Eur J Wildl Res (2015) 61:27–33
DOI 10.1007/s10344-014-0863-7

ORIGINAL PAPER

Extending the natural range of a declining species: genetic evidence for native great crested newt (*Triturus cristatus*) populations in the Scottish Highlands

C. D. O'Brien · J. E. Hall · D. Orchard · C. D. Barratt · J. W. Arntzen · R. Jehle

Received: 9 July 2014 / Revised: 19 September 2014 / Accepted: 23 September 2014 / Published online: 5 October 2014

Abstract Determining whether isolated populations of a species are native or introduced is important for conservation, as non-native occurrences are likely to be of lower priority for conservation organisations with limited resources. The great crested newt *Triturus cristatus* is an important wetland flagship species in the UK, and recent evidence suggested that putatively introduced isolated occurrences around Inverness (Scottish Highlands) might be of native origin. Here, we use six microsatellite loci and mtDNA sequence information (ND4 region) to genetically characterise eight Highland populations, comparing them with two populations from central Scotland (the northern limit of the species' continuous UK distribution) and central England (Leicestershire). Highland populations were characterised by low amounts of genetic variation at high degrees of differentiation, which can be best interpreted by demographic instability and isolation at the periphery of the species' range. We found no evidence for population bottlenecks in the last decades. All studied individuals possessed a single mtDNA haplotype previously described for British *T. cristatus*. Taken together, these results suggest that *T. cristatus* is native to the Scottish Highlands, with important implications for its local conservation status.

Communicated by C. Gortázar

C. D. O'Brien · J. E. Hall
Scottish Natural Heritage, Leachkin Road, IV3 8NW Inverness, UK

D. Orchard · C. D. Barratt · R. Jehle (✉)
School of Environment and Life Sciences, University of Salford, M5 4WT Salford, UK
e-mail: r.jehle@salford.ac.uk

J. W. Arntzen
Naturalis Biodiversity Center, P. O. Box 9517, 2300, RA Leiden, The Netherlands

Keywords Microsatellites · mtDNA · Scottish Highlands · *Triturus cristatus*

Introduction

Understanding patterns and processes at range margins is important for the conservation management of declining species. Near the edge of a species' geographical limit, populations are typically smaller and more unstable compared to the range centre, and show a more scattered local distribution (Gaston 2009; Sexton et al. 2009). Of particular interest are occurrences away from a species' continuous range, at distances exceeding natural dispersal abilities. They can be interpreted as signatures of range contraction, for example caused by human habitat destruction or longer-term climatic oscillations. Alternatively, they can also be the result of human-assisted colonisation. From a conservation perspective, it is important to address whether local occurrences are native or not, as indigenous populations usually warrant significantly more protection efforts than populations stemming from introductions.

Genetic tools can trace back the demographic history of populations, and are therefore also able to distinguish between native and introduced populations when historical knowledge on the origin of local occurrences is absent or ambiguous (Saltonstall 2002; Fitzpatrick et al. 2012; Avery et al. 2013; Pfenninger et al. 2014). In taxa with low powers of dispersal, such as amphibians, colonisation is expected to proceed along a broad front rather than through isolated populations separated by large distances from the main range (e.g. Arntzen and Wallis 1991; Urban et al. 2008; but see also White and Shine 2009). While dispersal abilities of amphibians are now thought to be higher than assumed in the past (Smith and Green 2005; Jehle and Sinsch 2007), they are on average several orders of magnitude less mobile than for example

mammals or birds (Wells 2007). Focusing on isolated occurrences, information drawn from genetic markers has already proven invaluable to identify the source of known introductions (Arntzen and Thorpe 1999; Ficetola et al. 2008), to discern between adjacent native and introduced populations (Johnson et al. 2011), to demonstrate that putatively native occurrences actually stem from introductions (Dubey et al. 2014), and to demonstrate that potentially introduced populations are actually likely to be native (Zeisset and Beebee 2001; Arntzen et al. 2010).

