A NARROW HYBRID ZONE BETWEEN TWO WESTERN AUSTRALIAN FROG SPECIES *RANIDELLA INSIGNIFERA* AND *R. PSEUDINSIGNIFERA*: THE EXTENT OF INTROGRESSION

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SUMMARY

Species compositions of populations along a transect across a narrow hybrid zone between *Ranidella insignifera*‡ and *R. pseudinsignifera*‡ were determined using diagnostic enzyme loci and multivariate analysis of skeletal parameters. These were compared with subjective assessments of male mating call choruses in the same populations. The three independent characters all demonstrated a sharp transition from one species to the other over a distance of 2·4 km indicating some barrier to gene flow between the species beyond this narrow hybrid zone. It is suggested that the contact between the two species is a relatively old and stable one. Possible explanations for the maintenance of the 480 km parapatric boundary between the two species are examined.

1. INTRODUCTION

MANY pairs of closely related species or races are now known to have narrow hybrid zones between contiguous allopatric distributions (Hewitt, 1975) and it has been suggested that some of these have remained stable for very long periods (Yang and Selander, 1968; Rising, 1970; Littlejohn, Watson and Loftus-Hills, 1971; Watson, 1972; Hunt and Selander, 1973; Key, 1974). In attempting to understand the dynamics of these hybrid zones and the mechanisms maintaining them the amount of gene flow across the zone must be measured. There is, however, some danger in judging the extent of introgression between two taxa by the width of the hybrid zone measured by a single set of characters. On the basis of body pattern and femoral pore counts Zweifel (1962), for example, considered that hybrids between two subspecies of the lizard *Cnemidophorus tigris* occupied a zone less than 5 miles in width. Using plasma protein characters Dessauer, Fox and Pough (1962) showed that introgression took place over much larger distances.

Similar results have subsequently been found in a variety of organisms using quite different characters. Gartside (1972) found that while the frequencies of six molecular transferrin types across a hybrid zone between the two frog species *Litoria ewingi* and *L. paraewingi* were consistent with artificial hybridisation data (Watson, 1972), the seventh showed a more gradual cline. Hunt and Selander (1973) found that the extent of introgression between two subspecies of house mice across a hybrid zone varied markedly between different biochemical loci. Atchley (1974) found that morphometric divergence between non-contact populations of chromosomal

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‡ Both species recently removed from the genus *Crinia* by Blake (1973).

races of morabine grasshoppers was much higher than between chromosomally pure populations closer to the hybrid zone.

Such differences in the degree of introgression across hybrid zones measured by different characters may be the result of selection against some characters in the heterozygous state. Rare backcross progeny remaining heterozygous at other loci would allow introgression to extend over a wider zone. Where several independent characters do demonstrate uniformity in the position and width of a hybrid zone, a more complete barrier to gene flow beyond the narrow zone is indicated. Such may be the case in the *Sceloporus* lizards studied by Hall and Selander (1973) in which the karyotypic hybrid zone was remarkably consistent with that measured biochemically and morphologically.

The narrow hybrid zones between the morphologically similar leptodactylid frog species *Ranidella insignifera* and *R. pseudinsignifera* in Western Australia have previously been described (Littlejohn, 1957a and 1959; Main, 1968; Bull, 1973) solely on the basis of male mating call and appear to have remained remarkably stable over a 16-year interval. The two species have a common boundary 480 km long following the scarp of the Darling and Whicher Ranges. *R. insignifera* is confined to the coastal plain, while *R. pseudinsignifera* extends its range eastwards on the inland plateau. Contact with narrow hybrid zones have been described at three localities: Vass (240 km south of Perth), Bullsbrook (40 km north of Perth) and the Helena River Valley (18 km east of Perth). One possible explanation for the sharp transitions from one parental call-type to the other across the hybrid zones could be that selection acts against hybrids with an intermediate mating call which is not attractive to either parent species. In this model the progeny of any rare backcrosses would be favoured only where most of the alleles of the other parent at loci determining mating call have been eliminated. Those backcross individuals which are favoured may, however, remain heterozygous at other independently assorting loci so that introgression may extend much further than indicated by the male mating call. It was therefore clearly necessary to measure the extent of introgression between the species using other independent characters before attempting further discussion on the possible mechanisms maintaining the zone.

