



# Hierarchical organization of genetic variation in the Costa Rican livebearing fish *Brachyrhaphis rhabdophora* (Poeciliidae)

JERALD B. JOHNSON\*

Department of Biology, University of Vermont, Burlington, VT 05405, USA

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I examined the geographic distribution of genetic variation in the livebearing freshwater fish *Brachyrhaphis rhabdophora* in northwestern Costa Rica as revealed by allozymes and mitochondrial DNA sequences. Allelic variability at 11 enzyme-coding loci surveyed across 12 localities revealed marked genetic differentiation among populations within drainages ( $\theta_p = 0.36$ ) and among drainages within regions ( $\theta_D = 0.17$ ), but not between northern and southern geographic regions ( $\theta_R = -0.02$ ). Allozyme variation was hierarchically organized such that populations found within stream drainages were more similar to each other than to populations found in adjacent drainages, a result confirmed by cluster analysis. In contrast to the allozyme data, there was extremely little DNA sequence variation among populations in the mitochondrial control region (3 variable nucleotide positions out of 444 bp examined). The difference in genetic divergence between allozyme and mtDNA markers was unexpected and is discussed in terms of biogeographical colonization events and a molecular selective sweep on the mitochondrial genome, both processes that could explain the lack of mitochondrial variability in this highly subdivided species.

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ADDITIONAL KEYWORDS: allozyme – biogeography – control region – gene flow – genetic distance – geographic variation – kin-structured colonization – mtDNA – population differentiation – polymorphism.

## INTRODUCTION

Studies of population subdivision are fundamental to understanding the potential for microevolutionary change within a species (Slatkin, 1985). In large panmictic species, high levels of gene flow can limit evolutionary diversification among populations (Felsenstein, 1976). Under such conditions, local adaptation can be achieved only if selection is sufficiently strong to overcome the effects of homogenizing gene flow (Haldane, 1930; Endler, 1977). Alternatively, species composed of small, genetically isolated populations that show low levels of gene flow are more likely to evolve independently, either by genetic drift or in response to more moderate selective pressures (Felsenstein, 1976; Slatkin, 1985). Hence, the degree to

which populations are genetically isolated largely determines the extent to which evolutionary diversification within a species can occur.

Population geneticists have long recognized that neutral genetic variation in a species can be geographically organized among potential breeding units, thus revealing the geographic nature of gene flow (Wright, 1978). Genetic differences can be found among individuals within populations, between populations within geographical regions, between populations from different geographic regions, and among geographic regions overall (Wright, 1978; Slatkin, 1985). Such hierarchical organization can be caused by historical vicariant events including geographic fragmentation and range expansion, and by contemporary isolation-by-distance due to limited migration (Templeton, 1998). How these biogeographical processes affect evolutionary change in natural systems is not well understood (Bossart & Prowell, 1998).

The livebearing freshwater fish *Brachyrhaphis rhabdophora* (Regan) represents a model study system to examine the relationship between local adaptation and

\* Present address: National Marine Fisheries Service, Conservation Biology Division, 2725 Montlake Boulevard East, Seattle, WA 98112-2013, USA. E-mail: [jerry.johnson@noaa.gov](mailto:jerry.johnson@noaa.gov)

population subdivision. This species is widely distributed throughout a hierarchical network of freshwater streams in northwestern Costa Rica (Bussing, 1987). In addition, there is a strong association between predation environment and life-history phenotypes, suggesting that populations could be locally adapted to differences in predator-mediated mortality (Johnson & Belk, 2001). If significant levels of population subdivision do exist in *B. rhabdophora*, it would indicate that populations are genetically isolated in a way that would permit independent, local evolutionary change.

In this study, I examine the geographic distribution of genetic variation in *B. rhabdophora* across five drainage systems in northwestern Costa Rica. I compare allelic variability surveyed in 12 populations as revealed by 11 polymorphic allozyme loci and by sequence variation in the mitochondrial control region. By comparing genetic structuring using both nuclear and mitochondrial markers, I address the following objectives: (1) I test for significant genetic subdivision in *B. rhabdophora* and evaluate whether such subdivision is conducive to local adaptation among geographically isolated populations; (2) I evaluate whether there is an association between geographic and genetic structuring; and (3) I contrast nuclear versus mitochondrial divergence to infer the relative importance of vicariant versus isolation-by-distance biogeographical processes.

