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TRACING ALIENS: IDENTIFICATION OF INTRODUCED WATER FROGS IN BRITAIN BY MALE ADVERTISEMENT CALL CHARACTERISTICS

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We have used sound analysis of male advertisement calls in a study of seven introduced populations of water frogs in Britain. Discriminant analysis of call characters identified five types of water frog, notably *Rana lessonae, R. esculenta, R. ridibunda, R. bergeri* and *R. perezi*. *Rana epeirotica* and *R. shqiperica* were not detected. Typical LE (*lessonae-esculenta*) systems were found at two sites, *R. ridibunda* occurred alone at two sites and *R. esculenta* occurred alone at one site. The remaining two sites were more complex. One had *R. ridibunda*, *R. perezi* and *R. esculenta* while the seventh site had four taxa of water frog (*R. lessonae, R. bergeri, R. esculenta* and *R. perezi*). The value of call analysis for the identification of water frog populations is discussed.

Key words: alien species, call frequency, green frogs, oscillograms, *Rana*

INTRODUCTION

Until recently it was generally accepted that six species of amphibian are postglacial natives of the British Isles (Smith, 1951). None of the European water frogs were included on this native list, although these amphibians are common and widespread in mainland Europe. Water frogs are a complex group and their taxonomy has been widely studied during recent decades (Berger, 1973; Wijnands, 1977; Balletto *et al.*, 1986; Nevo & Filipucci, 1988; Sjögren, 1991; Sinsch & Eblenkamp, 1994; Santucci *et al.*, 1996). It is now clear that three forms of water frog occur over much of northern and central Europe. These are the pool frog (*Rana lessonae*), the marsh frog (*R. ridibunda*) and the edible frog (*R. esculenta*). Edible frogs are fertile hybrids of pool and marsh frogs and are maintained in mixed populations of one or both parent species by the process of hybridogenesis (Berger, 1977, 1983). Water frogs commonly occur as mixed populations of *R. lessonae* and *R. esculenta* ("LE systems"), or of *R. ridibunda* and *R. esculenta* ("RE systems"), because *R. esculenta* usually requires one of its parent species to be available for back crossing (Graf & Polls-Pelaz, 1989). Other water frogs occur in southern Europe, including *R. perezi* in Iberia, *R. bergeri* in Italy, and *R. epeirotica* and *R. shqiperica* in the Balkans. Some of these southern species also hybridize with *R. ridibunda*, and another hybridogenetic cross (*R. ridibunda* x *R. perezi*) has produced *R. grafi* in southern France. Because some water frogs are true species whereas others are fertile hybrids, we have used the term 'taxon' for each of the main forms listed above (Graf & Polls-Pelaz, 1989).

Although not previously considered native, there have been many records of water frogs in Britain since the early nineteenth century (Smith, 1951). Recently, evidence has accumulated that some populations of *R. lessonae* were probably true natives of eastern England (Gleed-Owen, 2000; Zeisset & Beebee, 2001; Wycherley *et al.*, 2002*a*), though these are now extinct. However, it is clear that the great majority of water frog populations in Britain were introduced by humans in recent times. These introductions started at least as early as 1837 (Wolley, 1847) and some are well documented. Thus there are detailed reports on the introduction of the edible frog into East Anglia (Boulenger, 1884*a*, 1884*b*; Dutt, 1906; Buckley, 1986) and an introduction of the marsh frog into Kent (Smith, 1939; Menzies, 1962; Lever, 1980).

Most introductions, however, have uncertain origins and even the species present are often unknown. Populations of introduced water frogs in this category certainly occur in Greater London, and in the counties of Kent, Surrey, Sussex, Essex, Norfolk, Hampshire, Herefordshire, Worcestershire, Somerset and Yorkshire. There may well be others. Snell (1983, 1984) reported water frogs on the Isle of Sheppey in Kent and at Birdbrook, London. Elsewhere, populations have become established west of London around Heathrow airport and Staines Reservoirs (K. Morgan, *pers. com.)*, the River Longford, Barnes Nature Reserve (K. Morgan *pers. com.*), and to the east of London in the Lee Valley Navigation. Surrey has had many water frog introductions. Since 1903 Beambrook Nursery near Dorking has received repeated imports of water frogs from Belgium, Germany, France and Italy, and possibly elsewhere (Gillett, 1988). Another company near Redhill also imported many specimens for commercial purposes. Frogs escaped into local ponds and streams and have subsequently spread extensively. Other Surrey sites with water frog populations include Pyrford, Old Woking, the river Wey, the Royal Horticultural Society Gardens at Wisley, Burgh Heath, Ewell, Horley, Gatwick, Capel and Ewhurst (Wycherley & Anstis, 2001). *Rana ridibunda* has extended its range in both Kent and Sus-

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sex (Beebee & Griffiths, 2000), and there are certainly other water frog populations in both of these counties.