Initial attempts to differentiate between *R. insignifera* and *R. pseudinsignifera* included measurement of external body ratios, body marking patterns, and aceto-orcein stained karyotypes. None of these indicated any difference between the two species (Bull, 1973). This paper reports on the use of electrophoretic analysis of enzyme systems and multivariate statistical analysis of 13 skeletal parameters to distinguish between the species, and then uses these characters to examine a transect across the hybrid zone.

2. METHODS

(i) *Sampling localities*

Calling male frogs were collected in July and August 1972 from a series of populations along a traverse across the Helena Valley hybrid zone (fig. 1). From the chorus the First Dip population was judged to be pure *R. insignifera*, the Gravel Pit population almost entirely pure *R. insignifera* but with a low frequency of *R. pseudinsignifera* and intermediates, the Gutter

FIG. 1.—Map of the Perth region of Western Australia giving locations and species compositions based on male mating call chorus for *R. insignifera*-like (●) populations (1. Thornlie, 2. Welshpool-Williams Road, 3. First Dip); mixed (◖) populations (4. Gravel Pit, 5. Gutter Creek, 6. Wilkie-Coulston Road, 7. Wilkie Road Fenced Area); and *R. pseudinsignifera*-like (○) populations (8. Boya Quarry, 9. Glen Forrest, 10. Jacoby Street) used in this study. The inserted map shows the present distribution of *R. insignifera* (vertical stripes) and *R. pseudinsignifera* (horizontal stripes) and gives the position of the Bunbury region, where the remaining *R. insignifera* population was located, relative to the Perth region.

Creek population a mixture with slightly more influence from *R. insignifera*, the Wilkie-Coulston Road and Wilkie Road Fenced Area populations were mixtures with greater *R. pseudinsignifera* influence, and the Boya Quarry population was judged to be almost pure *R. pseudinsignifera*. Based on this evidence the hybrid zone is approximately 2·4 km wide and includes Wilkie-Coulston Road, Wilkie Road Fenced Area and Gutter Creek. The Gravel Pit population is just on the edge of the zone and First Dip and Boya Quarry are outside the zone on either side of it (Bull, 1973).

For comparison samples of frogs from pure allopatric populations of each species were included in the analyses. These were from Jacoby Street and Glen Forrest (10 km and 7·5 km east of the zone) for *R. pseudinsignifera* and from Thornlie and Welshpool-Williams Road (7·5 km and 4·5 km south-west of the zone) for *R. insignifera*. A further sample of *R. insignifera* was taken from a site near Bunbury approximately 140 km south of the Helena Valley hybrid zone and 15 km west of the closest *R. pseudinsignifera* population.

(ii) *Electrophoresis*

Livers were dissected from individual pithed frogs and homogenised in chilled distilled water at 15 per cent weight/volume. Homogenates were centrifuged at 18,000 g for 5 minutes at 4°C and the supernatents retained and stored at below −20°C.

Electrophoresis was carried out using vertical 7·5 per cent acrylamide gel slabs and a Tris-EDTA-borate buffer system in which the gel buffer was a 1 in 4 dilution of the electrode buffer (pH 8·6, I = 0·02). 5 ul samples of liver homogenate mixed with an equal volume of 20 per cent weight/volume sucrose solution containing Bromophenol blue were inserted into slots along the gel surface. Gels were run at 20 mA constant current for 2-2½ hours at 4°C and stained for non-specific esterases (Est) using the method of Lawrence, Melnick and Weimer (1960).

