variation is the Western grass snake (*Natrix natrix helvetica*) in relation to hypothesized phylogeny and conventional subspecies. *J. Zool.* **203**, 345-355.
-