Molecular Ecology (2004) **13**, 1047–1053 doi: 10.1111/j.1365-294X.2004.02119.x

Genetic diversity across a vertebrate species' range: a test of the central–peripheral hypothesis

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Abstract

Although it has been long presumed that population genetic variability should decrease as a species' range margin is approached, results of empirical investigations remain ambiguous. Sampling strategies employed by many of these studies have not adequately sampled the entire range. Here we present the results of an investigation of population genetic diversity in a vertebrate species, the Italian agile frog, *Rana latastei*, sampled comprehensively across its entire range. Our results show that genetic variability is not correlated with population location with respect to the range periphery. Instead, the model that best explains the genetic variation detectable across the range is based on an east-to-west gradient of declining diversity. Although we cannot state definitively what has led to this distribution, the most likely explanation is that the range of *Rana latastei* expanded post-glacially from a Balkan refugium.

Keywords: genetic diversity, microsatellites, population genetics, *Rana latastei*

Received 29 September 2003; revision received 2 December 2003; accepted 2 December 2003

Introduction

Biologists disagree concerning the importance of peripheral populations for the evolution of a species and their value for conservation. Peripheral populations have been viewed as unimportant for overall species and range persistence because of their small size, isolated locations and frequent occurrence in suboptimal habitat and the consequent increased likelihood of extinction (Hoffman & Blows 1994; Lesica & Allendorf 1995). Other authors, in contrast, have argued that peripheral populations provide a source of adaptively significant variation upon which natural selection may act, and are probably adapted to local conditions (Brussard 1984; García-Ramos & Kirkpatrick 1997). Furthermore, while population density may decline at range edges (Brown 1984), thus reducing the likelihood of the persistence of individual populations (Caughley 1994), recent analyses challenge the view that peripheral populations are demographically impaired and are likely to suffer local extinction. For example, peripheral populations experience fewer extirpations than centrally located populations because of range contractions that are not predicted from historical distributions and immigration rates (Channell & Lomolino 2000). A more thorough understanding of how genetic variation in nature is partitioned among peripheral and central populations would contribute much to this debate.

Theoretical studies have examined how population location should influence neutral genetic composition. Genetic correlation among subpopulations decreases with distance and the number of dimensions involved (Kimura & Weiss 1964). More specifically, the geographical proximity of populations strongly influences the likelihood of successful immigration and the number of immigrants is the largest external influence on population genetic variability (Caughley 1994; García-Ramos & Kirkpatrick 1997; Kirkpatrick & Barton 1997). The allelic diversity of a recipient population should therefore be determined primarily by the number of populations that contribute immigrants (Saccheri *et al*. 1998). Populations located at range margins are more isolated from sources of immigrants and are thus more prone to genetic bottlenecks (Karron 1987; Hamrick & Godt 1989; Rowe & Beebee 2003), a situation that should lead to depleted neutral genetic variation in peripheral populations.

Empirical evidence supporting this hypothesis remains ambiguous. Some data support the hypothesis that peripheral

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populations exhibit lower genetic diversity (Lammi *et al*. 1999; Yeh & Layton 1979; Jain *et al*. 1981), while others show no such relationship (Wendel & Parks 1985; Tigerstedt 1973). The results of these and other studies may not be representative because of sampling flaws and taxonomic bias (Hutchison 2003). Pseudoreplication as a result of unrepresentative or uneven sampling of species ranges is a common logistical or design problem (Tigerstedt 1973; Lammi *et al*. 1999), of that may originate in sampling species with prohibitively large ranges. No matter what the cause, studies of neutral genetic variation and its distribution across a species range have not incorporated samples representative of the total species distribution.

Here the population genetic diversity of the endangered Italian agile frog, *Rana latastei*, is investigated with the explicit purpose of determining if populations located on the periphery of the species range exhibit lower genetic diversity with respect to more centrally located populations. Previous work has shown that population genetic diversity varies greatly in the western part of the species distribution (Garner *et al*. 2003). Here, this previous work is extended by performing comprehensive sampling across the species range and by including a balanced sample of peripheral and central populations. The genetic diversity was assessed using microsatellites and several models were used to interpret the pattern of variability detected at these loci.

