Molecular Ecology (2004) **13**, 271–276 doi: 10.1046/j.1365-294X.2003.02057.x

Historical data refute recent range contraction as cause of low genetic diversity in isolated frog populations

ERIC A. HOFFMAN and MICHAEL S. BLOUIN
Oregon State University, Department of Zoology, 3029 Cordley Hall, Corvallis, OR 97331, USA

Abstract

This study tested whether low genetic diversity in remnant populations of a declining amphibian is best explained by recent bottlenecks or by a history of being peripheral. We compared diversity from eight microsatellite loci in historical and extant populations from the interior and former periphery of the species' range. We found that historic peripheral populations already had reduced levels of genetic variation before the range contraction. Therefore, low diversity in remnants could not be ascribed to recent range contractions. This study shows that a common conservation strategy for rescuing genetically depauperate populations, artificial gene flow, may often be unwarranted and detrimental to evolutionarily important peripheral populations.

Keywords: conservation, frog, peripheral populations, population supplementation, *Rana pipiens*, range contraction

Received 20 July 2003; revision received 25 September 2003; accepted 15 October 2003

Introduction

If a normally outbred population is isolated and reduced in size, it can be put at increased risk of extinction owing to genetic drift and inbreeding depression (Keller & Waller 2002). One management strategy for rescuing isolated and genetically depauperate populations is artificial gene flow, the introduction of individuals from other populations (Hedrick 1995; Land & Lacy 2000). Because of recent data on the success of population supplementation (e.g. Madsen *et al*. 1999) as a counter-active measure for decreasing genetic variation and the costs therein, artificial gene flow is now a popular method for 'rescuing' genetically depauperate populations. Artificial gene flow has drawbacks, however, such as the possibility of introducing diseases or genes that are maladapted to the local environment (Waples 1991; Hedrick *et al*. 2000). Thus, careful consideration of the causes of reduced variation in a population is necessary before supplementation is enacted.

Contraction of geographical range is a common occurrence for many species in today's global environment (Channel & Lomolino 2000). During the contraction process, isolated ('remnant') populations may persist outside the edge of the newly contracted range. If these populations are found to be less genetically diverse than populations inside the species' contiguous range ('interior' populations), it is often assumed that the remnant populations have lost diversity (see Matocq & Villablanca 2001 and references therein). However, there is another potential explanation. Because ranges usually contract from the periphery (Brown 1995), it may be that remnant populations were originally on the periphery of the species' continuous range. Peripheral populations tend to have lower diversity than interior populations owing to isolation, founder effects and chronically smaller population sizes (Lesica & Allendorf 1995). Thus, if remnant populations were originally peripheral populations, then they may have always had lower genetic diversity than interior populations. In these populations artificial gene flow may not be warranted. Although there have been a few studies that compared current vs. historical diversity in various populations (Bouzat *et al*. 1998; Rosenbaum *et al*. 2000; Matocq & Villablanca 2001; Pertoldi *et al*. 2001), there has never been a test of whether low diversity in a remnant population resulted from a recent range contraction or from a history of being peripheral.

Northern leopard frogs, *Rana pipiens*, have undergone a major range contraction in the Pacific Northwest since the 1980s (Leonard *et al*. 1999). However, recent surveys in the Pacific Northwest revealed a few isolated populations that

Correspondence and present address: Eric A. Hoffman, 310 Ferst Drive, School of Biology, Georgia Tech, Atlanta, GA 30332, USA. Fax: 404 385 4440; E-mail: eric.hoffman@biology.gatech.edu