A mixture of samples from different populations were run on each gel along with a standard 2 ul Quokka (*Setonix brachyurus*) serum sample. Duplication of the homogenates allowed at least one liver sample from each gel to be run on the succeeding gel. It was thus possible to cross-reference migration distances of bands both through the standard Quokka bands and through the frog liver samples themselves. The genetics of the esterase banding patterns in *R. insignifera* and *R. pseudinsignifera* have been described elsewhere (Blackwell 1974 and 1977). Migration distances for each allele at each locus were expressed as a proportion of the fastest migrating allele at the fastest migrating locus common to both species. Genotypes of animals from eight of the study populations (Wilkie-Coulston Road, Jacoby Street and Welshpool-Williams Road being omitted) were scored and gene frequencies for each allele at each locus calculated.

The genetic distance measure of Rogers (1972), which summarises the gene frequency data over all loci, was used to assess overall genetic similarity or difference between pairs of populations.

(iii) *Skeletal morphology*

Samples of at least 20 frogs from each population except Gutter Creek were used in the analysis of skeletal morphology. These were not always

the same animals as used for electrophoretic analysis. The frogs were initially preserved in 70 per cent alcohol, then cleared in 1 per cent NaOH and stained with alizarin.

The following 13 bone length parameters were measured in two of the pure species reference populations, Welshpool-Williams Road and Jacoby Street, using a micrometer eyepiece in a Wild binocular microscope: (i) total body length from anterior tip of nasal bone to posterior extension of the caudal process (B.L.); (ii) minimum between eye distance (B.E.); (iii) maximum width of nasal bones at posterior end (N.B.); (iv) length from anterior tip of nasal bones to the eye (N.E.); (v) length from anterior tip of nasal bones to posterior point of the otic capsule (N.O.); (vi) width of skull between the axis of the jaw (W.S.); (vii) length of vertebral column (V.C.); (viii) width of the anterior edge of the sacral diapophysis (S.D.); (ix) length of the femur (F); (x) length of the tibiofibular (T.F.); (xi) length of the tarsal (T); (xii) length of the first metatarsal (M.T.); (xiii) length of the radioulna (R.U.). Considerable within (over different years) and between population variation in the mean body length of calling males throughout the range of each species was found (Bull, 1973). This parameter was therefore used as a " normalising " factor, the remaining 12 parameters being treated as ratios of body length.

Multivariate, canonical and stepwise discriminant analyses were used in comparing the two reference populations. Following this, only the four parameters known to contribute most to the between species variability were measured in the remaining eight populations. Individuals from these populations were then projected on to the canonical vector derived in the initial analysis and assigned to one or other of the two species by comparing the resulting score against scores obtained for the reference populations.

3. Results

(i) *Electrophoresis*

Gene frequencies obtained for five esterase loci from the eight populations sampled appear in table 1. The loci most important in separating the two species were *Est-3*, which controls a multiallelic dimer present only in *R. pseudinsignifera*, and *Est-4*, which controls a multiallelic dimer present only in *R. insignifera*. Hybrid and introgressed individuals present in mixed populations possessed bands at both loci and in some cases were heterozygous at both loci. They could thus be distinguished from pure *R. insignifera* and pure *R. pseudinsignifera* individuals but not from each other. The composition of the eight populations determined by these two loci is given in table 2.

The *Est-1* locus is also partially diagnostic controlling a multiallelic dimer present only in *R. pseudinsignifera* but not in all animals. Some switching mechanism or silent alleles must be operating at this locus. Bands were never visualised in the homologous position in *R. insignifera*. The *Est-2* and *Est-5* loci control multiallelic dimers for which both species share alleles.

The matrix of genetic distance coefficients between pairwise comparisons of the eight populations using all esterase loci appears in table 3. First Dip (0·205, 0·188), Gravel Pit (0·093, 0·110) and Gutter Creek (0·221, 0·164) are closer to *R. insignifera* than Wilkie Road (0·310, 0·251) when

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TABLE 1

Allele frequencies at esterase loci in populations of R. insignifera *and* R. pseudinsignifera *including mixed populations of the Helena Valley Traverse. N = sample size. Alleles are designated by their relative migration distances (see text)*