## MATERIAL AND METHODS

### FIELD SAMPLING

In March 1999, I collected fish from 12 sites (Fig. 1) that span the northern geographic distribution of *B. rhabdophora* in northwestern Costa Rica. Sampling was conducted in five geographically isolated drainage basins that composed northern and southern regional groups within the Gulf of Nicoya. At each site, fish were randomly collected with respect to sex and size. Individuals used in the allozyme study ( $N=357$ ) were preserved in the field in liquid nitrogen, stored frozen at the University of Vermont ( $-70^{\circ}\text{C}$ ), and in April 1999, analysed at the Savannah River Ecology Lab. Individuals used in the DNA study ( $N=24$ ) were preserved in the field in 100% ethanol and sequenced at the University of Vermont.

### ELECTROPHORESIS

Thirty fish from each population (except population 43,  $N=27$ ) were examined for variation at 12 putative allozyme loci (Table 1). To prevent potential contamination, I removed the digestive tract from each sample and removed developing embryos from adult females. Fish were then manually homogenized in 100  $\mu\text{l}$  of 0.01 M Tris EDTA buffer (pH 7.5), ground

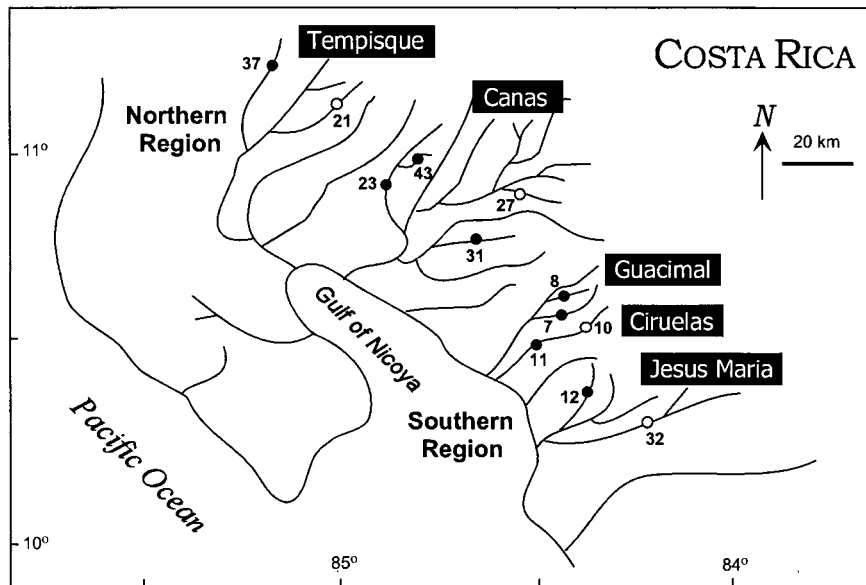
tissues were centrifuged, and supernatant was absorbed onto filter-paper wicks and moved by electrical current through a 10% starch gel using buffer systems optimized to resolve each target locus (Table 1). Electrophoresis and staining followed standard conditions (Harris & Hopkinson, 1976; Murphy *et al.*, 1996; staining recipes are available upon request). Loci were scored unambiguously and showed banding patterns identical to those expected by simple Mendelian inheritance (May, 1992; Murphy *et al.*, 1996). Of the 12 loci examined, all were polymorphic (0.95 criterion) with respect to the 12 populations evaluated (Appendix).

### DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Two individuals from each population ( $N=24$  total) were examined for mtDNA variation. Genomic DNA was extracted from ethanol-preserved muscle tissue using a proteinase K digestion, ammonium acetate separation of proteins, and cold-ethanol precipitation. To detect contamination, I carried a control tube through the extraction and amplification procedures. I used PCR to amplify the downstream half of the mitochondrial control region using primers 12R (reverse of H16498 in Shields & Kocher, 1991; Meyer, Morrissey & Schartl, 1994) and MRT2 (Ptacek & Breden, 1998). The forward primer is located in the highly conserved central domain of the control region (Lee *et al.*, 1995) and the reverse primer is positioned in the flanking tRNA-Phe gene. The thermal profile ( $94^{\circ}\text{C}$  for 1 min,  $52^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 1.5 min) was repeated 35 times. Purified double-stranded DNA was used as template (150 ng) for automated sequencing reactions. To ensure accuracy, I sequenced each sample in both forward and reverse directions resolving 444 bp of data. Sequences are available from GenBank (accession numbers AF343713–AF343724).

