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GENETIC STRUCTURE OF THE FROGS *GEOCRINIA LUTEA* AND *GEOCRINIA ROSEA* REFLECTS EXTREME POPULATION DIVERGENCE AND RANGE CHANGES, NOT DISPERSAL BARRIERS

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Abstract.—I describe the genetic structure of two frog species, *Geocrinia rosea* and *Geocrinia lutea*, using allozyme electrophoresis to understand population structure and thereby possible mechanisms of divergence and speciation. The sampling regimes represented the entire range of both species and provided replicated tests of the impact of ridges, rivers, and dry forest on gene flow. *Geocrinia rosea* and *G. lutea* were highly genetically subdivided (F_{ST} = 0.69, 0.64, respectively). In the extreme, there were fixed allelic differences between populations that were only 4 km (*G. rosea*) or 1.25 km (*G. lutea*) apart. In addition to localized divergence, two-dimensional scaling of genetic distance allowed the recognition of broad-scale genetic groups, each consisting of several sample sites. Patterns of divergence were unrelated to the presence of ridges, rivers, or dry forest. I argue that range contraction and expansion, combined with extreme genetic divergence in single, isolated populations, best accounts for the genetic structure of these species.

Key words.—Allozyme electrophoresis, barriers to dispersal, frog, genetic divergence, population genetic structure, range change, southwestern Australia, speciation.

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Goldman and Barton (1992) suggested that we will learn more about evolution by investigating patterns of genetic variation within species than by comparing different species. That is because patterns of genetic variation reflect the population structure of a species, which can influence the likely mechanisms of divergence and speciation. For example, Wright (1982) described the population structure most conducive to his shifting balance model of speciation as one that is geographically subdivided into many small demes that are subject to frequent extinction and recolonization. Other authors have developed speciation models that incorporate particular population structures, and often involve radical genetic change in small, isolated populations (e.g., Mayr 1954; Carson 1975; Lande 1980; Templeton 1980; Wright 1982).

The *Geocrinia rosea* complex consists of four frog species with allopatric distributions in the extreme southwest of Australia (Roberts et al. 1990). The four species occur within a very small geographic range, spanning only 200 km (for map, see Driscoll 1998). This group has provided evidence for speciation within the southwest, opposing the long accepted model of multiple invasions from eastern Australia (Wardell-Johnson and Roberts 1993; Roberts and Wardell-Johnson 1995). Wardell-Johnson and Roberts (1993) suggested that unsuitable habitat between swamps may prevent *Geocrinia* from dispersing. Subtle geographic barriers may contribute to separating the four species and may have contributed to their original speciation (Wardell-Johnson and Roberts 1993). This hypothesis does not specify the mechanism of speciation: whether by vicariance, which is approximated by the classic dumbell model, or other mechanisms that involve critical changes in small populations.

I investigated intraspecific genetic structuring of *G. rosea* and *G. lutea* to elucidate population structure. This enables a comparison of the likely importance for speciation of small, isolated populations versus broad-scale divergence around a geographic barrier. I also examine genetic structuring across three types of geograpic barrier (rivers, ridges, and dry forest) to help determine their impact on gene flow.

METHODS

Geocrinia rosea Complex

Geocrinia rosea, G. lutea, G. alba, and *G. vitellina* together form a monophyletic clade, the *G. rosea* complex in the family Myobatrachidae (Roberts et al. 1990). Adults vary from 17 mm to 28 mm in snout–vent length. Eggs are deposited in frog-sized burrows in soil or rotting wood, beneath leaf litter, or beneath moss. The nonfeeding tadpole stage develops through to metamorphosis entirely within the burrow. Tadpoles are never free swimming and die if their burrow is flooded (Driscoll 1996). In most areas, breeding is confined to distinctive riparian vegetation along drainage lines, although *G. rosea* occasionally breeds in rotting logs in upland sites (Main 1965; Wardell-Johnson and Roberts 1991). All four species have a very patchy distribution across the landscape and generally do not inhabit the entire length of creeks. The density of calling males varies along occupied sections of creeks, with clusters of tens to hundreds of males in seepage zones.

Sampling

Geocrinia rosea.—Twenty-seven populations were sampled from throughout the range of *G. rosea* (Fig. 1). The average sample size was n = 23 (for a full list of sample sizes, see Results). Sites were chosen to represent both the entire range of the species and to investigate possible relationships between forest type and genetic structure. In each of four major drainage systems (Donnelly, Warren, Dombakup, Gardner), two sets of three populations were sampled. The three populations within a set were chosen to be approximately 5 km from one another (range: 2.5–7.5 km; average = 4.75 km). One of the sets in each drainage system had 45–70% tall jarrah (*Eucalyptus marginata*) and marri (*E. calophylla*), or low jarrah and heathlands between sample sites (populations 1, 2, 3; see Results for details). The other set had 95–100% karri (*E. diversicolor*) or karri-marri forest

