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Do geographically widespread species of tropical amphibians exist? An estimate of genetic relatedness within the neotropical frog *Leptodactylus fuscus* (Schneider 1799) (Anura Leptodactylidae)

A. WYNN and W.R. HEYER

Division of Amphibians & Reptiles, National Museum of Natural History, NHB stop 162, Smithsonian Institution, Washington, DC 20560-0162, USA

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Leptodactylus fuscus (Schneider 1799) as currently understood has a broad geographic range, extending from Panama to Argentina east of the Andes and on the islands of Trinidad and Tobago. We obtained 16 samples throughout its distributional range for electrophoretic analysis to obtain estimates of genetic differentiation within the taxon. Twenty-four loci were scored for analysis. Analytical techniques were used that were appropriate for analyzing inter-population variation of open genetic systems and genetic systems with reduced or no gene flow among populations. The techniques used are: multidimensional scaling; correlation of geographic and electrophoretic distances; gene flow estimates; phylogenetic techniques. The results indicate that the series of samples from Trinidad, Tobago, French Guiana, and Roraima, Brazil have low genetic distances that correspond to an isolation-by-distance model of differentiation, thus comprising a system of populations linked by gene flow within a single species. However, the samples from Panama and those south of the Amazon River demonstrate genetic partitioning, such that there is insignificant gene flow among some sets of these samples as well as with samples north of the Amazon River. *Leptodactylus fuscus* is a "weedy" species, characteristic of open habitats and able to colonize and survive in human altered habitats. Such a "weedy" species would be expected to have relatively low levels of genetic diversity, contrasting strikingly with the levels of genetic differentiation discovered in our study. If these results are typical for other neotropical frogs, we are currently grossly underestimating the amount of diversity in tropical frogs, which has obvious conservation consequences.

KEY WORDS: *Leptodactylus fuscus*, genetic differentiation, zoogeography, neotropics, conservation.

INTRODUCTION

Geographically widespread species play an important zoogeographic role in documenting faunal affinities among geomorphologically distinctive units. As our understanding of the systematics of amphibian species has improved, many species that were considered to have broad geographic distributions have proven to be comprised of two or more closely related species, with resultant reduced geographic ranges. For amphibians, there have been two technological breakthroughs that have been applied to resolve problems of species boundaries.

Molecular techniques that estimate or directly analyze genetic differentiation among populations have uncovered many examples of populations differing genetically with no concordant or noticeable morphological differentiation (e.g., LARSON & HIGHTON 1978, GOOD & WAKE 1992). Molecular techniques when applied to many species that were considered to be geographically widespread have demonstrated that there are two or more genetically isolated population systems (species) involved, which often geographically replace one another. One good example is the study of biochemical evolution within what was considered to be a single, geographically widespread species of salamander, *Plethodon glutinosus* (Green 1818) (HIGHTON 1989, HIGHTON et al. 1989). There are at least 16 genetically differentiated population systems identified within *P. glutinosus* sensu lato, representing full species or semispecies.

The second technological breakthrough pertains only to frogs among the amphibians – quantitative study of advertisement calls. Advertisement calls have been demonstrated to be species-specific sexual attractants (see FRITZSCH et al. 1988 for a good introduction to the general problem and GERHARDT 1988 specifically for aspects of call recognition). For example, advertisement calls have demonstrated that populations thought to demonstrate moderate geographic variation in *Rana pipiens* Schreber 1782 actually represented a series of geographically complementary species (demonstrated initially by PACE 1974). Other advertisement call studies have demonstrated the existence of sibling species of frogs, differing markedly in properties of advertisement calls, but not differing morphologically. Two such examples within the frog genus *Leptodactylus* Fitzinger 1826 are the sibling species pairs *L. plaumanni* Ahl 1936 and *gracilis* (Duméril & Bibron 1841) (BARRIO 1973, as *L. geminus* Barrio 1973 for *L. plaumanni*) and *L. didymus* Heyer et al. 1996 and *mystaceus* (Spix 1824) (HEYER et al. 1996).

Leptodactylus fuscus (Schneider 1799) is a widespread neotropical frog found from Panama and the islands of Trinidad and Tobago southward through South America (east of the Andes) to northern Argentina and southern Brazil (Fig. 1). The species was analyzed morphologically (based on considerable data) and acoustically

(based on only a few recorded advertisement calls) (HEYER 1978) and showed no significant variation throughout its extensive distributional range. HEYER & MAXSON (1982) presented immunological distance data for three samples of *L. fuscus* from

Fig. 1. — Distribution of the frog *Leptodactylus fuscus* (circles) with sites of 16 samples used for electrophoretic analysis (squares).

the central Amazon, southeast Brazil, and Argentina. The Brazilian and Argentine samples differed considerably from each other, indicating the need for further study involving more geographic samples. The purpose of this paper is to use estimates of genetic differentiation among populations of *L. fuscus* throughout its range to evaluate the competing hypotheses: (i) is *L. fuscus* a single, geographically widespread species, or (ii) is *L. fuscus* actually a composite of two or more sibling species, each with smaller geographic distributions?

MATERIALS AND METHODS

Samples

We obtained samples from the entire geographic range of *Leptodactylus fuscus* (Fig. 1). Individuals from point localities were grouped into 16 geographic samples for analysis. Museum abbreviations are those recommended by LEVITON et al. (1985) with the addition of CBF = Colección Boliviana de Fauna. Coordinates were either part of the locality data or determined from gazetteers or maps. Some coordinates are for a nearby city as noted, not for the specimen locality itself.

Sample 1. Panama. Panama: Panama City, about 20 km ENE of, where Inter-American Highway crosses the Río Pacora (9°05'N, 79°17'W). USNM 335650-335652. Panama: Panama; near Tocumen, E of Nuevo Belem, on road to Chepo, 3 miles from Cerro Azul road crossing (9°05'N, 79°23'W for Tocumen). USNM 306189-306190.

Sample 2. Tobago. Tobago: St Paul; Roxborough, on Roxborough-Bloody Bay Road, at or 0.1 km W of junction with Windward Road (11°15'N, 60°35'W for Roxborough). USNM 306044, 306066-306068. Tobago: St Paul; Roxborough, W of, on Roxborough-Bloody Bay Road, 0.6 km W of junction with Windward Road in Roxborough. USNM 306070.

Sample 3. Arima. Trinidad: St George; Arima, about 7 km E of, Wallerfield road, 1.4 km E of junction with Churchill Roosevelt Highway and Antigua Road (10°38'N, 61°13'W). USNM 306148-306152. Trinidad: St George; Arima, S of, on Tumpuna Road, 0.1 km S of junction with Tecoma Blvd, and 1.6 km S of junction with Churchill Roosevelt Highway (10°35'N, 61°16'W). USNM 306155. Trinidad: St George; Arima, about 10 km SSE of, Arena dam road, 2.35 km from junction with Cumuto Tumpuna Road, 2.3 km W of Cumuto and 4.75 km from junction with Cumuto Tumpuna road in San Rafael, Arena Forest Reserve (10°34'N, 61°14'W). USNM 287008.

Sample 4. Nariva. Trinidad: Nariva; Nariva Swamp on Manzanilla Mayaro Road, between 45.5 milepost and curve in road before Bailey bridge over Nariva River, 15.0 km S of junction with Eastern Main Road (10°25'N, 61°04'W). USNM 306123-306126, 306130-306141.

Sample 5. Icacos. Trinidad: St Patrick; Southern Main Road, 8.0 km E of junction with Perseverence Road in Bonasse (10°06'N, 61°48'W). USNM 287015. Trinidad: St Patrick; Chatham Beach (on Erin Bay) (10°05'N, 61°44'W). USNM 314624-314626, 319174. Trinidad: St Patrick; Icacos Point, Icacos Erin Beach Road, 0.8 km S of Coral Point and 2.0 km N of junction with Icacos Savannah Road (10°04'N, 61°46'W). USNM 287011-287013. Trinidad: St Patrick; Icacos Point, Icacos Erin Beach Road, 1.9 km S of Coral Point and 0.8 km N of junction with Icacos Savannah Road (10°03'N, 61°45'W). USNM 287014.

Sample 6. Igarapé Cocal. Brazil: Roraima; Igarapé Cocal (03°45'N, 61°44'W). MZUSP 76019-76022, USNM 302410-302414.

Sample 7. Normandia. Brazil: Roraima; Caracaranã, near Normandia (03°50'N, 59°47'W). MZUSP 67073, USNM 302457.

Sample 8. Bôa Vista. Brazil: Roraima; Bôa Vista (02°49'N, 60°40'W). MZUSP 67039-67043, USNM 302108, 302385-302389.

Sample 9. French Guiana. French Guiana: Cayenne; Sinnamary, about 2 km W of, Sinna Rive Motel grounds (05°23'N, 52°57'W for Sinnamary). USNM 291363. French Guiana: Cayenne; Sinnamary, 1.75 km S of, 9 km W of, on route D21 (St Elie Road). USNM 291367.

French Guiana: Cayenne; Sinnamary, SW of, 7.2 km S of junction of Route D21 (St Elie Road) and Route N1, on Route D21 (St Elie Road). USNM 291368.

Sample 10. Pará. Brazil: Pará; Aldeia Ukre (07°41'S, 51°52'W). MZUSP 70915-70916. Brazil: Pará; Serra de Kukoinhoken, Kenpore (07°49'S, 51°56'W). MZUSP 69954-69955, 70914. Brazil: Pará; Rio Vermelho (07°15'S, 51°43'W). MZUSP 70074.

Sample 11. Pernambuco. Brazil: Pernambuco; between Serra dos Cavalos and Caruarú (8°17'S, 35°58'W). USNM 284551.

Sample 12. São Paulo. Brazil: São Paulo; Luis Antonio, about 5 km S of, Fazenda Jatai (21°33'S, 47°43'W). USNM 303149-303171, 303174.

Sample 13. Beni. Bolivia: Beni; Beni Biosphere Reserve, El Porvenir, about 300 m elevation (14°30'S, 66°00'W). CBF 02902-02903, 02905-02909, 02911, 02913-02916, USNM 498283-498288, 498290-498294.

Sample 14. Palos Blancos. Bolivia: La Paz; Palos Blancos, 1 km WNW of, on road just prior to second stream crossing, about 250 m elevation (15°30'S, 67°30'W). USNM 498280-498282, field numbers USNM-FS 174019-174020 deposited in CBF.

Sample 15. Embarcación. Argentina: Salta; Embarcación, 0.4 km NE of junction with road into, on National Route 34 (23°13'S, 64°06'W for Embarcación). FML 04789, USNM 319636-319637. Argentina: Salta; Embarcación, 4.0 km NE of junction with road into, on National Route 34. USNM 319644-319645. Argentina: Salta; Embarcación, 4.3 km NE of junction with road into, on National Route 34. USNM 319655-319656. Argentina: Salta; Embarcación, SW of, on unnumbered road 2.6 km N of junction with National Route 34 at La Quena (near Río Bermejo bridge). FML 04790(5), USNM 319673-319677.

