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ARTICLE

Rapid diversification of colouration among populations of a poison frog isolated on sky peninsulas in the central cordilleras of Peru

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ABSTRACT

Aim Comparison of *Epipedobates bassleri* (Myers, 1987), which occurs on high-altitude mountain ridges ('sky peninsulas') in the Andean transition zone and demonstrates high levels of divergence in colouration among populations, and *Epipedobates hahneli* (Schulte, 1999), which occurs throughout the lowland regions of the Amazon basin and is morphologically conserved, using phylogenetic analysis of mitochondrial sequence data and comparison of colour pattern.

Location Central cordilleras of Peru (near Tarapoto, San Martin).

Methods DNA was extracted from individuals of *E. bassleri* from the central cordilleras of Peru, and from individuals of *E. hahneli* from across Peru. The cytochrome *b* mitochondrial gene region was amplified and sequenced for individuals of each species, and phylogenetic analysis was carried out using Bayesian inference. Genetic distances among populations and geographic distances of each species were examined and compared using Mantel tests. Parametric bootstrapping was used to test the monophyly of *E. bassleri*.

Results *Epipedobates bassleri* formed a well-supported monophyletic group and showed higher levels of genetic divergence among populations than was shown among populations of *E. hahneli* from the same region. Distinct clades representing different geographic regions were recovered for *E. hahneli*. Levels of divergence among more geographically distant populations of *E. hahneli* were higher than levels of divergence among *E. bassleri* populations. We found a significant correlation between genetic divergence and geographic distance as measured along a 1000-m contour line, but not as measured by direct routes (crossing putative biogeographical barriers).

Main conclusions Levels of genetic divergence were higher among populations of morphologically conservative *E. hahneli* than among populations of morphologically variable *E. bassleri*, suggesting rapid divergence in colouration among populations of *E. bassleri*. These patterns support previous arguments concerning the role of the montane transition zone between the high mountains and lowlands in divergence and speciation. High levels of both genetic and phenotypic divergence among populations of *E. bassleri* indicate that ecological or behavioural factors may be responsible for the high levels of colour variation seen among *E. bassleri*, but not among *E. hahneli*, populations.

Keywords

Amazonia transition zone, divergence, *Epipedobates*, Peru, poison frogs, sky islands, systematics.

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INTRODUCTION

The Amazon basin contains the highest diversity of species on Earth, and the factors generating this diversity have been the subject of numerous hypotheses (e.g. Bush, 1994; Colvinaux, 1993; Endler, 1982; Haffer, 1969; Patton *et al.*, 1994). Frogs are spectacularly abundant within this region, and show particularly high diversity in the transition zone between the Andes and the lowlands (Duellman, 1982; Lynch & Duellman, 1997).

In Peru, the transition zone encompasses a large region from 50 to 250 km wide linking the east Andes versant and lowland Amazonia. This region includes a series of interrupted, smaller mountain ranges, or cordilleras, which were generated from secondary orogenies and subsequent erosion postdating the formation of the Andes (Sauer, 1971). In the central range of this zone, from Tarapoto, Peru north past Moyobomba, Peru lie the central cordilleras. Within these central cordilleras the elevation rarely exceeds 2000 m, and the mountains further west progressively increase in altitude and decrease in rainfall, probably as a rain shadow effect from the cordilleras adjacent to lowland Amazonia. Unlike lowland Amazonia, the lowland areas in the central cordilleras constitute tropical dry forest and have prolonged periods without rain. These habitats differ dramatically from premontane cloud forest and cloud forest, which are found throughout the upper regions of the central cordilleras and remain moist year round.

Several researchers have hypothesized that this transition zone generates diversification and speciation in many taxonomic groups, through divergence across ecological gradients (Endler, 1982) or through repeated bouts of separation and introgression (Colinvaux, 1993; Bush, 1994). Diversification in some groups has been found to be consistent with these hypotheses (e.g. Fjeldsá, 1994). With respect to frogs, Duellman (1982) proposed that the formation of refugia in the montane transition zone created repeated opportunities for isolation, divergence, and speciation. Lynch & Duellman (1997) also emphasized the importance of ecological variation among montane habitats as well as between montane and lowland habitats in generating population divergence and speciation. These hypotheses predict that the transition zone between the Andes and the lowlands will be a centre of diversification among populations, as well as a centre of species diversity (Graham *et al.*, 2004).

Although the diversity of frog species in the transition zone has been recognized for some time, there has been little research investigating differentiation among populations of single species from both phenotypic and genetic perspectives. Research with this focal point is important, however, in revealing the causes of diversity. Here we compare genetic divergence with colour variation among populations that provisionally constitute a valid species: the pleasing poison frog, *Epipedobates bassleri* (Myers, 1987), in the cordilleras that span the transition from the Andes to the lowlands in north central Peru.