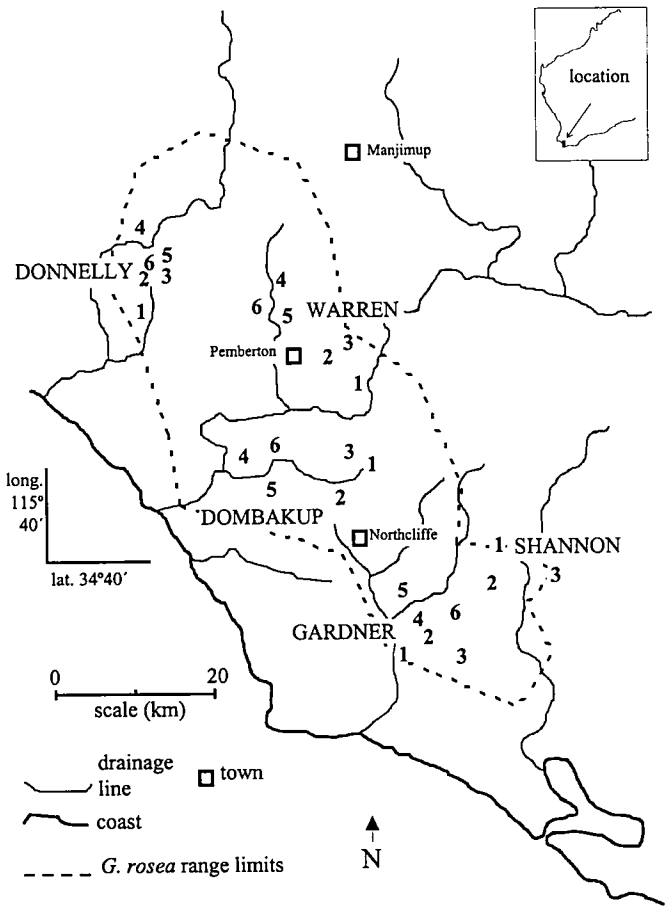

FIG. 1. Distribution of *Geocrinia rosea* and sites sampled in the southwest of Western Australia. Six populations were sampled from each of four river catchments (Donnelly, Warren, Dombakup, and Gardner). Three populations were sampled from the Shannon River catchment. Populations numbered 1, 2, and 3 have 45–75% of the area between them occupied by jarrah, marri, or heathlands. Populations numbered 4, 5, and 6 have 95–100% of the area between them occupied by karri or karri-marri forest.

between its populations (populations 4, 5, 6). The proportion of forest type between each set of populations was estimated from maps of soil types and landforms, each of which corresponds to a particular vegetation type (Churchwood et al. 1988; Churchwood 1992). The litter layer builds up two to three times faster and is wet for a longer period of the year in karri forest compared with jarrah (Christensen and Annels 1985). Karri and karri-marri forest may therefore provide greater opportunities for dispersal, assuming surface moisture level is a factor that influences the success of dispersal. Populations 4, 5, and 6 from each river system can therefore be considered the "wet" sites, whereas 1, 2, and 3 can be considered the "dry" sites. Only three populations were sampled from a fifth drainage system (Shannon River) because there is no continuous karri forest within the catchment. These populations (Shannon 1, 2, 3) fall into the dry forest category, with 50% of the area between populations dominated by jarrah, marri, or heathland.

Geocrinia lutea.—Twenty-eight populations of *G. lutea* were sampled (average n = 24, see Results for all sample sizes). Sites were chosen both to represent the geographic range of this species and as tests of the impact of ridges and rivers as barriers to gene flow (Fig. 2). Nine populations were sampled along the lower reaches of the Frankland River to determine the impact of the river on gene flow. These populations were located in three sets of three (a,b,c; d,e,f; and g,h,i). Each set had one population on the eastern side of the river, and two populations on the western side. The populations within each set were approximately the same geographic distance apart (all less than 2.5 km, see Fig. 2).

Four replicates of sets of three populations were used to investigate the impact of ridges on gene flow (populations j,k,l; m,n,o; p,q,r; s,t,u). Populations within each set were located an approximately equal distance apart (range 0.8–1.5 km). Two of the populations of each set had direct stream connections, whereas the third population was separated from the other two by a ridge. Seven other populations were sampled to ensure the entire range of the species had been examined (populations 1–7).

Frogs of both species were collected from breeding sites between July and December 1993. Three female *G. rosea* and nine female *G. lutea* were captured by chance and the rest were adult males. Two toes were removed from each frog and the toes stored in liquid nitrogen in the field before being transferred to a −70°C freezer. All frogs were returned alive to their point of capture.

Electrophoresis

I performed horizontal starch gel electrophoresis to investigate genetic structuring, using standard techniques (modified from Richardson et al. 1986; Murphy et al. 1990). Thirty-six enzyme systems were screened and 12 had sufficient activity for scoring. These represent 17 presumptive loci (Table 1).

Tables of allele frequencies for all populations at all loci were produced and provided the basis for analysis. Allozymes representing alleles were assigned letters, beginning with the most anodal allozyme. The same lettering system was used in the tables of each species. Enzyme and locus nomenclature follow Murphy et al. (1996).

Analysis

To identify patterns in the distribution of allele frequencies, a two-step approach was used. In the first step, two-dimensional scaling (Belbin 1992) of a Bray and Curtis (1957) distance matrix between all populations was used as an indication of genetic structuring within each species. Scaling methods can be used to examine both hierarchical and linear geographic structuring and are therefore used here, rather than clustering methods such as UPGMA, which impose a hierarchy regardless of whether one exists (Lessa 1990). In the second step, any groups that were ascertained visually using the two-dimensional scaling were compared against the table of allele frequencies to examine their genetic basis.

The magnitude of genetic differentiation was investigated with Wright's (1965) F_{ST}, using the methods of Weir and Cockerham (1984). Jackknifing was used to obtain standard deviations. F_{ST}-values were calculated over all *G. rosea* populations, and over all *G. lutea* populations. In addition, F_{ST}-