Sample 16. Joaquín V. González. Argentina: Salta; Joaquín V. González, 54.0-59.9 km NE, on Provincial Route 41 (24°59'S, 64°23'W). FML 04788(7), 04791(5), USNM 319562, 319582-319586, 319607-319612.

Electrophoretic analysis

Muscle from the hind limbs, liver, and kidney were removed from each specimen and maintained at – 70 to – 80 °C, and the carcass was preserved and deposited as a voucher specimen in collections at CBF, FML, MZUSP, or USNM, detailed above.

Liver and kidney were combined and homogenized at a 2:1 (buffer volume:tissue weight) ratio with grinding buffer (SELANDER et al. 1971). Muscle was homogenized separately, also at a ratio of 2:1. After homogenization, the samples were centrifuged to obtain an aqueous protein extract, then stored at – 80 °C.

Muscle and liver/kidney homogenates were analyzed separately using standard horizontal gel electrophoresis protocols (SELANDER et al. 1971). Both Sigma and Connaught starch were used, with no obvious differences in results. Buffer systems used are given in SELANDER et al. (1971). The buffers, protein systems assayed on each buffer, recipe sources, and tissues used are given in Table 1. Some stains were modified as follows (these modifications exceed minor recipe adjustments): for Aat, the amount of Fast Blue BB was increased to 0.1 g; for Fumh, the amount of fumaric acid was increased to 0.25 g; for Idh, 1.0 ml of 0.1M $MgCl_2$ was added to the stain; for Ldh-2, up to 15 mls of LiLactate was added; for Pep-2, 70 mg of Leu-gly-gly was substituted for leucyl alanine; for Lgl, a 0.2 M phosphate buffer (pH 7.0) was used; for Pgm, no glucose-1,6-diphosphate was used (see MURPHY et al. 1996: 112) and 0.1 M potassium phosphate buffer, pH 7.0, was substituted for 0.2 M potassium phosphate buffer. Sod was seen with most dehydrogenase stains, but was most consistently scored off G3pdh, Idh, and Mdhp.

All mobility assignments are based on side-by-side comparisons. When a stain produced more than one presumed genetic locus, the loci were numbered sequentially from anode to cathode. Allelemorphs at each locus are designated similarly, with the most anodal designated as "a," and the others assigned letters in sequence cathodally.

Genetic Data Analysis software (LEWIS & ZAYKIN 1999) was used to produce descriptive statistics for the electrophoretic data.

Table 1.

Enzymes, presumptive loci, tissues, and buffer systems used in the analysis of genetic differentiation of 16 samples of the neotropical frog *Leptodactylus fuscus*.

Protein	Locus	Enzyme#	Tissue[a]	Buffer[b]	Reference[c]
Aconitate hydratase	Acoh-1	4.2.1.3	lk,m	4	Murphy et al. 1996
Aconitate hydratase	Acoh-2	4.2.1.3	m	4	Murphy et al. 1996
Aspartate aminotransferase	Aat-1	2.6.1.1	m	2	Murphy et al. 1996
Aspartate aminotransferase	Aat-2	2.6.1.1	m	2	Murphy et al. 1996
Fumarate hydratase	Fumh	4.2.1.2	m	2	Murphy et al. 1996
General Protein	Gp-1		m	1,2,4	Hedges 1986
General Protein	Gp-2		m	1,2,4	Hedges 1986
General Protein	Gp-3		m	1,2,3,4	Hedges 1986
Glutamate dehydrogenase	Gtdhp	1.4.1.4	lk	3	
Glutathione reductase	Gr	1.6.4.2	lk	1	Harris & Hopkinson 1976
Glycerol-3-phosphate dehydrogenase	G3pdh	1.1.1.8	m	4	Selander et al. 1971
Isocitrate dehydrogenase	Idh-1	1.1.1.42	m	4	Selander et al. 1971*
Isocitrate dehydrogenase	Idh-2	1.1.1.42	m	4	Selander et al. 1971*
Lactoylglutathione lyase	Lgl	4.4.1.5	lk	1	Harris & Hopkinson 1976
L-Lactate dehydrogenase	Ldh-1	1.1.1.27	m	1	Hedges 1986
L-Lactate dehydrogenase	Ldh-2	1.1.1.27	lk	1	Hedges 1986
Malate dehydrogenase (NAD dependent)	Mdh	1.1.1.37	m	1	Hedges 1986
Malate dehydrogenase (NADP dependent)	Mdhp	1.1.1.40	m	3,4	Siciliano & Shaw 1976
Peptidase	Pep-1	3.4.-.-	m	3	Hedges 1986
Peptidase	Pep-2	3.4.-.-	m	3	Hedges 1986*
Phosphoglucomutase	Pgm	5.4.2.2	m	3,4	Selander et al. 1971*
Phosphogluconate dehydrogenase	Pgdh	1.1.1.44	m	4	Selander et al. 1971*
Superoxide dismutase	Sod	1.15.1.1	m[d]	4[d]	*

[a]: m = muscle; lk = liver and kidney.
[b]: Buffers are numbered as follows: 1 = Lithium Hydroxide; 2 = Poulik; 3 = Tris-citrate pH 8.0; 4 = Tris-versene-borate.
[c]: References noted with an asterisk were modified as explained in the text.
[d]: SOD was observed with the stains for several protein systems, primarily using muscle on TVB. See text.

Hypothesis testing analyses

To test our two competing hypotheses, we use analytical techniques that are appropriate for both analyzing intra- and inter-specific variation. The order of the technique descriptions more or less follows the intra- to inter-specific appropriate continuum.

Ordination. Three-way multidimensional scaling is performed on Rogers' (1972) distance values using the Kruskal method in SYSTAT (version 8.0 for Windows). Rogers' distances were generated from the electrophoretic data using the BIOSYS-1 program (Swofford & Selander 1981). Rogers' distances are used for this analysis as they meet the triangle inequality condition, a statistical assumption of multidimensional scaling.

The technique tries to minimize stress, a goodness of fit statistic. Stress values vary between 0 and 1, with values near 0 indicating a better fit. Stress values are determined for

each iteration, that is, one movement of all the points in the plot to a better solution. The iterations should proceed smoothly to a minimum. (WILKINSON 1996).

Geographic-genetic distance correlation. For genetic differentiation, due to small sample sizes, we use NEI's unbiased genetic distances (NEI 1978) generated by the BIOSYS-1 program (SWOFFORD & SELANDER 1981). Geographic distances are linear map distances in kilometers between sample mid-points. The Mantel test is used to determine whether the matrices being compared are statistically significant. The program used is that contained in NTSYSpc, version 2.0, using the raw Mantel statistic option (as per advice of Lee-Ann C. Hayek). One thousand permutations were run per test. Additional information was extracted from regression analyses using SYSTAT (versions 7.0.1 and 8.0 for Windows).

Gene flow estimates among populations. SLATKIN's (1993) $\hat{M}$ values were obtained for genetic cohesion estimates among all pairwise combinations of samples. Slatkin's "$\hat{M}$" program (SLATKIN 1993), downloaded from his Web site (http://ib.berkeley.edu/labs/slatkin), was used to calculate the values for our data. The program calculates genetic subdivision of population pairs using both WEIR & COCKERHAM's (1984) $\hat{\theta}$ and NEI's (1973) G_{ST}. Both statistics provide an estimate of WRIGHT's (1951) F_{ST} measure of genetic difference between a pair of populations. SLATKIN's program uses the $\hat{\theta}$ and G_{ST} values to estimate gene flow among all pairwise combinations of populations. Because values of $\hat{\theta}$ between two pairs of populations were negative, resulting in meaningless estimates of gene flow when $\log_{10}$ transformed, only G_{ST} values were used for Mantel test analysis. The same programs were used for the Mantel test and regression analyses as described in the preceding paragraph.

WHITLOCK & MCCAULEY (1999) pointed out that the assumptions made in estimating the number of migrants (Nm) entering a population per generation from F_{ST} (or similar) measures are unrealistic for most population systems, ours included. BOHONAK (1999) found that dispersal ability was consistently related to population structure (as indicated by measures such as F_{ST}), even though many of the statistical model assumptions were not met with actual biological data. We acknowledge the problems identified by WHITLOCK & MCCAULEY (1999) and as a consequence interpret estimates of dispersal ability (Nm) derived from $\hat{\theta}$ and G_{ST} values very conservatively.

Phylogenetic techniques. Phylogenetic techniques are appropriate to evaluate relationships among populations that have restricted or no gene flow among them. We use the FREQPARS program (SWOFFORD & BERLOCHER 1987) to produce a tree based on the linear programming method for allele frequency data. The PHYLIP program (FELSENSTEIN 1993) was used to produce a maximum likelihood tree (FELSENSTEIN 1981) and to produce a neighbor-joining tree. Each of the trees was drawn with TREEVIEW version 1.5 (PAGE 1996).

RESULTS

Electromorph distributions at the 24 loci resolved are shown in Table 2. Twenty one of the 24 electrophoretic loci screened are polymorphic for one or more populations. Descriptive statistics are typical of those observed in other studies (Table 3).

Ordination

Three-way multidimensional scaling using ROGERS' distance values (Table 4) result in a relatively smooth and linear Shepard diagram, with stress values for each consecutive iteration with lower values, with a final stress configuration of

Table 2.

Electromorph frequencies, expressed as a percentage, in 16 samples of *Leptodactylus fuscus* at 24 loci. Where the electromorph frequencies are not given, the frequency is 100. The number of individuals sampled from each species is given at the head of each column. The following loci were invariant: Gp-1, Gp-3, Gtdhp.