### ANALYSES

The Hardy–Weinberg principle is used to predict genotypic frequencies at a locus in the absence of mutation, migration, selection, nonrandom mating, and small population sizes (Hartl & Clark, 1989). Deviations from Hardy–Weinberg expectations could indicate that one or more of these processes is operating. Hence, I tested each allozyme locus within each population for deviation from the Hardy–Weinberg prediction using the Genetic Data Analysis (GDA) software package (Lewis & Zaykin, 2000). Goodness-of-fit was evaluated by Fisher's exact tests as described in Weir (1996: 92–112). Of the 144 tests made, 11 were significant at the Bonferroni-corrected critical level of  $\alpha=0.0004$ . However, nine of the 11 significant tests were at the *Ck-2* locus, which I consequently removed from all subsequent analyses; the remaining two (*6-Pgd* and



**Figure 1.** Geographic locations of 12 populations of *Brachyrhaphis rhabdophora* evaluated in this study (○ = predator-free sites, ● = predator sites; as detailed in Johnson & Belk, 2001). Numbers identify populations as follows: 37 = Tizate; 21 = Colorado; 23 = Piedras; 43 = Pital; 27 = Grande; 31 = Javilla; 7 = Acapulco; 8 = Surturbal; 11 = Ciruelas (lower); 10 = Ciruelas (upper); 12 = San Rafael; 32 = Vargas. Five river drainages are identified by black boxes. The northern geographic region encompassed the Tempisque and Canas drainages and the southern geographic region encompassed the Guacimal, Ciruelas, and Jesus Maria drainages. Specific map coordinates for each locality are available upon request.

**Table 1.** Enzyme systems evaluated, their abbreviations, number of alleles observed at each locus, and buffer systems. Buffers used were tris citrate pH 8.0 (TC 8.0) and tris citrate EDTA pH 7.1 (JRP)

Enzyme system	Locus abbreviation	Number of alleles	Buffer system
Aconitase	<i>Acon</i>	2	TC 8.0
Adenosine deaminase	<i>Ada</i>	3	TC 8.0
Creatine kinase	<i>Ck-1</i>	2	JRP
	<i>Ck-2</i>	2	JRP
Alpha-glycerophosphate-dehydrogenase	<i>αGpd</i>	2	JRP
Glucose phosphate isomerase	<i>Gpi-1</i>	2	TC 8.0
	<i>Gpi-2</i>	2	TC 8.0
Malate dehydrogenase	<i>Mdh-1</i>	2	JRP
	<i>Mdh-2</i>	2	JRP
Manose-6-phosphate isomerase	<i>Mpi</i>	3	TC 8.0
6-Phosphogluconic-dehydrogenase	<i>6Pgd</i>	2	TC 8.0
Phosphoglucomutase	<i>Pgm</i>	4	JRP

*Ada* from populations 21 and 43, respectively) were retained as their inclusion did not qualitatively affect any of the results presented here.

I estimated population structure in *B. rhabdophora* in a nested hierarchical framework using Cockerham's coancestry technique (Weir & Cockerham, 1984; Weir, 1996). This approach allowed me to partition the total (T) genetic variation across an increasing geographic

scale into variation among individuals within population (I), among populations within drainage basins (P), among drainage basins within geographic regions (D), and between geographic regions (R). This method yields results similar to traditional *F*-statistics (Weir & Cockerham, 1984) but allows additional levels of populations subdivision to be evaluated. Results can be interpreted as the coancestry of alleles within in-

dividuals from the same population ( $f$ ), within individuals at large ( $F$ ), and between individuals within a designated geographic level ( $\theta$ ). Here, I examined three levels of subdivision: among populations within shared drainages ( $\theta_p$ ); among drainages within shared regions ( $\theta_D$ ); and between regions relative to total variation ( $\theta_R$ ). This geographical hierarchy corresponds to the natural network of rivers depicted in Fig. 1. I also evaluated population subdivision separately within regions and within drainages by running the same analyses on geographical subsets of the entire data matrix. Confidence intervals for estimates of  $f$ ,  $F$ , and  $\theta$ 's were determined by bootstrapping (1000 replicates) over loci as outlined in Weir (1996). Evidence for subdivision is indicated when the 95% confidence interval for a particular statistic does not overlap zero. All analyses of population structure were performed with GDA software (Lewis & Zaykin, 2000).

