

mtDNA perspective of chromosomal diversification and hybridization in Peters' tent-making bat (*Uroderma bilobatum*: Phyllostomidae)

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Abstract

We compared sequence variation in the complete mitochondrial cytochrome-*b* gene with chromosomal and geographical variation for specimens of Peters' tent-making bat (*Uroderma bilobatum*). Three different chromosomal races have been described in this species: a $2n = 42$ race from South America east of the Andes, a $2n = 44$ from NW Central America and $2n = 38$ from the rest of Central America and NW South America. The deepest nodes in the tree were found within the South American race (42 race), which is consistent with a longer history of this race. Average distance among races ranged from 2.5 to 2.9%, with the highest amount of intraracial variation found within the $2n = 42$ race (1.7%), intermediate values within the $2n = 38$ race (0.9%) and lowest within the $2n = 44$ race (0.5%). Variation among chromosomal races accounted for over 55% of molecular variance, whereas variation among populations within races accounted for 6%. The $2n = 38$ and $2n = 44$ races hybridize in the coastal lowlands of Honduras, near the Gulf of Fonseca. Introgression between these two races is low (two introgressed individuals in 45 examined). Clinal variation across the hybrid zone for the cytochrome-*b* of *U. bilobatum*, is similar to clinal variation reported for chromosomes and isozymes of this species. Mismatch distribution analyses suggests that geographical isolation and karyological changes have interplayed in a synergistic fashion. Fixation of the alternative chromosomal rearrangements in geographical isolation and secondary contact is the most likely mechanism accounting for the hybrid zone between the $2n = 38$ and $2n = 44$ races. If a molecular clock is assumed, with rates ranging from 2.3 to 5.0% per million years, then isolation between these races occurred within the last million years, implying a relatively recent origin of the extant diversity in *Uroderma bilobatum*. None the less, the three chromosomal races probably represent three different biological species.

Keywords: chromosomal rearrangements, cytochrome-*b*, population divergence, speciation

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Introduction

The role of chromosomal rearrangements in the speciation process is controversial. Based on the observation that most animal species differed in their karyotype, White (1978) held that chromosomal changes were central in speciation events. The potential of chromosomal rearrangements for establishing barriers to gene flow in a relatively short time

has been well illustrated by theoretical models (White 1978; Templeton 1981; Baker & Bickham 1986; Noor *et al.* 2001) and empirical studies (Britton-Davidian *et al.* 2000). Recent reviews (Britton-Davidian 2001; Rieseberg 2001) call for examining how chromosomal rearrangements cause reductions of gene flow. The alternative, and more commonly held, view is that chromosomal changes are a by-product of diversification (Coyne & Orr 1998). While it is clear that speciation need not involve chromosomal changes, the fact that chromosomal rearrangements can cause divergence remains open. An understanding of how chromosomal variation is partitioned with respect to

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population structure and phylogeography provides an opportunity to examine the potential influence of chromosomal change on the speciation process. Chromosomal rearrangements in combination with geographical isolation can reinforce each other, as has been shown in the rapid establishment of chromosomal races in *Mus musculus* in Madeira (Britton-Davidian *et al.* 2000), accelerating the diversification processes.

Important insights into the process of intraspecific differentiation may be obtained by comparing chromosomal variation with matrilineal genealogies inferred from mitochondrial markers. The mitochondrial cytochrome-*b* gene has been extensively used to elucidate phylogenetic relationships among species within the same genus (Johns & Avise 1998; Avise & Johns 1999; Bradley & Baker 2001); in particular this gene has proved to be an important tool for the investigation of patterns of intraspecific phylogeographical variation (Avise 2000; Avise *et al.* 1998; Avise & Walker 1999; Ditchfield 2000; Hoffmann & Baker 2001; Costa 2002; Matocq 2002). A combination of perspectives, involving both phylogenetic and population genetics, may help to discriminate between the independent and combined effects of gene flow and historical factors e.g. migrations and population expansions (Templeton *et al.* 1992;

Templeton & Sing 1993). Study of the distribution of pairwise differences among haplotypes, within an intraspecific group, can promote a better understanding of demographic history (Slatkin & Hudson 1991; Rogers & Harpending 1992; Rogers *et al.* 1996) and distinguish between the predictions of alternative evolutionary histories (Smith *et al.* 2001). Incorporating these tools into studies of chromosomal variation may illuminate the population processes that lead to the fixation of alternative rearrangements.

Three chromosomal races (cytotypes) have been described (Baker *et al.* 1972, 1975; Baker 1979) for Peters' tent-making bat, *Uroderma bilobatum* (Chiroptera: Phyllostomidae). This species is distributed widely in the New World tropics (Fig. 1) and two of the races hybridize in Central America, making this system a suitable choice to study the role of chromosomal change in diversification and geographical variation. Specimens with $2n = 42$ (42 race) occur in South America east of the Andes, specimens with $2n = 44$ (44 race) are found along the Pacific versant of El Salvador, Guatemala, Honduras, and Mexico and specimens with $2n = 38$ (38 race) can be found in the remainder of Central America and along the Pacific versant of Colombia and northern Ecuador. There are no shared rearrangements