The great crested newt *Triturus cristatus* has a wide distribution across northern parts of Europe, including England and Scotland (e.g. Jehle et al. 2011). In the Scottish Highlands, occurrences around Inverness are separated by more than 80 km of unfavourable habitat from the northern limits of its main British distribution in the central belt of Scotland. As a consequence, they were in the past regarded as stemming from introductions (e.g. Langton and Beckett 1995), resulting in a situation where legislation would make it in effect illegal to re-release animals, for example those caught during surveys (Wildlife and Natural Environment (Scotland) Act 2011). However, a recently formalised alternative biogeographic scenario outlines a possible colonisation route from the south between 3000 and 7000 years ago (O'Brien and Hall 2012). Corroborated by evidence from historical records (Harvie-Brown and Buckley 1895) and the existence of several clusters of populations divided by tens of kilometres in fragmented landscapes, this scenario suggests that the majority of the Scottish Highland populations might actually be native. Here, we present data from genetic markers (mtDNA and microsatellites) across the distribution of *T. cristatus* in the UK (Scottish Highlands, central Scotland, central England), to provide evidence that the populations around Inverness are indeed indigenous. *T. cristatus* is an important wetland flagship species protected under European law (Annex II and Annex IVa of the European Habitats Directive), and this finding has profound consequences for the species' local conservation management. It also extends the known natural range of the species at its northwestern part of the range.

Materials and methods

Twelve sites were sampled in total: eight in the Scottish Highlands, two in central Scotland (representing the northern limit of the continuous distribution of *T. cristatus* in the UK), and two in central England (Leicestershire, representing populations from the core area of *T. cristatus* in the UK, Fig. 1). Fieldwork took place between 23 May and 29 May 2012 (Scotland), and in the early 1990s (Leicestershire). DNA sampling was based on the collection and immediate storage of embryos (eggs) in ethanol in the field. Egg sampling is a standard method to retrieve DNA for newts (e.g. Jehle et al. 2005a); it has minimal effects on population dynamic processes (each female lays about 200–400 eggs per season at high natural mortality rates, e.g. Jehle et al. 2011) and avoids pond perturbation through trapping and netting for DNA sampling from adults. The Highland sites included at least one site from each of O'Brien and Hall's (2012) proposed clusters of populations; for more details of the study area in Leicestershire, see Scribner et al. (2001).

DNA extractions were performed using standard phenol–chloroform procedures (Bruford et al. 1998). For an analysis based on nuclear microsatellites, we genotyped individuals at six polymorphic loci (*Tcri*27, *Tcri*29, *Tcri*35, *Tcri*36, *Tcri*43 and *Tcri*46; Krupa et al. 2002). Polymerase chain reactions (PCRs) were carried out in 10-μL reaction volumes under the following conditions: 1 or 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 U polymerase (GoTaq) and 0.1 mM of each primer in the manufacturer's buffer. Thermal profiles for *Tcri*27, *Tcri*35, *Tcri*36 and *Tcri*43 were 39 cycles of 30 s at 93 °C, 30 s at the primer-specific annealing temperature, 45 s at 72 °C; for *Tcri*29 and *Tcri*46, we used a 'touchdown' thermal profile (for more details, see Krupa et al. 2002). Primers were labelled with fluorochromes AT550, HEX and FAM. PCR products were separated on an ABI3130 Genetic Analyser (Applied Biosystems) and scored using Peakscanner v1.0 (Applied Biosystems) and Tandem v1.08 (Matschiner and Salzburger 2009). Individuals for which more than two loci failed to amplify were discarded.

Allele frequencies, Hardy–Weinberg equilibria and measures of genetic differentiation (F_{ST}) were calculated using Genepop on the Web (Rousset 2008). The possible existence of past population bottlenecks was investigated with the software Bottleneck (Luikart et al. 1998), using the Wilcoxon sign rank test and a mixed model of stepwise and infinite allelic mutations of 95 and 5 %, respectively (as determined previously for the employed *T. cristatus* microsatellites; Jehle and Arntzen 2002). Because multiple tests were conducted across study sites and loci, Bonferroni-adjusted significance levels were applied when applicable. For the Scottish Highland populations, isolation-by-distance scenarios were tested using Mantel tests to correlate F_{ST} and log-transformed geographic distances as implemented in the software IBD (Bohonak 2002); geographic distances were calculated along shorelines if applicable, as dispersal through salt water is impossible for *T. cristatus*. The partitioning of genetic variation among populations was addressed using the hierarchical Bayesian approach implemented in the software BAPS v3.2 (Corander et al. 2004), which clusters a predefined substructure (sites) into a more meaningful structure as reflected in the dataset.