I used cluster analysis to generate phenograms to show genetic similarity among populations. Phenograms were constructed using Nei's uncorrected (1972) and corrected (1978) genetic distances using the UPGMA clustering algorithm (Swofford *et al.*, 1996). The order of population entry into the analyses did not affect the outcomes. Results of both distance measures were qualitatively identical, so I report only the UPGMA tree for Nei's corrected distance. DNA sequences were aligned using Sequencher ver. 3.0 (Gene Codes Corp. 1995, Ann Arbor, MI); haplotype divergence among populations was calculated by hand.

## RESULTS

Genetic variation based on 11 allozyme markers was pronounced across the 12 populations evaluated in this study (Appendix). The mean proportion of loci polymorphic ( $P$ ) evaluated across all localities was 0.36; however, there was considerable variation among populations with values ranging from  $P=0.18$  in populations 43 and 11 to  $P=0.72$  in population 21. The average number of alleles per locus was 1.46 and the average number of alleles per polymorphic locus was 2.16. The observed proportion of heterozygotes ( $H_o$ ) averaged across all populations was 0.13, a value essentially the same as the expected proportion of heterozygotes ( $H_e=0.14$ ). Three private alleles (those found only in a single population) were found in population 21 and a single private allele occurred in population 10.

### HIERARCHICAL ORGANIZATION OF GENETIC VARIATION

I found high levels of genetic subdivision in *B. rhabdophora* at almost all geographic levels examined as revealed by allozyme markers. Considering all 12 localities together, there was evidence for structuring

among populations within drainages ( $\theta_p$ ) and among drainages within regions ( $\theta_D$ ); however, there was no genetic differentiation between northern and southern regions within the Gulf of Nicoya ( $\theta_R$ ) (Table 2). Evaluating regions separately, I found subdivision among drainages in the southern region but not in the northern region; population-level subdivision persisted in both regions. At the finest level of resolution (each drainage examined separately) there was significant genetic differentiation in four of five cases between localities within the same stream. I also found evidence for non-random mating within populations ( $f$ ) in the Tempisque and Ciruelas drainages, and a high degree of overall genetic structuring due to the combined effects ( $F$ ) of geographic subdivision and inbreeding.

### GENETIC SIMILARITY

Alleles differed markedly among populations both in frequency and composition as summarized by Nei's genetic distance measures (Table 3). The UPGMA phenogram derived from Nei's (1978) corrected distance showed that populations collected from the same drainages were most similar (Fig. 2). Moreover, populations from shared drainages clustered independently and in a manner concordant with geographic structuring of streams with one exception; two populations from the Canas drainage (23 and 43) were more similar to populations from the Ciruelas drainage (10 and 11) than to other populations from their same drainage. Despite the genetic affinity of populations within drainage systems, there was still considerable divergence among these samples. Only populations in the Jesus Maria drainage (12 and 32) showed a lack of population subdivision ( $\theta_p$ ) within a stream system (Table 2).

### MITOCHONDRIAL DNA

I found very little molecular variation in the mitochondrial control region among the 12 geographically dispersed populations of *B. rhabdophora* examined here. Of the 444 bp of mtDNA examined, I found only three variable nucleotide positions. Two of these variable sites were unique to population 21, leaving only a single point mutation that varied across the remaining 11 populations (=0.2% divergence). This single mutation delineated populations across a latitudinal cline separating four northern sites from eight southern sites; a single exception was population 12 from the south, which had the same haplotype as the four northernmost populations.