Locus	1. Panama 5	2. Tobago 4-5	3. Arima 7	4. Nariva 14-16	5. Icacos 5-9	6. Ig. Cocal 9	7. Normandia 2	8. Bôa Vista 9-11	9. Fr. Guiana 3	10. Pará 6	11. Pernambuco 1	12. São Paulo 24	13. Beni 17-23	14. Palos Blancos 3, 5	15. Embarcación 17	16. J.V. González 24
Aat-1			b(21)		b(06)								b(02)			a(08)
	c	c	c(79)	c	c(94)	c	c	c	c(83)	c	c	c	c(96)	c	c	c(88)
																d(04)
													e(02)			
									f(17)							
Aat-2																a(02)
															b(94)	b(96)
	d	d	d	d	d	d	d	d	d	d	d	c(04) d(96)	d	d(80) e(20)	d(06)	d(02)
Acoh-1						a(06)		a(04)				a(12)			a(12)	
	b	b	b	b	b	b(94)	b	b(91)	b		b	b(42)	b	b	b(82)	b(94)
										c		c(46)			c(06)	c(06)
								d(04)								
Acoh-2						a(06)										b(10)
															c(03)	c(04)
												d(02)				
	e	e	e	e	e	e(94)	e	e	e	e(92)		e(19)	e(33)		e(97)	e(85)
										f(08)	f	f(71)	f(67)	f		
												g(04)				
												h(04)				

(continued)

Table 2. *(continued)*

Locus	1. Panama 5	2. Tobago 4-5	3. Arima 7	4. Nariva 14-16	5. Icacos 5-9	6. Ig. Cocal 9	7. Normandia 2	8. Bôa Vista 9-11	9. Fr. Guiana 3	10. Pará 6	11. Pernambuco 1	12. São Paulo 24	13. Beni 17-23	14. Palos Blancos 3, 5	15. Embarcación 17	16. J.V. González 24
Fumh	a	b(90) d(10)	a(86) d(14)	a(59) b(41)	a(50) b(50)	b	b	b	b	a(25) b(67) c(08)	b	a(35) b(65)	a(68) b(32)	b	a(21) b(79)	a(08) b(92)
Gp-2	*a*	*a*	*a*	*a*	*a*	*a(22)* b(78)	*a(25)* b(75)	*a(15)* b(85)	b	*a*	*a*	*a*	*a*	b	*a*	*a*
Gr	a	a	a	a	a(94) b(06)	a	a	a	a	a	a	a	a	a	a	a
G3pdh	b	b	b	b	b	b	b	b	b	b	b	b	a(02) b(98)	b	b	b
Idh-1	b	a	a(93) b(07)	a(47) b(53)	a(56) b(44)	a(94) b(06)	a	a(96) c(04)	a	a(25) b(75)	a	a(98) b(02)	a(80) b(20)	a	a(94) b(06)	a
Idh-2	c	c	c	c	c	a(06) b(06) c(89)	c	c(96) d(04)	c	c	c	c	b(02) c(98)	c	c	c
Ldh-2	e	e	c(21) e(79)	c(81) e(19)	e(67) f(33)	c(39) e(61)	c(50) e(50)	c(32) e(54) f(14)	c	d	d	b(06) d(94)	a(40) c(40) d(19)	a(60) d(40)	a	a

(continued)

Table 2. *(continued)*

Locus	1. Panama 5	2. Tobago 4-5	3. Arima 7	4. Nariva 14-16	5. Icacos 5-9	6. Ig. Cocal 9	7. Normandia 2	8. Bôa Vista 9-11	9. Fr. Guiana 3	10. Pará 6	11. Pernambuco 1	12. São Paulo 24	13. Beni 17-23	14. Palos Blancos 3, 5	15. Embarcación 17	16. J.V. González 24
Ldh-1	a	a	a	a	a	a	a	a	a	a	a	a	a(96) b(04)	a	a(97) c(03)	a
Lgl	b	b	a(14) b(86)	a(44) b(56)	a(56) b(44)	a(22) b(78)	b	b(96) c(04)	b	b	b	b	b	b	b	b
Mdh	c	c	c	c(97) f(03)	c(67) f(33)	c	c	c	c	c(83) d(08) f(08)	f(50) g(50)	a(02) c(31) e(19) f(44) g(04)	a(09) b(28) c(15) e(48)	c(20) e(80)	b(82) e(18)	b(58) e(42)
Mdhp-1	a	a	a	a	a	a	a	a(82) c(18)	a	a	a	a(94) b(06)	a	a	a	a
Mdhp-2	c	c	b(64) c(36)	b(69) c(31)	b(83) c(17)	a(22) c(78)	c	a(23) c(77)	a(50) c(50)	c(50) d(50)	c	b(06) c(94)	b(09) c(87) e(04)	c	c(50) e(50)	c(40) e(60)
Pep-1	a	a	a	a	a	a	a	a	a	a(92) b(08)	a	a(67) b(33)	a(70) b(26) c(04)	a	a	a

(continued)

Table 2. *(continued)*

Locus	1. Panama 5	2. Tobago 4-5	3. Arima 7	4. Nariva 14-16	5. Icacos 5-9	6. Ig. Cocal 9	7. Normandia 2	8. Bôa Vista 9-11	9. Fr. Guiana 3	10. Pará 6	11. Pernambuco 1	12. São Paulo 24	13. Beni 17-23	14. Palos Blancos 3, 5	15. Embarcación 17	16. J.V. González 24
Pep-2	c	c	c	c	c	c	c	c	c(83) d(17)	b(92) c(08)	b(50) c(50)	a(67) b(25) c(08)	b(09) c(82) d(09)	c	b(74) c(26)	a(04) b(92) c(04)
Pgdh	c	d	d	d	d	d	d	d	d	c(83) e(17)	c(50) f(50)	a(15) c(06) f(77) g(02)	b(04) c(50) f(46)	c	c(03) f(97)	f
Pgm	b	a(30) b(70)	b	b	b	b	b	a(09) b(91)	b	b	b	b(94) c(06)	b	b	b	b
Sod	b	a	a	a	a	a(61) b(39)	a	a(82) b(18)	a	b	b	b	b(80) d(20)	d	a(12) b(88)	a(10) b(88) c(02)

Table 3.

Descriptive statistics over all loci.

Sample	N	P	A	Ap	He	Ho	F
Panama	5.000	0.000	1.000	***	0.000	0.000	0.000
Tobago	4.958	0.083	1.083	2.000	0.028	0.017	0.429
Arima	7.000	0.250	1.250	2.000	0.079	0.071	0.100
Nariva	15.667	0.250	1.250	2.000	0.098	0.085	0.136
Icacos	8.792	0.333	1.333	2.000	0.126	0.090	0.304
Igarapé Cocal	9.000	0.375	1.417	2.111	0.111	0.088	0.214
Normandia	2.000	0.083	1.083	2.000	0.049	0.021	0.667
Bôa Vista	10.833	0.417	1.500	2.200	0.104	0.080	0.239
French Guiana	3.000	0.125	1.125	2.000	0.053	0.042	0.250
Pará	6.000	0.333	1.417	2.250	0.109	0.069	0.383
Pernambuco	1.000	0.125	1.125	2.000	0.125	0.125	0.000
São Paulo	24.000	0.542	1.958	2.769	0.174	0.177	-0.018
Beni	22.500	0.583	1.917	2.571	0.195	0.194	0.010
Palos Blancos	4.917	0.125	1.125	2.000	0.052	0.067	-0.333
Embarcación	17.000	0.458	1.500	2.091	0.104	0.113	-0.092
Joaquín V. González	24.000	0.375	1.583	2.555	0.093	0.092	0.007
Mean	10.354	0.279	1.354	2.170	0.094	0.083	0.123

N = mean sample size; P = proportion of polymorphic loci; A = alleles/locus; Ap = alleles/polymorphic locus; He = expected heterozygosity; Ho = observed heterozygosity; F = fixation index.

0.055 and a proportion of variances (r^2) of 0.98. The data are appropriate for the statistical model.

Perspective can be deceiving when viewing multidimensional scaling results, depending on the orientation of the axes. The perspective figured (Fig. 2) is the perspective obtained without modification in SYSTAT. The discussion of the results is based on rotation of axes, which in some instances, provide information quite different from that obtained from the orientation illustrated (Fig. 2).

Three sets of samples consistently cluster together: (1) the three Trinidad samples (3-Arima, 4-Nariva, 5-Icacos); (2) the three Roraima samples (6-Igarapé Cocal, 7-Normandia, 8-Bôa Vista); and (3) the two Argentine samples (15-Embarcación, 16-Joaquín V. González). The Trinidad samples, the Roraima samples, and the samples from Tobago (2) and French Guiana (9) consistently cluster together as well, with the French Guiana sample always closer to the Roraima and Tobago samples than the Trinidad samples (as shown in Fig. 2). The Tobago sample is more intermediate in position between the Trinidad and Roraima samples in most perspectives than shown in Fig. 2. The two Bolivian samples (13-Beni, 14-Palos Blancos) are farther apart from each other in almost every other perspective than as shown in Fig 2. There are no other consistent clustering patterns. That is, the sample from Panama (1) and the samples from below the Amazon River (10-16) show no consistent clustering patterns among each other or with the cluster of samples Trinidad + Tobago + Roraima + French Guiana with the exception of the two Argentina samples (15-16), which always form a cluster with each other.

Table 4.

ROGERS' 1972 distance values for 16 samples of *Leptodactylus fuscus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	0.177	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	0.179	0.103	0	—	—	—	—	—	—	—	—	—	—	—	—	—
4	0.202	0.140	0.083	0	—	—	—	—	—	—	—	—	—	—	—	—
5	0.218	0.140	0.094	0.066	0	—	—	—	—	—	—	—	—	—	—	—
6	0.224	0.111	0.140	0.155	0.173	0	—	—	—	—	—	—	—	—	—	—
7	0.219	0.069	0.127	0.139	0.165	0.051	0	—	—	—	—	—	—	—	—	—
8	0.238	0.099	0.148	0.168	0.181	0.046	0.048	0	—	—	—	—	—	—	—	—
9	0.285	0.135	0.162	0.156	0.200	0.097	0.066	0.085	0	—	—	—	—	—	—	—
10	0.202	0.290	0.306	0.276	0.293	0.297	0.305	0.310	0.325	0	—	—	—	—	—	—
11	0.244	0.235	0.298	0.307	0.304	0.255	0.244	0.269	0.285	0.215	0	—	—	—	—	—
12	0.280	0.279	0.315	0.324	0.319	0.299	0.297	0.306	0.334	0.203	0.132	0	—	—	—	—
13	0.196	0.242	0.234	0.234	0.251	0.249	0.248	0.266	0.276	0.252	0.154	0.165	0	—	—	—
14	0.286	0.261	0.324	0.334	0.336	0.227	0.207	0.228	0.238	0.340	0.190	0.277	0.210	0	—	—
15	0.297	0.280	0.303	0.313	0.313	0.286	0.294	0.300	0.314	0.265	0.228	0.241	0.227	0.316	0	—
16	0.322	0.290	0.318	0.333	0.329	0.300	0.303	0.313	0.315	0.275	0.230	0.253	0.237	0.311	0.049	0

Sample 1 = Panama, 2 = Tobago, 3 = Arima, 4 = Nariva, 5 = Icacos, 6 = Igarapé Cocal, 7 = Normandia, 8 = Bôa Vista, 9 = French Guiana, 10 = Pará, 11 = Pernambuco, 12 = São Paulo, 13 = Beni, 14 = Palos Blancos, 15 = Embarcación, 16 = Joaquín V. González.