We also characterised two random individuals from each population from Scotland (Highland and central Scotland) by sequencing a 658-bp-long ND4 (mtDNA) fragment using existing PCR primers and protocols (F: AGCGCCTGTCGC CGGGTCAATA, R: GTGTTTCATAACTCTTCTTGGT,

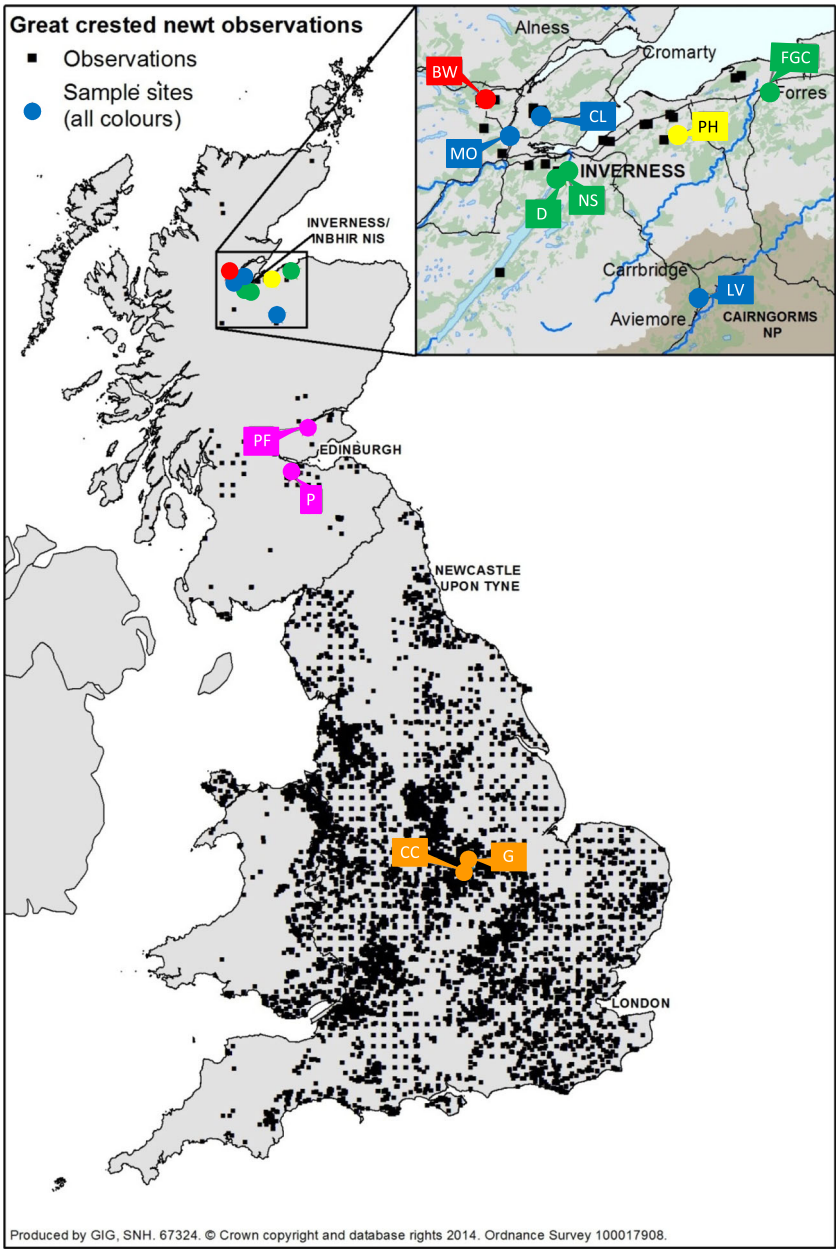

Fig. 1 Distribution of the great crested newt (*Triturus cristatus*) in the UK (NBN 2014), and location of sampling sites. The different colours of *T. cristatus* locations represent genetic clusters as identified using the software BAPS (for more details see text)