## DISCUSSION

### GEOGRAPHIC DISTRIBUTION OF VARIATION

I found strong evidence for genetic subdivision among *B. rhabdophora* populations ( $\theta_p$ ) within drainages,

**Table 2.** Partitioning of genetic variation in *Brachyrhaphis rhabdophora* over 11 polymorphic loci from 12 populations in northwestern Costa Rica. Genetic subdivision is evaluated over all locations combined, separately within northern and southern regions, and separately within each of five physically isolated stream drainages – this geographic hierarchy of river systems is depicted in Fig. 1. Reported values are coancestry parameter estimates with upper and lower limits of the 95% confidence intervals in parentheses. The interpretation of each parameter is given in the text. Bolding indicates coancestry parameter estimates that are statistically different from zero

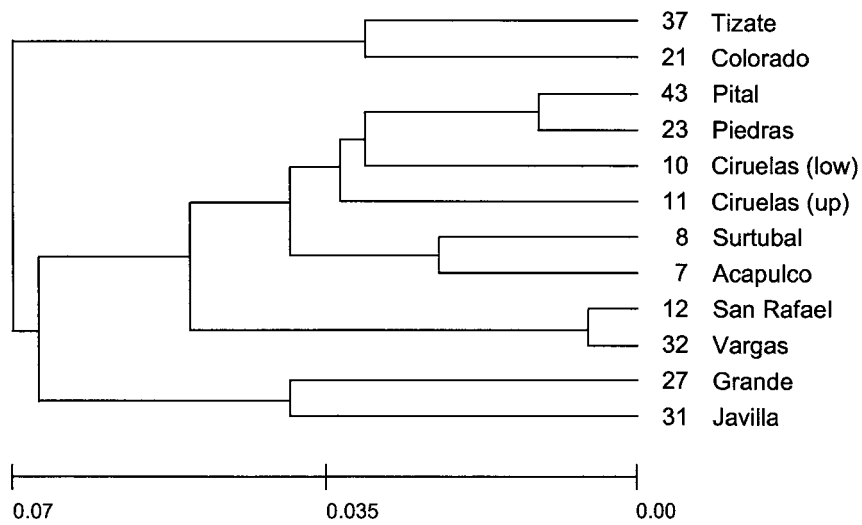
Geographic level of resolution	Parameter				
	<i>f</i>	<i>F</i>	$\theta_P$	$\theta_D$	$\theta_R$
Overall	0.19 (-0.02, 0.51)	<b>0.49</b> (0.26, 0.72)	<b>0.36</b> (0.24, 0.52)	<b>0.17</b> (0.02, 0.37)	-0.02 (-0.08, 0.03)
Regions					
Northern	0.25 (-0.20, 0.38)	0.37 (-0.01, 0.74)	<b>0.35</b> (0.13, 0.60)	0.14 (-0.07, 0.45)	—
Southern	0.13 (-0.03, 0.27)	<b>0.47</b> (0.30, 0.61)	<b>0.40</b> (0.19, 0.58)	<b>0.30</b> (0.01, 0.50)	—
Drainages					
Tempisque	<b>0.21</b> (0.01, 0.48)	<b>0.37</b> (0.10, 0.62)	<b>0.19</b> (0.03, 0.34)	—	—
Canas	-0.12 (-0.39, 0.35)	0.19 (-0.24, 0.69)	<b>0.29</b> (0.04, 0.52)	—	—
Guacimal	0.09 (-0.10, 0.23)	<b>0.18</b> (0.01, 0.33)	<b>0.09</b> (0.03, 0.17)	—	—
Ciruelas	<b>0.26</b> (0.09, 0.45)	<b>0.43</b> (0.32, 0.56)	<b>0.23</b> (0.16, 0.27)	—	—
Jesus Maria	0.00 (-0.09, 0.14)	<b>0.05</b> (0.01, 0.14)	0.05 (-0.01, 0.10)	—	—

**Table 3.** Mean genetic distances (lower diagonal) and identities (upper diagonal) between 12 *Brachyrhaphis rhabdophora* populations as revealed by allozyme markers. Values are based on 26 alleles at 11 polymorphic loci. Population identification numbers correspond to those presented in Figure 1