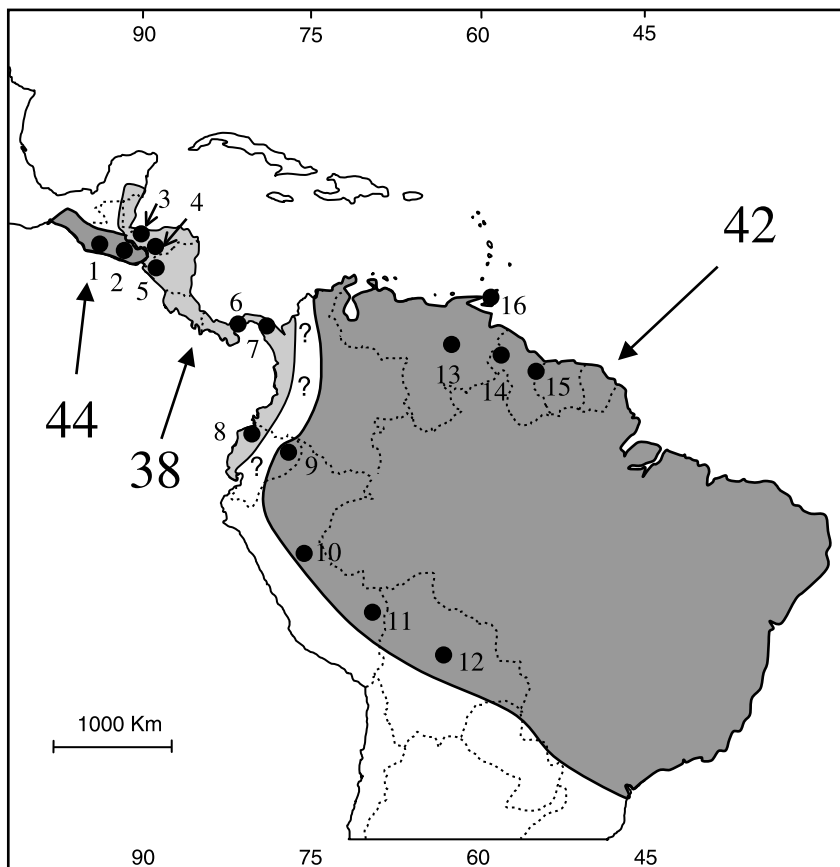


Fig. 1 Geographic distribution of Peters' tent-making bat, *Uroderma bilobatum*, modified from Davis (1968). Distribution of each chromosomal race drawn based on Baker *et al.* (1972). Specimens with $2n = 42$ (42 race) and a fundamental number of 50 occur in South America east of the Andes and on the island of Trinidad. Individuals with $2n = 44$ (44 race) and a fundamental number of 48 are distributed along the Pacific versant of El Salvador, Guatemala, Honduras and Mexico. Individuals with $2n = 38$ (38 race) and a fundamental number of 44 occur along the Atlantic versant of Guatemala, Honduras and the Yucatan Peninsula of Mexico; this form also occurs along both versants of Central America from Nicaragua southward through Costa Rica and Panama, and along the Pacific versant of Colombia and northern Ecuador in South America. Sample localities (1–16) are described in the Appendix. Question marks indicate areas where a potential contact between races 42 and 38 might exist.

among any two of the chromosomal races (Baker *et al.* 1972, 1975; Baker 1979). The 38 and 44 chromosomal races come into contact and hybridize in Honduras. These two parental cytotypes have been documented in sympatry at only one locality (Honduras, Departamento Valle, 17 km SSW of Nacaome). Individuals with karyotypes showing evidence of backcrosses have been found in localities that are 400 km apart (localities 2 and 9 of Fig. 2 in Baker 1981) but estimates of the 20/80 width, defined as the distance between the point where the frequency of a given marker is 20% to the point where its frequency reaches 80% (following May *et al.* 1975; Endler 1977), range between 30 and 40 km (Owen & Baker 2001).

Several authors have proposed different evolutionary scenarios to account for the patterns of variation observed across this hybrid zone. The main points of controversy were the primary or secondary origin of the zone, the role of selective forces in its maintenance and the amount of gene flow across this zone. Baker (1981) analysed karyological variation across 13 populations from Mexico to Costa Rica. He considered the zone to be the product of secondary contact and proposed negative heterosis against F_1 hybrids as the force maintaining it. Geographical patterns in the data suggested that the 38 race might be replacing the 44 race to the northwest. Based on the same data collected by Baker (1981), Barton (1982) concluded that the population was near Hardy–Weinberg equilibrium and that there was a deficit of backcross individuals in the zone of hybridization. He considered the zone to be maintained through negative heterosis against F_1 hybrids and backcrosses. Greenbaum (1981) documented a low level of electrophoretic variation (Rogers' similarity value ≥ 0.967), and suggested the occurrence of little or no introgression between the two parental cytotypes. Greenbaum (1981) interpreted the zone to have developed through primary contact, where reproductive isolation between the two races was favoured by selection. Hafner (1982) challenged Greenbaum's (1981) interpretation, based on the low frequency of marker alleles, and concluded that the data were consistent with a model of random diffusion, involving secondary neutral contact. Lessa (1990) reanalysed Greenbaum's (1981) data using multidimensional scaling and found that the clines coincided for isozymes and the three karyotypic rearrangements. Lessa (1990) concluded that both the karyological and electrophoretic data fitted the expectations of a narrow zone where selection mechanisms play a role in isolating the 38 and 44 races. Owen & Baker (2001) studied the zone with new data collected between 1990 and 1991, about 15 years after the collection of the original data used by Baker (1981), and Greenbaum (1981). Owen & Baker (2001) established that the clines derived from the additional chromosomal data were coincident with the previously calculated clines for same pattern of variation, regardless of the data analysed, and concluded

that the data indicated a stable hybrid zone in both space and time during this 15-year interval.

In this study we use data from the mitochondrial cytochrome *b* gene and compare it with evidence available from the nuclear genome (chromosomes and isozymes), in order to explore the expectations from the previous studies, therefore allowing us to evaluate the impact of chromosomal changes on diversification, whereas the assumption of a molecular clock would allow the estimation of the time of divergence among these chromosomal races. In particular, we focus upon (i) exploring how chromosomal races are grouped into phylogenetic analyses based on the mitochondrial cytochrome *b* gene; (ii) comparing how variation at the cytochrome *b* level partitions in relation to chromosomal races; (iii) using mismatch distributions to evaluate whether primary or secondary contact is the most plausible explanation for the origin of the hybrid zone in Honduras and (iv) using phylogenetic results as a starting point for a reconstruction of the evolution of the three races. If this hybrid zone mentioned above was the product of allopatric divergence, we would expect the two Central American races to form reciprocally monophyletic groups. In addition, we would expect a bimodal mismatch distribution when we combine samples from these two races. If there was either male- or female-biased dispersal, we would expect nuclear and mitochondrial patterns of variation to be different.