Wielstra et al. 2010). PCR reactions were carried out in a 10-μL reaction volume with the following constituents: 10 ng genomic DNA, 1 μL GoTaq×5 reaction buffer (1.5 mM $MgCl_2$ in ×1 concentration), 1 μM of each primer, 0.25 mM of each dNTP and 1 unit of GoTaq DNA Taq polymerase. Amplified products were cleaned using a standard ethanol/salt precipitation method and commercially sequenced in both directions. Sequences were examined using Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2013).

Field work and sample collecting was conducted under licences issued by Scottish Natural Heritage (Licence 13374) in Scotland.

Results

In total, 253 individuals across 12 sites and three British regions were analysed (Table 1). Five populations showed significant deviations from the Hardy–Weinberg equilibrium at one locus each (largely heterozygote deficiencies: D, MO, NS, LV, CC, Table 2). As these deviations are not based on specific loci, we argue that it is unlikely that they are caused by locus-specific non-amplifying alleles.

The amount of genetic variation found for each population was markedly lower in Scottish populations compared to central England. The central England populations displayed about three times higher amounts of alleles per locus than

Table 1 Details of sample sites

	Pond name	Abbreviation	Grid reference	Sample size (*n*)
Highland	Blackmuir Wood	BW	NH480572	28
	Cinquefoil Loch	CL	NH593538	19
	Dunain	D	NH632416	27
	Forres Golf Club	FGC	NJ049587	5
	Muir of Ord	MO	NH530501	26
	Ness Side	NS	NH643426	22
	Piper Hill	PH	NH865503	14
	Loch Vaa	LV	NH910177	21
Central Scotland	Pitmedden Forest	PF	NO195136	18
	Pumpherston	P	NT065702	13
Leicestershire	Corn Close	CC	SK655025	30
	Gaddesby	G	SK684132	30

populations from Scotland; observed heterozygosities of the two central Scotland populations were between the values of the eight Highland and the two central England populations (Table 2). In the Highlands, otherwise polymorphic loci were observed as fixed for specific populations in several instances (*Tcri*27: BW, *Tcri*29: PH, CL, *Tcri*36: D, PH; due to low sample size we do not consider population FGC here), whereas all loci were polymorphic in central Scotland and central England. In line with their higher level of genetic variation, the central England populations also possessed the highest numbers of private alleles, which were rare for Highland populations (Table 2). There was no consistent signature of past population bottlenecks across the study populations; the lowest p value shown by population P in central Scotland (0.02) is non-significant after applying a Bonferroni-adjusted threshold (Table 2).

Measures of genetic differentiation (F_{ST}) revealed that most of the study populations were highly genetically differentiated from each other (Table 3). F_{ST} values between Highland and central Scotland, and those between Highland and central England sites, were above 0.24 throughout. F_{ST} between the two central Scotland and the central England sites were 0.11 and 0.03, respectively. F_{ST} values between Highland sites had a wide range between 0.03 and 0.52, with a marginally significant pattern of isolation by distance (Z=11.314, r=0.338, p=0.055). The hierarchical analysis implemented in BAPS v3.1 revealed an optimum partition comprising six clusters. Both central England and central Scotland sites were assigned to one cluster each (PF/P and CC/G); the remaining clusters consisted of the merged Highland populations, CL/MO/LV and D/FGC/NS, and the single Highland populations BW and PH (Fig. 1).

