# Male advertisement call characters as phylogeographical indicators in European water frogs

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Anuran vocalizations are valuable in the determination of species identity and have the potential to discriminate intraspecific variation. We developed novel bioacoustic sampling techniques, based on Fast Fourier Transforms, to increase the precision and sensitivity of male advertisement call analysis and applied the method to European water frogs. This approach robustly separated the three types of north European water frogs (*Rana ridibunda, R. lessonae* and their viable, fertile hybrid *R. esculenta*) by their call subunit characteristics. The hybrid frog exhibited a high frequency call component absent from both the parental species. Furthermore, call analysis demonstrated significant intraspecific differences among populations of all three frog types. Call characters of *R. ridibunda* changed systematically as a function of longitude. This trend may reflect either clinal variation in selection pressures across Europe, or the consequences of drift following postglacial colonization from eastern refugia. High resolution vocalization analysis therefore provides a potentially useful method for investigating intraspecific differentiation and the phylogeographical origins of anuran distributions.   © 2002 The Linnean Society of London. *Biological Journal of the Linnean Society*, 2002, **77**, 355–365.

ADDITIONAL KEYWORDS: intraspecific variation – call characters – Rana esculenta – Rana lessonae – Rana ridibunda.

## INTRODUCTION

Sound-based communication is widespread among several invertebrate and vertebrate groups, and is achieved by a variety of different mechanisms. Although sound characters are typically species-specific, regional (intraspecific) variation has also been demonstrated for a wide range of organisms including crickets (Zuk, Rotenberry & Simmons, 2001), birds (Haftorn & Hailman, 1997; Wright & Wilkinson, 2001), mammals (Terhune, Healey & Burton, 2001) and amphibians (Ryan & Wilczynski, 1991; Kasuya & Shiobara, 1996; Hasegawa, Ueda & Sumoda, 1999; Rafinski & Babik, 2000). Vocalization in amphibians is a common component of breeding behaviour. Male anurans call to advertise their breeding status, defend territories and attract females (Brzoska, 1982; Gerhardt, 1994; Gerhardt *et al.*,

2000). These calls are distinctive and are of widespread interest in studies of behaviour and sexual selection.

The north European water frogs are a particularly fascinating group because they exhibit hybridogenetic reproduction, and several different hybrid systems have been identified (Graf & Polls-Pelaz, 1989; Lode, 2001). The pool frog *Rana lessonae* (Camerano) and the marsh frog *Rana ridibunda* (Pallas) are true species whereas the edible frog, *Rana kl. esculenta* (Linnaeus)*,* is their hybridogenetic progeny. *Rana esculenta* is both viable and fertile, but normally needs to back-cross with one of the parent species for successful reproduction. In this paper we refer for convenience to all three of these frogs as species, despite the hybrid nature of *R. esculenta*. All three are widespread across much of Europe and often coexist in mixed communities. They can be distinguished by morphological criteria (Schneider, Sofianidou & Kyriakopoulu-Sklavounou, 1984; Pagano & Joly, 1999) though not always with high confidence. Recently the development of biochem-

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ical (Sinsch & Eblenkamp, 1994), genetic (Sjogren, 1991; Zeisset & Beebee, 1998) and bioacoustic (Radwan & Schneider, 1988; Sinsch & Schneider, 1996) techniques have improved the reliability of identification within this group, and led to the discovery of further cryptic species in southern Europe (Schneider & Haxhiu, 1994; Dubois, 1998). However, standard analytical methods have not sufficed to demonstrate regional (intraspecific) variations in water frog calls (Sinsch & Schneider, 1996). We have therefore developed a novel analytical procedure designed to increase the resolving power of call analysis (Wycherley, Beebee & Doran, 2001). In this paper we describe the new method fully and employ it to test the hypothesis that call signatures of European water frogs vary not only between the three main forms but also among populations of each form. Our expectation was that the distribution of any such 'regional accents' could provide new insights into the phylogeography of these frogs.