Rapid diversification in conspicuous colouration among species and populations remains an intriguing and contentious issue in evolutionary biology (Summers *et al.*, 1997; Summers & Clough, 2001; Summers, 2003; Summers *et al.*, 2003).

Poison frogs of the family Dendrobatidae exhibit dramatic variation in colour hue, intensity, and pattern. Recent comparative analyses suggest that some of this variation is correlated with differences in toxicity and associated aposematism (Summers & Clough, 2001; Santos *et al.*, 2003).

Within the genus *Dendrobates*, dramatic variation in colouration occurs among populations of some species (Daly & Myers, 1967). *Dendrobates pumilio* (Schmidt, 1857) (the strawberry poison frog) in the Bocas del Toro Archipelago of Panama exhibits some of the most dramatic variation in colouration among populations known in vertebrates (Myers & Daly, 1983). Populations on different islands vary dramatically in hue, from red to yellow to green to blue to black and white, with substantial variation in pattern as well (Summers *et al.*, 2003). In contrast to the genus *Dendrobates*, few examples of extreme variation in colouration exist for species in the genus *Epipedobates* (the second largest dendrobatid genus).

However, one putative species, *E. bassleri* in northern Peru, provides an example of extreme variation in colouration among populations. Colour variation is evident among populations on high-altitude mountain ridges that are separated from each other by valleys. *Epipedobates bassleri* does not occupy the valleys, which contain unsuitable habitat (e.g. dry forest). In effect, these isolated populations inhabit 'sky peninsulas', as they are linked by regions of elevations consistent with hospitable habitat, with two exceptions (see Results). This distribution is reminiscent of some montane taxa in the American west (e.g. Knowles, 2001; Masta, 2000), although the populations in those studies were completely isolated on 'sky islands'.

Here we illustrate the colour morphs of *E. bassleri* using a topographic map to demonstrate the association of colouration and DNA sequence variation with specific montane regions. We present an analysis of mitochondrial DNA sequence variation that demonstrates the rapid nature of the divergence in colouration among these populations. We also carry out a phylogenetic analysis confirming the monophyly of these populations (to the exclusion of closely related congeners). These populations are characterized by several synapomorphies (e.g. call parameters) and, hence, provisionally constitute a valid species under the phylogenetic species concept (Cracraft, 1989). We test this hypothesis using parametric bootstrapping (Goldman *et al.*, 2000). We also compare genetic divergence between populations within *E. bassleri* with variation among populations of *E. hahneli* (Boulenger, 1883), a closely related species of Peruvian poison frog with both montane and lowland populations.

METHODS

Tissue collection

Ranges for the colour morphs of *E. bassleri* were established by the third author (R. Schulte) during multiple surveys over the course of the 30 years that he has lived in Tarapoto, Peru (Schulte, 1999). Samples for genetic analysis were collected in the field during the summers of 2003 and 2004 (Table 1; Figs 1 & 2).

Table 1 Localities and GenBank accession numbers for species included in the analysis. Long: longitude, Lat: latitude, Alt: altitude. A.V.R.: available upon request.

Species	Location	Accession no.	Long	Lat	Alt (m)
<i>E. pongoensis</i>	Huallaga Canyon	DQ339051	S06.54870'	W75.96169'	390
<i>E. pongoensis</i>	Convento	DQ339053	S06.25107'	W76.31459	200
<i>E. bassleri</i>	Saposa	DQ339049	A.V.R.	A.V.R.	781
<i>E. bassleri</i>	Altoshima	DQ339058	A.V.R.	A.V.R.	670
<i>E. bassleri</i>	Sisa 1a	DQ339059	A.V.R.	A.V.R.	1118
<i>E. bassleri</i>	Sisa 1b	DQ339060	A.V.R.	A.V.R.	1118
<i>E. bassleri</i>	Sisa 2	DQ339062	A.V.R.	A.V.R.	550
<i>E. bassleri</i>	Tara-Moyo	DQ339050	S06.632393'	W76.73437'	320
<i>E. bassleri</i>	Huallaga	DQ339052	A.V.R.	A.V.R.	390
<i>E. bassleri</i>	Chazuta	DQ339054	A.V.R.	A.V.R.	560
<i>E. bassleri</i>	Tarapoto	DQ339055	S06.47152'	W76.30297'	760
<i>E. bassleri</i>	Tarapoto	DQ339057	S06.43536'	W76.35011'	770
<i>E. bassleri</i>	Sauce	DQ339061	A.V.R.	A.V.R.	720
<i>E. hahneli</i>	Ivohote	DQ339064	S12.47086'	W73.09924'	658
<i>E. hahneli</i>	Chazuta	DQ339056	S06.52818'	W76.13942'	560
<i>E. hahneli</i>	Saposa	DQ339074	S06.77107'	W76.94120'	850
<i>E. hahneli</i>	Tarapoto	DQ339071	S06.47772'	W76.32274'	600
<i>E. hahneli</i>	Sisa	DQ339072	S06.58229'	W76.50974'	658
<i>E. hahneli</i>	Porto Walter	DQ339077	S08.25'	W72.74'	200
<i>E. hahneli</i>	Alto Purus	DQ339067	S10.90'	W73.17	300
<i>E. hahneli</i>	Rio Amigos	DQ339065	N.A.	N.A.	200
<i>E. hahneli</i>	Boca Manu	DQ339068	S12.25'	W70.9'	250
<i>E. hahneli</i>	Convento	DQ339069	S06.25107'	W76.31459'	207
<i>E. hahneli</i>	Itaya 1	DQ339076	S04.45'	W73.57'	100
<i>E. hahneli</i>	Itaya 2	DQ339075	S04.45'	W73.57'	100
<i>A. femoralis</i>	Saposa	DQ339066	S06.77107'	W76.94120'	850
<i>C. talamancae</i>	Panama	DQ339073	N.A.	N.A.	N.A.
<i>C. sp</i>	Bonilla	DQ339070	S06.21007'	W76.27226'	200