Enzyme locus and N	Allele	Populations							
		Bunbury	Thornlie	First Dip	Gravel Pit	Gutter Creek	Wilkie Road	Boya Quarry	Glen Forrest
N		45	31	17	24	10	20	18	19
Est-1	1.11	0	0	0	0	0	0·03	0	0·03
	1·08	0	0	0	0	0·05	0·03	0·09	0·05
	1·03	0	0	0	0	0·15	0·05	0·15	0·33
Est-2	1·00	0·01	0·11	0·06	0·08	0·20	0·32	0·50	0·63
	0·98	0·24	0·23	0·26	0·29	0·10	0·18	0	0·16
	0·96	0·12	0·23	0·06	0·07	0·35	0·18	0·47	0·21
	0·94	0·59	0·36	0·56	0·56	0·30	0·32	0	0
	0·91	0·02	0·02	0·03	0	0	0	0	0
	0·86	0·02	0·05	0·03	0	0·05	0	0·03	0
Est-3	0·83	0	0	0	0·02	0	0·23	0·15	0·32
	0·78	0	0	0	0·07	0·15	0·23	0·31	0·18
	0·76	0	0	0·15	0·04	0·25	0·47	0·27	0·26
	0·74	0	0	0	0·04	0·20	0·07	0·27	0·24
Est-4	0·72	0·12	0·19	0·50	0·11	0·30	0	0	0
	0·68	0·88	0·59	0·27	0·61	0·55	0·15	0	0
	0·65	0	0·22	0·23	0·28	0·05	0	0	0
Est-5	0·51	0·45	0·45	0·12	0·50	0·30	0·23	0·06	0
	0·50	0·42	0·42	0·59	0·27	0·45	0·24	0·63	0·47
	0·48	0·04	0	0	0·04	0	0·07	0·06	0·08
	0·46	0·06	0·10	0	0·06	0·05	0·23	0	0·05
	0·44	0·03	0·03	0·29	0·13	0·20	0·23	0·25	0·40

TABLE 2

Species composition of eight populations of R. insignifera *and* R. pseudinsignifera *including the Helena Valley Traverse as determined by diagnostic enzyme loci. N = sample size. Hybrids = hybrid or introgressed individuals*

Populations		N	R. insignifera (%)	Hybrids (%)	R. pseudinsignifera (%)
Reference R. insignifera	Bunbury	45	45 (100)	0 (0)	0 (0)
	Thornlie	31	31 (100)	0 (0)	0 (0)
The Helena Valley Traverse	First Dip	17	14 (82)	3 (18)	0 (0)
	Gravel Pit	24	20 (83)	4 (17)	0 (0)
	Gutter Creek	10	4 (40)	5 (50)	1 (10)
	Wilkie Road	20	0 (0)	3 (15)	17 (85)
	Boya Quarry	18	0 (0)	0 (0)	18 (100)
Reference R. pseudinsignifera	Glen Forrest	19	0 (0)	0 (0)	19 (100)

compared against reference *R. insignifera* populations.   Gutter Creek (0·236, 0·290) and Wilkie Road (0·221, 0·223) are closer to *R. pseudinsignifera* than First Dip (0·312, 0·346) or Gravel Pit (0·385, 0·405) when compared against Boya Quarry and the reference *R. pseudinsignifera* populations.   In fact, the First Dip and Gravel Pit distances are equal in magnitude to the reference *R. insignifera* populations, Bunbury (0·418,

TABLE 3

Matrix of Rogers' Genetic Distance Coefficients for pairwise comparisons between eight populations of R. insignifera and R. pseudinsignifera including mixed populations of the Helena Valley Traverse

		Bunbury	Thornlie	First Dip	Gravel Pit	Gutter Creek	Wilkie Road	Boya Quarry	Glen Forrest
Reference *R. insignifera*	Bunbury	0·000							
	Thornlie	0·099	0·000						
The Helena Valley Traverse	First Dip	0·205	0·188	0·000					
	Gravel Pit	0·110	0·093	0·176	0·000				
	Gutter Creek	0·221	0·164	0·214	0·207	0·000			
	Wilkie Road	0·310	0·251	0·271	0·259	0·210	0·000		
	Boya Quarry	0·418	0·351	0·312	0·385	0·236	0·221	0·000	
Reference *R. pseudinsignifera*	Glen Forrest	0·450	0·387	0·346	0·405	0·290	0·223	0·137	0·000

0·450) and Thornlie (0·351, 0·387), compared against Boya Quarry and the reference *R. pseudinsignifera* populations.