In East Anglia there are water frogs in Essex near Hadleigh Castle and Two Tree Island, at Witham, at West Mersea (J. Cranfield, *pers. com.*) and at Ardleigh near Colchester. In Norfolk there are two recent reports of water frog populations, one at Wolterton Hall near Itteringham, and a second, first mentioned by Buckley (1986), adjacent to the Steam Museum at Forncett St Mary. Isolated water frog populations occur near Bramshill in Hampshire, Bodenham Moor in Herefordshire, Holt in Worcestershire (W. Watson, *pers. com.*), and at Shapwick Heath and West Sedgemoor in Somerset (D. Westbrook, *pers. com.* 1996). The most northerly reports of waterfrog populations are from Swinemoor Common and Hedon in Yorkshire (R. Atkinson, *pers. com.*).

Identification of the frogs in these introduced populations is important if we are to understand the origins of the invaders and their likely future spread in the UK. Although genetic analysis is possible (Zeisset & Beebee, 1998), this is time-consuming, expensive and, by necessity, laboratory-based. It is highly desirable to develop a quick, inexpensive and relatively easy methodology that can identify all types of frog occurring in mixed populations. This, in turn, should enable examination of numerous populations within a relatively short time-scale. In this paper, we demonstrate the application of sound frequency analysis to male advertisement calls and its value in determining the species composition of introduced water frog populations. With this approach it is not necessary to handle or even catch the frogs, and identification can be carried out using only a tape recorder and a personal computer. The use of this technique for identifying *R. lessonae*, *R. esculenta* and *R. ridibunda* has been demonstrated previously (Wycherley *et al.*, 2001; Wycherley *et al.*, 2002*a*).

MATERIALS AND METHODS

DATA COLLECTION

We obtained recordings of water frog advertisement calls from a selection of British sites during May and June 1999-2001 (Fig. 1). Several calls from each individual frog were recorded for analysis. The following list gives the site name, site location (as national grid reference), the numbers of frogs and calls (*x*, *y*) analysed: Somerset Levels ST 424412 (5, 22); Bramshill, Hampshire SU 759614 (13, 34); Newdigate, Surrey TQ 226421 (14, 31); Romney, Kent TR 08 21 (5, 10); Sheppey, North Kent TQ 933697 and TQ 906685 (14, 34); Wisley, Surrey TQ 064585 (5, 29); Wolterton, Norfolk TG 166317 (9, 26). Recordings were made using a Sony Electronic Condenser Microphone ECM-BMS-957 and a Sony Professional Walkman WM-D6D. The PC program Cool Edit 96™ (Syntrillium Inc., Phoenix, AZ) was used in "record mode" to transfer short samples of sound from the original audiocassettes to a PC via a 16-bit sound card (Addonics, Freemont, CA). Individual sub-units (Wycherley *et al.*, 2001; Wycherley *et al.*, 2002*a*) were selected using the editing features of Cool Edit 96™ and transferred to the program IDL® (Interactive Data Language, Research Systems Inc., Boulder, CO), where they were analysed using a set of custom-written procedures. These procedures are available free of charge, together with instructions for use, from J. Wycherley (julia.wycherley@virgin.net).

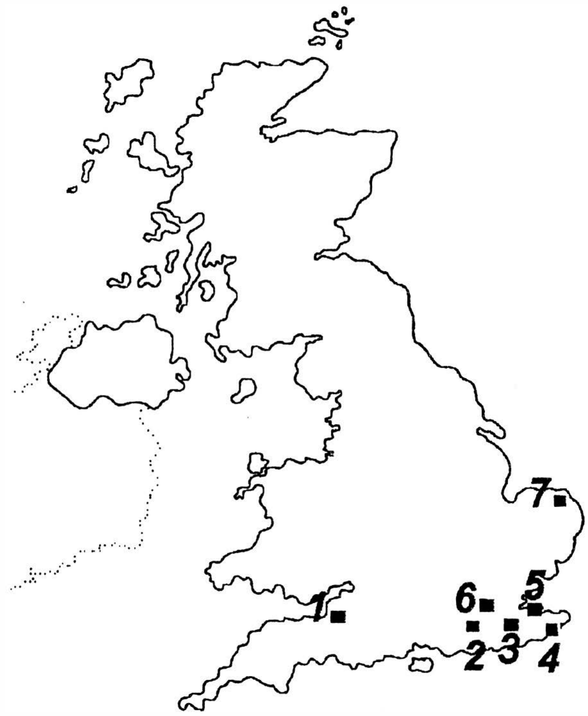

FIG 1. Distribution of British water frog sampling sites used in call frequency analyses. 1: Shapwick, Somerset; 2: Bramshill, Hampshire; 3: Newdigate, Surrey; 4: Romney, Kent; 5: Sheppey, North Kent; 6: Wisley, Surrey; 7: Wolterton Hall, Norfolk