Geographic-genetic distance correlation

For the overall data set, the Mantel test comparing Nei unbiased genetic distance values with linear geographic distances (Table 5) has a matrix correlation of 0.74 and the one-tail probability [random Z ≥ observed Z] is 0.002. There is thus a statistically significant correlation between these matrices. TILLEY (1997: 307-308) provides a clear explanation of the meaning of comparing genetic and geographic distances:

"D.A. GOOD (unpublished manuscript) and GOOD & WAKE (1992, 1993) have shown that the relationships between NEI's (1978) standardized genetic distance and geographic distance among populations can provide insight into patterns of differentiation within and among species. The null model for such analyses is an array of populations in which genetic differentiation occurs in response to mutation at rate u and is retarded by gene exchange at rate m. In such a system, it can be shown that the relationship between NEI's genetic distance and geographic distance eventually reaches an equilibrium, at which that relationship is expected to be linear to ($2u/m$, where u and m are the mutation and migration rates, respectively (NEI 1972). Deviations from this simple relationship can illuminate the processes and history of genetic fragmentation (D.A. GOOD unpublished manuscript; GOOD & WAKE 1992, 1993).

These deviations might take two general forms. Deviations from linearity indicate that opposing forces that promote differentiation and cohesion have not yet

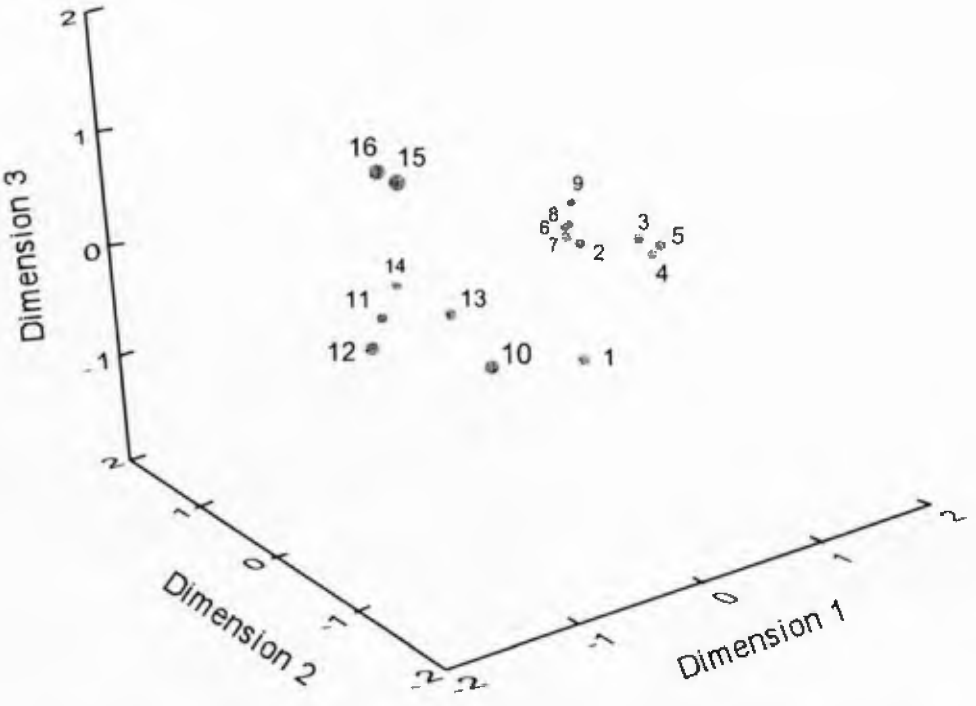

Fig. 2. — Kruskal method three-way scaling plot of ROGERS' genetic distance values. 1 = Panama, 2 = Tobago, 3 = Arima, Trinidad, 4 = Nariva, Trinidad, 5 = Icacos, Trinidad, 6 = Igarapé Cocal, Roraima, Brazil, 7 = Normandia, Roraima, Brazil, 8 = Bôa Vista, Roraima, Brazil, 9 = French Guiana, 10 = Pará, Brazil, 11 = Pernambuco, Brazil, 12 = São Paulo, Brazil; 13 = Beni, Bolivia, 14 = Palos Blancos, Bolivia, 15 = Embarcación, Argentina, 16 = Joaquín V. González, Argentina.

Table 5.
Nei's 1978 distance (upper matrix) and geographic distance (lower matrix, kilometers) for 16 samples of *Leptodactylus fuscus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0	0.183	0.156	0.174	0.178	0.196	0.222	0.222	0.308	0.172	0.255	0.257	0.129	0.318	0.304	0.352
2	2117	0	0.058	0.089	0.074	0.046	0.032	0.044	0.104	0.285	0.236	0.252	0.192	0.269	0.272	0.297
3	2053	100	0	0.034	0.028	0.092	0.083	0.095	0.131	0.303	0.306	0.286	0.177	0.342	0.305	0.338
4	2053	105	40	0	0.025	0.089	0.083	0.100	0.102	0.273	0.306	0.300	0.179	0.344	0.310	0.343
5	1979	193	93	103	0	0.095	0.099	0.105	0.148	0.290	0.291	0.297	0.204	0.343	0.304	0.334
6	2053	851	766	745	713	0	0.002	0.001	0.027	0.270	0.228	0.248	0.182	0.196	0.269	0.293
7	2170	819	755	734	723	226	0	0.000	0.016	0.297	0.244	0.266	0.193	0.195	0.284	0.307
8	2181	915	851	830	799	146	168	0	0.021	0.293	0.252	0.270	0.206	0.199	0.287	0.311
9	2968	1074	1085	1053	1106	979	745	894	0	0.338	0.300	0.320	0.236	0.225	0.325	0.343
10	3543	2298	2266	2234	2234	1649	1532	1521	1394	0	0.177	0.138	0.194	0.340	0.256	0.271
11	5117	3479	3489	3457	3500	3096	2915	2957	2415	1681	0	0.057	0.084	0.164	0.202	0.207
12	4777	3872	3830	3798	3798	3149	3085	3032	3032	1596	1883	0	0.091	0.239	0.195	0.202
13	2968	2872	2787	2766	2723	2032	2128	1979	2585	1670	3213	2032	0	0.137	0.151	0.173
14	3011	3032	2947	2925	2872	2191	2287	2149	2766	1862	3372	2117	200	0	0.317	0.323
15	3915	3798	3723	3702	3670	2957	3021	2894	3351	2128	3308	1649	1000	915	0	0.005
16	4096	3957	3883	3851	3819	3117	3170	3043	3479	2223	3319	1606	1170	1096	191	0

Sample 1 = Panama, 2 = Tobago, 3 = Arima, 4 = Nariva, 5 = Icacos, 6 = Igarapé Cocal, 7 = Normandia, 8 = Bôa Vista, 9 = French Guiana, 10 = Pará, 11 = Pernambuco, 12 = São Paulo, 13 = Beni, 14 = Palos Blancos, 15 = Embarcación, 16 = Joaquín V. González.

achieved an equilibrium. A relationship between genetic distance and geographic distance requires two things: sufficient time and geographic isolation to produce genetic fragmentation, and sufficient time and gene flow to generate higher levels of similarity among geographically proximal demes than among more distant ones. If levels of gene flow are sufficiently low, then there will be no discernable relationship between genetic and geographic distance. In an array of semi-isolated demes whose genetic structures are initially identical, the effects of gene flow will initially be evident at the smallest geographic distances, and the relationship between genetic and geographic distance will initially be asymptotic. Over time, genetic differentiation raises the asymptote while gene flow increases the geographic distance at which the asymptote is reached, until the relationship becomes linear over the entire range of geographic distances.

The second type of deviation from the null model concerns the *y* intercept of the relationship, that is, the genetic distance expected between two demes separated by zero geographic distance. This intercept is expected to be zero within a single array of populations that share a simple history of isolation by distance. However, the array of populations might include multiple groups descended from ancestors that underwent an initial period of differentiation of their constituent demes in response to isolation by distance. The *y* intercept for comparisons between populations of two genetically differentiated subgroups of populations should lie above the origin. The intercept's value in this case represents the additional genetic differentiation accomplished by factors other than isolation by distance. These might include a history in which populations ancestral to the subgroups differentiated in allopatry or current nongeographic barriers to gene exchange".

The relationship between genetic and geographic distances for the *Leptodactylus fuscus* data (Table 5) do not fit the linear model precisely (Fig. 3). Clearly, there has been sufficient time and geographic isolation to produce some genetic fragmentation. There is a general pattern of higher levels of genetic similarity among geographically proximal demes than among more distant demes. The *y*-intercept for the entire data set is at 0.06, not at zero (Fig. 3). The *y*-intercept is sufficiently offset to suggest some level of genetic differentiation among subgroups of populations.

The data that do not fit the strict linear model of differentiation fall into two classes: (1) those samples that show greater genetic differentiation than predicted by their geographic distances (Fig. 3, data points contained in ovals), and (2) those populations which show less genetic differentiation than predicted by geographic distances. Only the latter (2) data could be seriously impacted by the fact that the electrophoresis techniques we utilized would not be expected to uncover all genetic variants among alleles. Thus, some alleles scored as having the same electrophoretic mobility may in fact be genetically differentiated. All of the data points showing greater genetic differentiation than predicted by geographic distance (those values indicated by ellipses in Fig. 3) are accounted for by three samples: Tobago, Palos Blancos, and Pará.

The Tobago sample has higher than expected genetic differentiation from three of the Trinidad samples (Arima, Icacos, Nariva, lowest three values within ellipse on left of Fig. 3) compared to the Tobago-Roraima genetic distances.

In contrast to the Tobago sample, the data for the Pará and Palos Blancos samples support the conclusion that these samples have greater genetic differentiation from one another than predicted by geographic distance.

The nine samples north of the Amazon River demonstrate a stronger relationship between Nei and geographic distances than for the data set as a whole (Fig 3).

The Mantel test matrix correlation is $r = 0.88$ and the one-tail probability is P [random $Z \geq$ observed Z] = 0.002. The y-intercept is 0.01. Thus, the samples north of the Amazon River fit the linear model of differentiation rather well. When the Panama sample data are deleted from the samples north of the Amazon River, the matrices still have a statistically significant relationship, but the correlation is not as strong. The Mantel test matrix correlation for the eight samples is $r = 0.51$ and the one-tail probability is P [random $Z \geq$ observed Z] = 0.029. The y-intercept is 0.026. There are three data points indicating greater genetic distances than predicted by geographic distances, which are the Tobago-Trinidad data discussed above. Six data points show less genetic differentiation than predicted by geographic distance. Of these six, three points are closer to the straight-line relationship and involve comparisons between Tobago and the three Roraima samples. The other three data points, which are the farthest outliers, are data for the French Guiana sample compared with the three Roraima samples. This suggests that there may have been less time available for differentiation to occur or slower rates of differentiation between the French Guiana and Roraima localities than for the rest of the data set.