Table 2 Descriptive population genetic parameters for the sites under consideration

Site	Mean H_o	Mean H_e	Loci out of HWE	Mean *n* alleles/locus	Number of private alleles	*P*
Populations from Scottish Highlands						
BW	0.25	0.26	–	2.67	1	0.15
CL	0.39	0.40	–	3.33	0	0.22
D	0.37	0.44	*Tcri*27	2.67	0	0.22
FGC	0.41	0.35	–	1.6	0	0.25
MO	0.30	0.38	*Tcri*29	3.67	0	0.08
NS	0.36	0.52	*Tcri*35	3.33	1	0.69
PH	0.28	0.33	–	2.50	1	0.31
LV	0.37	0.42	*Tcri*36	3.17	0	0.62
Populations from the continuous range						
PF	0.43	0.53	–	3.67	2	0.44
P	0.56	0.53	–	2.83	0	0.02
CC	0.84	0.80	*Tcri*36	8.17	6	0.56
G	0.82	0.80	–	8.83	7	0.84

H_o observed heterozygosity, H_e expected heterozygosity, *HWE* Hardy–Weinberg equilibrium, *P* significance levels for past population bottlenecks (Wilcoxon test, for details see text)

Table 3 Pairwise genetic differentiation (F_{ST} values) across sampling sites

	BW	CL	D	FGC	MO	NS	PH	LV	PF	P	CC
CL	*0.18*										
D	*0.51*	*0.36*									
FGC	*0.52*	*0.22*	*0.27*								
MO	*0.22*	*0.05*	*0.41*	*0.24*							
NS	*0.40*	*0.22*	*0.03*	*0.14*	*0.27*						
PH	*0.42*	*0.29*	*0.27*	*0.36*	*0.32*	*0.21*					
LV	*0.32*	*0.11*	*0.36*	*0.14*	*0.12*	*0.21*	*0.43*				
PF	0.45	0.35	0.30	0.36	0.39	0.25	0.33	0.37			
P	0.53	0.37	0.27	0.36	0.42	0.25	0.47	0.40	0.11		
CC	0.37	0.29	0.31	0.30	0.32	0.27	0.35	0.32	0.23	0.23	
G	0.39	0.31	0.28	0.29	0.32	0.24	0.32	0.31	0.24	0.21	0.03

Values rendered in italics are the populations from the Highlands, values rendered upright are populations from the continuous range in the UK

All of the 20 Scottish individuals examined possessed the single ND4 haplotype previously found in British populations (GenBank accession numbers GU982383, Wielstra et al. 2010, and HQ697273, Wielstra and Arntzen 2011), confirming that, in the UK, *T. cristatus* comprises a single phylogeographic lineage depauperate in variation at the mtDNA level. Given that the haplotype is widespread across large parts of the species' range (including its glacial refugia in Southeastern Europe, Wielstra et al. 2013), it is, however, of limited diagnostic value to infer the native status of the Highland populations.

Discussion

The primary purpose of this study was to use molecular means to investigate the origin of isolated *T. cristatus* populations in the Scottish Highland region. Although these occurrences were previously assumed to be introduced (with, however, unknown sources, e.g. Langton and Beckett 1995), we argue that the most parsimonious interpretation of the gathered population genetic evidence is that they actually represent native populations.

The examined populations showed no evidence of a recent founder event followed by a demographic expansion, which would be expected for populations which have experienced an introduction based on a small propagule pool. The employed method is able to detect a bottleneck signature for up to about ten generations after its occurrence (Luikart et al. 1998). Typical average generation times for *T. cristatus* were estimated to be around 4–5 years (for a summary, see Jehle et al. 2011) and, due to the colder climate, are expected to be higher at northern range limits. Thus, our approach covered a period of about four to five decades back in time (~1960s). However, more than six loci would be necessary for a high statistical power to detect past bottlenecks (Luikart et al. 1998). Nevertheless, there were no noticeable differences between Highland populations and known native populations from central Scotland and England, suggesting that all groups of populations have experienced comparable recent demographic histories.