(ii) *Skeletal morphology*

The multivariate analysis of variance carried out on the pure species reference populations Welshpool-Williams Road and Jacoby Street showed a significant difference between the species (Wilks lambda = 0.24×10^{-6}; $P < 0.001$). Canonical analysis of the normalised skeletal measurements carried out to determine groupings produced the first set of coefficients of the canonical variates shown in table 4. Stepwise discriminant analysis demonstrated that most of the information separating the two species populations was contained in only four of the normalised parameters (B.E., N.E., N.O., and S.D.). Further canonical analysis considering only these four variables resulted in the second set of coefficients shown in table 4. From the summary of skeletal measurements data presented in table 5 it can be seen that the discrimination between the species is the result of a relatively wider sacral diapophysis, and greater between eye, nasal bone to eye, and nasal bone to otic capsule distances in *R. pseudinsignifera*.

TABLE 4

Coefficients of the canonical variates resulting from analysis of (A) all twelve normalised skeletal measurements compared between the Welshpool-Williams Road R. insignifera populations and the Jacoby Street R. pseudinsignifera populations, and (B) the four measurements containing most information in separating the two species. Variables = skeletal measurements abbreviated as in the text

Variables	S.D.	B.E.	N.O.	N.E.	T.	V.C.	R.U.	M.T.	W.S.	N.B.	F.	T.F.
Analysis A	23·62	37·08	−44·19	77·21	44·58	18·84	−25·03	−27·12	5·41	−17·03	6·56	1·54
Analysis B	35·515	54·323	−33·201	51·830								

When the four normalised skeletal measurements from inidviduals of the Welshpool-Williams Road and Jacoby Street populations are transformed by the analysis, the scores given in the first two columns of table 6 are obtained. Whilst the scores for *R. pseudinsignifera* are generally higher than those for *R. insignifera* some overlap does occur. Although perfect discrimination is thus not possible, scoring individuals above the midpoint (4·644) between the mean scores of the two samples as *R. pseudinsignifera* and those below as *R. insignifera* results in three misclassifications in the Jacoby Street sample and four in the Welshpool-Williams Road sample (individuals marked with an asterisk in table 6).

The reliability of this method of classifying individuals was further tested

TABLE 5

Data on skeletal measurements of samples taken from allopatric reference populations of R. insignifera *and* R. pseud-insignifera. *Results for the four measures contributing most to the discrimination between species are given together with body length (B.L.) against which these measures were normalised.* N = *sample size.* S.E. = *standard error.* B.E., N.E., N.O., *and* S.D. *are abbreviations for skeletal measures (see text)*

Population	N		Skeletal measurement				
			B.L.	B.E.	N.E.	N.O.	S.D.
R. insignifera							
Bunbury	20	Mean	18·717	1·338	2·173	6·354	2·867
		Range	16·50-21·50	1·13-1·50	1·83-2·38	5·71-7·00	2·42-3·33
		Variance	1·464	0·009	0·018	0·109	0·067
		S.E.	0·271	0·021	0·030	0·074	0·058
Thornlie	20	Mean	18·079	1·310	2·125	6·192	2·779
		Range	16·67-19·50	1·17-1·46	1·83-2·54	5·71-7·00	2·50-3·25
		Variance	0·599	0·006	0·034	0·092	0·032
		S.E.	0·173	0·018	0·041	0·068	0·040
Welshpool-Williams Road	20	Mean	19·633	1·425	2·233	6·469	2·875
		Range	17·30-22·30	1·25-1·58	1·75-2·67	5·50-7·17	2·33-3·67
		Variance	1·575	0·009	0·072	0·227	0·132
		S.E.	0·281	0·021	0·060	0·107	0·081
R. pseudinsignifera							
Jacoby Street	20	Mean	21·342	1·650	2·485	6·806	3·525
		Range	18·42-23·50	1·50-1·92	2·13-2·96	6·00-7·83	2·92-4·08
		Variance	2·825	0·014	0·073	0·297	0·113
		S.E.	0·376	0·027	0·060	0·122	0·075
Glen Forrest	20	Mean	22·096	1·627	2·654	7·185	3·500
		Range	19·33-26·00	1·50-1·79	2·29-3·25	6·21-8·58	3·00-4·25
		Variance	2·811	0·007	0·062	0·300	0·105
		S.E.	0·375	0·019	0·056	0·122	0·072

