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Genetic Variation among Insular Populations of the Sleepy Lizard, *Trachydosaurus rugosus* Gray (Squamata : Scincidae)

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Abstract

Seven island populations of the sleepy lizard, *Trachydosaurus rugosus*, in South Australia were studied to establish the genetic effects of isolation. These effects were assessed by comparing genetic characteristics (using allozyme electrophoresis) of the island populations with those of three adjacent mainland populations. Heterozygosity levels did not vary significantly among the populations although the island populations exhibited reduced allelic diversity. Alleles that were rare on the mainland were not present in the island populations. Genetic divergence among the island populations was much greater than among populations on the mainland, reinforcing the notion that evolutionary forces, probably genetic drift, were greatest among the insular populations. This study demonstrates that the intra-specific component of variation can be significant, and that the importance of this component will increase with the fragmentation and isolation of populations. This finding serves to emphasise the importance of considering the population as the unit of conservation.

Introduction

Many Australian species that were once widespread now occupy remnants of habitat fragmented by clearing for agriculture, pastoralism or forestry. Others are restricted to a few small offshore islands by the combination of habitat modification, and introduced predators and competitors. Conservation of these species requires an understanding of the genetic processes that follow isolation (Frankel & Soulé 1981; Schoenwald-Cox *et al.* 1983). Of particular concern is the effect genetic drift and inbreeding have on levels of heterozygosity in small insular populations. Heterozygosity has been linked to fitness (reviewed by Danzmann *et al.* 1988) so processes that erode heterozygosity must be considered in the management of wildlife populations. Reductions in the level of genetic diversity will also reduce the ability of populations to adapt to environmental change, possibly causing the future extinction of currently viable populations. The distribution of genetic variation among conspecific populations may also be important by providing a basis for priority listings in conservation strategies directed at single species.

Island populations provide an opportunity to study the long-term effects of isolation on genetic variation in natural populations. In the present study, genetic variation within and among seven island populations of the sleepy lizard, *Trachydosaurus rugosus*, was examined using allozyme electrophoresis, and compared to genetic variation in three adjacent mainland populations. *T. rugosus* is a large viviparous lizard that is widely distributed over the southern half of the Australian continent, inhabiting all states except the Northern Territory

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and Tasmania (Cogger 1986). The species also occurs on a series of small continental islands off the coast of South Australia, many of which were isolated from the mainland by rising sea levels 6000–8050 years ago. The aim of this study was to test the predictions that divergence will be higher among island populations, and heterozygosity lower within the island populations than for populations on the adjacent mainland.

Materials and Methods

Study Site

The island and mainland populations examined in the present study are located on or near the coast of Eyre and Yorke Peninsulas, South Australia (Fig. 1). The islands are characteristically low in topography, with vegetation that has been substantially modified since European settlement. The vegetation generally comprises low shrubs (such as *Atriplex* spp. and *Olearia* spp.) and herbs on shallow, sandy soils. Except for Troubridge and Weeroona, all islands in the study were isolated by rising sea levels 6000–8050 years ago (Robinson *et al.*, in press). Troubridge I. formed as the result of the entrapment of sand by a lighthouse placed on Troubridge Shoal in the 1850s. Sleepy lizards are assumed to have been introduced, probably this century, because the island was completely destroyed by an earthquake in 1902 (R. Symons, personal communication). Weeroona I. is separated from the adjacent mainland by several hundred metres of mangrove swamps inundated by sea water at high tide. Sleepy lizards exist on the adjacent mainland, and the island has been connected to this area since the 1940s by a narrow causeway which runs across the swamp. The size and time since isolation for each island are given in Table 1.