A broad phylogenetic analysis of *Epipedobates* in Peru (Roberts *et al.*, 2006) revealed that *E. pongoensis* (Schulte, 1999) is the closest relative of *E. bassleri*. Hence, we collected specimens of this species for comparison. *Epipedobates pongoensis* has a very restricted range, so we also collected specimens of the most closely related widespread species (*E. hahneli*) from the central cordillera region and other regions in Peru to use as a second outgroup in the phylogenetic analysis, and to compare with *E. bassleri* in the context of population divergence. Samples from each population consisted of a single toe from each individual. Toes were preserved in a buffer solution of 20% dimethylsulfoxide (DMSO) saturated with salt (sodium chloride). Several individuals were sampled from a population when possible. Voucher specimens representing each species were also collected and deposited in the University of San Marcos Museum of Natural History in Lima, Peru. Collecting and export permits were obtained from the Ministry of Agriculture (INRENA) in Lima, Peru (Authorization No. 061-2003-INRENA-IFFS-DCB, Permit No. 002765-AG-INRENA and CITES Permit No. 4326). Samples of *E. hahneli* from Porto Walter, Brazil were collected by J. P. Caldwell and were obtained by means of a tissue grant to the corresponding author from the Louisiana State University Museum of Natural Sciences Collection of Genetic Resources. Tissues obtained by J. P. Caldwell were collected during expeditions funded by the National Science Foundation (DEB-9200779 and DEB-9505518 to L. J. Vitt and J. P. Caldwell).

DNA extraction, DNA amplification, sequencing

Genomic DNA was extracted from tissue samples preserved in high-concentration salt buffer (DMSO/NaCl/EDTA) using the Qiagen DNeasy Tissue Kit. The cytochrome *b* mitochondrial gene region was amplified using DNA primers and protocols described in Summers *et al.* (1999), Clough & Summers (2000), and Symula *et al.* (2001) for a total of 891 base pairs. We used the following primer sets: CB1-L, CB2-H (Palumbi *et al.*, 1991); KSCYB1(A)-L, KSCYB(C)L, KSCYB1-H (Clough & Summers, 2000); KSCB1L1 (GCCAATGGCGCTT-CATTTTCT), KSCBARH1 (GGGGTAAAATTGTCTGGTCT), CytbAR-H (Goebel *et al.*, 1999). Polymerase chain reaction (PCR) amplifications were purified using the Qiagen QIAquick PCR Purification Kit. Products were sequenced using an Applied Biosystems (ABI) Prizm Sequencing Kit (Perkin-Elmer Corporation, Foster City, CA, USA).

Sequence analysis

Each sample was sequenced in both directions, and complementary sequences were aligned using AUTOASSEMBLER version 1.4.0 (Applied Biosystems, Inc., 1995). Consensus sequences were transferred to GENE JOCKEY (Taylor, 1990) for alignment with a sequence of the same region from a different individual. We translated all sequences to confirm



Figure 1 Map of Peru showing collection localities for *Epipedobates hahneli* (Boulenger, 1883) sequenced for this study: 1. Itaya, 2. Convento, 3. Tarapoto and Sisa, 4. Porto Walter, 5. Alto Purus, 6. Rio Amigos, 7. Boca Manu, 8. Ivochote. Areas above 1000 m are shaded. Top-left dotted box depicts the geographic area shown in Fig. 2.

that they were in the proper reading frame and did not contain stop codons. We aligned the DNA sequences using CLUSTALX (Thompson *et al.*, 1997). Completed sequences have been submitted to GenBank (Table 1).