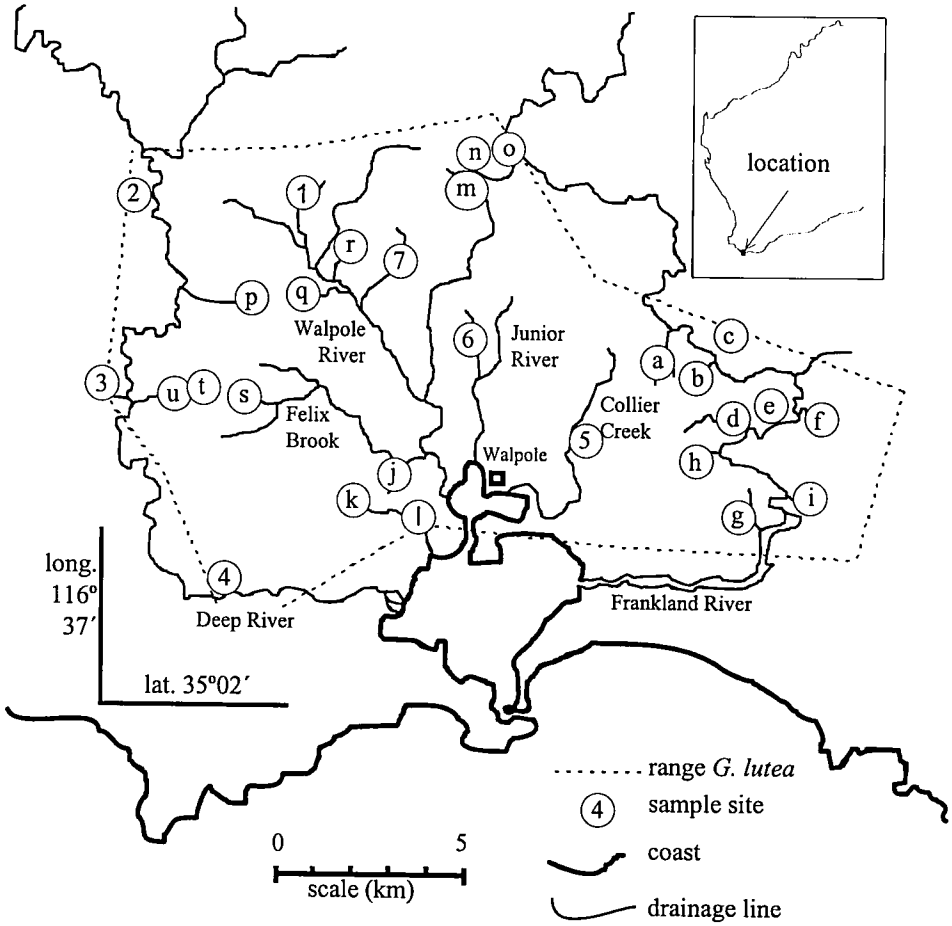

FIG. 2. Distribution of *Geocrinia lutea* sites sampled for genetic analysis in the southwest of Western Australia. Three sets of three populations were used to examine the impact of the Frankland River on genetic structure (sites a–i). Four sets of three populations were used to examine the impact of ridges on genetic structure (sites j–u). Seven additional populations (1–7) were sampled to complete the survey of the range of *G. lutea*.

values were calculated separately for the nine sets of three populations sampled within *G. rosea* to address the magnitude of subdivision at a fine scale. It was also used to investigate the impact of different forest types on gene flow, using the four paired wet and dry forest sets. The significance of differences between paired karri and jarrah sets of populations were estimated using Welche's approximate t for unequal variances in t-tests (Zar 1984).

TABLE 1. Enzyme systems used in electrophoretic study of *G. rosea* and *G. lutea*. TEB = tris-EDTA-borate; TM = tris-maleate.

Enzyme	Locus	Buffer
Leucyltyrosine peptidase	*Ltp*	TEB
Leucylproline peptidase	*Lpp*	TEB
Leucylglycylglycine peptidase	*Lgg*	TEB
Glycerol-3-Phosphate dehydrogenase	*G-3-pdh*	TEB
Glucose-6-phosphate isomerase	*Gpi*	TM
Phosphoglucomutase	*Pgm*	TM
Malate dehydrogenase	*Mdh-1*	TM
	Mdh-2	TM
Phosphogluconate dehydrogenase	*Pgdh*	TM
Malic enzyme	*Me-1*	TM
	Me-2	TM
Aspartate aminotransferase	*Aat-1*	TM
	Aat-2	TM
Lactate dehydrogenase	*Ldh-1*	TM
	Ldh-2	TM
Isocitrate dehydrogenase	*Idh-1*	TM
	Idh-2	TM

Welche's approximate t was also employed in the analysis of the impact of ridges and creeks on dispersal in *G. lutea*. For each set of three populations, an F_{ST} (weighted mean over variable loci) was calculated for populations on either side of a ridge and compared with the average F_{ST}-value from populations on the same side. In addition, allele frequencies at each locus were tested for significant differences using a Monte Carlo procedure (Engles 1988) for populations on either side of a ridge and for those on the same side. This procedure tests for heterogeneity among frequencies: when used on samples from two sites it provides a specific test for differences between them. Monte Carlo tests were used in preference to chi-squared tests due to the low frequency of many alleles. If the allele frequencies at a locus were significantly different, the F_{ST}-value for that locus was considered significantly different from zero. This permits a locus-by-locus assessment of the patterns, thereby allowing the significance of overall trends to be assessed more fully than could be achieved using the averaged F_{ST}-values.

TABLE 2. F_{ST}-values for each locus calculated over each of the 27 *G. rosea* and 28 *G. lutea* populations. The weighted means (Weir and Cockerham 1984) are shown.

Locus	*G. lutea*	*G. rosea*
Ltp	0.228	0.422
Lpp	0.042	0.993
Lgg	0.071	0.021
G-3-pdh	0.030	0.072
Gpi	0.872	0.218
Pgm	0	0.750
Mdh-1	0	0.729
Mdh-2	0.088	0
Pgdh	0.692	0.843
Me-1	0	0.521
Me-2	0	0.344
Aat-1	0	0.760
Aat-2	0.601	0
Ldh-1	0	−0.004
Ldh-2	0.669	0
Idh-1	0	0.679
Idh-2	0	0
Mean	0.644	0.690
SD	0.087	0.070

The impact of a river on dispersal by *G. lutea* was analysed by visual examination of the table of allele frequencies for the nine Lower Frankland River populations. No further analysis was possible because there was no genetic variation.

Throughout this paper F_{ST} was used as an estimate of gene flow, based on the relationship between F_{ST} and Nm, the product of effective population size and average number of immigrants (Wright 1931). In view of the inherent inaccuracies in estimating Nm (Slatkin and Barton 1989; Whitlock 1992), F_{ST}-values were used as a qualitative indication of the magnitude of gene flow. Following Porter's (1990) general guide: $F_{ST} < 0.2$ ($Nm > 1$), gene flow is important in promoting genetic similarity; $0.2 < F_{ST} < 0.33$ ($0.5 < Nm < 1$), gene flow is weak, but would permit exchange of alleles; $F_{ST} > 0.33$ ($Nm < 0.5$), gene flow is unimportant and populations are more or less completely isolated. Slatkin's (1981, 1985) private alleles method was not used to estimate gene flow because there were a limited number of loci (Slatkin and Barton 1989), there were only a small number of private alleles (Slatkin 1985), and the potential confounding problems caused by relictual private alleles (Whitkus and Crawford 1987). Calculations of F_{ST}-values and their standard deviations were made using Biosys-1 (rel. 1.7; Swofford and Selander 1981; Swofford 1989).

RESULTS

Magnitude of Genetic Differentiation

The weighted mean F_{ST}-values for *G. rosea* and *G. lutea*, respectively are 0.690 (SD = 0.070) and 0.644 (SD = 0.087; Table 2).

The F_{ST}-values within each of the catchments of *G. rosea* indicate that there is substantial genetic subdivision at a very small geographic scale (Table 3). This is particularly highlighted by the Donnelly dry (1–3) and Gardner dry (1–3) *G. rosea* populations, which have F_{ST}-values of 0.54 (SD = 0.24) and 0.68 (SD = 0.23), respectively. The geographic distances between populations in these groups range 2.5–7.5 km and 4.3–7.5 km, respectively.