	Population											
	37	21	23	43	27	31	8	7	10	11	12	32
37	—	0.94	0.96	0.96	0.86	0.89	0.90	0.94	0.92	0.92	0.88	0.91
21	0.06	—	0.90	0.88	0.87	0.82	0.84	0.86	0.86	0.84	0.79	0.82
23	0.04	0.11	—	0.99	0.93	0.94	0.95	0.98	0.97	0.58	0.91	0.94
43	0.04	0.13	0.01	—	0.91	0.93	0.93	0.97	0.97	0.98	0.90	0.94
27	0.15	0.14	0.07	0.10	—	0.92	0.87	0.88	0.92	0.88	0.81	0.84
31	0.11	0.20	0.07	0.07	0.08	—	0.88	0.91	0.89	0.89	0.85	0.88
8	0.10	0.17	0.05	0.07	0.14	0.13	—	0.98	0.94	0.88	0.86	0.90
7	0.06	0.15	0.02	0.03	0.13	0.09	0.02	—	0.97	0.93	0.90	0.93
10	0.08	0.15	0.03	0.03	0.09	0.11	0.06	0.03	—	0.96	0.92	0.94
11	0.08	0.18	0.06	0.02	0.12	0.12	0.13	0.08	0.04	—	0.85	0.89
12	0.13	0.24	0.09	0.10	0.21	0.17	0.16	0.11	0.08	0.16	—	0.99
32	0.10	0.19	0.06	0.06	0.17	0.12	0.11	0.07	0.06	0.12	0.01	—

among drainages within geographic regions, and across geographic regions (Table 2). The level of divergence was relatively constant across this increasing geographic scale as evidenced by a concomitant decrease

in genetic similarity (Fig. 2; Table 3). There were two notable exceptions to this trend: populations 12 and 32 in the Jesus Maria drainage showed no genetic subdivision, and population 21 in the Tempisque drain-



**Figure 2.** UPGMA phenogram of genetic similarity among sampled populations. The genetic distance measure used was Nei's corrected distance (Nei, 1978). The column adjacent to the tips of the phenogram shows population identification numbers and site names; these localities correspond to those presented in Fig. 1.

age was markedly different from other populations (a point also supported by the three private alleles and unique mitochondrial haplotypes found in this population). Nonetheless, there was a strong overall concordance between genetic and geographic divergence. Are these levels of genetic divergence sufficient for populations to undergo independent evolutionary change?

Slatkin (1985) reviewed this general question and suggested that effective migration rates ( $Nm$ ) calculated from  $\theta$  values (or other  $F$ -statistic analogs) could be used to gauge the effects of gene flow on population differentiation. This approach requires a specified model of gene flow (typically an 'infinite island' or 'stepping stone' model) and assumes that genetic markers are neutral and that subdivision is due solely to migration between populations. Whether or not effective migration rate can be accurately calculated from genetic markers and be biologically meaningful has been extensively debated (reviewed in Bossart & Prowell, 1998). Nonetheless, general predictions have emerged that suggest that when effective migration rates ( $Nm$ ) are below 0.5, local populations are largely unconnected under any model of gene flow dynamics (Felsenstein, 1976; Nagylaki, 1983). I calculated the effective migration rate among the 12 populations of *B. rhabdophora* evaluated here (under an island model following Weir, 1996: 183) and found  $Nm = 0.44$ . My results strongly suggest that *B. rhabdophora* populations are adequately isolated for local adaptation or drift to occur.

#### MECHANISMS OF POPULATION DIFFERENTIATION

Population subdivision can be influenced by two distinct processes: (1) historical events such as range expansion or fragmentation; and (2) isolation-by-distance due to limited gene flow. Few studies that examine allozyme data have attempted to distinguish between these alternatives (Felsenstein, 1982; Bossart & Prowell, 1998). However, recent theoretical work suggests that observed patterns of genetic variation across a geographic landscape might be used to infer cases where these processes are most plausible (Bossart & Prowell, 1998; Templeton, 1998). Limited gene flow among populations, for example, should result in a genetic hierarchy that conforms to the geographic landscape – in stream fishes, this means that populations from shared drainages, although genetically distinct, should be more similar to each other than to populations from different drainages. This pattern is evident in *B. rhabdophora*.