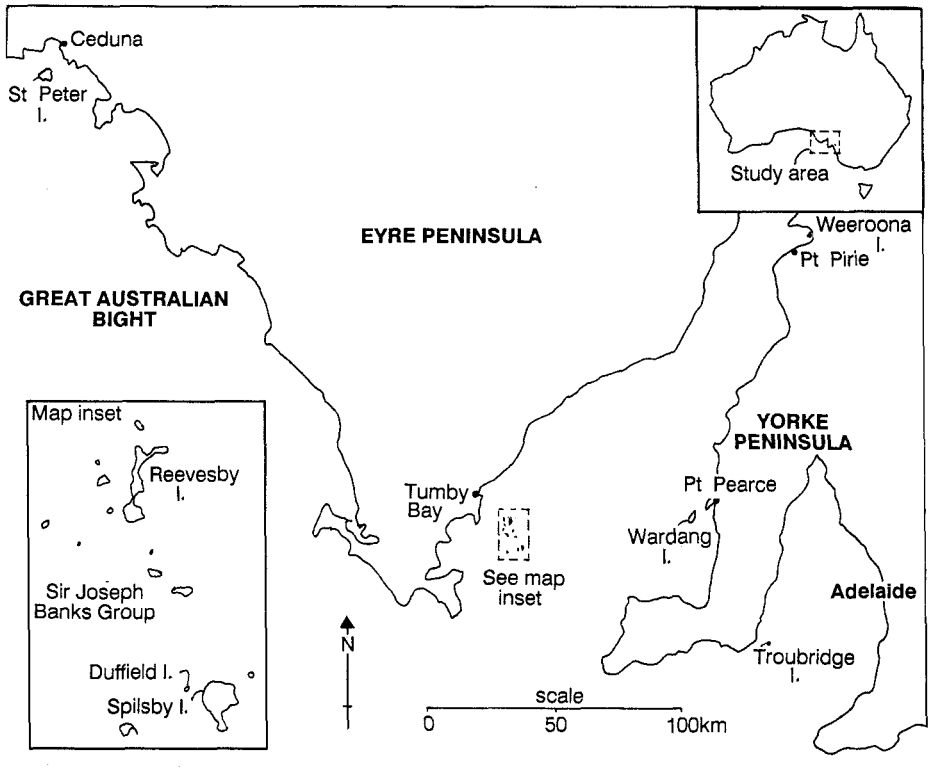

Fig. 1. The location of all populations of *T. rugosus* sampled in this study.

Sample Collection and Preparation

The population ecology of the sleepy lizard has been described by Bull (1987, 1988) and Dubas (1987). In all, 272 sleepy lizards were sampled between December 1986 and March 1988 along random transect lines from three mainland sites of Tumby Bay on Eyre Peninsula ($n=24$), Pt Pearce on Yorke Peninsula ($n=30$), and Pt Pirie also on Yorke Peninsula ($n=16$); and from seven island sites of St Peter I. ($n=9$), Spilsby I. ($n=19$), Duffield I. ($n=27$), Reevesby I. ($n=47$), Weeroona I. ($n=25$), Wardang I. ($n=53$), and Troubridge I. ($n=22$).

Table 1. Size, isolation time and population estimates (four islands) for the islands included in the study

All times of isolation are from Robinson *et al.*, in press. Superscript letters indicate other information sources: [A]Stevenson, unpublished data; [B]Robinson *et al.* 1985; [C]Mt Remarkable Council; [D]Owers, unpublished data; [E]Schwaner 1988

Island	Size (ha)	Time of isolation from the mainland (years before present)	Time of complete isolation (years before present)	Population estimate (total)
Wardang	20400[A]	6000	6000	$10{\cdot}6 \times 10^{4}$[E]
St Peter	4028[B]	6000	6000	–
Spilsby	425[B]	8050**	6000	$200 \pm 63{\cdot}0$
Reevesby	358[B]	8050	6000	–
Weeroona	210[C]	–	–*	–
Duffield	8[B]	8050**	6000	$50 \pm 27{\cdot}4$
Troubridge	8[D]	<150	<150	$53 \pm 17{\cdot}4$

*Separated from the mainland by mangrove swamp.

**Spilsby and Duffield were separated from Reevesby at approximately 7700 years before present.

Between 0·5 and 1·0 mL of blood was extracted from each lizard using a fine-tipped, 1-mL insulin syringe (laced with 5–10 μL heparin) to pierce the soft skin under the left arm and enter the heart. The whole-blood samples were frozen in liquid nitrogen within 30–60 seconds of extraction, returned to the laboratory and stored at −100°C in an ultrafreezer. Animals selected for release were marked individually by toe clipping, and released at the point of capture. Field blood samples were supplemented with blood taken from museum specimens as early as 1980 and stored at −100°C.

When tissue collection was complete, approximately 100 μL of each frozen sample was thawed, mixed with two drops of lysing solution (100 mL water, 10 mg nicotinamide adenine dinucleotide phosphate (NADP), 100 μL β-mercaptoethanol) and spun to remove insoluble debris. The supernatant was pipetted into capillary tubes, which were sealed and frozen.

Electrophoresis

Samples were assayed for electrophoretic variation in 16 systems, comprising the 14 enzymatic proteins carbonate anhydrase (CA), glucose-6-phosphate dehydrogenase (G6PD), glucose phosphate isomerase (GPI), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), mannose-phosphate isomerase (MPI), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), superoxide dismutase (SOD), umberlyferyl acetate esterase (UAE), and the two non-enzymatic proteins albumin (ALB) and transferrin (TRF), providing 17 informative loci. All proteins except TRF were assayed using cellogel (Chemetron, Milan) electrophoresis following the methods of preparation, buffering systems and staining procedures of Richardson *et al.* (1986). Vertical starch-gel electrophoresis (Harris & Hopkinson 1976) was used to type TRF because its activity was low. The vertical starch gels ('Electrostarch', Madison, Wisconsin, lot 392, 9%) were loaded with 15 μL of sample, 8 μL of tris-HCL buffer (pH 8) and 2 μL of Fe^{59} (code IFS.1, Amersham). Each gel was run for 18 h at 200 V, sliced, wrapped in Ceranwrap, and placed against an X-ray film (Kodak XRP-5 100, Cat. 4079158) for 48 h at −100°C, before being developed and scored. Controls were included on each gel, and appropriate lineup gels run for individual comparisons. Alleles were referred to by alphabetic characters, with the most anodal electromorph designated as 'A'.