Phylogenetic analysis

Phylogenetic analyses were carried out using Bayesian inference (Huelsenbeck & Ronquist, 2001). The data set was partitioned into codon position-specific sets of nucleotides (first, second, third positions), and MRMODELTEST version 2.0 (Nylander, 2004) was used to identify optimal priors for the following parameters: nucleotide frequencies, substitution model, gamma parameter, and proportion of invariant sites. Data may be explained better by partitioning a data set than by applying an average model across genes and codon positions, as indicated by higher model likelihood scores in partitioned analyses (Nylander *et al.*, 2004).

We applied the models indicated by MRMODELTEST, and used MRBAYES version 3.0b4 (Huelsenbeck & Ronquist, 2001) for both the *E. bassleri* data set and the *E. hahneli* data set. We ran four simultaneous Markov-chain Monte Carlo (MCMC) chains for one million generations, saving trees every 100 generations. We examined the plot of log likelihood scores and discarded all trees sampled before the chains stabilized (i.e. all trees from the burn-in phase). We created a 50% majority rule consensus tree from the remaining trees in PAUP* (Swofford, 2004), and then repeated the Bayesian analysis to ensure consistency of topology and posterior clade probabilities for the consensus tree (Fig. 3).

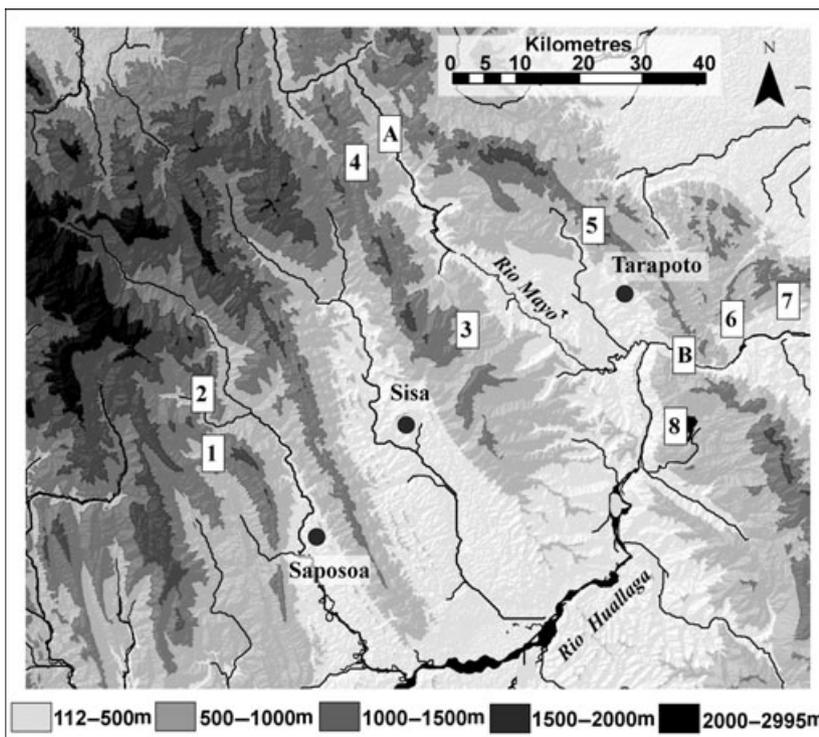


Figure 2 Map of the north central cordilleras showing collection localities for *Epipedobates bassleri* (Myers, 1987) sequenced for this study: 1. Saposoa, 2. Altoshima, 3. Sisa, 4. Road from Tarapoto to Mayobamba (Taramoyo), 5. Tarapoto, 6. Chazuta, 7. Huallaga, 8. Sauce. Letters A and B depict major riverine and low-elevation (< 10 km) barriers probably crossed if populations of *E. bassleri* were constrained and diverged through high-elevation habitats.