We previously established a reference set of calls from *R. lessonae* (Germany), *R. esculenta* (France) and *R. ridibunda* (France). For each of these three taxa in turn we obtained several advertisement call repetitions from a number of individual frogs. (Wycherley *et al.*, 2002*b*) The calls of additional European water frog species were obtained for reference comparison from a CD by Jean C. Roché: *Au pays des Grenouilles, Sittelle*™ 1997. These additional calls were from *Rana bergeri* (northern Corsica), *Rana epeirotica* (southern Albania), *Rana perezi* (central Spain) and *Rana shqiperica* (Durrës, Albania).

DATA ANALYSIS

We selected several sub-units from each frog call and for each one obtained the Fourier transform (Wycherley *et al.*, 2002*b*). For each principal peak in the Fourier transform we measured the peak frequency, peak width at half the maximum height, and relative amplitude as independent variables. Data from the multiple subunits of each call were averaged and these averages then used as data points (i.e. one datum point per variable per call) in subsequent analyses. The data set for each call was considered collectively as a 'case' (Wycherley *et al.*,

TABLE 1. Differentiation among seven taxa of water frog assessed by discriminant analyses. Calls used in the analysis were from *R. bergeri*, *R. epeirotica*, *R. perezi*, *R. shqiperica*, *R. ridibunda*, *R. lessonae* and *R. esculenta*.

	Eigenvalue	Canonical correlation	Wilks' lambda	χ^2	df	*P*
Discriminant Function 1	725.688	0.999	0.000	2522.36	70	<0.0001
Discriminant Function 2	456.289	0.999	0.000	1916.22	54	<0.0001
Discriminant Function 3	80.971	0.996	0.000	1352.69	40	<0.0001

2001, 2002*a,b*). All data were tested for normality of distribution using the Shapiro-Wilks test in the Statistix7™ Analytical Software package. No transformations were necessary and we carried out further analyses as described below using the statistical program SPSS, Chicago.

We analysed each British population using discriminant analysis, initially with all the independent variables. However, the peak-width variable did not improve discrimination significantly and we therefore excluded it in the full analyses. There was therefore a maximum of 10 variables, each having up to five peaks and with a frequency and amplitude relative to the largest peak (Wycherley *et al*., 2002*b*). However, the number of peaks varied between species and where peaks were absent, zero values were entered for the associated variables. For each British site we included data from all frogs recorded using the classification methods in discriminatory analysis to determine how well call samples separated and could be assigned to a particular species. We compared the call frequency characteristics from each selected British water frog population with those of our reference populations of *R. ridibunda*, *R. lessonae* and *R. esculenta*. When we were unable to identify all the calls from a population by comparison with these three taxa, we also made comparisons with the further four water frogs on the Roché CD (see above). Seven taxa were therefore available as standards for comparison with British frogs. The "leave-one-out" method of classification (Wycherley *et al*., 2002*b*), which provided cross-validation of the success of the classifications, gave a further measure of the effectiveness of the analyses in differentiating water frogs.

In order to ensure that the unknown populations were assigned to the nearest reference taxon we subjected each data set, comprising data from the standards and the selected unknown population, to discriminant analysis using SPSS but only selected the standards for discrimination. The unknown cases are then marked as 'ungrouped' but their nearest group membership is predicted. In this way the probable composition of each British water frog population was determined.

RESULTS

SEPARATION OF REFERENCE VARIETIES

The call-data sets from seven taxa of water frog (*R. bergeri*, *R. epeirotica*, *R. perezi*, *R. shqiperica*, *R. ridibunda*, *R. lessonae* and *R. esculenta*) were pooled and subjected to discriminant analysis as described above. Canonical discriminant functions showed very significant separations and results from the first three functions are shown in Table 1. The classification success of the discriminant functions for each of these seven taxa was 100% in both the original grouped cases and the cross-validated grouped cases. The extent of separation among these reference frog calls is demonstrated in Fig. 2a. Clear separations were achieved using only the first three discriminant functions although seven functions were derived in the analysis. *Rana perezi* and *R. ridibunda* aligned particularly closely, but were nevertheless fully resolved. These standards were then used as references for the identification of British water frog populations.