Materials and methods

We sequenced the entire cytochrome-*b* gene for 42 specimens of *Uroderma bilobatum* collected from populations adjacent to and within the hybrid zone. Additional specimens from Panama and South America, both east and west of the Andes, brought the total number of specimens sequenced to 57 (GenBank Accession nos AY169900–AY169957). This material covered as much of the geographical range of the species as was possible with the available data. In addition to the 57 specimens of *U. bilobatum*, two samples of *Uroderma magnirostrum* were included as part of the ingroup. A specimen of *Chiroderma doriae* was used as the outgroup (GenBank Accession no. AY169958), based on results from Baker *et al.* (2000). Karyotypic information was obtained from Owen & Baker (2001). Chromosomal affinities of samples where cytotypic information was not available were determined by extrapolations from cytotypic data belonging to the corresponding population (Owen & Baker 2001; Owen, pers. com.). All specimens from La Paz, El Salvador (locality 3T in Owen & Baker 2001) were reported to have a diploid number of 43 or 44. In Choluteca, Honduras, a potential F_1 was discovered ($2n = 41$); the remaining individuals had diploid numbers of either 38 or 39 (locality 7T in Owen & Baker 2001). All karyotyped

individuals from Atlantida, Honduras had a diploid number of 38 (data from Owen pers. com.).

DNA was extracted from liver, kidney or muscle tissue that were preserved frozen in lysis buffer or in ethanol. Extraction methods were either a PCI/phenol protocol (Longmire *et al.* 1997) or a SDS/proteinase K/NaCl extraction, and alcohol precipitation protocol (Miller *et al.* 1988; Maniatis *et al.* 1992). Complete mitochondrial cytochrome-*b* DNA fragments were amplified via PCR (Saiki *et al.* 1988), using primers and conditions reported in Hoffmann & Baker (2001). Polymerase chain reaction (PCR) products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, CA, USA), following the manufacturer's instructions. Double-stranded sequencing was accomplished with either ABI Prism dRothamine Terminator Ready Mix (PE Applied Biosystems) or ABI Big Dye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems). We used primers reported in Hoffmann & Baker (2001); and additional sequencing primers: gi1L and Uro3L (Hoffmann & Baker in press) and Phyllo3L (5'-GGA GCA TCT ATA TTC TTT ATC TGC C-3'), Uro1L (5'-AGA CAA AGC TAC CCT CAC TCC-3'). Sequencing products were ethanol precipitated and analysed with an ABI Prism 310 PE Applied Biosystems automatic sequencer. Sequences were edited and aligned using SEQUENCHER (version 3.01, Gene Code Corporation, Ann Arbor, MI, USA).

Pairwise distance comparisons were calculated as the uncorrected percentage of sequence divergence (p-distance). Phylogenetic relationships were estimated using neighbour-joining (Saitou & Nei 1987), maximum parsimony, maximum likelihood and Bayesian estimation. Bayesian phylogenetic searches were implemented using MrBayes version 2.01 (Huelsenbeck & Ronquist 2001). Four chains were run for 2×10^5 generations, trees were sampled every 100 generations, with convergence after 2.5×10^3 generations and only the last 500 trees used to build a strict consensus. Both parsimony and maximum-likelihood heuristic searches were performed using the tree-bisection-reconnection (TBR) branch-swapping algorithm, and 15 replicates of random addition of taxa. For comparative purposes we also checked the routine suggested by Takahashi & Nei (2000) of stepwise addition of taxa and nearest-neighbour-interchange branch swapping (NNI). MODELTEST (Posada & Crandall 1998) was used to select the best-fitting model of nucleotide substitution (Tamura-Nei, with a proportion of invariant sites, $\text{pinv} = 0.55$ and among-sites rate variation following a Γ distribution, $\alpha = 0.75$). Support for the nodes was evaluated using 1000 bootstrap (bs; Felsenstein 1985) replicates, in the full mode in case of parsimony, and Bayesian posterior probability values from MrBayes. In order to compare the likelihood scores of competing phylogenetic hypotheses, we carried out Kishino-Hasegawa (KH, Kishino & Hasegawa 1989)

and Shimodaira-Hasegawa (SH, Shimodaira & Hasegawa 1999) topology tests, as implemented by PAUP* 4.0b10 (Swofford 2002).

Tree-building methods are often inadequate to reconstruct intraspecific relationships. This is because within a species, an ancestral haplotype may generate multiple different derived haplotypes through mutations and both ancestral and derived forms are likely to coexist, creating ambiguity (Posada & Crandall 2001). Statistical parsimony was developed to address the aforementioned problems (Templeton *et al.* 1992) by grouping haplotypes into networks. Such networks are based on 95% statistical confidence estimates that differences among haplotypes are the result of a single mutation event. Statistical parsimony, as implemented by the program tcs (Clement *et al.* 2000), was used to explore how the different haplotypes in *U. bilobatum* can be arranged into 95% confidence networks and to assess the number of haplotypes that are placed into internal nodes in the networks.

Samples were arranged in two levels in order to carry out hierarchical analysis of molecular variation (AMOVA). Individuals grouped into populations, and populations nested within the corresponding chromosomal race. Estimates of Φ_{ST} (Hudson *et al.* 1992) values were obtained from the haplotype data. Genetic structure was explored using analyses of molecular variation (Excoffier *et al.* 1992, as implemented by ARLEQUIN version 2000, Schneider *et al.* (2001). This procedure focused on how genetic variation is partitioned within and among populations and chromosomal races.