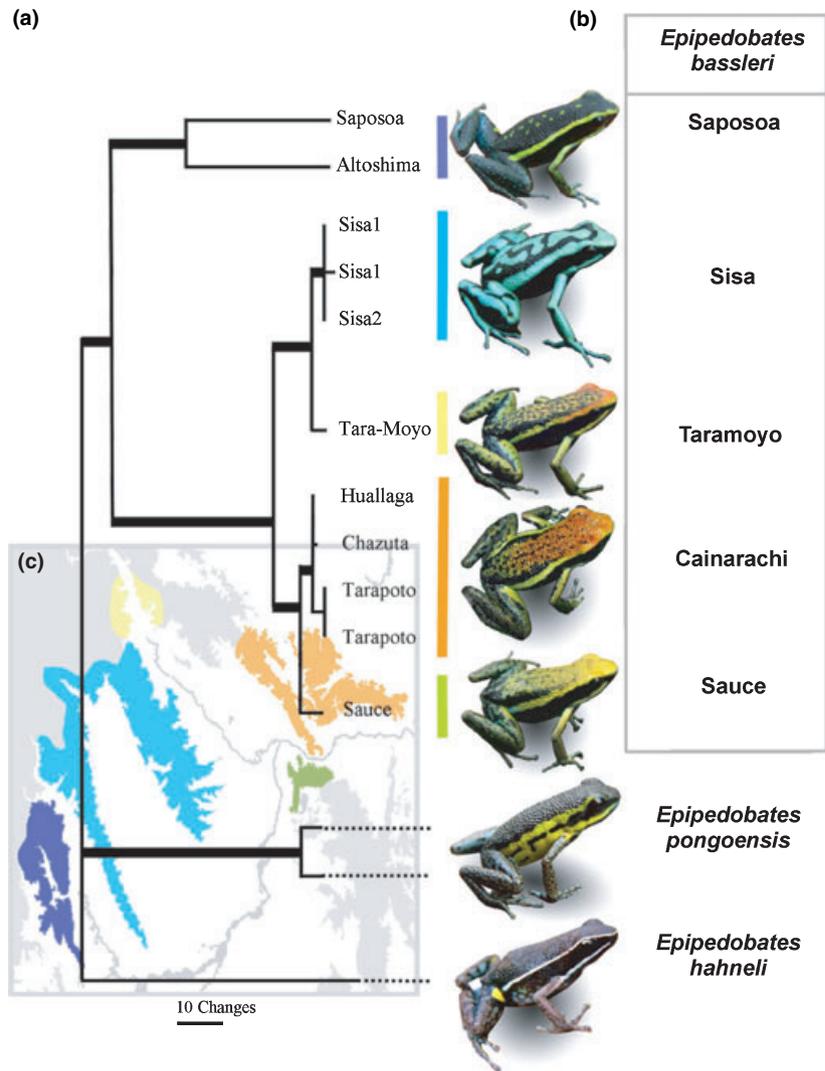


Figure 3 (a) Phylogram showing phylogenetic relationships among populations of *Epipedobates bassleri* (Myers, 1987). Thick branches indicate Bayesian posterior probabilities above 90. (b) Colour morphs of *E. bassleri* (Myers, 1987) and related species. (c) Ranges of colour morphs. Areas above 1000 m are shaded.

To test the monophyly of the populations of *E. bassleri*, parametric bootstrapping was carried out with routines implemented in the Batch Architect module of the program MESQUITE (Maddison & Maddison, 2004). Parametric bootstrapping provides a powerful method for testing hypotheses of monophyly (Goldman *et al.*, 2000). We used parsimony to construct the trees used in the test because of the long periods of time required for maximum likelihood analyses. The most parsimonious tree was determined by means of PAUP* (Swofford, 2004), under the constraint that the Saposoa populations of *E. bassleri* (the most basal lineage) are actually more closely related to populations of *E. pongoensis* (the sister taxon) than to other populations of *E. bassleri*. This tree was then used as a base with which to calculate a series of data matrices. First, we used PAUP* to estimate the following substitution model parameters on the most parsimonious constrained tree: nucleotide frequencies, proportion of invariant sites, gamma parameter, and transition probabilities for the general time-reversible model. These parameters were used to simulate 500 data (nucleotide sequence) matrices in the Batch Architect module in

MESQUITE. These matrices were then executed in PAUP*, and each one was used to infer two trees: one under the constraint (see above), and one without the constraint. PAUP* was used to calculate the difference in length between the constrained and unconstrained trees for each matrix. This file was then imported into MESQUITE and used to calculate the probability of obtaining a tree as different in length from the constrained tree as the original unconstrained tree.

Genetic distances were calculated under the Kimura two-parameter model using the program MEGA 2.1 (Kumar *et al.*, 2001). We designated samples from particular clades that corresponded to particular geographic areas (see Figs 2–4) as members of a population. We designated five populations for *E. bassleri* as follows: Tarapoto (Tarapoto, Chazuta, Huallaga); Saposoa (Saposoa and Altoshima); Sauce (Sauce); Tara-Moyo (Tara-Moyo); Sisa (Sisa 1 and Sisa 2). We designated four populations of *E. hahneli* as follows: Southern (Purus, Porto Walter, Rio Amigos); Northern (Itaya, Convento); Cordillera (Santa Rosa, Saposoa, Chazuta, Tarapoto); South Central (Ivochote).