The tables of allele frequencies of both species also highlight the enormous genetic differences found between populations at a scale of less than 7.5 km (Tables 4, 5). The most extreme example in *G. rosea* is the fixed difference for the *Aat-1* locus at Shannon 1, compared with 2 and 3, which are only 4 km and 7.5 km from Shannon 1, respectively. In *G. lutea* there is a fixed difference at the *Gpi* locus between populations q and r, which are only 1.25 km apart. In addition, the large differences are demonstrated by the restricted distributions of some alleles. Within *G. rosea* (Table 4) there are eight alleles unique to a single population (*Aat-1*[*a*], *Idh-1*[*f*], *Ldh-1*[*d*], *Ldh-1*[*e*], *Lgg*[*b*], *Me-2*[*a*], *Gpi*[*c*], *Pgm*[*c*]). All except the *Aat-1*, *Me-2*, and *Gpi* alleles are rare (frequency < 0.05) and so may occur at other sites but were not detected. In addition, *Idh-1*(*g*) and *Lpp*(*a*) occur in only two populations (*Lpp*[*a*] is rare in both populations) and four alleles are found in only three populations (*G-3-pdh*[*c*], *Aat-1*[*d*], *Idh-1*[*a*], *Me-1*[*e*]) none of which were rare in all three populations. For *G. lutea* (Table 5), there are two alleles found in only one population (*Lgg*[*b*], *Lpp*[*c*], both rare), five alleles that occur in only two populations (*Lgg*[*e*], *Mdh-2*[*c*], *Pgdh*[*d*], *G-3-pdh*[*a*], *Lpp*[*a*], the latter two are rare in both populations), and one allele found in three populations (*G-3-pdh*[*c*], rare in all three).

Pattern Analysis and Its Genetic Basis

Geocrinia rosea.—Populations from the Gardner and Shannon River catchments form a distinct group (Fig. 3). Differ-

TABLE 3. Proportion of forest type in the area separating the three sample sites in each of the nine sample areas (from Churchwood et al. 1988; Churchwood 1992) and magnitude of differentiation (F_{ST}) among the nine sets of three populations (see Methods for description of area codes and Fig. 1). The probability that the F_{ST}-values of paired wet (populations 4, 5, 6) and dry (populations 1, 2, 3) sites are the same is shown. The F_{ST} for the Shannon populations is based on the single variable locus (*Aat-1*).

River	Pop.	Karri	Karri-marri	Tall jarrah-marri	Low jarrah-heath	Mean F_{ST}	SD	P (*t*-test)
Donnelly	1, 2, 3	0%	55%	45%	0%	0.540	0.236	
	4, 5, 6	60%	35%	5%	0%	0.242	0.074	$P < 0.1$
Warren	1, 2, 3	10%	20%	45%	25%	0.214	0.166	
	4, 5, 6	50%	50%	0%	0%	0.171	0.067	$P > 0.5$
Dombakup	1, 2, 3	0%	50%	30%	20%	0.067	0.055	
	4, 5, 6	15%	80%	0%	5%	0.221	0.055	$P < 0.001$
Gardner	1, 2, 3	0%	30%	15%	55%	0.684	0.228	
	4, 5, 6	0%	100%	0%	0%	0.086	0.050	$P < 0.05$
Shannon	1, 2, 3	0%	50%	35%	15%	1.000	—	

TABLE 4. Allele frequencies and heterozygosity (direct count) for populations of *G. rosea*. All populations were fixed for *Mdh-2(b)*, *Aat-2(b)*, *Ldh-2(b)*, and *Idh-2(b)* (not shown on table). Dl = Donnelly River, Wr = Warren River, Db = Dombakup Brook, Gr = Gardner River, S = Shannon River.