DNASP version 3.53 (Rozas & Rozas 1999) was used to obtain estimates of haplotype and nucleotide diversity within each group to perform neutrality tests (McDonald & Kreitman 1991) and to estimate the mismatch distribution expected under constant population size and exponential growth. For the purpose of neutrality tests, each chromosomal race was considered as a species, and substitutions within and among the different races were compared in the test. Mismatch distributions are expected to be unimodal in populations expanding from a single source or recovering from a bottleneck and multimodal in populations with a stable effective size (Slatkin & Hudson 1991; see Smith *et al.* 2001 for an application of this approach). Two statistical tests were performed to detect deviation from neutrality and the probable factors behind them, following a procedure suggested by Fu (1997). Fu and Li's D^* (Fu & Li 1993) is more powerful to detect deviations from neutrality due to background selection, whereas Fu's F_S (Fu 1997) is more sensitive to changes in population size and genetic hitchhiking. In the two cases we obtained 99% confidence intervals by comparing the observed values with 1000 simulations in DNASP, using the observed sample size, number of segregating sites and observed parameter estimate as the input.

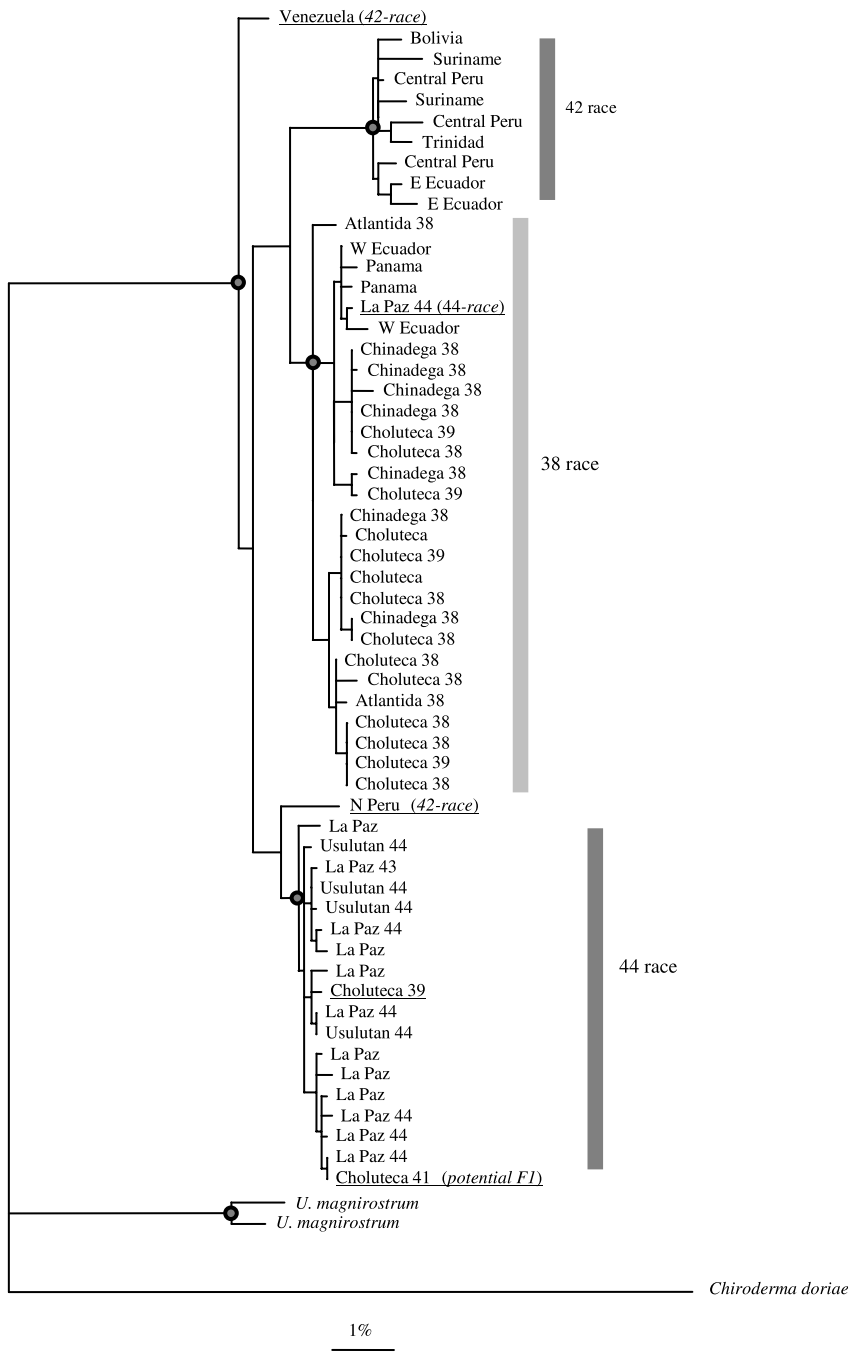


Fig. 2 Majority rule consensus phylogenetic tree based on the last 500 trees from Bayesian analyses. Dots correspond to nodes with parsimony bootstrap support $\geq 65\%$ and Bayesian posterior probabilities $> 80\%$. Specimen localities are given, with the corresponding chromosomal number if available; haplotypes with unstable position in the phylogeny or that are grouped with chromosomal races that do not match their own diploid number are underlined.

Results

Of 1140 nucleotides sequenced from the entire mitochondrial cytochrome-*b* gene for the 57 specimens examined, 116 were polymorphic. There were 16, nine and 91 polymorphic sites at the 1st, 2nd and 3rd codon positions, respectively. This resulted in 18 polymorphic amino acid positions. Haplotype diversity, calculated as gene diversity, was high (0.989 ± 0.007 SD, Nei 1987) with 46 unique haplotypes in the 57 specimens examined. Intraspecific variation ranged from 0 to 3.7%, with an average uncorrected pairwise distance (p-value) of 1.9%.

Differences in the cytochrome-*b* gene between the two chromosomal races (38 and 44) that hybridize involve fixed nucleotide changes in 15 positions, all of them synonymous, with an average distance between the two races of 2.5%. The McDonald & Kreitman (1991) tests did not detect significant departures from neutral predictions in the evolution when each of the races was compared against the other two.