Geographic distances between populations were calculated in ESRI's ARCTOOLS 9.0 (www.esri.com) using a digital elevation model created from Shuttle Radar Topography Mission–NASA–NGA (SRTM) data (GLCF, 2005). GPS points were plotted, and distances between points were calculated under two scenarios: Euclidian distance (distance between two points), and distance between points on the 1000-m contour line. Mantel tests were calculated in GENALEX 6 (Peakall & Smouse, 2005) using 10,000 random permutations.

RESULTS

We sampled 11 individuals from five distinct regions (Table 1 and Fig. 3). Although the number of individuals was low, we believe (based on a number of expeditions in the area) that we have sampled all of the distinct colour morphs of *E. bassleri*. The Bayesian phylogenetic analysis clearly supported the monophyly of *E. bassleri* (Fig. 3) and identified populations from the Saposoa range (green-spotted morph) as the basal lineage within the species. Individuals from Chazuta and Huallaga (orange–yellow morph) were closely related to the individuals from Tarapoto (orange–yellow morph), which is consistent with their shared colouration and their presence in the same range (Fig. 2). The sample from Sauce (yellow-striped morph) was most closely related to the Huallaga–Tarapoto clade, suggesting that this population may be derived from a recent colonization across the Huallaga Canyon, or that gene flow continues to occur between these populations. The Sisa samples (blue morph) were most closely related to the individual from the Tara-Moyo population.

For *E. hahneli*, we recovered distinct clades representing the southern part of Peru and neighbouring regions of Brazil (Rio Amigos, Rio Purus, and Porto Walter in Brazil), the central cordilleras (Saposoa, Santa Rosa, Chazuta, Tarapoto), the northern region (Itaya), and the south central region (Ivohote). The individual from Convento, which is in the lowlands at the base of the north central cordilleras, fell out in the northern clade with the Itaya River populations, in spite of the close proximity to populations from the central cordilleras (Fig. 4).

The genetic divergence observed in *E. bassleri* is correlated with the geographic distance across a high-elevation belt ($P < 0.009$, Mantel test, Fig. 5). When a 1000-m contour line is plotted within the cordilleras occupied by *E. bassleri*, and distances between capture points are measured along this line, this explains 94% of the genetic divergence (Fig. 5b). Euclidian distances, which imply that no physical barriers have limited the distribution of *E. bassleri*, only explain 52% of the genetic variation (Fig. 5a). Because the latter is a much lower value, this suggests that larger low-elevation areas are major dispersal barriers in this species. In contrast, large riverine barriers and lowland barriers less than 10-km wide (Fig. 2, points A and B) appear to have had little impact on the distribution of *E. bassleri*. Hence, populations of *E. bassleri* are partially isolated on sky peninsulas, rather than being totally isolated on sky islands.

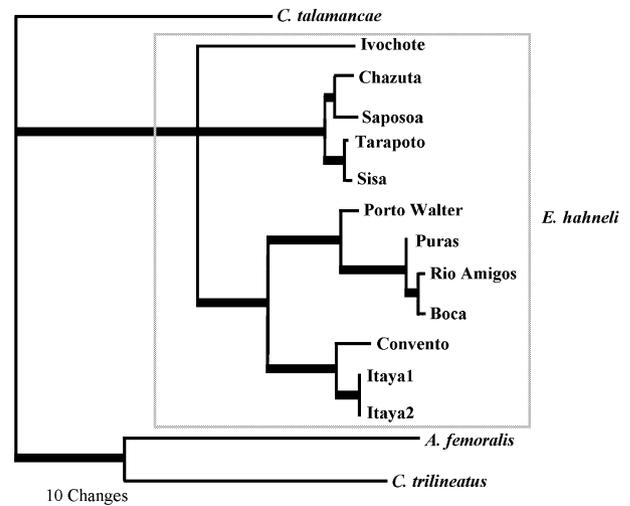


Figure 4 Phylogram showing phylogenetic relationships among populations of *Epipedobates hahneli* (Boulenger, 1883). Thick branches indicate Bayesian posterior probabilities above 90.

The analysis by parametric bootstrapping clearly demonstrated that the monophyly of populations of *E. bassleri* with respect to the nearest sister taxon (*E. pongoensis*) is strongly supported. The probability of obtaining a tree that grouped the basal lineage of *E. bassleri* (the Saposoa and Altoshima populations) with other *E. bassleri* populations under the hypothesis that that basal lineage actually grouped with the sister taxon (*E. pongoensis*) was $< 0.01\%$. To put it another way, the number of steps separating the best tree under an unrestricted analysis of the sequence data from the best tree under the hypothesis that *E. bassleri* is not monophyletic is too high to be ascribed to random error.

Genetic distances among populations of *E. bassleri* varied between 0.5% and 6.3% (Table 2). Genetic distances among populations of *E. bassleri* in the cordilleras were higher than those for equivalent comparisons for *E. hahneli* within the central cordilleras (Table 3). However, genetic distances between distant populations of *E. hahneli* were higher than the genetic distances between any pair of *E. bassleri* populations (Table 4).