In contrast, the seven samples south of the Amazon River do not demonstrate a statistically significant relationship between Nei unbiased genetic distances and geographic distances (Fig. 3). The Mantel test has a matrix correlation of $r = 0.08$ and the one-tail probability P [random $Z \geq$ observed Z] is 0.38. The fact that 14 of the 21 data comparisons have Nei distances > 0.15 indicates that there has been considerable genetic differentiation among these southern populations.

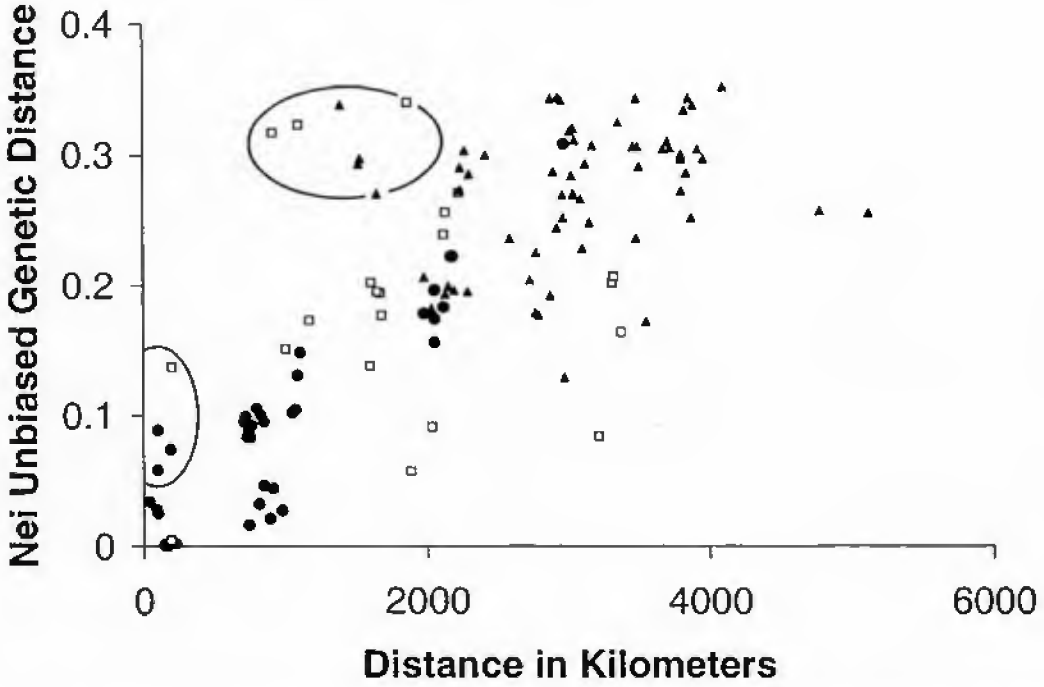

Fig. 3. — Nei unbiased genetic distance values plotted against geographic distance for all pair-wise comparisons of the 16 samples. Ellipses identify data discussed in text. Dots are between sample comparisons north of the Amazon River; squares are between sample comparisons south of the Amazon River; triangles are between sample comparisons north and south of the Amazon River.

Gene flow estimates among samples

SLATKIN (1993) indicated that a log-log plot of $\hat{M}$ values with geographic distance would demonstrate isolation by distance if there were a linear relation of the data. SLATKIN (1993) used two statistics to estimate F_{ST}, the measure of genetic difference between a pair of populations: $\hat{\theta}$ (WEIR & COCKERHAM 1984) and G_{ST} (NEI 1973). However, as two of the $\hat{\theta}$ values are negative, only the log G_{ST} values can be used when the entire data set is needed for analysis (Table 6).

When the entire data set is considered, the Mantel test results for comparison of the log $\hat{M}$ values based on G_{ST} values are statistically significant, with a matrix correlation of – 0.66 and a one-tail probability of P [random Z ≥ observed Z] = 0.001. The $\hat{\theta}$ and G_{ST} based values of $\hat{M}$ are very similar (Table 6), with the $\hat{\theta}$ based values typically smaller than the G_{ST} based values. The overall plot of values for $\hat{M}$ based on the two statistics are almost identical (note that the two pairs with negative $\hat{\theta}$ values are for sample pairs Igarapé Cocal-Normandia and Normandia-Bôa Vista, which are the second and third highest G_{ST} values in the data set).

The log plot of the G_{ST} based $\hat{M}$ values with geographic distance (Fig. 4) indicates over-all low genetic cohesion among sample pairs.

The genetic cohesion $\hat{M}$ values should be highest for the geographically closest sample pairs if isolation by distance is accounting for the variation observed. Some values fit this explanation, others do not. The data point for the two Argentina samples (Fig. 4, single point lying between clusters A and B) and the data point for the two Bolivian samples (Fig. 4, one of two overlapping circles on right in cluster C) neither fit the model extremely well nor extremely poorly. Somewhat surprisingly, the data for the three Trinidad samples (Fig. 4, cluster B), which are the geographically closest three samples to each other in the data set, demonstrate less genetic cohesion than the three Roraima samples (Fig. 4, cluster A). We know of no dispersal barriers to *Leptodactylus fuscus* either among the three Trinidad site samples or the three Roraima site samples. A possible explanation is that the Trinidad samples are not in equilibrium due to gene flow from the South American mainland across the strait at the tip of the Icacos Peninsula (see READ 1986 for a discussion of amphibian rafting from Venezuela to the Icacos Peninsula). The three data points involving Trinidad-Tobago comparisons show less genetic cohesion than predicted by the isolation by distance model (Fig. 4, cluster C).

There is a set of data points that show somewhat greater genetic cohesion for samples separated by considerable geographic distances than predicted by the isolation by distance model (Fig. 4, cluster D). The two points on the left involve French Guiana and Roraima samples. The most central point within cluster D and the rightmost point involve the Pernambuco sample (with the São Paulo and Beni samples, respectively). The $\hat{\theta}$ based log $\hat{M}$ value results differ for the Pernambuco-São Paulo comparison, in that the $\hat{\theta}$ results show much more genetic cohesion than the G_{ST} results (surpassed only by the Roraima comparison). Caution should be used with the Pernambuco data, however, as they are based on only one individual. The remaining point in cluster D is for the São Paulo-Beni samples. The $\hat{\theta}$ based result differs for this comparison, but in this instance, the results have a lower genetic cohesion and fall within the large cluster of points comprised by the bulk of the data.

The points within cluster E (Fig. 4) have extremely low genetic cohesion values – there is probably no gene flow among these samples. Given that all the comparisons involve the Panama sample (with the Tobago, Normandia, French Guiana,

Table 6.

$\hat{\theta}$ based (upper matrix) and G_{ST} based (lower matrix) $\hat{M}$ values for 16 samples of *Leptodactylus fuscus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0	0.021	0.084	0.126	0.136	0.109	0.015	0.097	0.018	0.100	0.015	0.180	0.382	0.024	0.083	0.065
2	0.037	0	0.272	0.262	0.357	0.506	0.256	0.523	0.096	0.078	0.049	0.192	0.276	0.044	0.099	0.079
3	0.129	0.422	0	0.765	1.051	0.305	0.254	0.285	0.158	0.097	0.091	0.178	0.305	0.063	0.101	0.079
4	0.153	0.357	1.204	0	1.199	0.333	0.339	0.290	0.263	0.117	0.113	0.160	0.276	0.081	0.105	0.083
5	0.188	0.503	1.510	1.968	0	0.369	0.363	0.321	0.225	0.134	0.160	0.185	0.285	0.095	0.120	0.093
6	0.152	0.690	0.519	0.612	0.643	0	-6.76	22.07	1.136	0.130	0.176	0.210	0.306	0.139	0.126	0.098
7	0.045	0.404	0.328	0.400	0.403	3.002	0	-3.46	0.849	0.103	0.091	0.214	0.333	0.076	0.111	0.086
8	0.129	0.696	0.489	0.538	0.578	7.705	4.644	0	1.442	0.116	0.147	0.187	0.263	0.133	0.116	0.092
9	0.042	0.172	0.240	0.361	0.304	1.150	0.781	1.363	0	0.087	0.071	0.173	0.257	0.068	0.096	0.077
10	0.160	0.131	0.177	0.220	0.237	0.233	0.137	0.213	0.132	0	0.226	0.370	0.301	0.078	0.130	0.102
11	0.071	0.108	0.137	0.161	0.195	0.220	0.117	0.196	0.107	0.264	0	5.977	1.986	0.116	0.183	0.137
12	0.201	0.237	0.273	0.290	0.323	0.355	0.238	0.323	0.214	0.572	1.112	0	0.650	0.209	0.237	0.185
13	0.410	0.334	0.451	0.492	0.481	0.501	0.344	0.443	0.304	0.462	0.846	1.205	0	0.393	0.326	0.226
14	0.043	0.078	0.108	0.129	0.153	0.223	0.117	0.212	0.115	0.134	0.188	0.279	0.497	0	0.093	0.078
15	0.100	0.139	0.178	0.201	0.232	0.238	0.145	0.219	0.138	0.240	0.240	0.431	0.588	0.144	0	0.952
16	0.108	0.149	0.179	0.198	0.226	0.234	0.154	0.218	0.149	0.242	0.251	0.413	0.502	0.160	2.082	0

Sample 1 = Panama, 2 = Tobago, 3 = Arima, 4 = Nariva, 5 = Icacos, 6 = Igarapé Cocal, 7 = Normandia, 8 = Bôa Vista, 9 = French Guiana, 10 = Pará, 11 = Pernambuco, 12 = São Paulo, 13 = Beni, 14 = Palos Blancos, 15 = Embarcación, 16 = Joaquín V. González.

and Palos Blancos samples), the lack of gene flow is not surprising. The $\hat{\theta}$ based results add one more comparison to this cluster, Panama-Pernambuco. Not all distant Panama comparisons yield such low $\hat{M}$ values, however. The Panama-Beni samples, with a geographic distance value of 2968 and a $\hat{M}$ value of 0.410 fall within the upper portion of the dense cloud of data points in Fig. 4.

When the regions are analyzed separately, isolation by distance is supported only for the samples north of the Amazon River, not for the samples south of the Amazon River (results not shown).

SLATKIN (1993) indicated that the y-intercept of the linear regression for the logarithmically plotted data can be used to estimate *Nm* (the product of the effective population number and rate of migration among populations), which can also be thought of as a neighborhood size when calculated by this method. For the entire data set, the G_{ST} based $\hat{M}$ values yield a *Nm* value of 26; the $\hat{\theta}$ based values 18.