In all cases, phylogenetic analyses arranged 55 of the 57 specimens of *U. bilobatum* into three separate, almost equidistant monophyletic groups that correspond well to the three chromosomal races (Fig. 2). The two remaining

	<i>n</i>	No. of haplotypes	θ_{π}	θ_s	Fu and Li's <i>D</i> *	Fu's <i>F</i> _s
44-Lineage	17	15	0.070	0.012	-2.34 NS	-8.01 <i>P</i> < 0.001
La Paz, El Salvador (population 1 in Fig. 1)	13	12	0.005	0.007	-2.06 NS	-5.98 <i>P</i> < 0.001
Usulután, El Salvador (population 2 in Fig. 1)	4	4	0.002	0.002	-0.21 NS	-1.41 NS
38-lineage	29	20	0.010	0.014	-0.38 NS	-3.37 NS
Atlántida, Honduras (population 3 in Fig. 1)	2	2	0.007	0.007	NA	2.08 NS
Choluteca, Honduras (population 4 in Fig. 1)	16	11	0.011	0.013	0.01 NS	0.06 NS
Chinandega, Nicaragua (population 5 in Fig. 1)	7	6	0.007	0.008	-0.24 NS	-0.16 NS
Panama & W Ecuador (populations 6 & 7 in Fig. 1)	4	4	0.004	0.004	-0.49 NS	0.62 NS
42-Lineage (populations 8–14 in Fig. 1)	11	11	0.015	0.020	-1.25 NS	-2.71 NS

Table 1 Genetic diversity estimates within the different phylogroups and populations of *Uroderma bilobatum*

samples, both from South America east of the Andes (one from Venezuela and one from northern Peru) changed their position on the phylogeny depending on the method and model of phylogenetic reconstruction implemented. All the different analyses agree on grouping 16 of 17 individuals from 44-like populations in one clade, nine of the 11 samples from South America east of the Andes (42 race) into a second clade, and 26 of 28 individuals from 38-like populations into a third clade. One sample of the 44 cytotypic from La Paz, El Salvador had a strong cytochrome-*b* sequence affinity with 38 cytotypic samples from Panama and western Ecuador. Two samples from Choluteca, Honduras (diploid numbers 39 and 41) have cytochrome-*b* haplotypes that are related more closely to samples from the 44 chromosomal race.

In parsimony and likelihood, random addition of taxa and TBR yielded slightly better trees than simple addition and NNI (two steps in parsimony, 2 units in likelihood). There is limited support for the nodes resolving the relationships among the three cytotypes at the base of *U. bilobatum*, with particular topologies depending on the method and parameter values selected. In the best ML tree ($-\ln L = 3509.91$, tree not shown), the two specimens of *U. magnirostrum* were grouped inside the 44 chromosomal race. In the majority rule consensus from Bayesian analyses *U. bilobatum* was found to be monophyletic (Fig. 2). In this tree, the sample from Venezuela is the basal branch of *U. bilobatum*; the sample from northern Peru is the sister lineage to the 44 lineage, and the remaining samples from South America, east of the Andes, were sister to the 38 race. Under the ML criterion, a tree that enforced *U. bilobatum* monophyly

was not significantly different from the best ML tree ($-\ln L = 3510.85$, $P < 0.93$ in a SH test), nor with the best tree from Bayesian analysis (Fig. 2, $-\ln L = 3521.69$, $P < 0.47$ in a SH test). In parsimony and Bayesian analyses the monophyly of *U. bilobatum* had high bootstrap and posterior probability values.

Statistical parsimony (as implemented by *tcs*, Clement *et al.* 2000), grouped haplotypes into four unconnected networks (not shown). The sample from Venezuela was the sole sample within the first network, the sample from northern Peru was placed with the 44-like haplotypes in the second network (44 network), all 38-like haplotypes were placed in the third network (38 network) and the remaining samples from eastern South America in the fourth (42 network). Connections among the four networks exceeded the 95% confidence level. In the 38 and 44 networks geographical localities are scattered, with no apparent geographical structuring; there is weak geographical structuring in the 42 network (results are available upon request).

We compared intrarace polymorphism with inter-race changes for each chromosomal race setting the other two as outgroups in McDonald and Kreitman's tests (McDonald & Kreitman 1991). These tests did not find statistically significant deviations from neutrality for any of the comparisons. Each chromosomal race was considered as a phylogroup in the hierarchical analyses of genetic variation and to obtain population parameter estimates and simulations. Within each chromosomal race, samples were pooled together into phylogroups based on their sampling localities and results from the phylogenetic analyses. The first phylogroup consisted of all samples from South

Table 2 Uncorrected distances among chromosomal races of *Uroderma bilobatum* below the diagonal, distances within races in the diagonal and net divergences among races above the diagonal. Average distance (p-distance) ± 1 SE

	44	38	42
44	0.5 ± 0.1%	1.9 ± 0.4%	1.9 ± 0.3%
38	2.5 ± 0.4%	0.8 ± 0.2%	1.3 ± 0.3%
42	2.9 ± 0.4%	2.5 ± 0.3%	1.5 ± 0.2%
<i>U. magnirostrum</i>	8.0 ± 0.3%	8.2 ± 0.4%	8.3 ± 0.8%

America east of the Andes (populations 9–15 in Fig. 1). The second phylogroup included samples of the 38 race from two populations in Honduras (3 and 4 in Fig. 1), one in Nicaragua (5 in Fig. 1), two in Panama (6 and 7 in Fig. 1) and one from western Ecuador (8 in Fig. 1), which were pooled together. The third phylogroup was composed of samples of the 44 chromosomal race from two populations in El Salvador (populations 1 and 2 in Fig. 1). Variation among chromosomal races accounted for 55.4% of the observed molecular variance in the AMOVA ($P < 0.001$). Variation

among these populations within each chromosomal race accounted for 5.3% ($P < 0.001$) and variation within populations accounted for the remaining 39.3% ($P < 0.001$) of the observed molecular variation in the AMOVA. Haplotype diversity, calculated as gene diversity (Nei 1987), was high in all three phylogroups, ranging from 0.95 in the 38 group to 1.00 in the 42. Estimates of F_u and Li's D^* did not detect departures from neutral expectations in any case, whereas the estimate of F_u 's F_S for the 44 race was significantly different ($P < 0.001$) from the values predicted under neutral conditions (Table 1). Nucleotide diversity (θ_n) was relatively low, ranging from 0.0048 in the 44–0.0146 in the 42 race (Table 1). Average genetic distance was 0.5% in the 44 phylogroup, 0.8% in the 38 and 1.5% in the 42. Comparisons of genetic distance among groups ranged from 2.5 to 2.9% (Table 2).