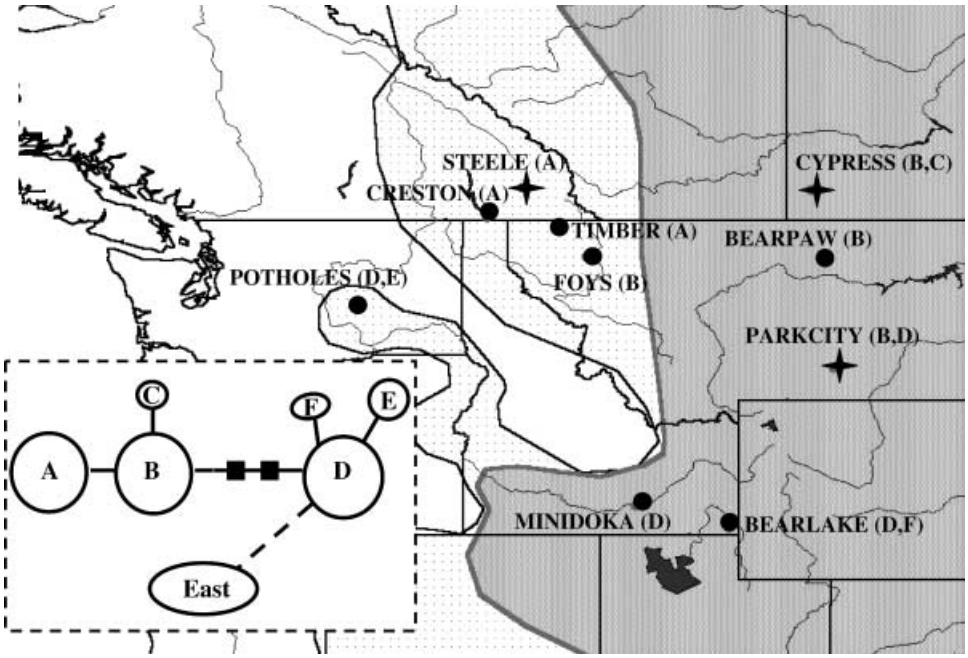

Fig. 1 Map of the northwest edge of the species range of *Rana pipiens*. Historic western reaches of the species range (Stebbins 1985) and the new species range (inferred from Csuti *et al*. 1997; Leonard *et al*. 1999; Waye & Cooper 2000) after a major contraction are both marked (historic range: stippled area; current range: shaded area). Population locations are marked with population name and letter designation of haplotypes present. Extant populations are designated by circles and historical samples are designated by stars. The dashed line box contains the mitochondrial haplotype network. For exact locations of haplotypes see Table 1. Circles represent haplotypes, with size roughly estimating relative frequency found among all samples. Straight lines connecting haplotypes represent single base pair changes and dark rectangles represent inferred haplotypes. Dashed line connecting haplotype D with East represents 18 mutational steps.

persist outside what is now the species' contiguous range (e.g. Leonard *et al*. 1999; Fig. 1). Therefore, a comparison of genetic diversity in remnant (formerly peripheral) and interior populations with that found in historical populations taken from those same regions before the range contraction makes for a model system to test hypotheses about the causes of low genetic diversity in these remnant populations.

In this study we tested whether low genetic diversity in remnant populations of Pacific Northwest northern leopard frogs is explained best by a recent bottleneck or by a history of being peripheral. First, we assayed mitochondrial DNA (mtDNA) variation to verify that the putative remnant populations were actually native remnants, and not introduced populations from laboratory stock or the pet trade. Second, we used eight microsatellite loci to compare genetic diversity in remnants with diversity in historical samples from the same region (i.e. the former periphery of the species range) that were collected before the population decline. We also compared genetic diversity in historical and extant interior populations. We conclude by discussing the implications of our results for management of the evolutionary legacy of *R. pipiens* and of peripheral populations in general.

Materials and methods

Leopard frog samples were collected from 10 populations in the Pacific Northwest (Fig. 1). Populations were categorized according to location (peripheral or interior) and age of samples (extant or historic). 'Remnant' populations were originally at the edge of the historic range of *R. pipiens*, but are now located outside what is currently the contiguous species range (Fig. 1). 'Interior' sites are still within the continuous range of the species. Historic and extant population locality information is provided in Table 1. Additionally, a *R. pipiens* individual from the eastern part of the species range (Ohio) was also included in the phylogenetic analysis both as an outgroup and as a representative from the region where most commercial frogs are collected (Hoffman unpublished data). All extant samples consisted of toe clips collected during the summers of 1999–2000 and preserved by desiccation in 1.5 mL tubes filled with drie-rite desiccant. The historic population samples consisted of frozen liver in 95% ethanol from the Museum of Vertebrate Zoology, UC Berkeley (collected in 1975) or dried skins from the Canadian Museum of Nature (collected in 1971). Total genomic DNA was extracted following a standard phenol/chloroform technique (Sambrook