## METHODS

### DATA COLLECTION

Recordings of male advertisement calls of *Rana ridibunda, R. lessonae* and *R. esculenta* were obtained from a selection of countries across Europe (Fig. 1).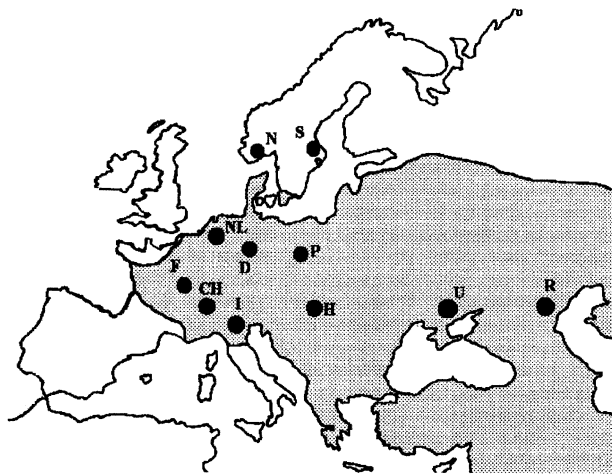 Calling frogs were identified to species in the field by morphology and by ear, because with experience it is possible to discriminate between the three forms. The following list gives country, site location and the numbers of calls ( ) and subunits as defined below {} that were analysed: France 46°N 1°E Migne (10){28}; Netherlands 52°N 6°E Diever (10){77}; Germany from CD Naturschutzbund Deutchland, Landesverband, Brandenburg (8){16}; Norway 58°N 8°E Arendal (11){88}; Sweden 60°N 17°E Hallnas (14){112}; Hungary 46°N 13°E Lake Balaton, Bockeret Forest 48°N 24°E (7){10}; Ukraine 48°N 33°E Mykolayiv (11){18}; Poland 51°N 15°E Wroclaw (5){15}; Switzerland 46°N 7°E Bern (7){14}; Italy 45°N 7°E Torino (5){35}; Russia 46°N 48°E Astrakhan (16){38}.

Recordings at all sites except those in Germany, Ukraine and Russia were made by J. Wycherley and Kevin Morgan using a Sony Professional Walkman WM-D6D and a Sony Electronic Condenser Microphone ECM-BMS-957. At each site, the calls of several individual frogs were recorded. 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). Frogs in Russia and the Ukraine were recorded by Nigel Tucker using similar equipment. Individual subunits (see below) 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.

### CALL STRUCTURE

The hierarchical structure of frog calls is exemplified in Figure 2. For *R. ridibunda* and *R. esculenta* (not shown), each call consists of a number of repeating segments (pulse groups), each of which is made up of a number of repeating subunits. In the case of *R. lessonae* the call is made up only of repeating subunits with no distinct segments. The call subunit consists of a decaying oscillation (Bradbury & Vehrencamp, 1998). Although the initial amplitude of the signal for each subunit changes through a call segment, the oscillation frequency and decay rate remain approximately constant for a given call.

The sound generation apparatus in frog calls can be regarded as a system of damped oscillators. These lead to a small number of modes of vibration with characteristic frequencies and decay rates. The transient signal in each subunit decays either partially or completely before the next impulse. Such a system is well suited to analysis using Fourier transforms (Singleton, 1967; Boas, 1983; Mulgrew, Grant & Thompson, 1999) in which the signal is modelled as the sum of $N$ exponentially decaying oscillatory terms:



**Figure 1.** Water frog distributions in Europe. Shaded areas show the approximate distribution of the water frogs used in this study, and sampling sites where recordings were taken are indicated as solid circles. All three species of frogs do not occur in all areas, and only *R. lessonae* occurs in the isolated regions of Norway and Sweden. CH = Switzerland, F = France, D = Germany, H = Hungary, I = Italy, N = Norway, P = Poland, R = Russia, S = Sweden, U = Ukraine.

$$S(t) = \sum_{k=1}^{N} A_k e^{i\omega_k t} e^{-\beta_k t} \qquad [1]$$

where $S$ = Signal, $t$ = time and the parameters $A_k$, $\omega_k$ and $\beta_k$ are, respectively, the amplitude, characteristic angular frequency and decay rate of the $k^{\text{th}}$ component. We use the convention common in signal processing that the observed signal corresponds to the real part of the complex number $S$. A complex number can be written as $z = a + it$ where $i$ is an imaginary number equal to the square root of $-1$. The first part of this $[a]$ is the real part of the equation and $[it]$ is the imaginary part. Figure 3 shows the subunit from Figure 2 to which we have fitted a single decaying component. The goodness of fit, particularly towards the start of the decay, justified our chosen model. The Fourier transform of $S(t)$ is given by $\tilde{S}(\omega)$ and it can be shown that:

$$\tilde{S}(\omega) = \sum_{k=1}^{N} \frac{A_k}{\beta_k} \left\{ \frac{1}{1 + (\omega - \omega_k)^2 / \beta_k^2} - \frac{i(\omega - \omega_k)/\beta_k}{1 + (\omega - \omega_k)^2 / \beta_k^2} \right\} \qquad [2]$$

The first (real) part of this Fourier transform expression represents a set of peaks each with a Lorentzian form (Boas, 1983; Ulriksson, 1986). The position of the centre of the peak in the frequency domain corresponds to the characteristic angular frequency $\omega_k$ of the oscillation in the time domain, whilst the width of the peak at half its maximum value is equal to the decay rate $\beta_k$ (Fig. 3). We have plotted the spectral amplitude of $S$ as a function of frequency $f$, rather than of angular frequency $\omega$, where $f = \omega/2\pi$, as this is a more familiar variable to those working in bioacous-

tics. The Fourier transform of the call data was above the zero line at higher frequencies and this indicated the presence of 'white noise' in our recording.