DISCUSSION

Populations of *E. bassleri* reveal dramatic divergence in colouration, equivalent to that seen among island populations of *D. pumilio* in the Bocas del Toro Archipelago (Summers *et al.*, 2003). This variation is associated with populations that are restricted to particular montane ridges in the north central cordilleras of Peru, which constitute the transition zone from the Andes to the lowlands in that region. Our phylogenetic analysis reveals that, despite the dramatic divergence among populations, they form a monophyletic clade. Parametric bootstrapping demonstrates that these populations form a cohesive group to the exclusion of the most closely related species. Despite colouration differences, these populations

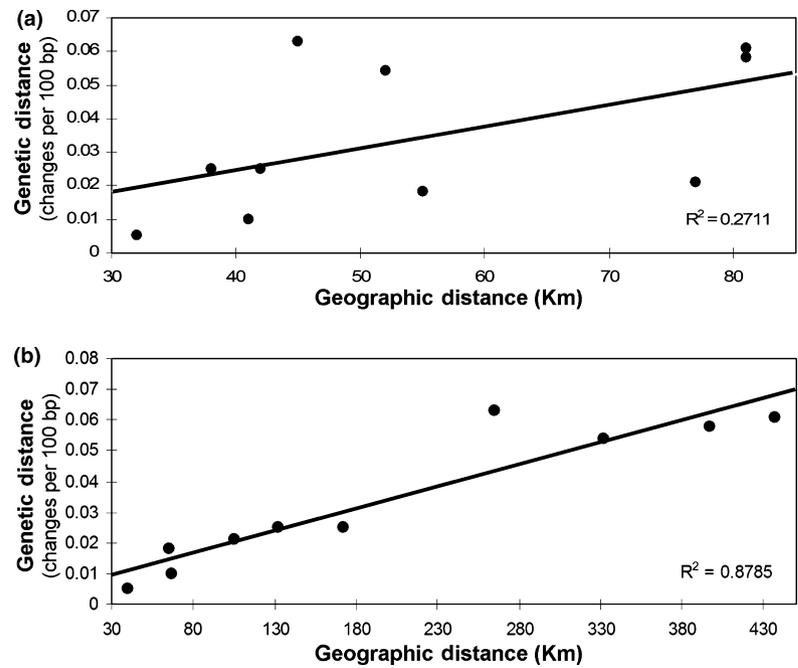


Figure 5 Mantel tests comparing genetic distances of populations of *Epipedobates bassleri* with geographic distances between sample collection points. (a) Euclidian (direct) geographic distances vs. genetic distances. (b) Geographic distances between capture points on a 1000-m contour line vs. genetic distances.

Table 2 Genetic distances among colour morphs of *Epipedobates bassleri*, calculated with the Kimura two-parameter model. Genetic distances among localities within colour-morph ranges were very low (0.1% on average).

Population	Saposoia	Tara-Moyo	Huallaga	Sisa
Tara-Moyo	0.054			
Huallaga	0.058	0.018		
Sisa	0.063	0.010	0.025	
Sauce	0.061	0.021	0.005	0.025

Table 3 Genetic distances among populations of *Epipedobates hahneli* in the north central cordilleras, calculated as in Table 2

Population	Chazuta	Tarapoto	Sisa
Tarapoto	0.013		
Sisa	0.015	0.003	
Saposoia	0.015	0.021	0.024

Table 4 Genetic distances among populations of *Epipedobates hahneli* in different regions of Peru, calculated as in Table 2

Region	North cordilleras	South cordilleras	South lowlands
South cordilleras	0.093		
South lowlands	0.141	0.127	
North lowlands	0.118	0.135	0.088

share several synapomorphies that indicate that they represent a single species [under some interpretations of the phylogenetic species concept (Agapow *et al.*, 2004)]. These synapomorphies include calling parameters and tadpole colouration.

The main (advertisement) call type in the *E. bassleri* populations consists of a slow single-note whistle chain (Schulte, 1999), whereas *E. pongoensis* has a double-note whistle call given in fast groups, and the main call of *E. hahneli* is a triplet-note whistle call. The tadpole of *E. bassleri* is beige with cream-white lateral mouth spots, in contrast to the grey colour of *E. pongoensis* tadpoles. In contrast to the relatively uniform colouration in populations of the lowland species *E. hahneli*, *E. bassleri* has experienced rapid divergence in population colouration. These results, in combination with evidence for high genetic divergence among lowland populations of *E. hahneli* (see below), support previous arguments that those montane regions spanning the transition between the high mountains and lowlands are conducive to divergence and speciation (Bush, 1994; Colinvaux, 1993; Duellman, 1982; Endler, 1982; Fjeldsá, 1994; Lynch & Duellman, 1997; Roy, 1997).