		Dl-1	Dl-2	Dl-3	Dl-4	Dl-5	Dl-6	Wr-1	Wr-2	Wr-3	Wr-4	Wr-5	Wr-6	Db-1	Db-2	Db-3	Db-4	Db-5	Db-6	Gr-1	Gr-2	Gr-3	Gr-4	Gr-5	Gr-6	S-1	S-2	S-3
	(*n*)	20	23	24	21	23	24	24	32	24	24	24	24	24	24	24	22	22	24	24	24	24	24	24	24	24	12	20
Locus																												
Aat-1	*a*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	c	1	1	1	1	1	1	1	1	1	1	1	1	1	0.92	1	0.55	0.93	1	1	1	1	1	1	1	0	1	1
	d	0	0	0	0	0	0	0	0	0	0	0	0	0	0.08	0	0.46	0.07	0	0	0	0	0	0	0	0	0	0
G-3-pdh	*b*	1	1	1	1	0.89	1	1	1	1	1	1	1	1	0.90	1	1	1	1	1	1	1	0.96	1	1	1	1	1
	c	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0.10	0	0	0	0	0	0	0	0.04	0	0	0	0	0
Idh-1	*a*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.14	0.14	0.21	0	0	0	0	0	0	0	0	0
	b	1	1	1	1	1	1	0.56	0.72	0.46	1	1	1	1	0.63	0.92	0.32	0.09	0.52	1	0.17	1	1	0.02	0	0	0	0
	e	0	0	0	0	0	0	0.44	0.28	0.54	0	0	0	0	0.38	0.08	0.36	0.66	0.27	0	0.79	0	0	0.98	1	1	1	1
	f	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0
	g	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.18	0.11	0	0	0	0	0	0	0	0	0	0
Ldh-1	*c*	1	1	1	1	1	1	1	0.98	1	1	1	0.98	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	d	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	e	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lgg	*b*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0
	c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.96	1	1	1	1	1	1	1
Lpp	*a*	0	0	0	0	0.02	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	1	0.98	1	1	1	1	1	1	1	0.98	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Ltp	*a*	0.80	1	1	1	1	1	0.15	0.97	0.60	0.75	0.23	0.44	0.79	0.71	0.75	1	0.75	0.54	1	1	1	1	1	1	1	1	1
	b	0.20	0	0	0	0	0	0.85	0.03	0.40	0.25	0.77	0.56	0.21	0.29	0.25	0	0.25	0.46	0	0	0	0	0	0	0	0	0
Mdh-1	*a*	0	0	0	0	0	0	0.02	0	0	0	0.15	0	0.02	0.04	0.04	0.07	0	0.06	0	0	0	0	0	0	0	0	0
	b	0	0	0.02	0	0	0	0.90	0.75	0.73	0.10	0.19	0.25	0.98	0.96	0.96	0.68	0.73	0.90	1	1	1	1	1	1	1	1	1
	c	1	1	0.98	1	1	1	0.08	0.25	0.27	0.90	0.67	0.75	0	0	0	0.25	0.27	0.04	0	0	0	0	0	0	0	0	0
Me-1	*a*	0.98	0.53	0.23	0.45	0.39	0.10	0.02	0	0	0.92	0.56	0.88	0.39	0.33	0.29	0.73	0.48	0.29	0.29	0.40	1	0.98	1	1	1	1	1
	b	0.03	0.43	0.42	0.29	0.54	0.85	0.98	1	1	0.08	0.44	0.13	0.61	0.67	0.71	0.27	0.52	0.71	0	0	0	0	0	0	0	0	0
	d	0	0.03	0.35	0.26	0.07	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.71	0.60	0	0.02	0	0	0	0	0
Me-2	*a*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.36	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.64	1	1	1	1	1	1	1	1	1	1
Pgdh	*f*	0	0	0	0	0	0	0.13	0.13	0.04	0	0	0	0	0.29	0.25	0.05	0	0.56	1	1	1	1	1	1	1	1	1
	g	1	1	1	1	1	1	0.88	0.88	0.96	1	1	1	1	0.71	0.75	0.96	1	0.44	0	0	0	0	0	0	0	0	0
Gpi	*b*	1	1	1	1	1	1	0.65	0.66	0.85	1	0.98	1	1	1	0.85	1	0.66	0.88	1	1	1	1	1	0.88	1	1	1
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0	0
	d	0	0	0	0	0	0	0.35	0.34	0.15	0	0.02	0	0	0	0.15	0	0.34	0.13	0	0	0	0	0	0	0	0	0
Pgm	*a*	0.98	0.09	0.10	0.74	0.13	0.50	1	0.98	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	b	0.03	0.91	0.90	0.26	0.87	0.50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H		0.02	0.04	0.05	0.05	0.04	0.04	0.11	0.08	0.09	0.04	0.06	0.06	0.03	0.11	0.10	0.13	0.13	0.15	0.02	0.06	0	0.01	0	0.01	0	0	0
SE		0.01	0.03	0.04	0.04	0.02	0.03	0.05	0.03	0.04	0.02	0.03	0.04	0.02	0.04	0.04	0.06	0.04	0.06	0.02	0.04	0	0.01	0	0.01	0	0	0

TABLE 5. Allele frequencies and average heterozygosity (direct count) for populations of *G. lutea*. All populations were fixed for *Pgm(a), Mdh-1(c), Me-1(a), Me-2(b), Aat-1(c)*. D = Deep River, I = Isle Creek, Fx = Felix Brook, F = Frankland River, W = Walpole River, C = Collier Creek, J = Junior River.

		D 2	D p	D t	D u	D 3	D 4	I k	I l	Fx j	Fx s	W q	W r	W l	W 7	W m	F o	F n	C 5	J 6	F a	F b	F c	F d	F e	F f	F g	F h	F i
	(*n*)	24	24	24	25	23	24	26	24	24	25	24	24	26	25	24	24	24	24	26	24	24	24	24	25	22	24	21	23
Locus																													
Aat-2	*a*	1	0.98	0.26	0.66	1	0.02	0.39	0.85	0.77	0.46	1	1	1	1	1	1	1	0.96	1	1	1	1	1	1	1	1	1	1
G-3-	*b*	0	0.02	0.74	0.34	0	0.98	0.62	0.15	0.23	0.54	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0
pdh	*a*	0	0	0	0.04	0	0	0	0	0	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	0.94	1	1	1	1	1	0.94	1	1	1	1	1	1	1	1	1	0.96	0.94	1	1	1	1	1	1	1
	c	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04	0.06	0	0	0	0	0	0	0
Ldh-2	*a*	0	0	0.19	0.24	0	0.08	0.29	0.06	0.44	0.06	0	0.31	0.35	0.86	0.29	0.13	0.25	0.06	0.79	1	1	1	1	1	1	1	1	1
	b	1	1	0.81	0.76	1	0.92	0.71	0.94	0.56	0.94	1	0.69	0.65	0.14	0.71	0.88	0.75	0.94	0.21	0	0	0	0	0	0	0	0	0
Lgg	*b*	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	0.94	1	1	1	1	1	1	1	1	0.96	1	0.88	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	e	0.06	0	0	0	0	0	0	0	0	0	0	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lpp	*a*	0	0	0	0	0	0.08	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	0.98	1	0.92	1	1	1	0.98	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	c	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ltp	*a*	0.94	0.63	0.60	0.72	0.96	0.69	0.62	0.92	0.73	0.62	0.83	0.58	0.50	1	1	1	1	1	0.77	1	1	1	1	1	1	1	1	1
	b	0.06	0.38	0.40	0.28	0.04	0.31	0.39	0.08	0.27	0.38	0.17	0.42	0.50	0	0	0	0	0	0.23	0	0	0	0	0	0	0	0	0
Mdh-2	*b*	1	1	1	1	1	0.88	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.93	1
	c	0	0	0	0	0	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.07	0
Pgdh	*a*	0	0	0	0.02	0	0	0.12	0.04	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	1	1	0.88	0.54	1	0.52	0.85	0.60	0.48	0.70	0.98	0.19	0	0.26	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0
	d	0	0	0	0.22	0	0	0	0	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	f	0	0	0.13	0.22	0	0.48	0.04	0.35	0.44	0.30	0.02	0.81	1	0.72	1	1	1	1	0.96	1	1	1	1	1	1	1	1	1
Gpi	*b*	1	1	0.98	0.98	1	0.88	0.98	0.92	0.81	0.98	1	0	0.02	0	0	0	0	1	0.69	1	1	1	1	1	1	1	1	1
	c	0	0	0.02	0.02	0	0.13	0.02	0.08	0.19	0.02	0	1	0.98	1	1	1	1	0	0.31	0	0	0	0	0	0	0	0	0
	H	0.02	0.02	0.08	0.10	0	0.10	0.08	0.06	0.12	0.09	0.02	0.09	0.07	0.04	0.02	0.02	0.02	0.01	0.05	0.01	0.01	0	0	0	0	0	0.01	0
	SE	0.01	0.02	0.04	0.04	0	0.04	0.04	0.03	0.05	0.04	0.02	0.04	0.04	0.03	0.02	0.02	0.02	0.01	0.03	0.01	0.01	0	0	0	0	0	0.01	0