## SOUND SAMPLING AND SUBUNIT ANALYSIS

A call or segment of a call (a 'long' sound sample) can be used to provide a sonogram, and this has been the approach generally used in earlier studies. By averaging this sonogram in the horizontal direction it is possible to obtain a set of broad frequency peaks. However, these broad peaks do not discriminate well between closely related species. The novel aspect of our analysis involved extraction of 'short' sound samples corresponding to individual subunits. Our procedure for the analysis of the subunit data was as follows:

(a) From each call segment, a representative number of subunits was selected. For *R. lessonae* eight subunits were selected throughout the whole call, and for *R. ridibunda* and *R. esculenta* two subunits from each of two or three call segments were selected. In *R. ridibunda* and *R. esculenta* the subunits selected were from the latter part of the call segment as these normally exhibited complete decay.

(b) For each subunit, the Fourier transform was obtained and smoothed by zero-filling (Mulgrew *et al.*, 1999) in the time domain. We considered the absolute value of the complex Fourier transform, rather than the real part as described above. Whilst this leads to a decreased frequency resolution because the individual peaks were broader, it simplifies the analysis in the case of more than one peak.
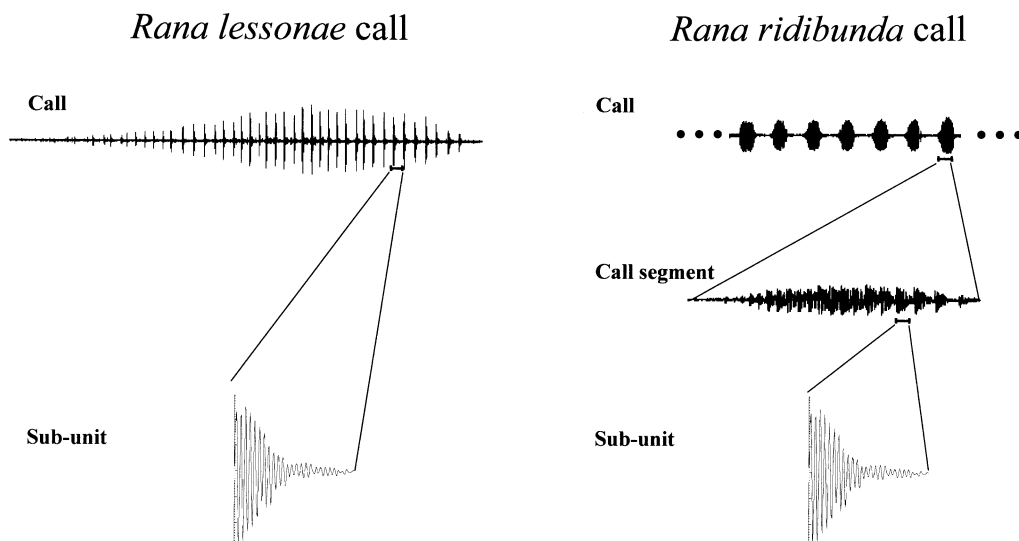
(c) For each of the principal peaks in the Fourier



## *Rana lessonae* call

Call

Sub-unit

## *Rana ridibunda* call

Call

Call segment

Sub-unit

**Figure 2.** Frog call structures Examples of hierarchical call structures in *Rana lessonae* and *R. ridibunda*.

transform the peak position, peak width at half-maximum height and relative amplitude were recorded. These parameters then formed the input data for the later statistical analysis.

## DATA ANALYSIS

Some components of amphibian acoustic signals vary according to temperature (Kuhn & Schneider, 1984) but frequency components usually show only weak or insignificant correlations (Littlejohn, 1977). As we extracted three variables for each of several frequency peaks and were analysing these together as a data set or 'case', we did not make any temperature compensations to our data. We did however, measure water temperatures at a selection of the recording sites and ascertained that there were no substantial or systematic (e.g. longitudinal) differences between them.

The averages of measurements taken from multiple subunits of each call were used as data points (i.e. one datum point per call) in subsequent analyses. All data were tested for normality of distribution using the Shapiro–Wilks test in the Statistix7 Analytical Software package. No transformations were necessary and further analyses of these data were carried out using the statistical program SPSS. Sigma Plot™ (SPSS, Chicago) was used to display data in the form of two-dimensional scatter plots.