Table 1 Table of population name, location, museum catalogue numbers, category (E = extant, H = historic, P = peripheral, I = interior, R = remnant), mtDNA data and microsatellite summary statistics

					MtDNA data		Microsatellite data			
									Mean heterozygosity (± SE)	
Location	Latitude	Longitude	Museum cat. no.	Category	Sample size	Haplotype	Mean sample size (± SE)	Mean alleles / locus (± SE) [rarified to sample size = 5]	Direct-count (± SE)	HWE expected (± SE)
Steele	49.711	−115.739	CMN 16081-1-15	H-P	15	A	14.88 (0.13)	2.13 (0.40) [1.9 (0.28)]	0.304 (0.10)	0.304 (0.10)
Cypress	49.658	−109.494	CMN 16089-1-10	H-I	10	B, C	10 (0)	5.5 (0.87) [4.2 (0.56)]	0.725 (0.08)	0.695 (0.06)
Park City	45.69283	−108.984	MVZ 501161-70	H-I	9	B, D	9 (0)	3.88 (0.77) [3.5 (0.61)]	0.556 (0.13)	0.585 (0.13)
Potholes	47.07613	−119.3536	N/A	R	12	D, E	29 (0)	2.63 (0.50) [1.9 (0.26)]	0.284 (0.08)	0.291 (0.09)
Creston	49.05	−116.5017	N/A	R	12	A	26.25 (0.37)	2.63 (0.38) [2.3 (0.28)]	0.413 (0.09)	0.435 (0.08)
Timber	48.814	−115.001	N/A	R	6	A	5.50 (0.27)	2.0 (0.33) [2.0 (0.32)]	0.392 (0.15)	0.320 (0.11)
Foys	48.178	−114.366	N/A	R	6	B	4.38 (0.26)	1.75 (0.25) [1.8 (0.25)]	0.331 (0.14)	0.246 (0.09)
Bear Paw	48.1591	−109.2003	N/A	E-I	6	B	4.38 (0.46)	3.0 (0.5) [3.0 (0.49)]	0.615 (0.12)	0.547 (0.11)
Bear Lake	42.1385	−111.262	N/A	E-I	12	D, F	21.75 (0.16)	6.88 (1.16) [4.0 (0.54)]	0.602 (0.10)	0.618 (0.11)
Minidoka	42.6198	−113.2837	N/A	E-I	12	D	29.88 (0.48)	5.25 (1.00) [3.4 (0.51)]	0.501 (0.10)	0.550 (0.11)

et al. 1989). All historic DNA samples produced high quality DNA from which we had no problems amplifying microsatellite loci.

For mtDNA sequence analysis the 5′ region of the mtDNA ND1 gene, including the upstream tRNA–leucine were amplified by polymerase chain reaction (PCR) using primer MB77 and primer MB145 and following the protocol in Hoffman & Blouin (2004). All sequences were aligned with a type sequence and checked for correct base calls in SEQED (1.0.3, Applied Biosystems, Inc.). Sequence from 644 base pairs (bp) of mtDNA was obtained for six to 15 samples in each population. TCS (Clement *et al*. 2000) was used to create an intraspecific network of haplotypes using the 95% statistical parsimony method of Templeton *et al*. (1992). For confirmation of network topology, an intraspecific phylogenetic tree of unique haplotypes was constructed with PAUP* (version 4.0b, Swofford 2002) using maximum likelihood (ML) optimality criterion (using the HKY model of sequence evolution (Hasegawa *et al*. 1985) as determined by MODELTEST version 3.0 (Posada & Crandall 1998).