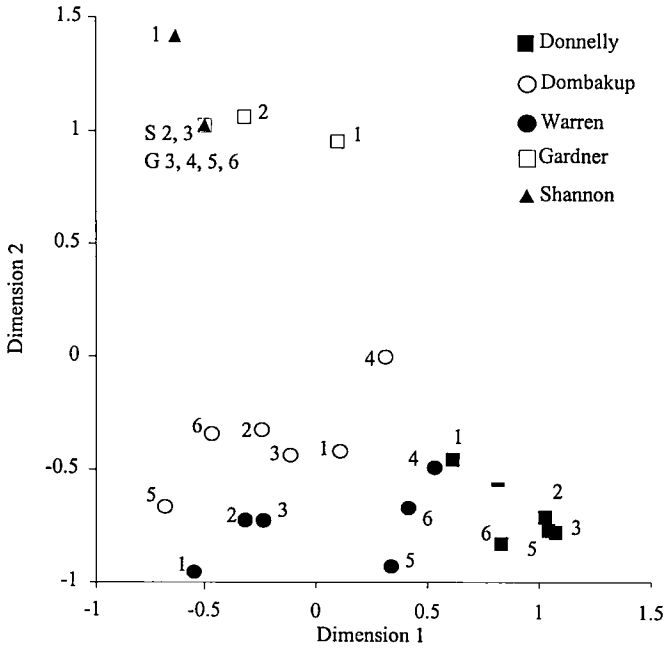

FIG. 3. Two-dimensional scaling of a Bray and Curtis distance matrix derived from allele frequencies for *Geocrinia rosea* populations from five river catchments. Population numbers are indicated. Stress = 0.086 (satisfactory, Belbin 1992).

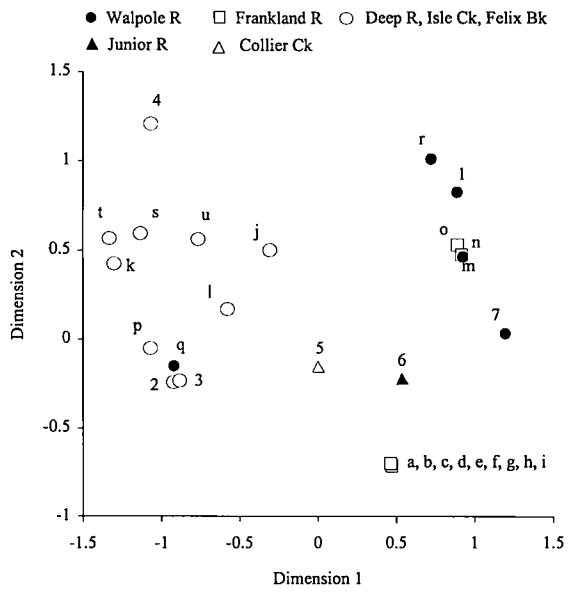

FIG. 4. Two-dimensional scaling of a Bray and Curtis distance matrix derived from allele frequencies for all *Geocrinia lutea* populations. Population numbers/letters are indicated. Stress = 0.087 (satisfactory, Belbin 1992).

ences at many loci have led to this dichotomy. The Gardner and Shannon populations are delimited from the rest of the species by a fixed difference at the *Lpp* locus and the absence of alleles found in other parts of the species range at most other variable loci (*Ltp*[*b*]; *Lpp*[*a*], *Lpp*[*b*]; *Gpi*[*d*]; *Pgm*[*b*], *Pgm*[*c*]; *Mdh-1*[*a*], *Mdh-1*[*c*]; *Pgdh*[*g*]; *Me-1*[*b*]; *Me-1*[*d*]; *Me-2*[*a*]; *Aat-1*[*d*]; *Ldh-1*[*d*]; *Ldh-1*[*e*]; *Idh-1*[*a*]; *Idh-1*[*g*]; Table 4). Most Gardner and Shannon populations have very low heterozygosity (Table 4). There are also fixed differences between all of the Gardner and Shannon populations compared with some populations from other catchments (locus *Mdh-1* for Donnelly 1, 2, 4, 5, 6; locus *Pgdh* for all six Donnelly populations, Warren 4, 5, 6, and Dombakup 1, 5; locus *Me-1* for Warren 2, 3).

Populations in the Donnelly River may group together with the Warren wet (4–6) populations, whereas Warren 1–3 may group with those from Dombakup Brook (Fig. 3). In support of this grouping, the absence of the *Idh*(*e*) and *Pgdh*(*f*) alleles and the rarity of *Gpi*(*d*) suggest Warren 4–6 are more similar to populations in the Donnelly River than to populations in the Warren and Dombakup catchments. However, there are also allelic characteristics that seem to oppose the grouping suggested in Figure 3. Warren 4–6 are missing *Me-1*(*d*) and *Pgm*(*b*) alleles, which are characteristic of populations in the Donnelly catchment (with the exception of Donnelly 1 for *Me-1*[*d*]). In addition, Warren 4–6 have *Ltp*(*b*) and *Mdh-1*(*b*) alleles, which are characteristic of populations in the Warren and Dombakup catchments and are generally absent from the Donnelly populations.

Although there may not be a second bifurcation among the *G. rosea* populations, the Donnelly, Warren, and Dombakup populations are not randomly distributed throughout the two-dimensional space (cf. Figs. 1 and 3). The genetic patterns reflect the geographic location of samples, although it appears that there has been some intergradation of allelic characters between the northern catchments of *G. rosea*. While the Donnelly populations have some characteristic alleles, population 1 is a notable exception with the absence of *Me-1*(*d*) and the presence of *Ltp*(*b*). These traits are commonly observed among Warren and Dombakup populations. In addition, Warren 4–6 are geographically and genetically located between the Donnelly and the other Warren populations (Fig. 1, Table 4).