The discounting of a recent introduction based on demographic analyses is supported by records of the species in the region of Inverness dating back to the end of the nineteenth century (Harvie-Brown and Buckley 1895). That the Highland populations possessed a common (and the only British) *T. cristatus* ND4 mtDNA haplotype did not entirely rule out an introduction from other phylogeographic lineages in mainland Europe, and allows the possibility of an introduction by, e.g. Victorian naturalists earlier in the same century. This, however, lacks historical evidence, as only two introductions of *T. cristatus*, both of which are over 75 km away from the populations near Inverness, are reported (Scottish Natural Heritage, unpublished). Assisted colonisations have high failure rates for *T. cristatus* (even when specifically conducted for conservation purposes, Oldham and Humphries 2000), and isolated populations are predicted to have high long-term extinction rates (Halley et al. 1996). The success of amphibian translocations also rises with the size of the donor population regardless of the adaptive genetic background (Zeisset and Beebee 2013), and it is unlikely that deliberate introductions are based on a large number of propagule individuals. Taken together, this suggests that tens if not hundreds of introductions would have been necessary for the local occupancy we see today, which is highly unlikely.

A main finding of the present study is the pronounced decline in microsatellite genetic diversity from central England northward to the Scottish Highlands. While the general pattern is in line with previous knowledge of the distribution of genetic diversity across the range of *T. cristatus* (Babik et al. 2009; Wielstra et al. 2013), a roughly three-fold decrease in microsatellite variation within a distal (island) area

about 2000 km away from the species glacial refugium is difficult to explain by post-glacial range expansion alone. Low levels of neutral variation for Highland populations would fit the pattern expected of an introduction. However, an introduction from core areas of the species' range (UK or Europe) would result in marked differences in the amount of genetic variation between the northern limit of the species' continuous range and the Highland populations, which was not observed. We argue that the observed patterns are rather a result of demographic processes in peripheral populations, which include reduced gene flow, small population size and higher extinction–recolonisation turnover (see also, e.g. Peterman et al. 2013 for North American frogs). The two populations from central England showed amounts of neutral variation equal to or higher than that of populations in central and Western Europe (Jehle et al. 2005a; Babik et al. 2009; Schön et al. 2011), reinforcing the interplay between local population processes and biogeographic history in shaping the amount of genetic variation at the level of microsatellites.

Within the Highland populations, several loci were monomorphic in some populations but polymorphic in others. The observed pairwise F_{ST} values for Highland *T. cristatus* exceed the values documented during studies conducted elsewhere at comparable spatial scales (0.07–0.11: Jehle et al. 2005a, 0.074–0.141: Schön et al. 2011). This confirms that a scenario of assisted colonisation of the Highlands is only plausible if multiple successful introductions have taken place, which we deem highly unlikely. Instead, we argue that local forces, such as low local population density combined with a terrestrial environment around ponds which is unfavourable for dispersal, lead to high degrees of genetic drift and fragmentation of otherwise natural populations, which is also reflected in the weak but existing pattern of isolation by distance. It is also noteworthy that the genetic data match with the barriers to dispersal proposed by O'Brien and Hall (2012). With an F_{ST} value of 0.03, NS and D have the lowest genetic differentiation between them, and indeed are situated in close geographic proximity. F_{ST} between MO and CL is also low (0.05), and nineteenth century maps suggest that they would formerly have been connected by favourable terrestrial habitat (O'Brien and Hall, unpublished data). That the algorithm in BAPS merged the two central Scotland and central England sites into one cluster each, and separated the ten Highland sites into four further clusters, once again supports the hypothesis that the Highland occurrences are of native origin. As found for a set of *T. cristatus* populations in Western Europe (Jehle et al. 2005b), high random genetic drift in the absence of connectivity between specific pairs of populations is likely to be responsible for clusters partially, but not entirely, representing the actual spatial settings. Due to our hierarchical sampling and problematic effects of isolation by distance (Frantz et al. 2009), we refrained from a further analysis of population assignments using, e.g. the software Structure.

The weight of evidence suggests that *T. cristatus* is native to the Highland region, and this has already given impetus to efforts to conserve the species, particularly site protection and habitat creation to link previously isolated populations. The genetic data further highlight that Highland populations might be at risk from loss of fitness arising from low genetic diversity (microsatellite heterozygosity is correlated with body size and age in *T. cristatus*, Herdegen et al. 2013). Ongoing fieldwork has already discovered several new sites (Miró, O'Brien and Hall, unpublished) and suggests that habitat requirements of Highland populations resemble those of *T. cristatus* at the northern range margins in Scandinavia (e.g. Skei et al. 2006). Taken together, this work has contributed to our set of case studies showing how genetic research can be used to clarify the origin of local populations, and has extended the known northwestern range of an important wetland flagship vertebrate.