Of the eight microsatellite markers used, six loci were developed for *R. pipiens* (Rpi100, Rpi101, Rpi103, Rpi104, Rpi107 and Rpi108) (Hoffman *et al*. 2003) and two loci were developed originally for *R. pretiosa* (RP193 and RP415; Blouin unpubl. data). PCR conditions were as follows: 3 min of initial denaturation, followed by 35 cycles of the following steps: 94 °C for 45 s, annealing temp. for 30 s and 72° for 1 min, followed by a 7 min final extension. Annealing temperatures and allele detection conditions are the same as described in Hoffman *et al*. (2003). The annealing temperatures of RP193 and RP415 were 44 °C and 51 °C, respectively. Microsatellite data were analysed with GENEPOP version 3.3 (Raymond & Rousset 1995) to test for Hardy–Weinberg equilibrium (exact tests, applying a sequential Bonferroni correction (Rice 1989)). *A* priori contrasts of within-population levels of genetic diversity were conducted between remnant vs. extant interior populations, historical peripheral vs. historical interior populations and remnant vs. historic peripheral populations. Differences in within-population levels of genetic diversity among sampling sites were assessed by one-way analysis of variance (ANOVA) using SYSTAT version 9.0 (for the PC, Systat Inc., Evanston, USA). Model parameters included either average expected heterozygosities or average number of alleles per locus as the main effect; interlocus variation was accounted for as a blocking variable. To account for differences among populations in sample size, alleles per locus were estimated at the lowest common sample size via rarefaction (Hurlburt 1971) using POPULATIONS version 1.2.28 (©Langella 2000). Unbiased heterozygosities were calculated according to Nei (1987) by FSTAT version 2.9.3 (©Goudet 2001). A sequential Bonferroni test (Rice 1989) was used to adjust significance levels for multiple comparisons.

Results

Statistical parsimony and ML networks from mtDNA sequence data exhibit identical topologies and show a northwest group (haplotypes A, B, C) and a southwest group (haplotypes D, E, F) within the Pacific Northwest (Fig. 1, Table 1). The sequence from Ohio was markedly different and provided evidence that no 'eastern' frogs were present in the populations sampled. Moreover, as would be expected for native frogs, no major population differences were detected between extant and historical populations. In fact, extant populations consisted of the haplotypes that would have been predicted given the closest historic populations. Therefore, we are confident that our remnant populations are indeed native remnants, rather than recent introductions from elsewhere.

Microsatellite data are summarized in Table 1. Only one locus in a single population showed a deviation from Hardy–Weinberg equilibrium (Rpi 103 in Creston), and all individuals amplified at this locus. Therefore, populations appear to be random mating, and there may be a low-frequency null allele at Rpi103 in Creston. Remnant populations had a significantly lower average number of alleles per locus, n_a ($F = 33.4_{1,47}$, $P < 0.001$) and lower expected heterozygosity, H_E ($F = 14.6_{1,47}$, $P < 0.001$) than extant interior populations. Similarly, the historic peripheral population had significantly lower n_a ($F = 22.0_{1,15}$, $P = 0.001$) and H_E ($F = 19.7_{1,15}$, $P = 0.001$) than historic interior populations. Within each region, historic and extant populations did not differ (n_a: $F = 0.016_{(1,31)}$, $P = 0.90$ for remnant vs. periphery, and $F = 1.69_{(1,31)}$, $P = 0.20$ for extant vs. historic interior; H_E: $F = 0.032_{(1,31)}$, $P = 0.86$ for remnant vs. periphery, and $F = 1.63_{(1,31)}$, $P = 0.21$ for extant vs. historic interior). A plot of per population expected heterozygosity vs. number of alleles per locus indicates a strong reduction in both variables in all remnant/peripheral populations (Fig. 2).

Discussion

Mitochondrial sequence data indicate that peripheral populations of the northern leopard frog, *R. pipiens*, in the Pacific Northwest are not introduced from other localities and are not likely to be liberated laboratory frogs. They appear to be native, remnant populations. Support for this conclusion stems from three sources of evidence. First, there is genetic similarity among all extant populations investigated from the Pacific Northwest. Second, there is genetic similarity among extant peripheral populations and historical populations from the same vicinity that were collected before the population contraction occurred. Third, there is a huge difference between populations in the Pacific Northwest and those from the East where most commercial frogs are collected (see also Hoffman & Blouin 2003).