We employed discriminant analysis to compare data among species and populations, with all the selected variables from each sample. Each sample was entered as a unit or 'case'. There was a maximum of 12 variables, with up to four peaks, each with a frequency and width at half maximum height and an amplitude relative to the largest peak. However, peak numbers varied between species. Where peaks were absent, zero values were entered for the associated variables in the analysis of interspecific comparisons. We included all data sets from all countries, using the classification methods in discriminatory analysis, in order to determine how well call samples separated and could be assigned to a particular country of origin. Independent statistical classification of calls and the use of the 'leave-one-out' method of classification, which provided cross-validation of the success of the classifications, gave measures of the effectiveness of the analyses in differentiating the calls. We also carried out hierarchical cluster analysis of the call characters, using distances between species or populations estimated by the 'proximities' procedure to quantify average linkage between groups and thus construct a dendrogram.

## RESULTS

### FOURIER TRANSFORMS OF MALE ADVERTISEMENT CALLS

Fourier transformation of the subunit sound samples provided clear parameters that could be used in subsequent analyses. Typical frequency analyses of the calls of each of the three species are shown in Figure 4. Each species had a characteristic number of frequency peaks, with two for *R. lessonae*, three for *R. ridibunda* and four for *R. esculenta*. Figure 4A demonstrates the
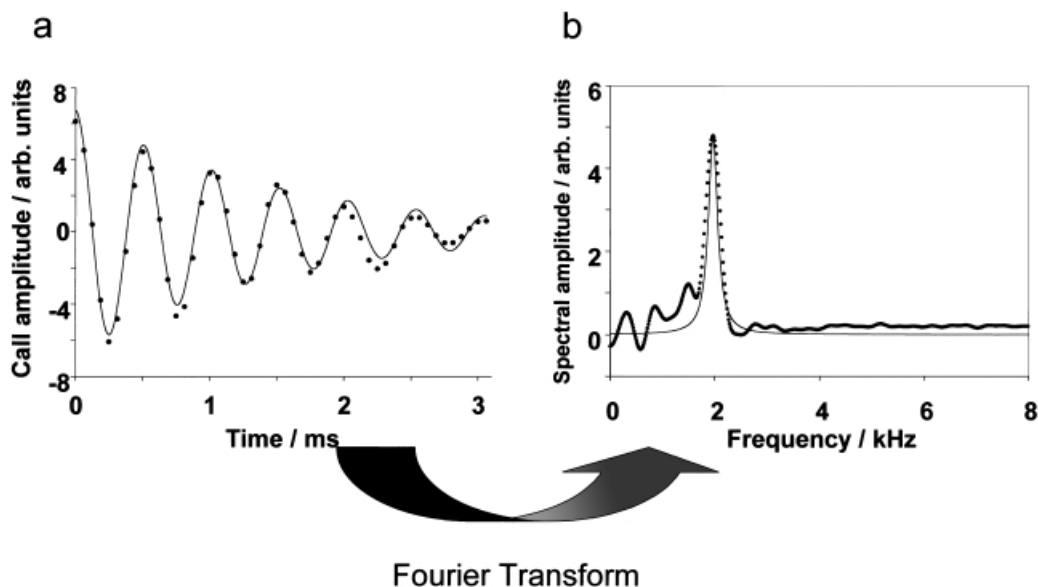