Mismatch distributions were unimodal in the case of the 44 race, multimodal in the 42 race, with an intermediate pattern in the 38 race (Fig. 3a–d). The peak closer to the origin (Fig. 3a) represents variation within each chromosomal race; the other peak represents variation among the three races. In the case of the 44 chromosomal race, there is agreement between the observed mismatch distribution (Fig. 3d) and

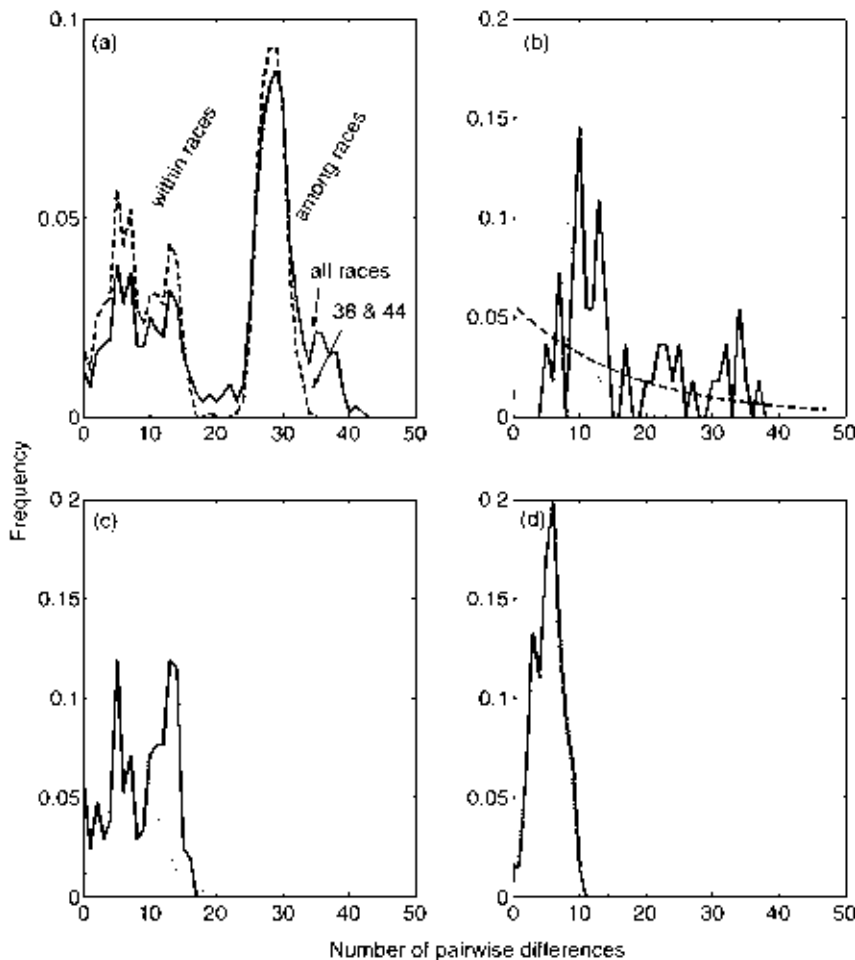


Fig. 3 Mismatch distributions corresponding to (a) all specimens of *Uroderma bilobatum* in a solid line, specimens from the 38 and 44 chromosomal races combined in a dashed line, (b) specimens from the 42 chromosomal race, (c) specimens from the 38 chromosomal race and (d) specimens from then 44 chromosomal race. In boxes b, c and d solid lines present the observed distribution, and dotted lines present the distribution expected under population growth, and in box b the dashed line plots expectations from constant population size.

the mismatch distribution expected under population growth. There is an intermediate pattern in the 38 group (Fig. 3c), which does not change if we restrict our analysis to samples from the vicinity of the hybrid zone (populations 4 and 5 in Fig. 1). A unimodal pattern is to be expected when a population stems from a few closely related ancestral haplotypes. Under the assumptions of the neutral theory, in time there will appear new variants, and given enough time the pattern would turn into a multimodal distribution.

Within the 44 phylogroup ($n = 17$) there was a specimen whose haplotype was shared by an individual from La Paz (El Salvador, population 1) and an individual from Usulután (El Salvador, population 2) localities approximately 50 km apart. In the 38 phylogroup ($n = 29$) we found four shared haplotypes. The first was shared by four specimens from Choluteca (Honduras, population 4) the second was shared by three specimens from Choluteca and one from Chinadega (Nicaragua, population 5), approximately 50 km apart, the third was shared by one specimen from Choluteca and two from Chinadega, and the fourth was shared by one specimen from Choluteca and one from Chinadega. One haplotype was shared between populations on different sides of the hybrid zone. These were an individual from Choluteca on the 38 side of the zone ($2n = 41$ and a potential F_1 , population 4) and an individual from La Paz ($2n = 44$, population 1) on the 44 side, localities that are over 150 km apart.

Discussion

Intraspecific variation in the cytochrome *b* gene is low within *U. bilobatum* (1.7%) relative to other widespread species of the family Phyllostomidae e.g. *Carollia brevicauda*, *C. castanea*, *Glossophaga commissarisi* and *G. soricina*; for additional examples see Ditchfield (2000).