Phylogenetic techniques

Phylogenetic techniques produce results similar to those seen with three-way multidimensional scaling. Neighbor joining (Fig. 5), maximum likelihood (Fig. 6), and FREQPARS (Fig. 7) produce overall similar results with the northern samples from Tobago, Trinidad, Roraima, and French Guiana forming a tight set of samples separate from a loose set containing the remaining samples from Panama and southern South America. Panama lies intermediate between the northern and

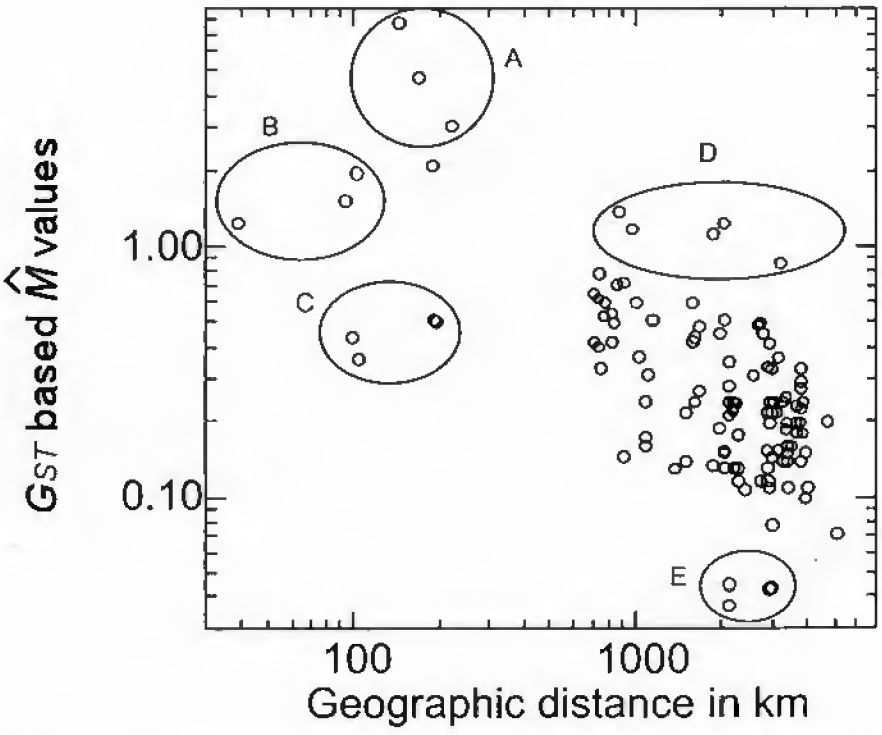

Fig. 4. — Log_{10} G_{ST} based $\hat{M}$ values plotted against $\log_{10}$ geographic distances. Clusters A-E identify data discussed in text.

southern samples, and the relationship of the Pará sample to the other South American samples is ambiguous. The Pará sample lies in the FREQPARS tree (Fig. 7), along with Panama, between the remaining northern and southern samples, or with the southern samples in the other two trees (Figs 5-6).

Within the northern samples, there are minor differences in the branching pattern, but overall the results make geographic sense. The samples from Trinidad appear together, and the samples from Roraima and French Guiana appear together. However, in none of the trees does the Tobago sample lie proximate with any Trinidad sample. Instead, the Tobago sample is near the Roraima/French Guiana assemblage, but at a basal position in each, reflecting the genetically intermediate position also seen with three-way scaling, gene flow, and geographic-genetic distance results.

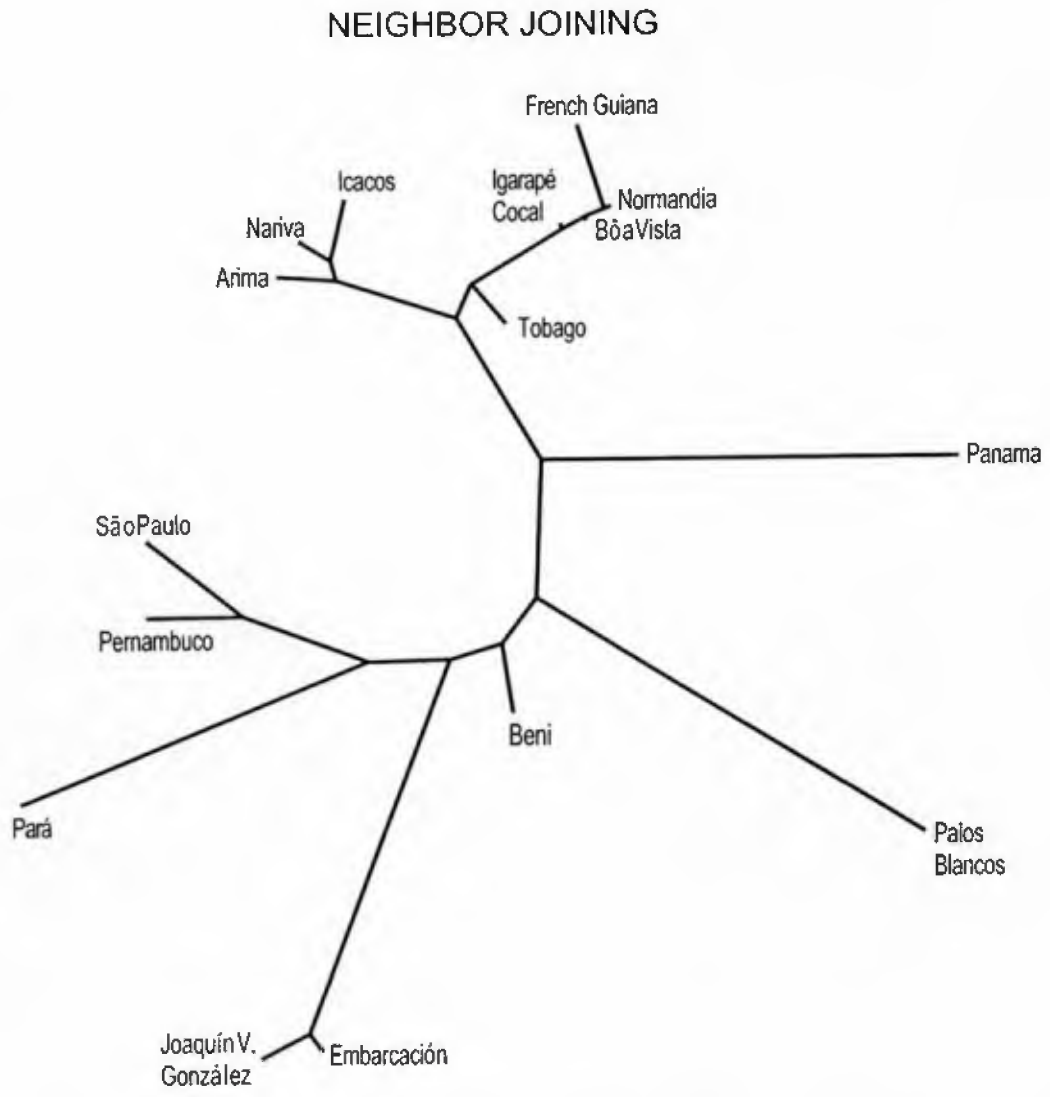

Fig. 5. — Neighbor-joining tree for 16 geographic samples of *Leptodactylus fuscus*.

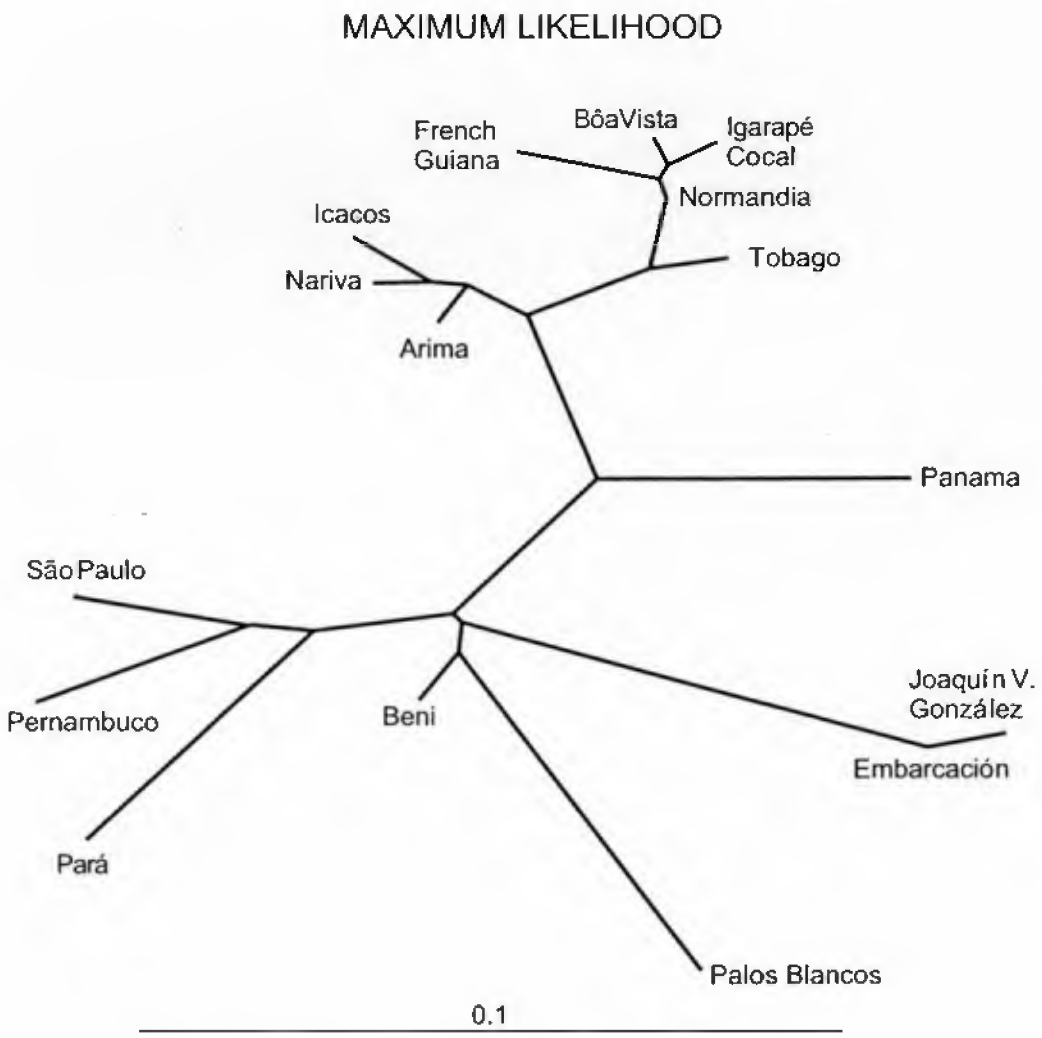

Fig. 6. — Maximum likelihood tree for 16 geographic samples of *Leptodactylus fuscus*.