Acknowledgments Andy Krupa, Sophie Ward and Robert Coles helped during the laboratory work. Àlex Miró and Katie and Danny O'Brien helped with field work in Scotland. Figure 1 was produced by Jake Hanson, based on data courtesy of the NBN with thanks to all the data contributors. The NBN and its data contributors bear no responsibility for the further analysis or interpretation of this material, data and/or information.

Conflict of interest The authors declare that they have no conflict of interest.

References

Arntzen JW, Thorpe R (1999) Italian crested newts in the basin of Geneva: distribution and genetic interaction with autochthonous species. Herpetologica 55:423–433

Arntzen JW, Wallis G (1991) Restricted gene flow in a moving hybrid zone of the newts *Triturus cristatus* and *T. marmoratus* in western France. Evolution 45:805–826

Arntzen JW, Burke T, Jehle R (2010) Estimating the propagule size of a cryptogenic crested newt population. Anim Cons 13(suppl.1):74–81

Avery JD, Fonseca DM, Campagne P, Lockwood JL (2013) Cryptic introductions and the interpretation of island biodiversity. Mol Ecol 22:2313–2324

Babik W, Pabijan M, Arntzen JW, Cogalniceanu D, Durka W, Radwan J (2009) Long-term survival of a urodele amphibian despite depleted major histocompatibility complex variation. Mol Ecol 18:769–781

Bohonak AJ (2002) IBD (Isolation by Distance): a program for analysis of isolation by distance. J Hered 93:153–154

Bruford MW, Hanotte O, Brookfield JFY, Burke T (1998) Multilocus and single-locus DNA fingerprinting. In: Hoelzel AR (ed) Molecular genetic analysis of populations: a practical approach, 2nd edn. IRL, Oxford, pp 287–336

Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics 20:2363–2369

Dubey S, Leuenberger J, Perrin N (2014) Multiple origins of invasive and 'native' water frogs (*Pelophylax* spp.) in Switzerland. Biol J Linn Soc 112:442-449

Ficetola GF, Bonin A, Miaud C (2008) Population genetics reveals origin and number of founders in a biological invasion. Mol Ecol 17:773–782

Fitzpatrick BM, Fordyce JA, Neimiller M, Reynolds RG (2012) What can DNA tell us about biological invasions? Biol Invasions 14:245–253

Frantz AC, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? J Appl Ecol 46:493–505

Gaston KJ (2009) Geographic range limits: achieving synthesis. Proc Roy Soc B 276:1395–1406

Halley J, Oldham RS, Arntzen JW (1996) Predicting the persistence of amphibian populations with the help of a spatial model. J Appl Ecol 33:455–470

Harvie-Brown JA, Buckley TE (1895) A vertebrate fauna of the Moray Basin. David Douglas, Edinburgh

Herdegen M, Nadachowska-Brzyska K, Konowalik A, Babik W, Radwan J (2013) Heterozygosity, sexual ornament and body size in the crested newt. J Zool 291:146–153

Jehle R, Arntzen JW (2002) Review: microsatellite markers in amphibian conservation genetics. Herp J 12:1–9

Jehle R, Sinsch U (2007) Wanderverhalten und Orientierung bei Amphibien: eine Übersicht. Zeitschr Feldherpetol 14:137–153

Jehle R, Wilson GA, Arntzen JW, Burke T (2005a) Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt populations (*Triturus cristatus*, *T. marmoratus*). J Evol Biol 18:619–628

Jehle R, Burke T, Arntzen JW (2005b) Delineating fine-scale genetic units in amphibians: probing the primacy of ponds. Conserv Genet 6:227–234

Jehle R, Thiesmeier B, Foster J (2011) The crested newt: a dwindling pond dweller. Laurenti, Bielefeld

Johnson JR, Thomson RC, Micheletti SJ, Shaffer HB (2011) The origin of tiger salamander (*Ambystoma tigrinum*) populations in California, Oregon, and Nevada: introductions or relicts? Conserv Genet 12:355–370