Geocrinia lutea.—Three clusters and two intermediate populations (5, 6) are evident in Figure 4 and these groups are supported by the distribution of allele frequencies (Table 5). The Lower Frankland populations (a–i) form a tight group because all nine populations are fixed or nearly fixed for the same allele at every locus. Two populations from the upper Frankland (n and o) clump together with most of the populations in the Walpole River (m, r, 1, and 7; Fig. 4). Members of this group (Walpole group) are fixed or nearly fixed for *Gpi*(*c*) and *Pgdh*(*f*), and are fixed for the *Aat-2*(*a*) allele. The third group encompasses populations from the Deep River, Felix Brook, and Isle Creek (2, 3, 4, j, k, l, p, s, t, u) and has one population from just over the ridge, in the Walpole River catchment (q; Fig. 4). These populations form the Deep River group. They all have relatively low frequencies of *Pgdh*(*f*), and most populations are fixed or have very high frequencies of *Gpi*(*b*). In addition, *Aat-2*(*b*) is present in most of the populations, whereas it is absent from members of the other two groups. All of the populations in this group have *Ltp*(*b*), which is absent from most of the Walpole group and all of the Lower Frankland group.

There is some intergradation between the Walpole and Deep groups. The *Ltp*(*b*), *Gpi*(*b*) and *Pgdh*(*c*) alleles occur in all members of the Deep group and are found in up to two of the geographically closest populations in the Walpole

TABLE 6. F_{ST}-values for each locus and the average F_{ST} for four sets of populations, each comparing genetic divergence across a ridge and along a creek (see Fig. 2 for location of each *G. lutea* population). Allele frequencies within each pair were tested for significant differences using a Monte Carlo procedure (ns = not significant). Dashes indicate allele frequencies were identical. Average weighted mean F_{ST}-values (Weir and Cockerham 1984) were tested for significant differences using a *t*-test and probabilities are shown.

	Ridge j–k	Creek k–l	Ridge m–n	Creek n–o	Ridge p–q	Creek q–r	Ridge s–t	Creek t–u
Ltp	0.01 ns	0.21	—	—	0.08	0.12 ns	−0.02 ns	0.01 ns
Lpp	—	—	—	—	—	—	−0.00 ns	0.00 ns
Lgg	—	—	—	—	—	0.11	0.02 ns	—
G-3-pdh	—	—	—	—	—	—	0.04 ns	0.00 ns
Gpi	0.13	0.02 ns	—	—	—	1.00	—	—
Pgdh	0.26	0.16	—	—	0.00 ns	0.78	0.06	0.15
Aat	0.24	0.35	—	—	0.00 ns	—	0.05 ns	0.24
Ldh	0.03 ns	0.14	−0.02 ns	0.03 ns	—	0.30	0.06 ns	−0.01 ns
Average F_{ST}	0.15	0.23	−0.03	0.03	0.13	0.73	0.03	0.11
SD	0.07	0.06			0.07	0.23	0.02	0.07
P (*t*-test)		ns		—		0.05		0.02

group. In addition, populations in the Junior (6) and Collier (5) Rivers exhibit allelic characteristics of both groups. Population 5 has a high frequency of *Pgdh(f)* and does not have *Ltp(b)*, like the Walpole group. However, it is also fixed for *Gpi(b)* and has *Aat-2(b)* like the Deep group. Population 6 has a high frequency of *Pgdh(f)* and does not have *Aat-2(b)*, like the Walpole group, but it also has a high frequency of *Gpi(b)* and has *Ltp(b)*, like populations in the Deep group.

Barriers to Dispersal

The Influence of Forest Type on Gene Flow: Geocrinia rosea.—There are no consistent patterns of divergence among populations in wet and dry forest (Table 3). In the Dombakup Brook, populations within karri (wet) forest had a significantly higher F_{ST} than those with a high proportion of jarrah (dry) forest between them. However, the opposite pattern was observed among populations from the Gardner and Donnelly Rivers. The difference was significant in the Gardner River catchment. In the Warren River, populations were equally differentiated in both karri and jarrah forest. The results for the Shannon and Dombakup dry populations emphasize the lack of correlation between forest type and genetic differentiation. Very similar proportions of each forest type were encompassed within the Shannon and Dombakup dry areas, yet they had F_{ST}-values of opposite extremes (Table 3).

Ridges as Barriers to Dispersal: Geocrinia lutea.—There were no consistent patterns in the effects of dry ridges on allelic divergence. There were no significant differences in allele frequencies in the cross-ridge or along creek comparisons in the *G. lutea* m,n,o set of populations (Table 6). There were no consistent differences among loci in the k,j,l set of populations; therefore, the average F_{ST} across the ridge was not significantly different from the F_{ST} along the creek. In the p,q,r and s,t,u sets, the average F_{ST} along the creek was significantly higher than the F_{ST} across the ridge. However, in the s,t,u set the difference was based on divergence at only two loci (*Pgdh* and *Aat*). The p,q,r set included populations from two of the genetic groups identified above (Walpole and Deep).

Rivers as Barriers to Dispersal: G. lutea Lower Frankland River.—The nine Lower Frankland populations were fixed or almost fixed for the same allele at all loci (Table 5). It was therefore not possible to detect differences in gene flow over land as compared to across the river. Panmixis or complete isolation of populations would give the same result under these circumstances. However, genetic uniformity is not a phenomenon peculiar to the drainage system: the two northern Frankland River populations (n, o) were genetically more similar to frogs in the Walpole River.

DISCUSSION

Genetic Differentiation and Dispersal

Geocrinia rosea and *G. lutea* are among the most highly genetically subdivided species known. Their F_{ST}-values (0.69 and 0.64, respectively) are above the average F_{ST} (0.53, SD = 0.19) reported by Larson et al. (1984) for 22 species of salamanders, a group renowned for the magnitude of their genetic differentiation. Only four of the 22 salamander species exceeded 0.69, while nine exceeded 0.64. Of those, only one study was at a similar geographic scale to the present study (*Plethodon ouachitae*, 70 km, F_{ST} = 0.67; Duncan and Highton 1979). The remainder either had smaller F_{ST}-values or the studies were carried out at a scale one or two orders of magnitude larger than the tens of kilometers used in this study. Nevertheless there are species that are more subdivided: the annual plant *Limnanthes floccosa* has 96% of its total genetic diversity partitioned among populations and it occurs at a spatial scale comparable to *G. rosea* or *G. lutea* (Dole and Sun 1992).