Relative to other stenodermatine genera e.g. *Ardops*, *Ametrida*, *Artibeus*, *Dermanura*, *Platyrrhinus*, *Pygoderma*, *Stenoderma*, *Sturnira* and *Vampyroides*, the genus *Uroderma*, and in particular the species *U. bilobatum*, has undergone considerable chromosomal evolution. The nine genera cited above (excluding *Uroderma*) contain 17 species that have been karyotyped, all with a diploid number of 30 or 31, and a fundamental number of 56 (Baker 1979). Obvious rearrangements are restricted to the Y chromosome and to an autosome, which are either fused or represent separate elements. The $2n = 30$ –31 karyotype is probably the ancestral condition for the subfamily Stenodermatinae (Baker 1979; Baker *et al.* 1979). Within *U. bilobatum*, at least seven different autosomes have been rearranged (Baker *et al.* 1979), with several rearrangements required to derive any *U. bilobatum* karyotype from the proposed primitive type. Karyotypic differences suggest that each of the three races differs from the other two by three rearrangements and that shared derived chromosomal rearrangements are not present in any two races (Baker *et al.* 1972, 1975). Given the

available data, the diversification of the three races is best explained as a rapid, nearly simultaneous radiation.

Phylogenetic results based on cytochrome-*b* arrange samples into three groups that match well the chromosomal races with hybrids and backcrossed individuals as the exception. However, these analyses cannot resolve relationships among the three races, in congruence with inferences based on chromosomal data. Regardless of the method of phylogenetic reconstruction, in all cases the trees obtained suggest that the extant lineages of *U. bilobatum* last shared a common ancestor in South America east of the Andes, in the Pleistocene. Samples of the 42 chromosomal race (from South America east of the Andes) are placed at the base of the *U. bilobatum* radiation and representatives of this race are sister to both the 38 and 44 lineages. Further, statistical parsimony analyses divided the 42 race into three different networks, whereas the 38 and 44 races were restricted to one network each. These results suggest that the 42 race is the oldest of the three, and that the Central American races diverged from a 42-like ancestor in a relatively short time. During this time the cytochrome-*b* gene did not accumulate enough phylogenetic signal to resolve relationships among the three races. If we consider a molecular clock with rates that range from 2.3 to 5% per 10^6 years (Arbogast & Slowinski 1998; Smith & Patton 1999) and using net divergences to correct for variation within each race, then divergence among the three chromosomal races would have occurred between 0.9 and 0.2 Mya.

We found evidence of a low level of introgression in the hybrid zone in Honduras (only two specimens of 46 had an mtDNA haplotype not matching their karyotype, plus one potential F_1), which lends support to Greenbaum's (1981) view that gene flow between these two cytotypes is limited. These specimens, one from La Paz in El Salvador with a reconstituted 44 cytotype and one from Choluteca in Honduras with a $2n = 39$ cytotype, are the result of multiple introgression events. It is interesting that the specimen from La Paz is grouped with specimens from western Ecuador and Panama rather than with specimens from the populations of the 38 race adjacent to the zone (populations 3 and 4 in Fig. 1). It is placed in an internal position in statistical parsimony, suggesting that the hybridization event that gave rise to this specimen might have taken place during a time where genetic composition of the 38 chromosomal race was somewhat different from our sample from 1991 (Owen & Baker 2001).

Results from the AMOVA indicate that variation among chromosomal races accounts for over 55% of maternally inherited molecular variation, suggesting a strong association between chromosomal variation and molecular divergence in the mitochondrial genome. The low amount of variation accounted for by variation among populations within races suggest geographical structuring within

chromosomal races is weak. Our results indicate that chromosomal rearrangements have been central in shaping patterns of variation within this species, with limited gene flow among the chromosomal races, which probably are different biological species. Moreover, the bimodal mismatch distribution observed when *U. bilobatum* is considered as a whole, is consistent with the view that the three chromosomal races have evolved in isolation. Four aspects of our results suggest a relatively recent population expansion within the 44 race: (i) the observed mismatch distribution (Fig. 3d); (ii) the significant departures from neutrality detected by Fu's F_s , but not by Fu and Li's D^* ; (iii) the lack of geographical structure; and (iv) the low amounts of genetic divergence. Further sampling in the northernmost part of the range of the 44 race might help locate the area where the expansion started, thus providing further evidence for the geographical origin of the zone. It is apparent from our study that within the two Central American chromosomal races, reproductive conditions approximate panmixia with high gene flow among populations within each of them. This evidence includes: the number of shared haplotypes among different localities within the 38 and 44 lineages, and the weak geographical structuring within these lineages (which is also reflected in the value of the second component in the AMOVA). The hypothesis that the hybrid zone between these two races is a result of secondary contact is consistent with the reciprocal monophyly of these two races, the match between chromosomal and mtDNA data and the bimodal mismatch distribution observed when samples of these two races are combined together (Fig. 3a).

Phylogenetic results in combination with the patterns of population variation found within and among chromosomal races would suggest a scenario where geographical isolation and the fixation of chromosomal rearrangements were instrumental in shaping the extant patterns of phylogeographical variation in this species, with the Andes and the area close to the Gulf of Fonseca, which was under water throughout much of the Pleistocene, as barriers to gene flow. A similar scenario, where chromosomal rearrangements and geographical isolation reinforce each other, causing divergence in historical times has been presented for house mice from the Madeira island (Britton-Davidian *et al.* 2000). An inspection of the branch lengths leading to the two derived chromosomal races would also suggest rapid diversification in this case, but probably not as extreme as the Madeiran situation.