**Figure 3.** Fourier transform of a *Rana lessonae* subunit. (●) = Call data, ~ = single component fit.
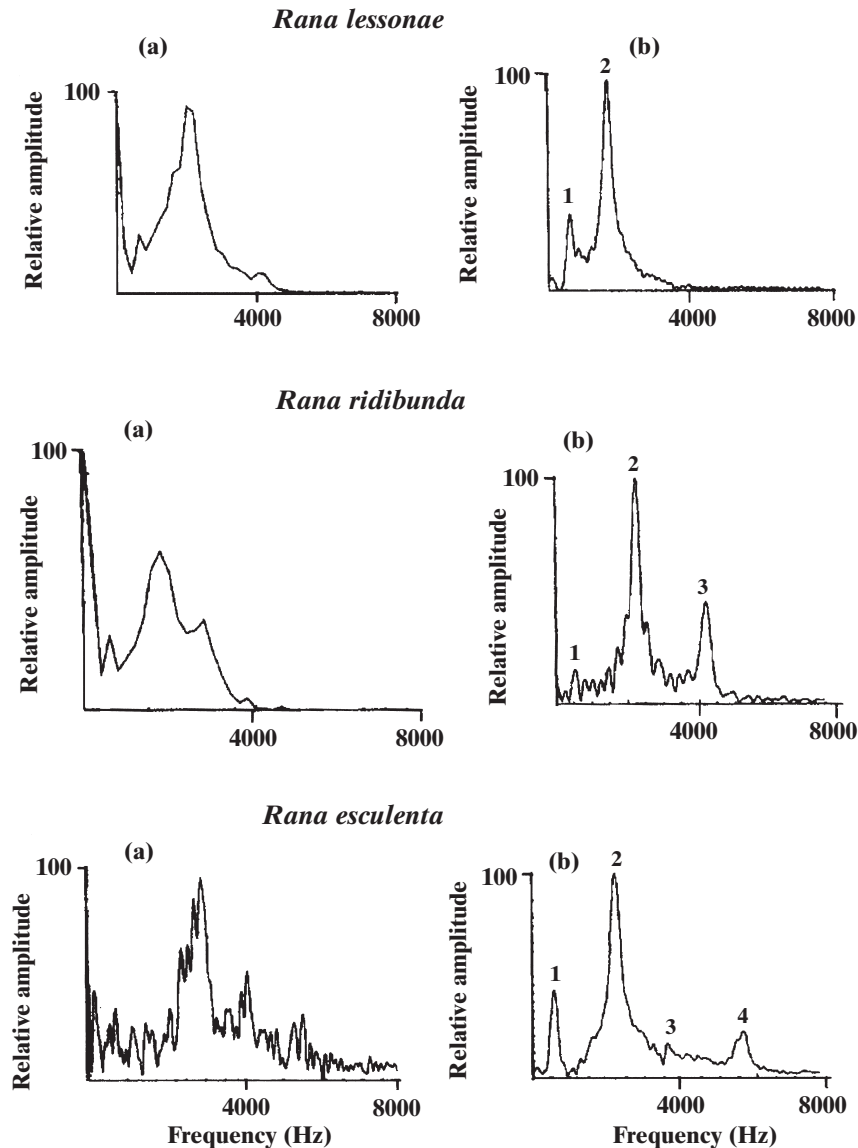
**Figure 4.** Frequency analyses of call subunits. (a) = 'long' sound samples (see text) (b) =subunit samples.

broader peaks obtained when 'long' sound samples were analysed in IDL™, but when subunits were selected we obtained the narrower, better defined peaks shown in Figure 4B. From these we extracted the peak frequency maxima, widths and relative amplitudes that were used later in the statistical analyses.

### SEPARATION OF SPECIES BY INTERSPECIFIC CALL DIFFERENCES

Call data from recordings taken at multiple sites across Europe (Fig. 1) were pooled for each species and subjected to discriminant analysis using the variables for peak frequency and relative amplitude described

in Methods. Canonical discriminant functions showed very significant separations of all the paired comparisons of frog species (Table 1). These functions are mathematical extractions from the set of independent variables and cannot be related simply to specific variables. A canonical discriminant functions plot of Functions 1 and 2, as shown in Figure 5, illustrates these separations. The individual samples also showed close clustering within each frog species, particularly in the case of *R. lessonae*. The cluster formed by the *R. esculenta* populations was roughly equidistant from those of the two parent species. Percentage classification success of the discriminant functions for pairwise discrimination of the three frogs is also summarized in Table 1. Classification success of the

**Table 1.** Differentiation among *Rana lessonae*, *R. ridibunda*, and *R. esculenta* by pairwise discriminant analyses

(a) Significance of discriminant functions for pairwise species comparisons

| Species pair | Eigenvalue | Canonical correlation | Wilks' lambda | $\chi^2$ | df | $P$ |
|---|---|---|---|---|---|---|
| *R. ridibunda* $\times$ *R. lessonae* | 62.1 | 0.992 | 0.016 | 37.3 | 6 | <0.0001 |
| *R. lessonae* x *R. esculenta* | 1967.3 | 1.000 | 0.001 | 60.7 | 8 | <0.0001 |
| *R. esculenta* $\times$ *R. ridibunda* | 217.8 | 1.000 | 0.005 | 21.6 | 8 | <0.006 |

(b) Classification success of discriminant functions

| | Predicted species membership (%) | | |
|---|---|---|---|
| Species | *R. ridibunda* | *R. lessonae* | *R. esculenta* |
| *R. ridibunda* | 100 (100) | 0 (0) | 0 (0) |
| *R. lessonae* | 0 (0) | 100 (100) | 0 (0) |
| *R. esculenta* | 0 (40) | 0 (0) | 100 (60) |