The average F_{ST}-values for both *G. rosea* and *G. lutea* far exceed the value that Porter (1990) and Wright (1931) suggested for little or no gene flow ($F_{ST} > 0.33$). *Geocrinia rosea* and *G. lutea* are very unlikely to disperse between sample sites. This conclusion is supported by direct estimates of dispersal in *G. alba* and *G. vitellina*. *Geocrinia alba* and *G. vitellina* have very low dispersal tendencies and they have a similar degree of genetic structuring to *G. rosea* and *G. lutea* (Driscoll 1997, 1998).

Extremely low dispersal between sites in *G. lutea* and *G. rosea* explains the lack of relationship between geographic barriers (dry forest, ridges, rivers) and genetic structure. The impact of barriers could only be assessed if dispersal rates were the key factor promoting or inhibiting divergence.

Therefore, population genetic structure is more likely to be the product of factors other than contemporary patterns of movement.

Evolution in Geocrinia

If current patterns of dispersal do not explain the intraspecific genetic groups, what does? Natural selection is an unlikely candidate. Although different selection regimes in two areas could juxtapose populations of different allele frequencies (Endler 1973, 1977; Johannesson and Tatarenkov 1997), I found no obvious selection gradient that would explain the genetic structure of *G. rosea* or *G. lutea*. Wardell-Johnson and Roberts (1993) found that soils were similar throughout the range of the *G. rosea* complex. All of the sites they examined had extremely low pH, high carbon and water content, and similar textural properties (Wardell-Johnson and Roberts 1993). The intraspecific genetic pattern also does not correspond with the main climatic gradient (Wardell-Johnson and Roberts 1993). Within the range of *G. lutea* there is a weak east-west rainfall cline (1250–1400 mm), which roughly corresponds to the major genetic groupings. *Geocrinia rosea* experiences a slightly stronger rainfall gradient, from 1100 mm on the northeast border, to 1400 mm along the southwest border. However, this rainfall cline is perpendicular to the major genetic changes. In addition, abrupt changes in alleles present, or in allele frequency do not correspond with changes in vegetation (Churchwood et al. 1988; Churchwood 1992) or other known environmental factors. Therefore, there is no evidence that spatially varying natural selection explains divergence within *G. rosea* or *G. lutea*. Although past selection cannot be precluded as a possible explanation for divergence, it seems unlikely that natural selection would have acted on so many presumably independent loci.

In the absence of strong natural selection and with very low contemporary dispersal rates, any patterns within the data may represent historic range changes (Arter 1990). Range changes commonly influence the genetic structure of species. Examples include patterns of chromosomal arrangements in a wingless grasshopper (Hewitt and Barton 1980); mitochondrial DNA haplotype variation in cicadas (Martin and Simon 1990), mountain sheep (Ramey 1995), kiwis (Baker et al. 1995), and rainforest birds (Joseph et al. 1995); morphological variants in frogs (Martin 1972); and allozyme divergence in kiwis (Baker et al. 1995), salamanders (Highton and Webster 1976), and frogs (Highton and Hedges 1995; Green et al. 1996).

Support for a model of range change is provided by the very low levels of heterozygosity in the Gardner and Shannon populations of *G. rosea* and in the Lower Frankland populations of *G. lutea*. Low heterozygosity over a broad geographic range probably indicates the area was recently colonized from one or a small number of populations that had low genetic variation (e.g., Highton and Webster 1976; Larson et al. 1984; Martin and Simon 1990; Highton and Hedges 1995). The source population for range expansion is more likely to have been a population isolate, rather than a central pool of individuals. Colonization from a central pool of populations could lead to lower genetic variation over substantial geographic areas if alternative alleles were excluded by selection (Hewitt and Barton 1980; Green et al. 1996). However, I argued above that natural selection is an unlikely explanation of genetic structuring. It is also doubtful that long-distance dispersal could explain propagule formation, given the extreme genetic structuring and inferred low dispersal reported here.

A likely evolutionary scenario for southern *G. rosea* includes range contraction into an isolated population that became fixed for *Lpp(c)* and lost variation at many other loci, followed by range expansion to form the contemporary distribution. Subsequent to the expansion phase, populations have become isolated and undergone divergence (e.g., *Idh-1* locus in Gardner River populations). A similar scenario is likely to explain the other genetic clusters of populations within both species. However, the model needs to be expanded to include exchange of alleles upon recontact of the Deep and Walpole groups of *G. lutea* and the Warren and Donnelly populations of *G. rosea*.

Although speculative, it seems likely that range changes have been driven by climatic fluctuations. Sturman and Tapper (1996) report that fluctuations in rainfall and temperature are common throughout history in southern Australia. However, it is difficult to assign particular climatic events and times to key range changes in *Geocrinia* because the impact of climatic shifts of particular magnitudes is not known and molecular clocks are unlikely to be accurate enough at the time scale of interest (Thorpe 1982; Driscoll 1998).

The genetically uniform Lower Frankland group of *G. lutea* implies that frogs must have traversed the Lower Frankland River at some time in the past. Therefore the river does not present a long-term barrier to dispersal. Howard et al. (1983) came to a similar conclusion in regard to four populations of the larch mountain salamander (*Plethodon larselli*). Although there was probably no recent gene flow between the four sample populations, low genetic distances between populations on opposite sides of the Columbia River implied there may have been dispersal across the river in the recent past. Dispersal may have occurred at a time of low river flow (Howard et al. 1983). In another example, Patton et al. (1994) concluded the Rio Jurua had not been a significant barrier to dispersal of arboreal spiny rats in the Amazon Basin. Like the *G. lutea* results, Patton et al. (1994) found that genetic groups spanned the river, implying dispersal had not been constrained by the water body. However, the possible impact of rivers on genetic structure should not be dismissed in other species: Easteal and Floyd (1986) suggested the Brisbane River may have slowed the spread of *Bufo marinus* following their introduction into Australia.

The extreme genetic structuring of *G. lutea* and *G. rosea* provides valuable insight into the evolution of the *G. rosea* species complex. Ridges, rivers, and dry forest appear to have little influence over divergence because these species have very low dispersal tendencies, regardless of surrounding geography. Despite low dispersal, both species appear to have undergone extensive range changes. It is likely that the evolutionarily potent combination of extreme population divergence and distribution changes have produced distinct genetic groups within these species and perhaps, ultimately, may be important in promoting speciation.

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