IDENTIFICATION OF BRITISH WATER FROGS

The advertisement calls obtained from each British site were analysed as previously described and the resulting data were compared with those from the seven standards. By this means we allocated British frog calls to one or more of the reference taxa. In every analysis the standards showed 100% classification success for grouped cases and cross validations. The nearest group membership (to a standard) of each of the unknown British cases was predicted. This indicated the range of species present at each British site. We then examined the distribution of the 'cases' in each British population to see how well they clustered with the standards. The first three discriminant functions for each British population analysis were plotted and the results from each site are shown in Fig. 2b-h. A classification summary is provided in Table 2.

Discriminant analysis of calls recorded from the frogs at Bramshill (Fig. 2b) indicated the presence of a mixed *R. lessonae* and *R. esculenta* population (i.e. a LE system). Close affinity to both *R. lessonae* (23.5% of calls) and *R. esculenta* (70.4% of calls) was observed in the two dominant clusters, with a few samples more typical of *R. perezi*. At Newdigate (Fig. 2c), the calls separated into four main clusters, including *R. perezi* (13.5%), *R. bergeri* (16.3%), *R. lessonae* (35.5%) and *R. esculenta* (34.8%). This combination could never occur without human intervention, because two of these frogs (*R. perezi* and *R. bergeri*) have widely separated natural distributions in Europe. Frogs on Sheppey were discriminated into three types (Fig. 2d), allocated to *R. perezi* (36.6%), *R. ridibunda* (19.5%) and *R. esculenta*