The samples from sub-Amazonian South America appear more disparate. Although the samples from São Paulo and Pernambuco consistently appear together, as do the two geographically close samples from Argentina, the two geographically close samples from Bolivia do not consistently appear together, and as noted above, the sample from Pará differs in the FREQPARS tree from the other two trees. The long branch lengths (Figs 5-7) also indicate the large genetic distances between many of these samples.

A bootstrap analysis was run on the neighbor joining tree results using 1000 iterations. The only convincingly supported relationship is that of the two Argentina samples (supported in 989 of the 1000 iterations for the seed number used). The only other relationships supported in more than 50% of the iterations are: (1) The three Trinidad samples (681); (2) the French Guiana, Roraima, Trinidad, and Tobago samples (639); (3) the Nariva and Icacos samples (542); and (4) the Pernambuco and São Paulo samples (523).

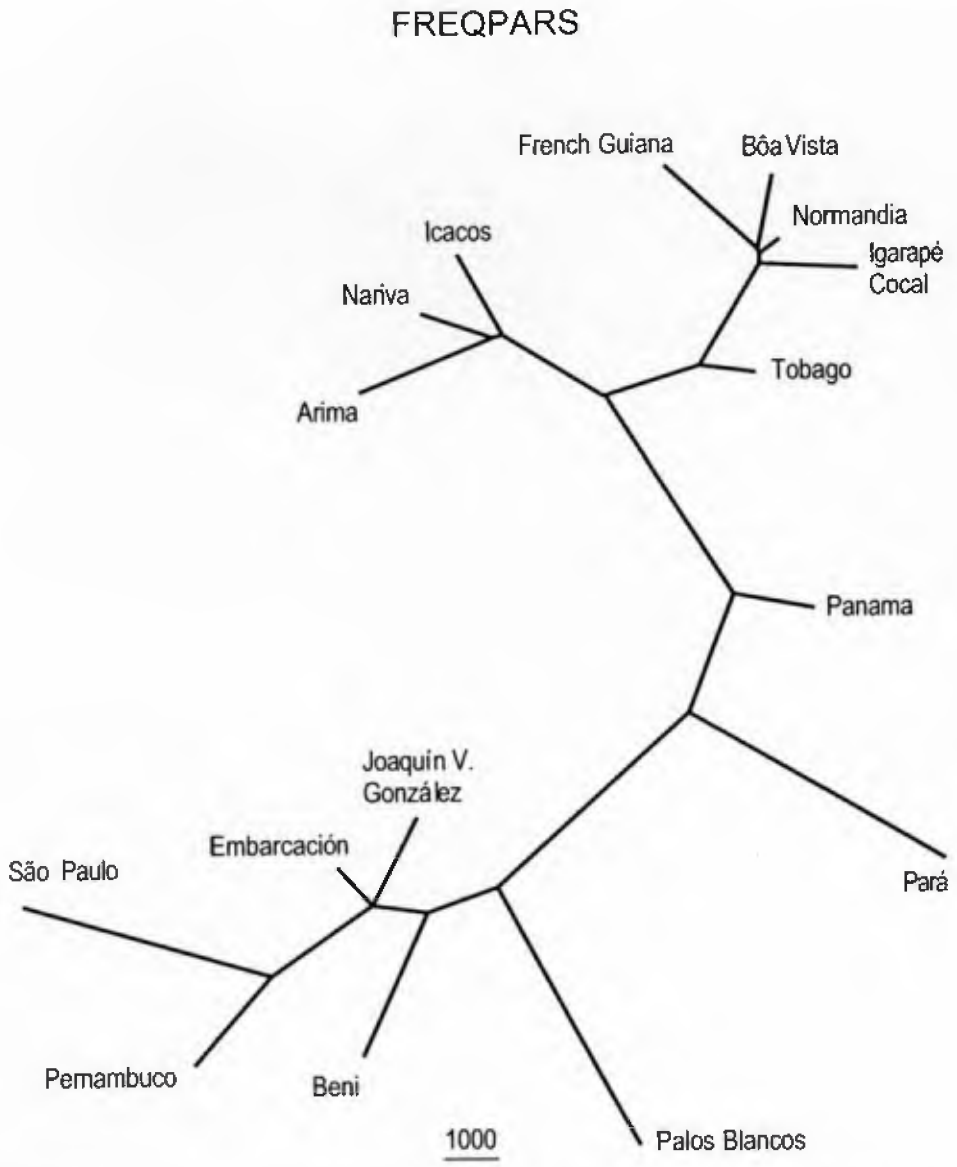

Fig. 7. — Linear programming method for allele frequency data (FREQPARS) for 16 geographic samples of *Leptodactylus fuscus*.

DISCUSSION

All of the analyses resulted in an apparently anomalous placement of the Tobago sample showing greater genetic affinities with the Roraima/French Guiana samples than with the geographically most proximate Trinidad samples. The greater similarity of the sample from Tobago to samples from Roraima Brazil (Igarapé Cocal, Normandia, Bôa Vista) than to samples from Trinidad (Arima, Icacos, Nariva) is the result of 14 variable loci at these localities. The sample from Tobago is variable at only two of these loci. Aside from two polymorphisms (at Fumnh and Pgm) shared with the Tobago sample, Trinidad and Roraima, Brazil are

polymorphic for 13 loci. At each of these loci, Tobago shares an allele found (either fixed or in the polymorphic state) in both the Trinidad and Roraima samples. Although the Trinidad samples are only polymorphic for eight of these loci, whereas the Roraima samples are polymorphic for nine loci, on average, the Roraima samples have higher frequencies for the alleles shared with Tobago. This accounts for the lower genetic distance between the Tobago and Roraima samples and the higher than anticipated genetic distances between the Tobago and Trinidad samples. The relatively low genetic cohesion values for the Trinidad-Tobago comparisons could be due to the water barrier in addition to the map distances involved with homozygosity of the Tobago sample resulting from either a founder event or some other genetic bottleneck.

Gene flow

PETERSON & DENNO (1998, see p. 428 for citations) list the following factors that have been hypothesized to influence gene flow: geographic distance; dispersal capability; ecological specialization; phenological isolation; habitat patchiness; habitat persistence; and frequency and nature of extinction/recolonization events. We use these as a background to discuss gene flow in amphibians in general and *Leptodactylus fuscus* in particular.

Amphibians demonstrate at least a moderate dispersal capability when suitable habitat is continuous, as witnessed by the recolonization of habitat that occurred after the last glaciation in North America. A good example is the salamander *Plethodon cinereus* (Green 1818), which has expanded into over half of its current range since the Wisconsin maximum ca 21,000 years B.P. (HIGHTON & WEBSTER 1976, HIGHTON 1995) with an average rate of linear dispersal from 0.04 to 0.07 km/year (GROBMAN 1944, WYNN 1986). *Plethodon cinereus* also provides a good example of an amphibian species showing considerable genetic differentiation in the portion of the range where population isolation would have occurred when habitats became fragmented and/or moved geographically during periods of global climatic changes (HIGHTON & WEBSTER 1976). In fact, many species of salamanders show such a large amount of genetic differentiation among populations that it is likely that there is no gene flow operating among many of them (LARSON et al. 1984, but see further discussion). This implies that although amphibians have reasonable dispersal ability, gene flow is limited. The high level of genetic differentiation among amphibian populations has been responsible for controversy in how these populations should be treated taxonomically (e.g., WAKE & SCHNEIDER 1998; HIGHTON 1998, 2000).

Leptodactylus fuscus occurs in open habitats (vegetation with either no canopy or an open canopy), such as the Gran Chaco of southern South America and the cerrados and caatingas of Brazil. The species occurs on riverbanks along the large rivers in the Amazon, but does not occur within the rainforest itself nor along smaller forested rivers (HEYER 1976, GASC & LESCURE 1981, ZIMMERMAN 1991). *Leptodactylus fuscus* also occurs in vacant lots, roadsides, and other disturbed habitats in urban and suburban settings. The species was the first amphibian to reach a 25 ha forest plot that had been clear-cut and burned, reaching the cleared plot by trail from the nearest small town (GASC & LESCURE 1981). Among amphibians, *L. fuscus* is a "weedy" species that would be expected to be a good disperser within any network of open habitats. During the last glacial, *L. fuscus* should have had a

more extensive distribution in Amazonia, as open habitats were more extensive in the Amazon basin than at present (AB'SÁBER 1977). From an ecological perspective, *L. fuscus* would be a good candidate species to demonstrate a single genetic system with any genetic differentiation among samples being accounted for by the isolation by distance model.

There are few studies that have examined gene flow in amphibians, and for the studies that have, several different statistics have been used making direct comparisons of results difficult. There are three studies that do allow rough calibration of gene flow for *L. fuscus*, however. SLATKIN (1993) used the *y*-intercept of $\hat{\theta}$ based $\hat{M}$ values to estimate *Nm* (the product of effective population number and rate of migration among populations), which also is an indication of neighborhood size. The *y*-intercept for the gull *Larus glaucescens* Naumann 1840, an excellent disperser, was approximately 1,300. SLATKIN (1993) did not give the *y*-intercept value for the pocket gopher, *Thomomys bottae* (Eydoux & Gervais 1836), but a visual inspection of his Fig. 11 puts the intercept between 10 and 20 for the set of populations that best fit the isolation-by-distance model. The *y*-intercept for the $\hat{\theta}$ based $\hat{M}$ results for *L. fuscus* is about 18 (for the samples North of the Amazon River that best fit the isolation-by-distance model, the *y*-intercept is about 23). SLATKIN (1981) previously characterized *Thomomys bottae* as having a medium apparent level of gene flow. PETERSON & DENNO (1998) used G_{ST} based $\hat{M}$ values to study genetic differentiation patterns in phytophagous insects. The *L. fuscus* log G_{ST} based $\hat{M}$ – log geographic distance slope for samples north of the Amazon River of – 0.772 and the *y*-intercept value of 1.8 are most consistent with the moderately mobile category of phytophagous insects (PETERSON & DENNO 1998: fig. 3).

The preceding discussion indicates that *Leptodactylus fuscus* would seem to be a species demonstrating moderate dispersal ability with potential for moderate gene flow among populations. Our analyses indicate that this potential for moderate gene flow among populations is only being realized currently for the northern samples that best fit the isolation-by-distance model of differentiation. Our results indicate that there is deeper genetic differentiation among the remainder of the samples included in the study. Gene flow is either reduced or absent among these latter samples.