Percentages in parentheses are percentages of correct classifications predicted using the leave-one-out method (see Methods).
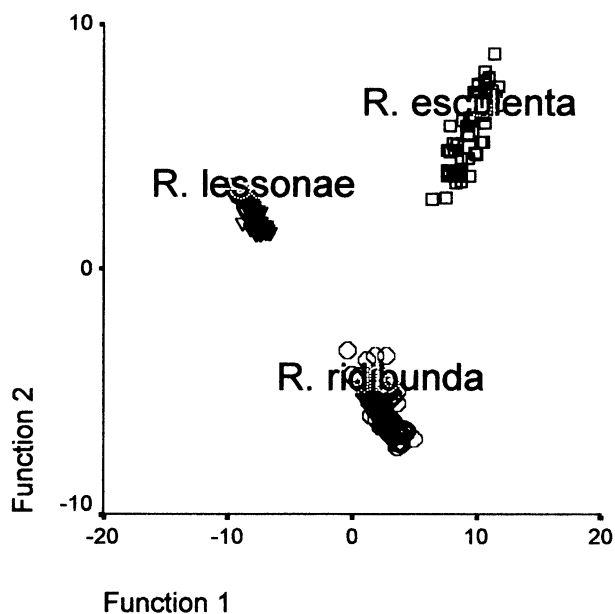


**Figure 5.** Discriminant function plots of water frog calls. ▼= *Rana lessonae,* ○= *R. ridibunda,* □= *R. esculenta*.

discriminant functions for all samples from all three species analysed together, measured by correct assignment, gave 100% correct classification. Correct assignment by cross validation was also 100% for *R. ridibunda* and *R. lessonae,* but just 60% for *R. esculenta* with the remainder wrongly classified as *R. ridibunda.*

### SEPARATION OF POPULATIONS BY INTRASPECIFIC CALL DIFFERENCES

We analysed populations of each species separately to determine whether regional differentiation of dialects was detectable among the frog calls. The call variables for each separate population were therefore subjected to pairwise discrimination. Table 2 shows arbitrarily selected examples of these analyses for a sample of *R. ridibunda* and *R. esculenta* population pairs across Europe. All population pairs, not just the ones shown, exhibited significant differences by this analysis. *Rana lessonae* data were omitted because they were analysed separately (Wycherley, Doran & Beebee, 2002), but pairwise differences were equally significant for this species. Canonical discriminant function comparisons for both *R. ridibunda* and *R. esculenta* showed very significant separation of all paired populations. When all populations of a single species were considered together (i.e. in the interspecific comparisons) the first four canonical discriminant functions accounted for 100% of the variance among the test variables. More discriminant functions were required, however, to account for the intraspecific differences between populations of the same species.

Classification success and predicted group memberships for populations of *R. ridibunda* and *R. esculenta* are also listed in Table 2. In the case of *R. ridibunda* 85.7% of original grouped cases and 82.9% of the cross-validated cases were correctly assigned to the population of origin. For *R. esculenta* 98.2% of original grouped cases and 89.3% of grouped cross-validated

**Table 2.** Intraspecific differentiation among Rana *ridibunda* and *R. esculenta* populations by pairwise discriminant analyses

(a) Significance of discriminant functions for pairwise population comparisons

| Population pair | Eigenvalue | Canonical correlation | Wilks' lambda | $\chi^2$ | df | $P$ |
|---|---|---|---|---|---|---|
| *Rana ridibunda* | | | | | | |
| Russia × Ukraine | 2.05 | 0.820 | 0.328 | 32.9 | 5 | <0.0001 |
| France × Hungary | 10.53 | 0.956 | 0.087 | 37.9 | 5 | <0.0001 |
| Russia × Germany | 11.13 | 0.958 | 0.082 | 73.6 | 5 | <0.0001 |
| France × Russia | 9.68 | 0.952 | 0.094 | 46.2 | 5 | <0.0001 |
| *Rana esculenta* | | | | | | |
| France × Poland | 28.15 | 0.983 | 0.034 | 62.4 | 7 | <0.0001 |
| France × Netherlands | 13.78 | 0.966 | 0.068 | 45.8 | 8 | <0.0001 |
| Hungary × Switzerland | 7.64 | 0.940 | 0.116 | 32.3 | 8 | <0.0001 |

(b) Classification success of discriminant functions

*R. ridibunda*

| Population | Predicted population membership (%) | | | | |
|---|---|---|---|---|---|
| | France | Russia | Ukraine | Hungary | Germany |
| France | 90 (90) | 0 (0) | 0 (0) | 10 (10) | 0 (0) |
| Russia | 0 (0) | 100 (93.8) | 0 (6.2) | 0 (0) | 0 (0) |
| Ukraine | 0 (0) | 5.6 (5.6) | 72.2 (66.7) | 16.7 (22.1) | 5.6 (5.6) |
| Hungary | 0 (0) | 10 (10) | 10 (10) | 80 (80) | 0 (0) |
| Germany | 12.5 (12.5) | 0 (0) | 0 (0) | 0 (0) | 87.5 (87.5) |

*R. esculenta*

| Population | Predicted population membership (%) | | | | |
|---|---|---|---|---|---|
| | France | Netherlands | Hungary | Poland | Switzerland |
| France | 100 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Netherlands | 0 (0) | 100 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hungary | 0 (0) | 0 (12.5) | 100 (75) | 0 (0) | 0 (12.5) |
| Poland | 0 (0) | 0 (0) | 0 (8.3) | 100 (91.7) | 0 (0) |
| Switzerland | 0 (0) | 0 (0) | 0 (0) | 7.7 (23.1) | 92.3 (76.9) |

cases were correctly assigned. Almost all the incorrectly assigned cases were assigned to an adjacent country to that of the sample.