Materials and methods

The Italian agile frog, *Rana latastei*, inhabits a small geographical range delimited by the Adriatic Sea and montane habitat inhospitable to the species (Fig. 1). Although other brown frog species inhabit wetlands along a broad altitudinal and latitudinal gradient and often exhibit adaptations to cold that have enabled range expansions, *R. latastei* is a lowland species and does not occur above approximately 600 m elevation (Ildos & Ancona 1994; Veith *et al*. 2003). This species is associated with slow-moving streams, ditches and other drainage channels and requires forest in close proximity to breeding sites (Ildos & Ancona 1994; Grossenbacher 1997).

Populations were categorized as peripheral or central as follows. The distribution map for *R. latastei* published by the Societas Herpetologica Italica (1996) which indicates the occupation of sites within a grid of 10×10 km squares was inspected. The range edge was defined by inspection as the least perimeter polygon that encapsulated all occupied squares (excluding marine habitat). Any populations in occupied squares falling on this line were peripheral, while those falling within the line were deemed central. Previously published data on seven populations of *Rana latastei* were augmented with information from 12 new populations sampled in the spring of 2001 and in the manner described in Garner *et al*. (2003) (Table 1, Fig. 1). Polymerase chain reaction (PCR) amplification was performed using seven microsatellite loci, but because of amplification failure at one locus using both published (RlatCa18; Garner & Tomio 2001) and redesigned primers the final data set was restricted to information derived from six loci. All amplification products were electrophoresed using the SEA 2000® Electrophoresis Apparatus and Spreadex® gels (Elchrom Scientific, Switzerland) following the conditions outlined in Garner & Tomio (2001). Gels were analysed using the Q-EL™ 330 Digital Recording and Analysis System (Elchrom Scientific, Switzerland).

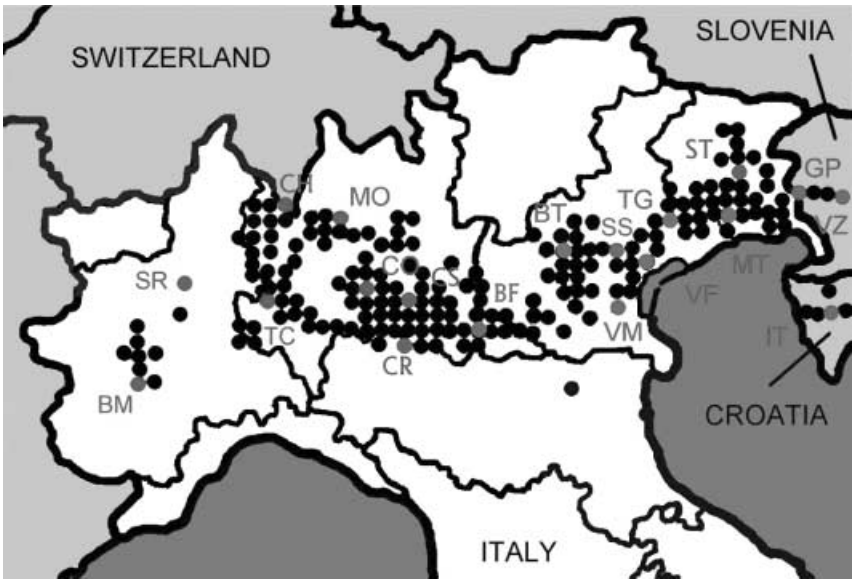

Fig. 1 Distribution map of *Rana latastei* with sampled populations in grey. Population abbreviations are as in Table 1.

Table 1 Location, sample size, measures of genetic variability and exact probability estimates of agreement with Hardy–Weinberg for 19 populations of *Rana latastei*