At higher nesting levels, where comparisons were made among drainages within regions ( $\theta_D$ ) and between regions overall ( $\theta_R$ ), genetic structuring in *B. rhabdophora* was often weak or absent (Table 2). Lack of genetic subdivision at this level could arise from historical or current ecological processes. For example, individuals could migrate through the marine environment in the Gulf of Nicoya resulting in panmictic gene exchange among drainages and regions; poeciliid fishes are extremely salt tolerant and capable of movement through brackish environments (Meffe & Snelson, 1989). Alternatively, lack of differentiation at

higher hierarchical levels could simply be due to the retention of shared ancestral polymorphisms following colonization by original founding populations.

#### NUCLEAR VERSUS MITOCHONDRIAL DIVERGENCE

In contrast to the allozyme markers examined in this study, I found almost no variation in the mitochondrial control region in *B. rhabdophora*. A less complete survey of the mitochondrial cytochrome *b* gene in *B. rhabdophora* across this same geographic region showed a similar lack of variability (Johnson, unpubl. data). These results are surprising. The mitochondrial genome typically mutates much more rapidly than the nuclear genome (Moritz, Dowling & Brown, 1987) and the control region is thought to be among the highest mutating of all mitochondrial DNA sequences (Lee *et al.*, 1995). Hence, at the outset of this study I predicted that the mitochondrial control region would show more neutral genetic variation than the nuclear allozyme loci. What could account for the discrepancies in variation between the nuclear and mitochondrial markers evaluated here?

Two hypotheses could explain a lack of mitochondrial diversity. The mitochondrial genome could recently have experienced a 'selective sweep' resulting in the fixation of a single optimal haplotype across all populations. Because the mitochondrial genome is linked and maternally transmitted as a cohesive unit, selection could have acted on any mitochondrial trait (or any maternally inherited factor) to produce the observed patterns (Ballard & Kreitman, 1995). Evidence for mitochondrial selective sweeps in natural systems is rare but not unprecedented (Ballard & Kreitman, 1994; Ballard *et al.*, 1996). An intriguing alternative hypothesis is that a single adult female (or a small group of females with identical mitochondrial haplotypes) could recently have founded the geographic region examined in this study resulting in a mitochondrial bottleneck. Like other poeciliids, female *B. rhabdophora* can store sperm from multiple males that can be used to produce offspring over several brood cycles (Constanz, 1989). If allozyme polymorphisms were present in the stored sperm of a migrant female, this type of founder effect could account for high nuclear variability with little mitochondrial variation (Wade, McKnight & Shaffer, 1994). High allozyme variability could also occur if male dispersal into the unoccupied habitat was high relative to female dispersal. In either case, the observed discrepancy between nuclear and mitochondrial variation would suggest that *B. rhabdophora* have only recently come to occupy the Pacific northwestern region of Costa Rica (Wade *et al.*, 1994). Colonization of *B. rhabdophora* into this region likely occurred from the southeast, the only other area

where *B. rhabdophora* is found (Bussing, 1987). Consequently, a survey of nuclear and mtDNA from across this expanded range could be used to test the kin-structured colonization hypothesis.

#### CONCLUSIONS

The results of this study have important implications for understanding life-history divergence in *B. rhabdophora*. I have previously shown that predation environment predicts divergent life-history phenotypes among geographically isolated populations (Johnson & Belk, 2001). The present study demonstrates that this pattern of life-history divergence is expressed across highly subdivided populations that show low levels of genetic exchange. It remains unknown whether life-history differences among populations are genetically-based; experiments are currently underway testing this. However, if a genetic basis for life-history divergence is found, then the results presented here clearly suggest that local adaptation to predation environment has evolved repeatedly and independently across isolated populations in multiple drainages.

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APPENDIX

Allele frequencies for 12 populations of *B. rhabdophora* from five stream drainage systems in northwestern Costa Rica. All 12 loci evaluated in this study are presented. For location of populations see Fig. 1. 37=Tizate; 21=Colorado; 23=Piedras; 43=Pital; 27=Grande; 31=Javilla; 8=Surtubal; 7=Acapulco; 11=Cireulas (downstream); 10=Ciruelas (upstream); 12=San Rafal; 32=Vargas. Alleles are given a superscript which denotes their anodal mobility relative to the most common allele in the Tizate population which is set at 100 units.