Hierarchical cluster analysis was used to investigate relationships between populations of the three frog species based on the call data. Sample site averages were used for the data variables in this analysis (Fig. 6), which resolved the three frog species into distinctly separate clusters. It was notable, however, that in this analysis the two parental species clustered more closely to each other than to the hybrid. At this level there was relatively little discrimination within species with respect to branch lengths, and none at all for *R. lessonae*. Nevertheless a clear phylogeographical cluster pattern was apparent among populations of

this species when analysed separately (Wycherley *et al.*, in press). A clear geographical pattern was also evident in the call characteristics of *R. ridibunda,* where a distinct and significant ($r = -0.9439$, df = 3, $P = 0.0158$) east–west clinal trend was observed for canonical discriminant function 1 (Fig. 7).

## DISCUSSION

In this study we analysed very short subunit sound samples, following Fourier transforms, to produce precise frequency peak data. Kuhn & Schneider (1984) and Schneider *et al.* (1984), also working with water frogs, proposed the name 'pulse group' for what we have described as a call segment, and considered each
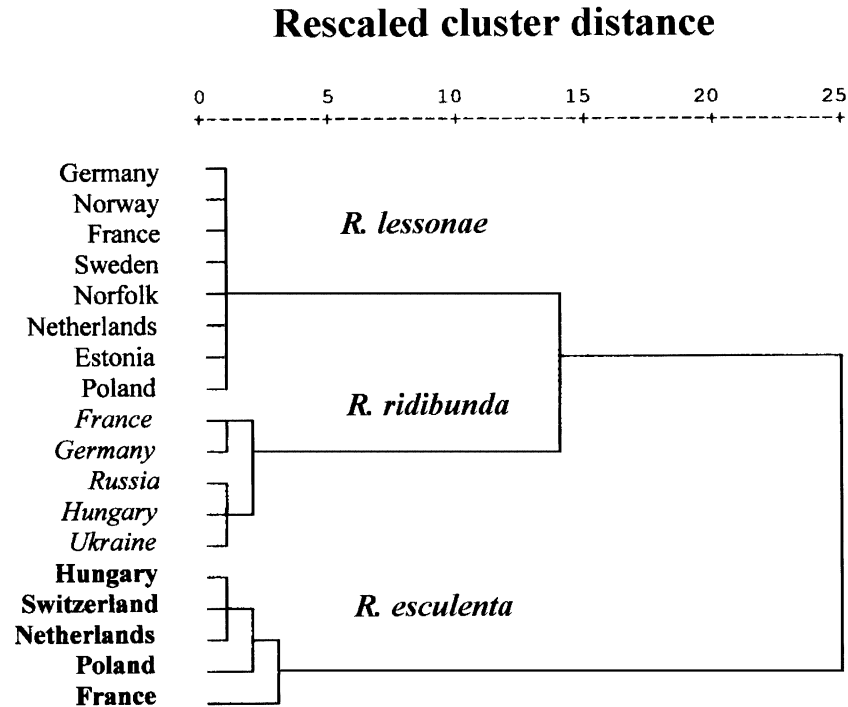
## Rescaled cluster distance



**Figure 6.** Hierarchical cluster analysis of water frog call characters.
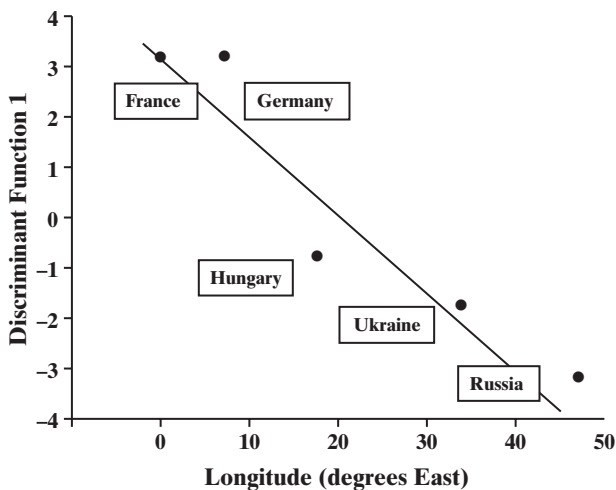


**Figure 7.** Longitudinal variation in *Rana ridibunda* call characters.

segment as a set of pulses with peaks and troughs. They characterized call segments rather than subunits, as have others in previous studies with a variety of anuran species (e.g. Sanchez-Herraiz *et al.*, 1995; Kasuya & Shiobara, 1996; Kaya & Simmons, 1999). Segment analysis has proved adequate for distinguishing between species, but less powerful with respect to intraspecific differentiation of call charac-

ters (Sinsch & Schneider, 1996). We have shown that subunit analysis permits robust separation of three closely related water frogs, and of populations within each of these groups. Such 'regional accents' have not previously been demonstrated in the European water frogs. Analysis of such accents in a geographical context might make possible a phylogeographical investigation of postglacial history independent of the more usual methods based on neutral molecular markers (Hewitt, 2000).