The close relationship between the widespread *E. hahneli* (with high genetic divergence among basal lineages) and *E. bassleri* suggests that *E. bassleri* is probably derived from an early expansion of populations of ancestral *E. hahneli* into the north central cordilleras. These ancestral populations probably occurred throughout the cordilleras during a period when climatic conditions were wet, but then became partially isolated in montane habitats when drier conditions caused the formation of dry forests in the lowland valleys between mountain ridges. This scenario would be consistent with previous hypotheses concerning the impact of climatic change on montane populations of frogs in Amazonia (e.g. Duellman, 1982).

Populations of *E. hahneli* that currently inhabit the lower slopes of the cordilleras are presumably derived from a more recent expansion of *E. hahneli* populations. This is consistent with our finding that genetic divergences among populations

of *E. bassleri* in the central cordilleras are generally higher than those among populations of *E. hahneli* from the same regions. The one exception involves a population of *E. hahneli* in Convento, which displays marked divergence from montane populations nearby. However, phylogeographic analysis of *E. hahneli* reveals that the Convento population is derived from a northern clade, whereas the adjacent montane populations are derived from a southern clade (Fig. 4). This scenario suggests that *E. hahneli* may be paraphyletic, an interpretation supported by a larger-scale phylogenetic analysis (Roberts *et al.*, 2006).

Our analyses indicate that the levels of genetic divergence among *E. bassleri* populations are closely associated with geographic distance along corridors of montane habitat (Fig. 5b). Hence, genetic divergence at the cytochrome *b* locus among these populations is consistent with an isolation-by-distance model. Comparison with the levels of genetic divergence among populations in a monomorphic species (*E. hahneli*) indicates that the levels of genetic divergence observed among *E. bassleri* populations are not necessarily associated with high levels of morphological divergence. Genetic divergence among geographically distant populations within a large monophyletic clade of *E. hahneli* (in the lowlands of northern and southern Peru), which are largely homogeneous in colour and pattern, exceeds that among populations of *E. bassleri*. This comparison suggests that some form of selection is driving the rapid divergence in colouration seen among *E. bassleri* populations.

The most likely explanation is that sexual selection is driving divergence in colouration among populations. *Epipedobates bassleri* is larger, more colourful, and more toxic than *E. hahneli* (Schulte, 1999). Males call out in the open during the day, and apparently have few predators. Although males perform parental care in this and other species of *Epipedobates*, behavioural data on related species indicate that there is strong sexual selection acting on males, and that females are highly selective about mating (e.g. Roithmair, 1994). Whether females in any species of *Epipedobates* respond to male colouration as part of mate choice is unknown, but previous research has demonstrated female choice for colour in the strawberry poison frog, *Dendrobates pumilio* (Summers *et al.*, 1999). Thus, it is possible that female choice for colour in *E. bassleri* could influence population variation in colouration. The dramatic variation in colouration among *E. bassleri* populations is not accompanied by high variation (relative to *E. hahneli*) among populations in other aspects of morphology, such as snout-vent length (Supplementary Tables S1 and S2). This result is consistent with the hypothesis that divergent sexual selection for colouration is acting on these populations. This hypothesis predicts that female *E. bassleri* will prefer their local morph in mate-choice tests (controlling for differences in other features of the phenotype, such as acoustic parameters of the mating call).

Alternatively, ecological factors could account for the observed divergence in colouration among populations of *E. bassleri*. *Epipedobates bassleri* is a montane specialist, living

at higher altitudes than *E. hahneli* and other related species, such as *E. trivittatus*. Habitat differences among populations of *E. bassleri* include distinct patterns of rainfall and humidity, which, in turn, are associated with differences in floristic composition. However, it is not clear why such differences should affect colouration. One possibility is that different suites of predators, with different visual sensitivities, occupy the different habitats. Divergence in colouration could then be driven by differences in the efficiency of specific colours and patterns as aposematic signals in the different habitats. This hypothesis predicts that other co-distributed aposematic taxa (e.g. insects or millipedes) will show similar patterns of variation.

Finally, it is possible that differences in ambient irradiance associated with habitat differences could interact with female choice to influence colouration (Gamble *et al.*, 2003). This hypothesis could be tested by measuring ambient irradiance in the different environments (e.g. Summers *et al.*, 2003) and comparing the spectral sensitivities of the frogs (e.g. Siddiqi *et al.* 2004) under the light regimes in the different habitats.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1 Variation in snout-vent length within populations of *Epipedobates bassleri* and *Epipedobates hahneli*.

Table S2 Variation among populations in snout-vent length, calculated as the mean of population one minus the mean of population two, divided by the mean of population one.

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