Genetic differentiation and species limits

In order to evaluate the significance of the genetic partitions or barriers within *Leptodactylus fuscus*, it is useful to compare the level of genetic differentiation in *L. fuscus* with that found in other amphibian species. HIGHTON (1995) has found that a NEI's distance value greater than 0.15 correlates well with morphologically distinct sympatric species of salamanders. SASA et al. (1998) found a threshold of NEI's distance equal to 0.30 for hybrid inviability in a study of 116 crosses of frogs of 46 species reported in the literature.

Several studies have reported considerable genetic differentiation within what were concluded to be single species. GOOD & WAKE (1992) concluded that NEI distance values somewhat over 0.4 were found between some populations of the salamander *Rhyacotriton variegatus* Stebbins & Lowe 1951. RYAN et al. (1996) found a maximum NEI distance value of 0.43 within what they considered a single neotropical species *Physalaemus pustulosus* (Cope 1864).

HIGHTON (2000) interpreted the genetic variation described by GOOD & WAKE (1992) for *Rhyacotriton variegatus* to be taxonomically congruent with recognition of four species, rather than one. The *Physalaemus pustulosus* genetic data of RYAN et al. (1996) indicate that there are four groups of populations with reduced gene flow among them (our interpretation of their data). That is, there are four geographically cohesive groups of samples with low intra-group genetic distances (mean intra-group NEI's distances range from 0.026 to 0.049) separated by abrupt increases in genetic distance (mean inter-group NEI's distances range from 0.150 to 0.360). Furthermore, if genetic and geographic distances are plotted by group, there is little or no evidence that the high inter-group genetic distances result from isolation by distance. If the arguments of HIGHTON (2000) and SASA et al. (1998) were applied to these *Physalaemus* data, at least two and up to four distinct species would be recognized, rather than one.

GASCON et al. (1996, 1998) examined population genetics of five species of frogs along a 1,000 km section of the Rio Juruá in Amazonian Brazil. The results of these studies, together with the *Physalaemus pustulosus* study described above are the only studies we know of that provide within-species genetic estimates of differentiation for neotropical frogs. We find differences between some of GASCON et al.'s (1996, 1998) results and results we obtain from reanalyzing their allozyme data. GASCON et al. (1996) reported a maximum NEI's 1978 distance (NEI 1978) of 0.293 for a pair of populations of *Vanzolinius discodactylus* (Boulenger 1883) based on their allozyme frequency data (GASCON et al. 1996, Table 4); for the same population pair, we obtain a NEI's distance of 1.154. Based on UPGMA phenograms published by GASCON et al. (1998), we interpret maximum NEI's 1978 distance values of just over 0.10 for *Scarthyla ostinodactyla* Duellman & de Sá 1988, about 0.13 for *Scinax rubra* (Laurenti 1768), about 0.20 for *Epipedobates femoralis* (Boulenger 1884 "1883"), and about 0.27 for *Physalaemus petersi* (Jiménez de la Espada 1872). However, we obtain maximum NEI (1978) distance values of 0.036 for *S. ostinodactyla*, 0.147 for *S. rubra*, 0.131 for *E. femoralis* and 0.078 for *P. petersi* (note, however, that for *E. femoralis*, they analyzed samples 2, 4, 5 [no data provided], 7, 9, 12, 17; based on data in Appendix 4, we analyzed samples 2, 4, 7, 9, 12, 13, 17).

The *Vanzolinius discodactylus* data provided by GASCON et al. (1996) are unusual. Excluding Samples 1 and 10, NEI (1978) distance values range from 0.0 to 0.135. Their Sample 10 from Altamira is genetically very distinct with seven genetically fixed differences from all other samples and major frequency differences at three other loci, with NEI's (1978) D values ranging from 1.02-1.15 (our values based on their data). The high NEI's (1978) D values for the Porangaba sample, their Sample 1 (0.18-0.26 excluding Sample 10, their data), also suggests the sample at Porangaba represents a distinct species. Given the exceedingly large NEI's distance value for the Altamira sample in particular, the *Vanzolinius* results might be due to identification, laboratory, experimental, or data transcription error. We think it inadvisable to compare our results with them.

Using our calculations of NEI (1978) distances for GASCON et al.'s (1998) data, *Scarthyla ostinodactyla*, *Scinax rubra*, *Epipedobates femoralis*, and *Physalaemus petersi* all have allozyme variation that falls within that typical of intraspecific variation and less than we find in *Leptodactylus fuscus*. This is not surprising, as the scales of sampling are different with *Leptodactylus fuscus* sampled over distances four times those sampled for the Rio Juruá study.

The levels of differentiation we observe in *Leptodactylus fuscus* approach those found in studies such as for *Rhyacotriton variegatus* (GOOD & WAKE 1992) and

Physalaemus pustulosus (RYAN et al. 1996). As pointed out above, however, there is a difference of opinion whether *R. variegatus* and *P. pustulosus* represent single species or complexes of species.

The *Physalaemus pustulosus* situation is particularly pertinent, as the variation found in RYAN et al.'s (1996) study is similar to that we find for *Leptodactylus fuscus*. *Physalaemus pustulosus* is comprised of between two to four distinct genetic units, which logically could be inferred to represent two to four species (data in RYAN et al. 1996, interpretation ours). *Physalaemus pustulosus* behaviorally comprises a single species, however. The variation in advertisement calls does not demonstrate sharp differences among the genetically isolated units and there is little variation exhibited in the call features that are used as species-coding information in *Physalaemus* (RYAN et al. 1996). The conflict between the genetic and behavioural data sets has at least two interpretations: (1) the behavioral information of advertisement calls provides more reliable assessment of species boundaries than genetic differentiation, with a single species being represented (this is the position taken by RYAN et al. 1996); (2) the genetic differentiation data provide a more reliable estimate of species boundaries (with the consequence that the ancestral advertisement call has been retained in the 2-4 species involved).

The data for *Leptodactylus fuscus* indicate that gene flow is either very restricted or non-existant among several of our samples, including one geographically close pair (Beni and Palos Blancos). Thus, more than one species is contained in the taxon currently known as *Leptodactylus fuscus*. With the exception of the two Bolivian samples, all other sample pairs that demonstrate high values of genetic differentiation from each other are separated by rather substantial geographic distances.

One convincing demonstration of defining species boundaries based on genetic differentiation is for the differentiated units to be characterized by having fixed, unique allele differences. The only cluster of samples in our study that can be defined by a fixed allele is the Trinidad + Tobago + Roraima + French Guiana cluster. Each of the samples in this cluster is fixed for Pgdh *d*, whereas Pgdh alleles *a*, *b*, *c*, *e*, *f*, or *g* are found throughout the remainder of the range of *L. fuscus*. A similar north-south dichotomy is also found with Ldh-1. Although Ldh-1 *c* occurs in the Beni sample, Ldh-1 alleles *c*, *e*, and *f* are otherwise restricted to the northern samples (including Panama), with Ldh-1 alleles *a*, *b*, and *d* restricted to the samples found south of the Amazon. Otherwise, no sample, or group of samples, is fixed for a unique allele, although several polymorphic alleles are geographically restricted.

Our efforts were directed toward obtaining samples throughout the geographic range of *Leptodactylus fuscus*. Many of our samples are separated by considerable geographic distances. Given our limited samples, we are unable to determine how many distinct genetic units are contained within *L. fuscus* and what their geographic distributions are; therefore, taxonomic interpretations based on our data are inappropriate. More detailed sampling is needed to resolve the taxonomy of how many species should be recognized for the populations currently contained in the composite taxon *L. fuscus*. Nevertheless, it is clear from our data that *L. fuscus* is a genetically diverse taxon, likely comprised of several species.

Zoogeography and conservation

The genetic data are consistent with one zoogeographical hypothesis: *Leptodactylus fuscus* (either as a single or multiple species) originated somewhere south

of the Amazon River and its presence north of the Amazon River is due to dispersal. The data that support this hypothesis are (1) the samples south of the Amazon demonstrate greater genetic distances among themselves than those north of the Amazon for comparable geographic distances; (2) the variation among samples north of the Amazon River is much better explained by the isolation-by-distance model than is true for samples south of the Amazon River, and (3) the phylogenetic results are consistent with this hypothesis. For the common ancestor to have originated north of the Amazon and dispersed southwards, the genetic relationships would require that the ancestor was either genetically homogeneous over a widespread area for a long period of time or had a small, genetically uniform population that dispersed southward considerably before expanding its distribution north of the Amazon.

The electrophoretic data can roughly put a time frame on when dispersal likely occurred north of the Amazon. HIGHTON & WEBSTER (1976) reported NEI distance values ranging from 0.000-0.014 (calculated from their reported NEI I values) among six population samples of *Plethodon cinereus* from glaciated areas of North America. Assuming similar rates of evolution, the ranges of NEI D values among the Trinidad samples (0.025-0.034) and Roraima and French Guiana samples (0.016-0.027) suggest that dispersal into this region predated the last glaciation; dispersal among the Roraima localities (0.000-0.002) was probably a post-glacial event. This, in turn, implicates *L. fuscus* being on Trinidad and Tobago when they were connected with the mainland, rather than dispersing there after they became separated from the mainland after the last glaciation event.

We wondered whether the Amazon River itself might be a dispersal barrier for *Leptodactylus fuscus*. Our previous analyses involving geographic distances used straight-line distances between sample pairs. If the Amazon River were a barrier, then dispersal should have been via the western portion of the Amazon. We took Leticia, Colombia as the point through which all north-south sample distances were measured. We also assumed that the Panama sample would disperse around the Andes and that the Tobago sample dispersed from Trinidad, and the Trinidad samples dispersed from the adjacent mainland. The comparison of the NEI distance value matrix with the recalculated geographic distance matrix is significant (Mantel test one tail probability P [random $Z \geq$ observed Z] = 0.002) with a matrix correlation of 0.78. While the matrix correlation is marginally better than that based on straight-line distances (0.74), the y-intercept is marginally higher (= worse for the isolation-by-distance model) at 0.07 (versus 0.06). We find the differences between the straight-line and dispersal distance results to be trivial. This analysis suggests that *L. fuscus* does not experience the Amazon River as a dispersal barrier.

Leptodactylus fuscus, as a "weedy" species among frogs, would be expected to have relatively low levels of genetic diversity. Our conclusions are in striking contrast to this expectation. *Leptodactylus fuscus* demonstrates considerable genetic diversity throughout its geographical range. We have assumed that a "weedy" species is a relatively good disperser and that good dispersers tend to homogenize populations through time. But good dispersal abilities could also enhance differentiation if dispersal occurs across barriers that result in reduced gene flow. If our findings apply to other neotropical frogs, we may be grossly underestimating the amount of diversity in frogs. This conclusion requires much more intensive efforts to measure and conserve biodiversity in the neotropics.

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