Allele	Population											
	37	21	23	43	27	31	8	7	10	11	12	32
<i>Acon</i> <sup>100 (C)</sup>	0.65	0.70	0.73	0.52	0.53	0.75	0.82	0.67	0.68	0.90	0.77	0.82
<i>Acon</i> <sup>82 (B)</sup>	0.35	0.30	0.27	0.48	0.47	0.25	0.18	0.33	0.32	0.10	0.23	0.18
N	27	30	26	30	30	10	25	21	25	25	30	30
<i>Ada</i> <sup>107 (E)</sup>	0	0.28	0	0	0.47	0.10	0	0	0	0	0	0.03
<i>Ada</i> <sup>100 (D)</sup>	0.68	0.50	0.65	0.50	0.33	0.53	0.67	0.80	0.68	0.30	0.80	0.60
<i>Ada</i> <sup>91 (C)</sup>	0.32	0.22	0.35	0.50	0.20	0.37	0.33	0.20	0.32	0.70	0.20	0.37
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Ck-1</i> <sup>100 (C)</sup>	1.0	0.77	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Ck-1</i> <sup>95 (D)</sup>	0	0.23	0	0	0	0	0	0	0	0	0	0
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Ck-2</i> <sup>111 (D)</sup>	0.43	0.15	0.17	0.77	0.72	0.88	0.25	0.18	0.35	1.00	0.31	0.43
<i>Ck-2</i> <sup>100 (C)</sup>	0.57	0.85	0.83	0.23	0.28	0.12	0.75	0.82	0.65	0	0.69	0.57
N	27	30	30	30	29	30	30	30	30	30	29	30
<i>αGpd</i> <sup>124 (D)</sup>	0.04	0.03	0	0.03	0.03	0	0.02	0	0	0	0	0
<i>αGpd</i> <sup>100 (C)</sup>	0.96	0.97	1.00	0.97	0.97	1.00	0.98	1.00	1.00	1.00	1.00	1.00
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Gpi-1</i> <sup>126 (C)</sup>	0.39	0.28	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Gpi-1</i> <sup>100 (B)</sup>	0.61	0.72	0	0	0	0	0	0	0	0	0	0
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Gpi-2</i> <sup>142 (D)</sup>	0	0	0	0	0.38	0.78	0	0	0	0	0	0
<i>Gpi-2</i> <sup>100 (C)</sup>	1.00	1.00	1.00	1.00	0.62	0.21	1.00	1.00	1.00	1.00	1.00	1.00
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Mdh-1</i> <sup>117 (D)</sup>	0	0	0	0	0	0	0.68	0.43	0.25	0	0	0.07
<i>Mdh-1</i> <sup>100 (C)</sup>	1.00	1.00	1.00	1.00	1.00	1.00	0.32	0.57	0.75	1.00	1.00	0.93
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Mdh-2</i> <sup>145 (D)</sup>	0	0.23	0.05	0	0	0.07	0.15	0	0	0	0	0
<i>Mdh-2</i> <sup>100 (C)</sup>	1.00	0.77	0.95	1.0	1.0	0.93	0.85	1.00	1.00	1.00	1.00	1.00
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>Mpi</i> <sup>109 (D)</sup>	0	0.02	0	0	0	0	0	0	0	0	0	0
<i>Mpi</i> <sup>100 (C)</sup>	1.00	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Mpi</i> <sup>91 (B)</sup>	0	0.48	0	0	0	0	0	0	0	0	0	0
N	27	30	30	30	30	30	30	30	30	30	30	30
<i>6Pgd</i> <sup>107 (D)</sup>	0	0.44	0.21	0	0.78	0.12	0.25	0	0.30	0	0	0
<i>6Pgd</i> <sup>100 (C)</sup>	1.00	0.56	0.79	1.00	0.22	0.88	0.75	1.00	0.70	1.00	1.00	1.00
N	26	27	29	30	30	30	30	30	30	30	30	30
<i>Pgm</i> <sup>100 (D)</sup>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00	0.06	0.27
<i>Pgm</i> <sup>76 (C)</sup>	0	0	0	0	0	0	0	0	0.19	0	0.89	0.69
<i>Pgm</i> <sup>47 (B)</sup>	0	0	0	0	0	0	0	0	0.10	0	0.06	0.04
<i>Pgm</i> <sup>35 (A)</sup>	0	0	0	0	0	0	0	0	0.08	0	0	0
N	18	15	18	28	25	30	30	30	24	30	27	28