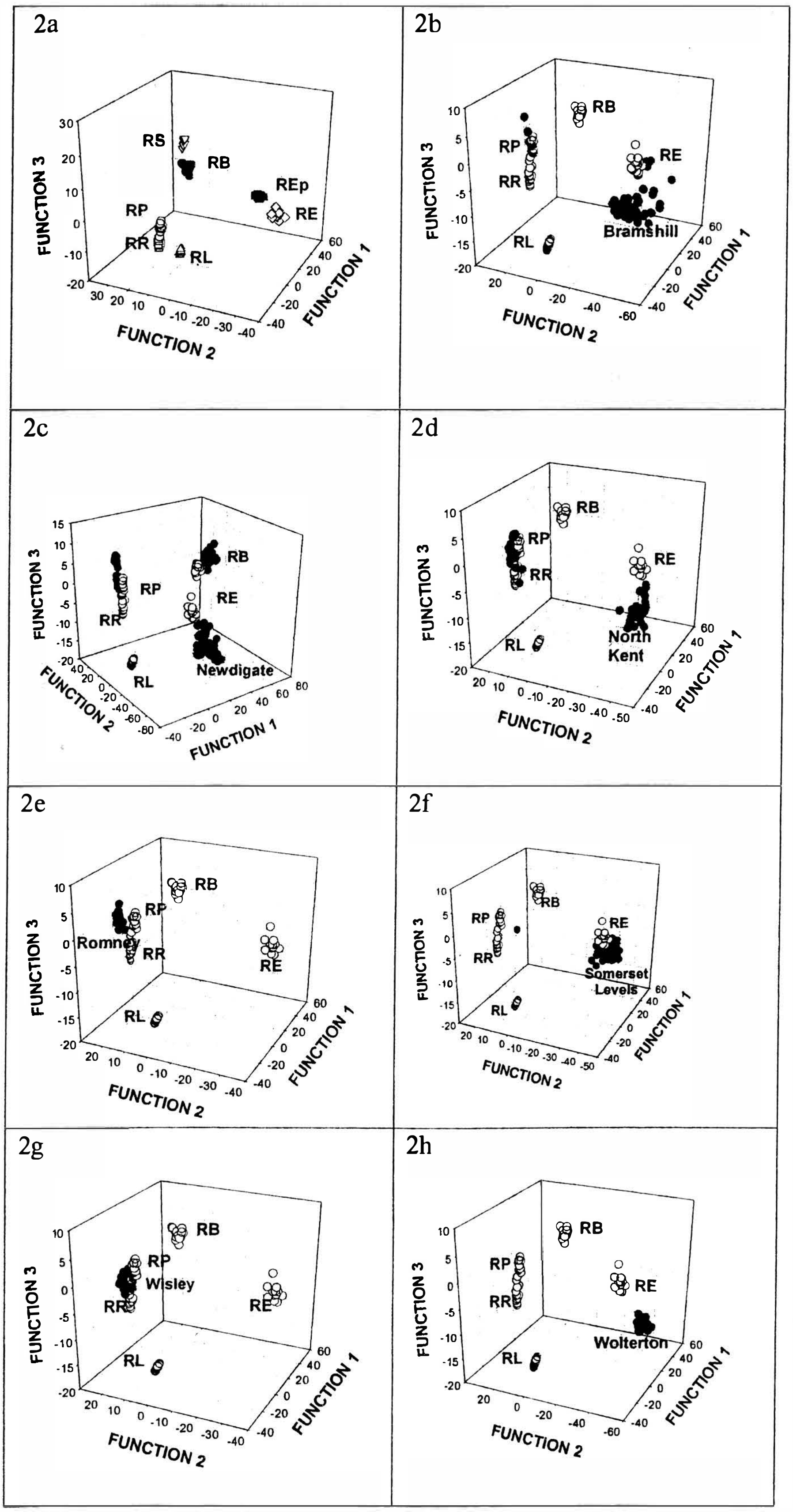

FIG 2. (a) Separation of frog taxa by call frequency analysis. RB (solid circles), *R. bergeri*; Rep (solid squares), *R. epeirotica*; RP, (circles) *R. perezi*; RS (inverted triangles), *R. shqiperica*; RR (squares), *R. ridibunda*; RL (triangles), *R. lessonae*; RE (diamonds), *R. esculenta*. (b-h) Scatter plots of discriminant functions 1, 2 and 3 derived from British water frog calls. Species eliminated by initial analyses [*R. epeirotica* and *R. shqiperica*] were not included in these scatter plots. References (open circles): *R. bergeri*, *R. perezi*, *R. ridibunda*, *R. lessonae*, *R. esculenta*; sample population (filled circles): b, Bramshill, Hampshire; c, Newdigate, Surrey; d, North Kent (Sheppey); e, Romney, Kent; f, Somerset Levels (Shapwick); g, Wisley, Surrey; h, Wolterton, Norfolk.

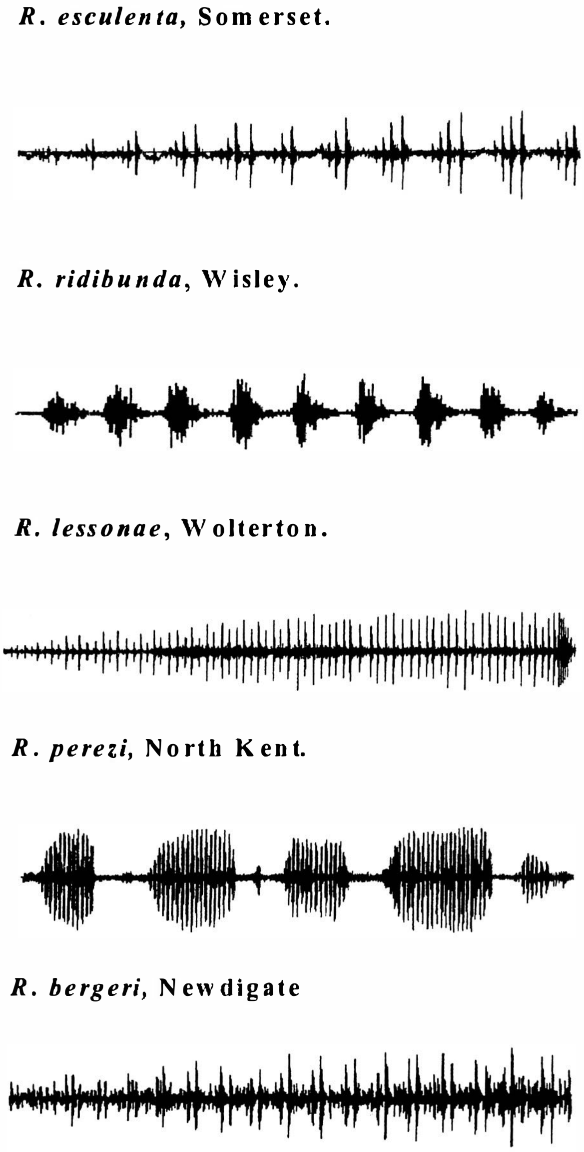

FIG 3. Advertisement call oscillograms of the five water frog taxa identified in Britain.

(43.9%). This is similar to an RE system, since *R. perezi* and *R. ridibunda* are closely related, and is a combination that could occur naturally in parts of south-eastern France. The introduction of *R. ridibunda* to the Romney Marsh area in 1935 was well documented. Discriminant analysis (Fig. 2e) confirmed that probably only *R. ridibunda* was still present at this site, although one individual was statistically assigned to *R. perezi*. By contrast there is no record of the source of the frogs introduced to the Somerset Levels, where they were first reported as recently as 1996. Canonical discriminant functions (Fig. 2f) placed all these call samples as *R. esculenta* in keeping with the morphology of individuals seen there. It is, however, very unusual to find *R. esculenta* by itself and this site clearly warrants further study. *Rana ridibunda* was first reported from Wisley about five years ago. The scatter-plot (Fig. 2g) of discriminant functions confirmed that this is indeed the species present in the Royal Horticultural Society gardens. Finally, call analysis showed that two distinct taxa of frog were present at Wolterton Hall in north-east Norfolk (Fig. 2h). These were *R. lessonae* (56.8%) and *R. esculenta* (43.2%), evidently constituting another LE system.