TABLE 6

Scores derived by projecting individuals from R. insignifera, R. pseudinsignifera, *and mixed populations on to the canonical vector originally derived from canonical analysis of normalised skeletal measurements of individuals from the pure species populations Welshpool-Williams Road* (R. insignifera) *and Jacoby Street* (R. pseudinsignifera). x̄ = *mean*

Populations

Welshpool-Williams Road	Jacoby Street	Thornlie	Bunbury	Glen Forrest	First Dip	Gravel Pit	Wilkie-Coulston Road	Wilkie Rd Fenced Area	Boya Quarry
3·360	5·395	4·423	5·093*	4·734	3·806	3·913	5·432	5·735	6·197
3·250	5·160	3·388	3·305	4·526*	4·128	3·572	4·795	4·646	4·703
3·467	5·270	3·960	3·910	4·526*	3·675	5·229	4·045	4·058	6·208
3·013	5·412	4·554	3·552	4·710	3·771	3·986	4·148	3·810	3·968
3·460	5·580	3·449	4·436	4·754	4·175	3·869	5·493	5·326	4·579
2·246	4·155*	4·186	3·934	5·078	5·109	2·675	5·763	4·884	4·382
4·550	5·580	4·635	3·221	4·754	4·244	3·424	3·955	4·965	4·193
3·671	5·612	3·264	4·177	5·131	3·668	3·289	4·649	4·522	6·322
4·253	6·245	3·796	3·000	4·773	3·551	5·231	4·003	4·839	6·122
3·203	6·074	4·366	3·944	5·096	3·076	2·487	4·101	4·150	5·145
3·585	5·638	3·943	3·682	4·900	3·942	3·720	5·031	4·533	4·481
5·076*	5·504	4·302	3·693	5·342	4·549	3·718	4·448	4·445	5·358
4·297	5·738	3·913	4·606	6·193	3·888	4·002	4·744	4·670	4·813
5·586*	4·568*	4·219	4·059	4·007*	4·332	3·661	4·274	5·340	5·411
4·094	5·131	4·053	3·748	4·504*	3·771	4·220	5·106	3·838	4·423
5·171*	4·782	3·771	4·424	4·785	3·722	3·378	4·981	4·224	5·046
5·097*	5·646	3·930	3·346	4·521*	3·332	3·442	4·372	4·627	5·644
3·263	5·842	4·182	3·646	4·781	4·557	3·676	4·282	4·385	5·204
4·258	4·182*	4·182	4·494	5·390	4·162	3·566	4·614	4·506	4·746
4·096	5·263	3·967	4·360	6·088	4·220	2·997	3·492	4·994	4·782
							4·892	4·838	
							4·411		
							5·179		
							4·170		
							4·255		
x̄ = 3·950	x̄ = 5·339	x̄ = 4·007	x̄ = 3·932	x̄ = 4·934	x̄ = 3·984	x̄ = 3·703	x̄ = 4·585	x̄ = 4·635	x̄ = 5·086

* Individuals from pure species populations misclassified by the analysis (see text).

by projecting individuals from two other *R. insignifera* reference populations (Thornlie and Bunbury) and one other *R. pseudinsignifera* reference population (Glen Forrest) on to the canonical vector derived in the initial analysis (table 6, columns three to five). In this case, the method proved more accurate for the *R. insignifera* populations in which only one individual (from Bunbury) was misclassified than for the *R. pseudinsignifera* population in which five individuals were misclassified. The difference between the two species in numbers misclassified is a consequence of the position of the randomly assigned cut-off point. Because of overlap in the ranges of the scores, the same number of individuals will always be misclassified. Altering the position of the point will only alter the population with most misclassifications. Over all reference populations up to 20 per cent misclassification occurred in *R. insignifera* and up to 25 per cent in *R. pseudinsignifera*.