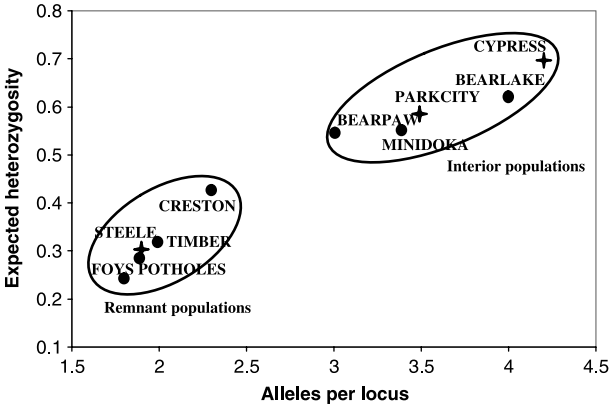

Fig. 2 Plot of unbiased expected heterozygosity and number of alleles per locus adjusted to a common sample size of five via rarefaction (Hurlburt 1971). Both variables are reduced strikingly in all remnant/peripheral populations compared to interior populations. Extant populations are designated by circles and historical samples are designated by stars.

The comparison of genetic variation in microsatellite DNA data between historical edge vs. historical interior populations suggests that peripheral populations were genetically depauperate before the range contraction occurred. Furthermore, Fig. 2 shows that historical populations fall exactly within the range of genetic variation within extant samples from the same region. Thus, it appears that peripheral populations of *R. pipiens* already had reduced levels of genetic variation. One limitation of this study to keep in mind is that we had access to only one historic peripheral population. Although the diversity of that population falls completely in line with that of the extant remnant populations (Fig. 2), the inference that historic peripheral populations in general had the same diversity as the remnants is based on a single sample. Additionally, sample sizes in three of our populations are relatively low. However, given the large differences between interior and peripheral populations in this study, larger sample sizes would probably not change the final conclusions (Nei 1987). Therefore, our data show that low diversity in the remnants cannot be most parsimoniously ascribed to the recent range contraction.

These results support the hypothesis that low diversity in remnant populations results simply because they were originally at the periphery of the species' range and not because of recent declines. This study illustrates the importance of using historical information to make accurate management decisions. The usual comparison involving only extant populations could have misled managers into proposing an unwarranted augmentation of the remnant populations using frogs from the interior.

Artificial gene flow into peripheral populations such as those in this study may be a particularly bad idea due to the potential costs associated with supplementation.

Ecological costs can include introduced diseases or competition between natives and non-natives. Genetic costs can include decreasing effective population size and reducing the genetic variation of the recipient population, as well as outbreeding depression (Tufto 2001). Recent studies investigating evolutionary models of the effects of gene flow from interior to peripheral populations have determined that supplementation can hinder adaptation (Kirkpatrick & Barton 1997) and increase the probability that peripheral populations will go extinct (Boulding & Hay 2001) by decreasing fitness in recipient populations.

Because peripheral populations are at the interface of species boundaries, they are essential for understanding the evolutionary processes involved in species boundary determination (Hoffmann & Blows 1994). Both empirical studies (Chapin & Chapin 1981) and genetic models (Garcia-Ramos & Kirkpatrick 1997) suggest that selection drives variation in species' fitness between interior and peripheral populations. Because of this adaptive potential, peripheral populations may be hotspots of evolution owing to their opportunity to drift and adapt to novel environments (Garcia-Ramos & Kirkpatrick 1997; Pertoldi *et al*. 2001). In sum, artificial gene flow and the subsequent loss of genetic distinctness of peripheral populations could do serious damage to the evolutionary legacy of a species.

Should we be concerned about the fate of peripheral populations even though the species as a whole is not in danger of extinction? The evolutionary potential of *R. pipiens* lies not only within interior populations but also within edge and remnant populations found across the periphery of the species' range. Although action should be taken to make sure that these remnant populations are not lost, population supplementation may not be the best strategy. Artificial gene flow to save small, isolated populations should be used only as a last resort and only when truly warranted.

Acknowledgements

We would like to thank C. Criscione, S. Arnold and A. Jones for helpful comments on various stages of the manuscript. We would also like to thank C. Cicero for providing tissue from the MVZ and F. Scheuler and the CMN for providing the tissues he collected in 1971. This research was funded by USGS contract no. 00HQAG0027 to MB and WDFW grant no. 58300726 to EH.

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This study is a chapter in the PhD dissertation of E. Hoffman, who has worked on various aspects the evolutionary history of the northern leopard frog including genetic structure, conservation issues and detecting selection on the green–brown colour polymorphism. M. Blouin's laboratory focuses on the causes and consequences of genetic structuring and on applications of methods for pedigree reconstruction in natural populations. He works on a variety of taxa including fish, amphibians and parasites.