DISCUSSION

This study extends our previous analyses (Wycherley *et al.*, 2002*a,b*) by successfully distinguishing seven rather than three taxa of European water frog on the basis of male advertisement calls. Earlier studies on the identification of water frog populations used other techniques including genetics (Graf *et al.*, 1977; Spolsky & Uzzell, 1986), morphology (Juszczyk, 1971; Ogielska *et al.*, 1998; Sinsch & Schneider, 1999), and sound analyses based on the call pulses and repetition rate (Schneider, 1997; Gerhardt *et al.*, 2000). We have also shown that identification of unknown populations is possible by using call frequency analysis followed by the 'predicted group membership' and 'display casewise results' facilities of discriminant analysis. Frogs previously identified on morphological grounds or by knowledge of the introduction history (Wisley and Romney) were classified in the expected way, giving independent support to the accuracy of call analysis. However, at both these sites a single sample was classified as *R. perezi*. Further examination of the call data showed that both of these samples had lower peak frequencies that were well above the population average, and close to the lower range of *R. perezi*. Visual inspection of the call oscillograms indicated that these frogs

TABLE 2. Statistical assignment of British water frog populations. In this analysis the cases from each population were assigned to the nearest taxon.

Population	Taxon allocation at individual UK sites (%)						
	R. bergeri	*R. epeirotica*	*R.perezi*	*R. shqiperica*	*R. ridibunda*	*R.lessonae*	*R. esculenta*
Bramshill, Hampshire	0	0	6.1	0	0	23.5	70.4
Newdigate, Surrey	16.3	0	13.5	0	0	35.5	34.8
Sheppey, North Kent	0	0	36.6	0	19.5	0	43.9
Romney, Kent	0	0	7.1	0	92.9	0	0
Shapwick, Somerset	0	0	0	0	1.9	0	98.1
Wisley, Surrey	0	0	9.6	0	90.4	0	0

were probably *R. ridibunda*, but it will be important to make further corroborative tests of our call analysis method at other sites in future.

Two LE systems were identified by our analysis, as well as one population with a mixture of four taxa (*R. lessonae, R. bergeri, R. esculenta* and *R. perezi*) probably not found together anywhere else in the world. Of particular interest was the Somerset site where only *R. esculenta* was detected. This hybrid normally requires sympatric populations of one or other parent species and can only survive in isolation as a triploid (Graf & Polls-Pelaz, 1989). Such triploid populations are known in parts of northern Europe, and it will interesting to discover whether the Somerset frogs are triploid or whether other water frogs occur as yet undiscovered in the area.

Call analysis by our method is sufficiently sensitive to resolve local dialects in *R. lessonae* (Wycherley *et al.*, 2002*a*) and *R. ridibunda* (Wycherley *et al.*, 2002*b*). This means that classifications at the species level might vary slightly in their concordance with reference samples, according to the population source of the reference. This probably explains why clustering (Fig 2) did not always show unknown samples precisely superimposed on the references. However, the sensitivity of our analysis also means that with more comprehensive reference material it might be possible to ascribe introduced populations not just to taxon but to likely areas of origin.

It is particularly interesting to note the variable distribution of the hybrid *R. esculenta* populations in the 3-D scatter plots of discriminant functions when compared to the standard for this taxon obtained from a population in France. We have previously shown that both *R. lessonae* and *R. ridibunda* demonstrate phylogeographic variation in call characteristics across Europe (Wycherley *et al.*, 2002*a,b*). This may also be true of *R. esculenta*, since British populations were probably founded by frogs from various origins across Europe. These may well include a variety of different *ridibunda* hemiclones in the hybrids (Semlitsch *et al.*, 1997).

The use of call analysis should enable further sites to be examined relatively easily, and therefore extend our knowledge of water frogs present in Britain. However, the number of frog taxa identified at any site is obviously limited by the range of advertisement calls recorded. Where mixtures are suspected, successful identification of all the frogs present requires repeated site visits to ensure that sampling is comprehensive. Our sample sizes were rather small and therefore these results may not reflect the total range of frogs present at every site. Nevertheless, the range of European water frogs identified in Britain now includes *R. ridibunda*, *R. lessonae*, *R. esculenta*, *R. bergeri* and *R. perezi*. Oscillograms of the advertisement calls of these five taxa are shown in Fig. 3. This study has sampled only a very small proportion, probably less than 5%, of the water frog introduction sites in Britain. It has become apparent that many more populations exist and are widely distributed across England, since new sightings are a regular occurrence.

