An investigation into the provenance of Bedford's midwife toads

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Introduction

The Common Midwife Toad *Alytes obstetricans* is a small amphibian species that has been introduced to Great Britain (Beebee & Griffiths 2000). Midwife toads get their name from the parental care displayed by the male, which wraps the fertilised eggs from a female around his hind legs and cares for them until they are ready to hatch (Fig. 1). It is at this point that he seeks out a suitable body of water to deposit them in, such as a garden pond. It is only during their time as a tadpole that midwife toads rely on water bodies, the rest of their life cycle is completed on land including breeding. This is completely different to the strategies employed by our native species that breed explosively in water bodies come the spring.

A number of feral populations are known throughout England and Wales, the first of these to be identified was in Bedford. This population was first recorded in 1903, although it is likely that the midwife toads had been present for some time prior to this (Beebee & Griffiths 2000). Midwife toad populations are usually identified when large tadpoles, which have overwintered, are discovered in resident's ponds, or through the conspicuous electronic-like beeping call the males make. It has long been assumed that

Fig. 1 A male midwife toad (from the Cambridge population) carrying a string of eggs, demonstrating how this species gets its name. *Steven Allain*

midwife toads were accidentally introduced to Bedford in the packing cases of plants from the south of France (Fitter 1959; Smith 1949). This makes logical sense as the original area where midwife toads were identified was a garden nursery operated by Horton & Smart.

The nursery and pond which housed the toads was demolished in 1922 to make way for housing, with the toads being moved nearby, although Palmer (1949) gives the date of this happening as 1919. A number of other populations are known to exist around Bedford (Fig. 2) and it is plausible that they all originate from Bedford stock, although midwife toads were once a popular pet so some of these may be separate introductions. Bedford's midwife toads have however been recorded as the founder stock for a number of other populations in Great Britain such as in York in 1933 (Blackwell 1985) and Worksop in 1947 (Smith 1950).

The aim of the project was to collect DNA samples from populations of midwife toads from Bedford and the surrounding villages (such as Bromham and Sutton) to determine if they shared the same introduction pathway and to investigate where the toads originated, by comparing the sequenced DNA to an online library of comparative sequences. Based on the literature it was expected that the populations sampled would be identical indicating the same introduction pathway and that their country of origin would be France.

Fig. 2 A map highlighting the sampled midwife toad colonies as part of this project (red diamonds). Additional sightings and records of other midwife toads in the Bedford area have been omitted.

Methods

Four populations of midwife toads were identified from Bedford and the surrounding villages based on the ease of access and therefore collection of specimens. Thankfully a number of captive collections exist from which midwife toads from Bromham and Biddenham were the founding stock. In order to collect the required genetic material for sequencing, MW-100 fine-tip dry swabs (Medical Wire & Equipment, Corsham, Wiltshire) were used to take buccal samples from individuals for each population. Wild midwife toads from Bedford Park and Sutton were also swabbed, following the same protocol under licence from Natural England.

DNA was extracted from each individual swab using either the Chelex method (Singer-Sam *et al*. 1989) or a modified CTAB method (Winnepenninckx *et al*. 1993), both of which have proven successful and cost efficient for obtaining quality sequences from non-tissue samples. The 16S global amphibian barcode and the COI global barcode were amplified via PCR for each extracted sample. These mitochondrial gene fragments were chosen based on the availability of comparative sequences covering the known distribution of *A. obstetricans*. Successfully amplified samples underwent Sanger sequencing at Macrogen Europe. The resulting sequences were then inspected and aligned. These sequences were then subjected to a BLAST (Altschul *et al*. 1990) search and compared to reference sequences held in the GenBank library (Benson *et al*. 2012). This process provided two functions: (i) it confirmed the identity of the species from which each sequence was derived and (ii) it created a list of reference sequences to which our sequences were most similar.

Results

All individuals from the four Bedford populations surveyed were confirmed to be *A. obstetricans* and genetically identical for both 16S and COI gene sequences. This provides significant support to the credence that Bedford toads had been used to found the populations of *A. obstetricans* in the surrounding towns and villages. However, when investigating the origins of those original toads the results were not what we expected and further research is required. This is due to a limitation in the reference sequences, where not all sequences are long enough for comparative purposes.

Discussion

Despite confirming that the *A. obstetricans* populations found in and around Bedford are genetically identical, we are still unsure of their origin. Our results also indicate that a single introduction of *A. obstetricans* to the area is most likely, with new populations being founded through the movement of the already established toads rather than multiple, distinct introductions. A cause of these man-mediated translocations is likely in-part due to residents having a fondness for the species and taking them with them when they move house (Blackwell 1985). Overall, this study increases our knowledge on the potential impacts of trade, population movements and human affinity for introducing non-native species. A similar protocol has previously been used to determine the provenance of introduced Alpine Newts *Ichthyosaura alpestris* to New Zealand and Smooth Newts *Lissotriton vulgaris* to Australia (Tingley *et al*. 2015), both countries who lack native urodele species (Arntzen *et al*. 2016).

Our results highlight the issues with relying on reference databases for comparative purposes, the results can only be as good as the reference sequences available. Despite the availability of sufficient sequence data for *A. obstetricans* for the Iberian Peninsula, there is a lack of comparative sequences available from the rest of their range north of the Pyrenees. It is hoped that these challenges can be overcome in the future by accessing mitochondrial sequences that are not currently in the public domain and is something we hope to implement in the future. This research is part of an ongoing national project to assess the origins of all known *A. obstetricans* populations in Great Britain, involving a number of citizen scientists from around the country. As Bedford is the siting of the oldest known introduction, it is important to establish as much

information as possible about the midwife toad populations there before any conclusions can be drawn on a national scale. Further research is still required, particularly in regards to the provenance of Bedford's midwife toads and if they were also used to found other populations within Great Britain. This study also demonstrates the benefits of scientist-led citizen science, with teams of people able to collect far more data over a given time period than just one researcher working alone.

Although genetic information is currently limited to a small number of sites in Great Britain, it is hoped that over the lifespan of the project we'll be able to provide potential insights into the invasion ecology and establishment potential of *A. obstetricans* on the island. Throughout their native range in Europe, *A. obstetricans* are declining due to factors such as disease and habitat loss (Bosch *et al.* 2001). However, in Great Britain, their populations appear to be stable although more monitoring is needed to confirm this. So far, the limited disease surveillance has shown that midwife toads are free from known pathogens such as the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, but more effort is needed in the future (Allain & Goodman 2018). Once the national project is complete, we'll deposit the sequences collected onto GenBank so that they are available for other researchers to use in future studies.

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Map produced using QGIS 3.12.

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Glossary

BLAST: The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches.

DNA: Deoxyribonucleic acid (DNA) is a molecule composed of two polynucleotide chains that coil around each other to form a double helix carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses.

GenBank: A comprehensive database that contains publicly available nucleotide sequences for over 280,000 formally described species.

PCR: Polymerase chain reaction (PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

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The British Midwife Toad Project has two main aims: (i) to determine the presence of midwife toad colonies in Great Britain and (ii) to determine their origin using comparative genetic sequences to populations throughout the species' natural range. It's likely that, with a number of populations spread throughout England and Wales, there have been multiple introductions of these distinctive toads. Where possible, populations are also screened for the amphibian chytrid fungus to determine whether or not the population may pose a threat to our native amphibians.