The reproductive unit of this species is a harem, consisting normally of a single male and about 10 females. Members of the unit build a tent which they occupy as a group (Fleming *et al.* 1972; Wilson 1979). If there was strong female philopatry, populations would consist of closely related haplotypes which, in a model of genetic isolation by distance, would result in geographical structuring. Our

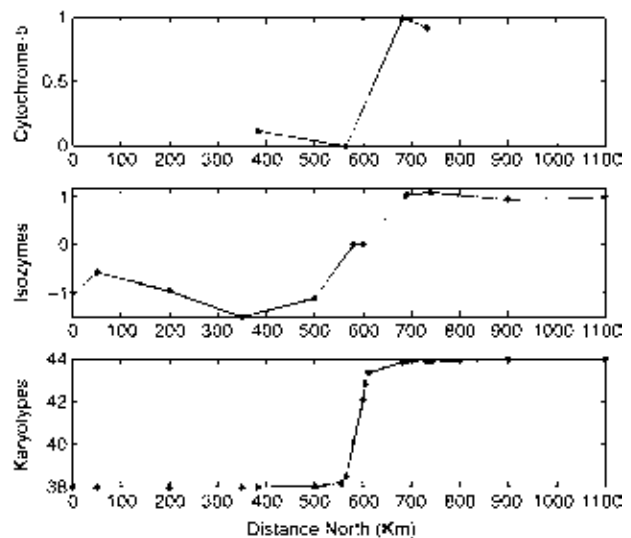


Fig. 4 Clinal variation across the hybrid zone between the 38 and 44 chromosomal races of *Uroderma bilobatum* for cytochrome *b* (relative frequency of 44 haplotypes), allozymes (multidimensional scaling scores modified from Lessa 1990), and mean diploid number.

results are not consistent with female philopatry, but are consistent with female vagility. This vagility is possibly driven by a genetic component but environmental components such as hurricanes or human disturbances cannot be eliminated by our data. Clinal variation across the hybrid zone is congruent for the cytochrome-*b*, allozymes and karyotype (Fig. 4), and given the coincidence between allozymes, chromosomes and maternally inherited mtDNA data, we found no evidence of either male or female biased dispersal.

Conclusions

We hypothesize an origin for *U. bilobatum* in South America, east of the Andes, followed by dispersal into Central America and the western slope of the Andes (Fig. 4). This stock was ancestral to the 38 and 44 chromosomal races. Divergence between the three races occurred over a relatively short time frame, but evidence for a common ancestry of any two races was not present in the genetic data. The available evidence suggests this divergence occurred in geographical isolation and posterior contact between two of them was established in an area that is now the hybrid zone in Central America. The lack of phylogenetic resolution among the three chromosomal races is the probable outcome of rapid diversification. The pattern of variation in cytochrome-*b* haplotypes among populations and within races is indicative of high levels of female dispersal within these races. In light of these results, we propose a history in which fixation of chromosomal differences was associated with episodes of geographical isolation, but maintenance of these differences is strongly associated with chromosomal rearrangements.

Views regarding the role of hybrid zones are diverse. Some authors view hybrid zones as windows to gain an understanding of the speciation process (Barton & Hewitt 1985, 1989; Hewitt 1988), while others (mainly plant researchers such as Abbot 1992; Arnold 1992; among others) suggest their potential for introducing evolutionary novelties (see Arnold 1997: 20–21 for a revision of different views on the hybridization process); these views are not mutually contradictory. Our results indicate that despite evidence of interbreeding between the 44 and 38 chromosomal races, within the hybrid zone, introgression is limited and that the genomes of the two parental cytotypes are essentially isolated from one another. This phenomenon has also been documented in mule deer and white-tailed deer (Carr *et al.* 1986; Cathey *et al.* 1998; Bradley *et al.* 2003). We found no evidence of replacement of the 44 race by the 38 race, as suggested by Baker (1981); in fact, his results and those of Owen & Baker (2001) suggest that the hybrid zone is stable under present conditions. These results are consistent with the selective mechanisms proposed by Baker (1981), Barton (1982) and Lessa (1990). For all practical matters, the 38 and 44 chromosomal races act as separate biological species, as suggested by Greenbaum (1981). Greenbaum (1981) also pointed out that these hybrid populations are restricted to a zone that may be ecologically less favourable for *U. bilobatum*, an observation that is consonant with predictions from cline models (Endler 1977). Demographic inferences also suggest that the zone is the product of secondary contact. Our results support the interpretation that the high degree of similarity among the 38 and 44 chromosomal races is a product of recent common ancestry rather than ongoing gene flow. Further, it seems each chromosomal race is behaving like a biological species.

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Appendix I*Specimens examined*

Specimens examined and their geographical localities are given below: TK numbers correspond to samples from the frozen tissue collection at the Natural Science Research Laboratory from Texas Tech University, Lubbock, Texas; MVZ numbers correspond to samples from the Museum of Vertebrate Zoology, Berkeley, California; ROM numbers correspond to samples from the Royal Ontario Museum, Ontario, Canada. Numbers in brackets correspond to localities in Fig. 1.

Chiroderma doriae — Brazil: Sao Paulo, Caetetus Biological Station TK16379

Uroderma magnirostrum — El Salvador: La Paz, La Herradura TK40046, TK40068

Uroderma bilobatum — Bolivia: La Paz, Puerto Linares TK14522 (12); Ecuador: Esmeraldas, San Lorenzo TK104603,

TK104630 (8); Napo, El Saladero ROM105930, Onkone Gare ROM105969 (9); El Salvador: La Paz, La Herradura, TK34552, TK34555, TK34557, TK34560, TK34567, TK34836, TK34842, TK34910, TK34914, TK34963, TK34964, TK40004, TK40011 (1), Usulután, Hacienda El Cañal, TK40043, TK40045, TK40082, TK40084 (2); Honduras: Atlantida TK40314, TK40318 (3) Choluteca, Valle TK40398, TK40399, TK40400, TK40401, TK40402, TK40403, TK40404, TK40405, TK40406, TK40408, TK40409, TK40410, TK40411, TK40412, TK40413, TK40414 (4); Nicaragua, Chinadega, Posoltega TK40095, TK40099, TK40107, TK40111, TK40114, TK40139, TK40140 (5); Panama: Panama, Altos de Campana National Park ROM104234 (6); Darien, Darien National Park ROM104368 (7); Peru: Huanuco, Leoncia Prado TK22594, TK22969 (10); Madre de Dios, Puerto Maldonado MVZ168867, MVZ192681 (11); Suriname: Nickerie, Sipaliwini TK10186 (14); Marowijne, Albina TK17624 (15); Trinidad and Tobago: Trinidad, Nariva TK25111 (16); Venezuela: Guarico, Calabozo TK15288 (13).