The future prospects of water frogs in Britain will no doubt depend on many factors. Perhaps the most significant of these are the recent climate changes that have been reported due to global warming effects (IPCC, 2001). These changes have already impacted upon many ecosystems (Walther *et al*., 2002). Amphibians, including water frogs, can be susceptible to changes in spring temperatures (Beebee, 1995) because higher temperatures advance the breeding season. This in turn improves reproductive success and may enable some species to expand their ranges. This has already occurred in the case of the British water frog populations. Records from localities radiating from the introduction sites at Romney and Newdigate have shown steady outward advance (Menzies, 1962; Beebee & Griffiths, 2000; Wycherley & Anstis, 2001). This has been particularly apparent at Newdigate during the 1980s and 1990s. Prior to this there are few records of outward expansion since the first introductions began in the earliest decades of the last century. Opinions differ on the risks, if any, that these expansions might pose to native fauna. It seems likely, though, that introduced water frogs will continue to spread in Britain for the foreseeable future.

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REFERENCES

Balletto, E., Cherchi, M. A., Salvidio, S. & Lattes, A. (1986). Area effect in south western European green frogs (Amphibia, Ranidae). *Bollettino di Zoologia* **53**, 97-109.

Beebee, T. J. C. (1995). Amphibian breeding and climate change. *Nature* **374**, 219- 220.

Beebee, T. J. C. & Griffiths, R. A. (2000). *Amphibians and Reptiles*. New Naturalist Series, Collins, London.

Berger, L. (1973). Systematics and hybridisation in European green frogs of *Rana esculenta* complex. *Journal of Herpetology* **7**, 1-10.

Berger, L. (1977). Systematics and hybridisation in the *Rana esculenta* complex. In *The Reproductive Biology of Amphibians*, 367-388. Taylor, D. H. and Guttman, S. I. (Eds). Plenum Press, New York and London.

Berger, L. (1983). Western Palearctic water frogs (Amphibia, Ranidae): systematics, genetics and population compositions. *Experientia* **39**, 127-130.

Boulenger, G. A. (1884*a*). On the origin of the edible frog in England. *Zoologist* **VIII**, 265-268.

Boulenger, G. A. (1884*b*). Notes on the origin of the edible frog in England. *Proceedings of the Zoological Society of London*, 573-576.

Buckley, J. (1986). Water frogs in Norfolk. *Transactions of the Norfolk and Norwich Naturalist Society* **27**, 199-211.

Dutt, W. A. (1906). The edible frog. In *Wildlife in East Anglia*, 165-173. Methuen & Co., London.

Gerhardt, H. C., Tanner, S. D., Corrigan, C. M. & Walton, H. C. (2000). Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behavioural Ecology* **11**, 663-669.

Gillett, L. (1988). Beambrook Aquatic Nurseries: an update. *British Herpetological Society Bulletin* **26**, 31.

Gleed-Owen, C. P. (2000). Subfossil records of *Rana* cf. *lessonae*, *Rana arvalis* and *Rana* cf. *dalmatina* from Middle Saxon (c. 600-950 AD) deposits in eastern England: evidence for native status. *Amphibia-Reptilia* **21**, 57-66.

Graf, J.-D. & Polls-Pelaz, M. (1989). Evolutionary genetics of the *Rana esculenta* complex. In *Evolution and Ecology of Unisexual Vertebrates*, 289-301. Dawley, R. M. and Bogart, J. P. (Eds.). Bulletin **466**, New York State Museum, New York, USA.

Graf, J.-D., Karch, F. & Moreillon, M-C. (1977). Biochemical variation in the *Rana esculenta* complex: A new hybrid form related to *Rana perezi* and *Rana ridibunda*. *Experientia* **33**, 1582-1584.

IPCC (2001). Technical summary of the working group I report. In *Climate Change 2001: The Scientific basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, 21-83. Houghton, J. T., Ding Y., Griggs D. J., Noguer M., van der Linden P. J., Dai X., Maskell K., Johnson C. A. (Eds.). Cambridge University Press, Cambridge, United Kingdom and New York, N.Y., USA.

Juszczyk, W. (1971). The morphometric structure of populations of green frogs from southern Poland. *Acta Biologica, Cracov* **14**, 197-209.

Lever, C. (1980). Naturalised Reptiles and Amphibians in Britain. *British Herpetological Society Bulletin* **1**, 27-29.