Individuals from the five populations across the Helena Valley Traverse were also projected on to the canonical vector as ungrouped data (table 6, columns six to ten). From these scores individuals were assigned to a species and the composition of boundary populations determined (table 7) and compared to the reference populations. A rapid change from First Dip and Gravel Pit populations, where the proportions are indistinguishable from the reference *R. insignifera* populations ($\chi_1^2 = 0.24$ and 0.05), to the

TABLE 7

Species composition of ten populations of R. insignifera *and* R. pseudinsignifera *including the Helena Valley Traverse as determined by morphometric analysis.* $N = $ *sample size*

Populations		N	" insignifera-like " (%)	" pseudinsignifera-like " (%)
Reference *R. insignifera*	Bunbury	20	20 (100)	0 (0)
	Thornlie	20	19 (95)	1 (5)
	Welshpool-Williams Road	20	16 (80)	4 (20)
The Helena Valley Traverse	First Dip	20	19 (95)	1 (5)
	Gravel Pit	20	18 (90)	2 (10)
	Wilkie-Coulston Road	25	14 (56)	11 (44)
	Wilkie Road Fenced Area	21	11 (52)	10 (48)
	Boya Quarry	20	6 (30)	14 (70)
Reference *R. pseudinsignifera*	Jacoby Street	20	3 (15)	17 (85)
	Glen Forrest	20	5 (25)	15 (75)

Boya Quarry population, which does not differ significantly from the reference *R. pseudinsignifera* population ($\chi_1^2 = 0.74$), occurred. The two intermediate populations, Wilkie-Coulston Road and Wilkie Road Fenced Area, had proportions which differed significantly from both the *R. insignifera* ($\chi_1^2 = 12.83$ and 15.91) and the *R. pseudinsignifera* reference sample ($\chi_1^2 = 8.90$ and 6.73).

4. DISCUSSION

Both Littlejohn (1957*a*) and Main (1968) discussed several theories for the origin of the boundary between *R. insignifera* and *R. pseudinsignifera*. One hypothesis is that recent intervention by man has provided suitable habitat to allow contact of the two previously isolated species. Littlejohn

also suggested that relatively recent climatic changes have altered conditions on the western boundary of the plateau occupied by *R. pseudinsignifera* allowing it to extend its range to contact *R. insignifera*. These hypotheses imply that the present distribution is recent and may represent only a transitory phase before overlap of the ranges occurs.

In the present study species compositions of the boundary populations of the Helena Valley Traverse determined by electrophoretic analysis agree closely with the original compositions determined by the subjective assessment of the male mating call chorus. The electrophoretic data suggest that the First Dip population, considered to be just outside the zone on the *R. insignifera* edge according to male mating call, should now be included within the zone. This does not, however, affect the sharpness of the transition from *R. insignifera*-like populations to *R. pseudinsignifera*-like populations which occurs over the 2·4 km distance between the Gutter Creek and Wilkie Road populations. This is substantiated by the genetic distance estimates which show both First Dip and Gravel Pit populations to be as genetically distant from pure *R. pseudinsignifera* populations on the *R. pseudinsignifera* edge of the zone as the pure *R. insignifera* reference populations are.