Although some frog call characters are affected by size of the calling male, and the three frogs studied here differ in mean size, our analysis is unlikely to be significantly affected by this variation. The samples were obtained from multiple sites and were random with respect to individuals at each, so should reflect the full size ranges for all three frog types. These sizes overlap substantially among *R. ridibunda*, *R. esculenta* and *R. lessonae* but call characters for each species were consistent and clearly different from each other. These differences extended not just to frequencies, which can be affected by body size, but overall call structure including peak numbers (Fig. 4).

Hybrid (*R. esculenta*) call characteristics appeared equally distinct from both parental species by discriminant function analysis (Fig. 5), but cluster analysis placed the hybrid distant from both parents (Fig. 6). Clearly this does not reflect the biological relationships between these frogs in the expected way. The

clustering pattern seems to have arisen because the *R. esculenta* call has an extra frequency component (i.e. the fourth peak shown in Fig. 3), at around 4000–5500 Hz, not present in either parent. Presumably this extra call component arises from some kind of pleiotroptic interaction between *lessonae* and *ridibunda* genomes in the hybrid. This hybrid feature could be of considerable evolutionary significance. Female *R. esculenta* and *R. lessonae* specifically avoid mating with male *R. esculenta* in mixed populations of frogs, and make their choice (of male *R. lessonae* in both cases) on the basis of as yet unspecified call characters (Abt & Reyer, 1993; Roesli & Reyer, 2000). Such mate choice is an essential requirement for the hybridogenetic system of *R. lessonae* and *R. esculenta* to persist as it does over much of Europe (Som, Anholt & Reyer, 2000). Mating with male *R. esculenta* results in a waste of reproductive effort by female *R. lessonae* because their genes are not passed on by the hybrid, and by female *R. esculenta* because their progeny contain clonal *R. ridibunda* genomes with very low viability (Graf & Polls-Pelaz, 1989). Because *R. esculenta* receive a new *R. lessonae* genome each generation, combined with a clonal *R. ridibunda* genome, there is no possibility for the *R. esculenta* call characteristics to evolve. Male hybrids are therefore compromised with a signal that females can seek to avoid, perhaps identifiable by the extra high frequency component around 4000–5500 Hz. Our analysis makes possible the generation of synthetic frog calls lacking specific components and testing the hypothesis that particular components trigger responses in females. The anuran auditory system is certainly capable of sensitive differentiation. It contains both basilar papilla for detecting high frequency (>1000 Hz) and amphibian papilla for detecting low frequency (100–1000 Hz) sound (Feng, Narins & Capranica, 1975). When two frequencies are presented together a large response is evoked in the thalamus (Mudry, Constantine-Paton & Capranica, 1977).

During the range expansions, founder effects (essentially genetic drift) could have led to the observed patterns of interpopulation differences in call characters as the frogs colonized Europe. Such founder effects are very likely as a consequence of leptokurtic distributions and range expansions mediated at each step by relatively few individuals (Ibrahim, Nichols & Hewitt, 1996). Alternatively, or perhaps additionally, selection may have operated differentially on call characters according to local conditions such as mean temperatures during the breeding season. However, advertisement call differences reflect genetic phylogeography, based on microsatellite analyses, in *R. lessonae* (Beebee & Zeisset, 2001; Wycherley *et al.*, in press). This does not necessarily demonstrate that drift rather than selection has dominated call

character differentiation, though we believe it to be the most likely explanation. Frogs normally respond to different local temperature regimes by altering the timing of their breeding season, rather than by breeding at different temperatures. Selection for call characters therefore may not vary greatly across the biogeographical ranges. The clinal change in *R. ridibunda* mating calls across the longitudinal range of 1°E to 48°E seems to reflect the likely pattern of postglacial re-colonization of Europe from easterly refugia, an observation that is also compatible with drift as the primary cause. However, the possibility now exists to investigate the significance of local call characters in experimental situations where temperature and other environmental factors can be varied systematically. Water frogs offer excellent prospects for elucidating the relative importance of drift and selection in the evolution of male advertisement calls.

## ACKNOWLEDGEMENTS

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