Menzies, J. I. (1962). The marsh frog (*Rana esculenta ridibunda* Pallas) in England. *British Journal of Herpetology* **3**, 43-54.

Nevo, E. & Filippucci, M. G. (1988). Genetic differentiation between Israeli and Greek populations of the marsh frog, *Rana ridibunda*. *Zoologischer Anzeiger Jena* **221**, 5/6, S. 418-424.

Ogielska, M., Kazana, K., Palczynski, M. & Tomaszewska, A. (1998). An influence of the Great Flood 1997 on amphibian and reptile populations in the Odra river valley near Wroclaw (Lower Silesia, Poland). In *Le Bourget du Lac / France*, 345-349. Miaud C. and Guyétant R. (Eds). Societas Europaea Herpetologica.

Santucci, F., Nascetti, G. & Bullini, L. (1996). Hybrid zones between two genetically differentiated forms of the pond frog *Rana lessonae* in southern Italy. *Journal of Evolutionary Biology* **9**, 429-450.

Schneider, H. (1997). Calls and reproductive behaviour of the water frogs of Damascus, Syria (Amphibia: Anura: *Rana bedriagae* Camerano, 1882). *Zoology in the Middle East* **15**, 51-66.

Semlitsch, R., Hotz, H. & Guex, G-D. (1997). Competition among tadpoles of coexisting hemiclones of hybridogenetic *Rana esculenta*: support for the frozen niche variation model. *Evolution* **51**, 1249-1261.

Sinsch, U. & Eblenkamp, B. (1994). Allozyme variation among *Rana balcanica*, *R.levantina*, and *R. ridibunda* (Amphibia: Anura). *Zeischurft fur Zoologische Systematic und Evolutions forschung* **32**, 35-43.

Sinsch, U. & Schneider, H. (1999). Taxonomic reassessment of Middle Eastern water frogs: morphological variation among populations considered as *Rana ridibunda*, *Rana bedriagae* or *Rana levantina*. *Journal of Zoological Systematics and Evolutionary Research* **37**, 67-73.

Sjögren, P. (1991). Extinction and isolation gradients in metapopulations: the case of the pool frog (*Rana lessonae*). *Biological Journal of the Linnean Society* **42**, 135-147.

Smith, E. P. (1939). On the introduction and distribution of *Rana esculenta* in East Kent. *Journal of Animal Ecology* **8**, 169-170.

Smith, M. (1951). *The British Amphibians and Reptiles*. New Naturalist Series. Collins, London.

Snell, C. (1983). Introduced frogs on the Isle of Sheppey. *British Herpetological Society Bulletin* **8**, 58.

Snell, C. (1984). Observations on the Birdbrooke site, London S.E.3. *British Herpetological Society Bulletin* **10**, 55-56.

Spolsky, C. & Uzzell, T. (1986). Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. *Molecular Biology and Evolution* **3**, 44-56.

Walther, G.-R., Post, E., Convey, P., Menzels, M., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldburg, O. & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature* **416**, 389-395.

Wijnands, H. E. J. (1977). Distribution and habitat of *Rana esculenta* complex in the Netherlands. *Netherlands Journal of Zoology* **27**, 277-286.

Wolley, J. (1847). Is the Edible Frog a true native of Britain? *Zoologist* **5**, 1821-1822.

Wycherley, J. & Anstis, R. (2001). *Amphibians and Reptiles of Surrey*. Surrey Wildlife Trust, Pirbright, Woking, Surrey.

Wycherley, J., Beebee, T. J. C. & Doran, S. (2001). Regional accents in the pool frog? Development of new computer analytical techniques aids bioacoustic separation of pool frog populations and may elucidate the status of the Norfolk pool frogs. *Mitteilungen aus* *dem Museum fur Naturkunde in Berlin, Zoologische Reiche* **77,** 25-30.

Wycherley, J., Doran, S. & Beebee, T. J. C. (2002*a*). Frog calls echo microsatellite phylogeography in the European pool frog *Rana lessonae*. *Journal of Zoology* **258**, 479-484.

Wycherley, J., Doran, S. & Beebee, T. J. C. (2002*b*). Male advertisement call characters as phylogeographic indicators in European water frogs. *Biological Journal of the Linnean Society* **77**, 355-365.

Zeisset, I. & Beebee, T. J. C. (1998). RAPD identification of north European water frogs. *Amphibia-Reptilia* **19,** 163-170.

Zeisset, I. & Beebee, T. J. C. (2001). Determination of biogeographical range: an application of molecular phylogeography to the European pool frog *Rana lessonae*. *Proceedings of The Royal Society, London B.* **268**, 933-938.

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