The sharpness of the transition from *R. insignifera*-like to *R. pseudinsignifera*-like populations is also demonstrated by the morphometric data. These were less accurate in classifying the allopatric reference samples but nevertheless described the zone within the same boundaries as the other two characters. Although it cannot be stated with absolute certainty that there is no wider introgression for some other characters, the present evidence from the three independent measures suggests (1) that the genes controlling these characters form unique coadapted complexes within each species and (2) that there is a strong barrier to any flow of genes between populations of *R. insignifera* and *R. pseudinsignifera* beyond this very narrow hybrid zone. If this is true, evaluation of male mating call chorus should be sufficient to describe the hybrid zone elsewhere, thus confirming the earlier reports (Littlejohn, 1957a and 1959; Main, 1968; Bull, 1973) of a long parapatric boundary between the two species which follows the scarps of the Darling and Whicher Ranges in Western Australia. If the original hypotheses of Littlejohn and Main about the origin of this boundary were true, it would be unlikely that at one time the whole 480 km of the extending front of the *R. pseudinsignifera* populations should coincide so closely with the edge of the *R. insignifera* range.

A third and more plausible suggestion made by Littlejohn (1957a) was that the contact between *R. insignifera* and *R. pseudinsignifera* is a relatively old and stable one, and that some interaction between the species, or between each species and the environment prevents either species extending its range. In previous studies two major barriers have been suggested for the maintenance of other such parapatric distributions. One is the barrier of ecological incompatibility (demonstrated by Dixon, 1955; Fisler, 1965; Hagen, 1967; Yang and Selander, 1968) and competitive interaction (invoked by Hairston, 1949; Miller, 1964; Bovbjerg, 1970; Jaeger, 1970) and the other is the barrier of hybrid inviability when a " tension zone " (Key, 1968) develops if the two populations have a low enough premating isolation to allow mixed mating and a high enough postmating isolation to put hybrids at a selective disadvantage.

In *Ranidella* none of these mechanisms seems wholly applicable. In

studying the ecological relationships of the two species Bull (1973) could find no positive evidence to support the idea that physiology or competitive differences were limiting the range of either species. The major substrate change across the *R. insignifera/R. pseudinsignifera* transect is from sand to clay-based ponds and this occurs within the *R. insignifera* range. Detailed geological substrate distribution across the boundary at Bullsbrook, 40 km north of Perth (Burbridge, 1963), failed to demonstrate any change coinciding with the species transition. The scarps of the Darling and Whicher Ranges may themselves represent a significant barrier to the extension of *R. insignifera* out of the flat coastal plain but the downhill dispersal of *R. pseudinsignifera* from the scarp to the plain should present no such problems. Plant associations of the coastal sandy swamps and the eucalyptus forests on the plateau are quite different but *R. pseudinsignifera* has a wide distribution covering a range of soil types and plant associations. It is therefore improbable that the one ecotonal change which inhibits further extension of its range should coincide so closely with the change preventing *R. insignifera* extending its range.

Laboratory crosses (Bull, 1973) also fail to show any postmating inviability between the species, at least to the extent that hybrids are viable and hybrid males fertile. The only mechanism which does seem to prevent some introgression is a behavioural premating isolation. There are many cases (for example, in other amphibians: Littlejohn, 1957*b*; Michaud, 1962; Watson and Martin, 1968; Haertel and Storm, 1970) where two genetically compatible species do not interbreed because they have developed effective premating isolation. However, between *R. insignifera* and *R. pseudinsignifera* even this mechanism is not completely effective. Under experimental conditions (Bull, 1973) gravid females of both species prefer to go towards the male call of their own species but do not do so exclusively. Nor are there ecological, spatial or temporal premating isolating mechanisms which would be completely effective in preventing interspecific matings.

It appears, therefore, that neither ecological or genetical interactions alone are sufficient to prevent *R. insignifera* and *R. pseudinsignifera* overlapping in their distributions. Possibly a combination of the two may be important so that it is the presence of one species which is the major barrier to the expansion of the other species range. A more detailed examination of the properties of the two species required to support this hypothesis and how the interactions of *R. insignifera* and *R. pseudinsignifera* at their contact compare with the general pattern of interactions of closely related populations on coming into secondary contact will form the basis of further communications aimed at elucidating this phenomenon.

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