Population	Location	*N*	*n*	*P*	*H*	Prob. HW
Croatia						
Istria						
Istarke Toplice (IT)	P	20	3.086	100.0	0.355	0.019
Slovenia						
Gozd Pavonec (GP)	P	20	3.555	100.0	0.593	0.000
Velike Zablje (VZ)	P	20	2.792	100.0	0.395	0.000
Italy						
Friuli						
Savorgnano della Torre (ST)	C	20	3.731	100.0	0.513	0.000
Muzzana del Turgnano (MT)	C	20	4.344	100.0	0.587	0.000
Veneto						
Villa Friedenbeg (VF)	C	20	3.07	100.0	0.403	0.000
Tavaràn Grando (TG)	C	19	3.392	100.0	0.365	0.000
Sorgenti del Sile (SS)	C	20	3.671	100.0	0.522	0.000
Via Montagnon (VM)	P	16	3.942	100.0	0.532	0.000
Belvedere di Tezze (BT)	C	14	3.425	100.0	0.421	0.009
Lombardia						
Bosco Fontana (BF)	C	20	3.076	100.0	0.422	0.005
Cascina Stella (CS)	C	20	3.099	83.3	0.368	0.042
Comazzo (CO)	C	20	2.115	83.3	0.227	1.000
Emilia-Romagna						
Chiavica Rossi (CR)	P	17	2.284	66.7	0.27	0.583
Lombardia						
Montevecchia (MO)	P	20	1.829	50.0	0.243	0.983
Tenuta Castagnolo (TC)	C	19	1.498	33.3	0.155	0.721
Switzerland						
Ticino						
Pozza Bosco Penz (CH)	P	20	1.807	50.0	0.243	0.905
Italy						
Piemonte						
Settimo Rottaro (SR)	P	18	2.205	33.3	0.187	0.002
Bosco Merlino (BM)	P	19	1.277	33.3	0.049	0.259

Abbreviations for population names are included in parentheses after full names. Order reflects location of the population across the range progressing from easternmost location to westernmost location. Location is either central (C) or peripheral (P), *N* is the sample size, *n* is the allelic richness averaged across all six loci, *P* is the percentage of loci exhibiting polymorphism at the 99% criterion level, *H* is the gene diversity averaged across all six loci and Prob. HW is the *P*-value for the probability estimate of agreement with Hardy–Weinberg equilibrium.

Each locus was tested in each population and across all populations for deviations from Hardy–Weinberg expectations using the exact probability test implemented using default settings in GENEPOP version 3.1c (Raymond & Rousset 1995). Measures of population genetic variability (allelic richness, gene diversity and percentage of polymorphic loci at the 0.99 criterion) were calculated using FSTAT (Goudet 2001) and BIOSYS-2 (Swofford & Selander 1981). Since these components of variation are highly correlated, they were analysed as a single vector using principle component analysis as implemented in SAS version 8 (PROC PRINCOMP, SAS Institute Inc. 1999). Then, the distribution of genetic variation was analysed across all populations using five models, again employing SAS version 8 (SAS Institute Inc. 1999) and PROC GENMOD. These models incorporated population location using one or both of the possible explanations for geographical variation in genetic diversity previously proposed by Garner *et al*. (2003): location with regards to the centre of the species' range, or along the east-to-west gradient. Model selection analysis was performed on all the candidate models using Akaike's Information Criterion (AIC, Burnham & Anderson 1998).

To assess the potential impact null alleles would have on the analysis, their frequency at each locus in each population

Table 2 Generalized linear models examined as potential explanations for genetic variation in *Rana latastei*

Model	Effects	d.f.*	Type III MS/ Error MS	*F*	*P*
1	position	1	6.0/2.59	2.31	0.147
2	km	1	34.74/0.586	59.34	< 0.0001
	position	1	2.74/0.586	4.68	0.046
3	km^2	1	37.13/0.437	85.03	< 0.001
	position	1	0.46/0.437	1.05	0.32
4	km^2	1	42.66/0.438	97.4	< 0.0001
5	km	1	38.0/0.712	53.34	< 0.001

*Total degrees of freedom for all models is 18.
Position refers to location with regards to centre vs. periphery, km refers to number of kilometers along the east to west gradient.

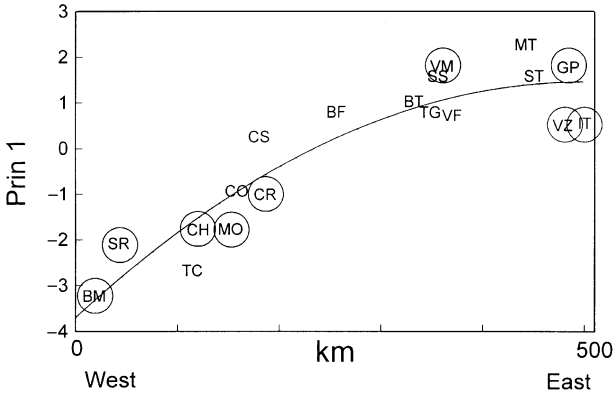

Fig. 2 The relationship between genetic variability and location along an east-to-west gradient for *Rana latastei*. Genetic variability is the first principle component of the three measures of genetic variation described in text. Peripheral populations are encircled.