Krupa AP, Jehle R, Dawson DA, Gentle LK, Gibbs M, Arntzen JW, Burke T (2002) Microsatellite loci in the crested newt (*Triturus cristatus*) and their utility in other newt taxa. Conserv Genet 3:87–89

Langton TES, Beckett CL (1995) A preliminary review of the distribution and status of great crested newt *Triturus cristatus* records in Scotland. HCI/SNH, Halesworth, Suffolk

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238–247

Matschiner M, Salzburger W (2009) TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics 25:1982–1983

NBN (2014) www.data.nbn.org.uk/Taxa/NHMSYS0000080156 [7 July 2014]

O'Brien CD, Hall J (2012) A hypothesis to explain the distribution of the great crested newt *Triturus cristatus* in the Highlands of Scotland. Herp Bull 119:9–14

Oldham RS, Humphries RN (2000) Evaluating the success of great crested newt (*Triturus cristatus*) translocation. Herp J 10:183–190

Peterman WE, Feist SM, Semlitsch RD, Eggert LS (2013) Conservation and management of peripheral populations: spatial and temporal influences on the genetic structure of wood frog (*Rana sylvatica*) populations. Biol Cons 158:351–358

Pfenninger M, Weigand A, Balint M, Klussmann-Kolb A (2014) Misperceived invasion: the Lusitanian slug (*Arion lusitanicus* auct. non-Mabille or *Arion vulgaris* Moquin-Tandon 1855) is native to Central Europe. Evol Appl 7:702-713

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Res 8:103–106

Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. Proc Natl Acad Sci U S A 99:2445–2449

Schön I, Raepsaet A, Goddeeris B, Bauwens D, Mergeay J, Vanoverbeke J, Martens K (2011) High genetic diversity but limited gene flow in Flemish populations of the crested newt, *Triturus cristatus*. Belg J Zool 141:3–13

Scribner KT, Arntzen JW, Cruddace N, Oldham RS, Burke T (2001) Environmental correlates of toad abundance and population genetic diversity. Biol Cons 98:201–210

Sexton JP, McIntire PJ, Angert AL, Rice KJ (2009) Evolution and ecology of species range limits. Ann Rev Ecol Evol Syst 40:415–436

Skei JK, Dolmen D, Rønning L, Ringsby TH (2006) Habitat use during the aquatic phase of the newts *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) in central Norway: proposition for a conservation and monitoring area. Amphibia-Reptilia 27:309–324

Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110–128

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729

Urban MC, Phillips BL, Skelly DK, Shine R (2008) A toad more traveled: the heterogeneous invasion dynamics of cane toads in Australia. Am Nat 171:E134–E148

Wells KD (2007) Ecology and behaviour of amphibians. University of Chicago Press, Chicago

White AW, Shine R (2009) The extra-limital spread of an invasive species via 'stowaway' dispersal: toad to nowhere? Anim Cons 12:38–45

Wielstra B, Arntzen JW (2011) Unraveling the rapid radiation of crested newts (*Triturus cristatus* superspecies) using complete mitogenomic sequences. BMC Evol Biol 11:162

Wielstra B, Themudo GE, Güclü Ö, Olgun K, Poyarkov NA, Arntzen JW (2010) Cryptic crested newt diversity at the Eurasian transition: the mitochondrial DNA phylogeography of Near Eastern *Triturus* newts. Mol Phyl Evol 56:888–896

Wielstra B, Crnobrnja-Isailović J, Litvinchuk SN, Reijnen BT, Skidmore AK, Sotiropoulos K, Toxopeus AG, Tzankov N, Vukov T, Arntzen JW (2013) Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modelling. Front Zool 10:13

Zeisset I, Beebee TJC (2001) Determination of biogeographical range: an application of molecular phylogeography to the European pool frog *Rana lessonae*. Proc R Soc Lond B 268:933–938

Zeisset I, Beebee TJC (2013) Donor population size rather than local adaptation can be a key determinant of amphibian translocation success. Anim Cons 16:359–366