was estimated using the program CERVUS (Marshall *et al*. 1998). CERVUS estimates the frequency of null alleles using the method proposed by Summers & Amos (1997) which, unlike the estimator proposed by Brookfield (1996), does allow double null homozygotes to be included in the data set. Then, the null allele frequency was averaged across all loci for each population and the null allele frequency was compared to the geographical distribution of populations using correlation analysis.

Results

A total of 362 individuals were included in the final data set (Table 1). Of the six loci used in this study, four exhibited deviations from Hardy–Weinberg across populations (Table 2). The first principal component described 93% of the variation in the response vector. Populations in the western portion of the range were less genetically diverse than eastern populations (Fig. 2). By itself, the central/peripheral position of the population failed to explain significant levels of genetic diversity (Table 3). When together with east-to-west location, centre/periphery proved to be a significant factor in only one model (Table 3). Model selection, using AIC, indicated that the model composed of only a quadratic term for east-to-west position was best-supported, while there was substantially less support for the hypothesis that position on the centre/periphery was important in explaining levels of genetic diversity in this species (Table 4).

Table 3 Results of tests for deviations from Hardy–Weinberg expectations for six loci used in this study

Locus	*P*
RlatCa27	0.0000
Rt2Ca9	0.1279
RlatCa9	0.0000
RlatCa21	0.0000
RlatCa17	0.0000
RlatCa41	0.7624

Frequencies of null alleles calculated using CERVUS and averaged across loci for each population ranged from an average frequency of 0.000 to 0.4375 (Table 5). Spearman rank correlation analysis showed that null alleles were more frequent in the eastern portion of the range, corresponding to the area of high genetic diversity (correlation coefficient –0.805, $P < 0.001$).

Table 4 Results of model selection analysis performed using five candidate models

Model	Effects	Log-likelihood	K	AICc	ΔAICc	Akaike weight
1	position	–34.96	3	76.64	33.8	3.0×10^{-8}
2	position, km	–20.24	4	50.0	7.16	1.9×10^{-2}
3	position, km^2	–17.45	4	44.42	1.58	0.31
4	km^2	–18.06	3	42.84	0.0	0.67
5	km	–22.68	3	57.52	14.68	4.3×10^{-4}

The smallest Akaike Information Criterion (AICc) or largest Akaike weight indicates the model with the highest explanatory power. Position and km as in Table 2.

Table 5 Average number of null alleles detected and population rank along the east-to-west gradient for 19 *Rana latastei* populations

Population	Null alleles	Rank
IT	0.3016	1
GP	0.3415	2
VZ	0.2987	3
ST	0.2010	4
MT	0.3337	5
VF	0.2851	6
TG	0.4375	7
SS	0.3040	8
VM	0.2431	9
BT	0.2255	10
BF	0.1899	11
CS	0.0933	12
CO	0.0068	13
CR	0.0726	14
MO	0.0000	15
TC	0.0244	16
CH	0.0033	17
SR	0.1380	18
BM	0.0305	19

Discussion

Genetic variability declined strongly west of the River Adige, reaching its lowest point at Bosco Merlino, located in the most western part of the species' distribution. The model that best described the distribution of genetic variability in *Rana latastei* was a simple quadratic term for population location along the east-to-west gradient, and our results provide little, if any, evidence that location with regards to the centre of the distribution explains any significant proportion of intrapopulation genetic variation. Since our overall sample comprehensively covers the range of *R. latastei*, it is unlikely that further sampling will alter this conclusion.

Although alternative mechanisms may be candidates for explaining the observed pattern of genetic variability in *R. latastei* (Hettyey & Pearman 2003), the most likely explanation would be that *R. latastei* expanded postglacially from a refugium located somewhere in the eastern part of the present-day range. The Balkan area is considered to be an important Pleistocene refugium for numerous plant and animal species (Hewitt 1999) and range expansions from such locations are inferred from present-day hybrid contact zones at points where expansion routes meet and, more importantly for our study, an increase in homozygosity and concurrent decrease in genetic variation along proposed expansion routes (Hewitt 1996). Mountainous regions located north of the present-day distribution would probably prevent expansion northward for *R. latastei*, and marine (but not brackish, see Seppä & Laurila 1999) regions are impenetrable for amphibian species. Expansion would therefore be restricted along the short latitudinal gradient presently inhabited by the species.

Such an expansion pattern illustrates the limitation of the hypothesis that peripheral populations exhibit lower genetic diversity. If range expansions and contractions occur along regular axes radiating from a core founder region, then populations located at range margins should exhibit similar patterns of decreased genetic diversity, barring the effects of mutation and selection. The majority of temperate-zone species, though, have been periodically subject to large-scale climatic fluctuations that have affected ranges asymmetrically (Webb & Bartlein 1992; Veith *et al*. 2003). Range expansions and contractions have generally followed a north–south gradient and/or have been channelled along routes proscribed by aquatic or alpine regions (Hewitt 1999). Although the margins of expansion routes may carry some signal of genetic depletion, the location of a population relative to the expansion source may be a more important factor in determining genetic variation than location relative to the core of the overall range. Although one of our models including both central–peripheral location and east-to-west location does show a significant effect of location at the range margin, the model incorporating both factors but with east-to-west location in the quadratic form does not. It is this latter model that explains substantially more variation than the other combined effects model (Table 4).

The presence of null alleles at substantial frequencies in many populations could militate against the reliability of our data and the accuracy of our interpretation. Null frequency, however, decreases along the east-to-west gradient, following the pattern observed in the allelic diversity that was detected directly. The effect of nulls on our data set is strongest in the areas with the highest detectable diversity and weakest in the areas with the lowest detectable diversity. Therefore, our estimates of the change in genetic variation along the east-to-west gradient are probably conservative but the general pattern would remain unchanged after the addition of nulls to the data set. In addition, it is conceivable that our data set is affected by strong gene flow among populations. Preliminary analyses of interpopulation genetic structure do not support this hypothesis, as global F_{ST} estimated using GENEPOP is 0.2915, suggesting low gene flow and strong population differentiation.

The adaptive consequences of genetic depletion that were detected in this species have yet to be determined, but other studies have shown that low population-level genetic diversity often has severe fitness consequences for amphibian populations. Low genetic diversity negatively affects reproduction, larval performance and overwinter survivorship (Samollow & Soulé 1983; McAlpine 1993; Hitchings & Beebee 1998; Rowe & Beebee 2003), while heterozygosity and fitness do not correlate when outbred

populations are examined (Rowe & Beebee 2001). *Rana latastei* offers a particularly inviting model system for investigating the consequences of decreased genetic variability on adaptive traits such as growth, fecundity, survivorship and disease resistance. Given the present attention being paid to amphibian population declines and the general lack of population genetic studies being incorporated into research on the causes of these declines (see Semlitsch 2003 and references therein), such a model is overdue.

Acknowledgements

We would like to thank Francesco Barbieri, Franco Bernini, Lucio Bonato, Nicola Bressi, Leonardo Carlotto, Augusto Gentilli, Damiano Ghezzi, Michele Menegon, Sergio Mezzardi, Katja Poboljsaj, Paolo Rezzani, Dino Scaravelli, Massimo Semenzano and Roberto Sindaco for their help with the field component of this study. This work was funded by a Swiss National Fund grant (SNF 31–40688.94) to H.-U. Reyer.

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Trent Garner studies the behavioural ecology, population genetics and adaptive genetic variation of reptiles and amphibians, but has also been known to work on other vertebrates and the odd invertebrate. He is specifically interested in the evolution and maintenance of polyandrous mating systems, how species distributions affect population genetic diversity and how genetic diversity affects resistance to the effects of pathogen exposure. Peter Pearman is broadly interested in the dynamics of viral infections of amphibians, and their impacts on both populations and community structure. Most recently he has used experimental approaches to investigate the relationship between genetic diversity and immunocompetence. His previous work has involved work on biodiversity patterns in tropical forests and the population ecology of tadpoles. Sonia Angelone is interested in population genetics. This study was part of her Diploma thesis. This manuscript is the fourth in a series investigating the interactions between neutral genetic diversity, reproductive ecology, population ecology, habitat and adaptive genetic variation in the endangered Italian agile frog.