Analysis

The inheritance of the electromorphs was not studied, but phenotypic characteristics (i.e. quaternary structure and isozyme number) were consistent with those found in other electrophoretic surveys of vertebrates. Although tests for linkage disequilibrium are rarely undertaken, and loci are generally assumed to be independent, an apparent association was observed between the loci *Alb* and *Sod*. The non-association between all loci within each population was therefore tested using Fisher's exact test (Zar 1984).

Average heterozygosity estimates were obtained for each population by direct count, and from those expected under Hardy–Weinberg equilibrium, using Nei's unbiased estimate (Nei 1978). Heterozygosity values were subjected to an arcsine transformation ($y' = \text{arcsine}\sqrt{y}$) to normalise the distribution (Archie 1985), and the significance of variation between populations was tested using a one-way ANOVA.

Other measures of genetic variation obtained were the mean number of alleles per locus and the percentage of polymorphic loci (95% and 99% criteria). Departures from Hardy–Weinberg equilibrium were assessed using the Fisher's exact test for 2×2 contingency tables because of the small samples available for many of the populations. When more than two alleles were present at a locus, the genotypes were pooled into three classes to avoid biases resulting from low expected frequencies. These classes consisted of homozygotes for the most common allele, heterozygotes for the most common allele and all other genotypes.

Distances between populations were determined using Rogers' genetic distance (Rogers 1972). In one analysis a heirachical cluster analysis was performed using the unweighted pair-group method with arithmetic mean averaging (UPGMA) (Sneath & Sokal 1973). In a second analysis, an unrooted Wagner tree was produced using the Distance Wagner Procedure (Farris 1972). The program WAGPROC (Version 3.3; Swofford 1981) was implemented on an IBM personal computer using Lahey Computer Systems implementation of FORTRAN 77. Options for randomly shuffling the populations before analysis and for beginning tree building at a random branch were added to the program. The heuristic approach of repeated runs of WAGPROC (with shuffling and random branch starts) in search of the shortest tree was adopted. On each iteration of WAGPROC, the length of the resulting tree was compared with the previously found shortest tree, and only a set of the shortest trees (tolerance 2%) was retained. Approximately 1250 trees were compared.

In the third analysis, chi-squared contingency analyses of allele frequencies were used to test for divergence between populations (Workman & Niswander 1970). If expected frequencies were low, the frequencies for rare alleles were pooled.

Finally, '*F*' statistics (Wright 1969, 1978) were calculated to determine the degree of differentiation between populations. These statistics include F_{ST}, F_{IS} and F_{IT}, where F_{ST} can be considered a measure of genetic differentiation among populations, and F_{IS} and F_{IT} are measures of departures from Hardy–Weinberg equilibrium within populations and over all populations respectively (Hamrick 1983). The '*F*' statistics for island and mainland populations were calculated both separately and together to determine the extent to which differentiation varied among these two groups.

Unless otherwise stated, all data analyses were carried out using the statistical packages BIOSYS-1 (Swofford & Selander 1981) and SAS Version 6.03 (SAS Institute 1987).

Population Estimates

Duffield I. was surveyed in February 1985, December 1986, and February 1988, and Baileys Triple Catch method (Caughley 1980) was used to estimate population size. Spilsby I. was surveyed in August 1987 and February 1988, and Troubridge I. was surveyed in October 1987 and March 1988. The Peterson method (Caughley 1980) was used to estimate population size for the areas surveyed on both islands. The Wardang I. population was sampled at 2-month intervals from November 1986 to November 1987. In all, 340 individuals were marked in an area of approximately 100 hectares (Schwaner 1988), and population size was estimated with the Jolly–Seber method.

For Duffield and Troubridge Is, sampling covered the entire island. Only partial areas were surveyed on Spilsby and Wardang, so density estimates were multiplied by island area to obtain rough estimates of total population size. Insufficient time was available for estimating population sizes from the mainland localities.

Results

Genetic Relationships

Eight of the 17 presumptive loci scored were polymorphic in at least one population. The number of loci observed (but not necessarily scored) per system, and the quaternary structure of those loci examined, were comparable to those generally found in vertebrates (Richardson *et al.* 1986). Allele frequencies for all polymorphic loci are given in Table 2 with no more than four alleles observed at any one locus. There was a consistent pattern of reduced numbers of alleles in the insular populations compared to the adjacent mainland populations. Alleles that were rare in the mainland populations were absent in the island populations.

Of 180 Fisher's exact tests of association between loci (Table 3), 13 (approximately 7%) were significant. This is little more than would be expected by chance alone at the 95% level of significance. However, seven of these were observed between *Alb* and *Sod* ($P<0{\cdot}01$). This suggests that these loci are linked, and that the assumption of independence between them is questionable. Consequently, the locus *Sod* was excluded from the analyses of genetic relationships and heterozygosity.

Table 2. Allele frequencies for all populations at all polymorphic loci

Abbreviations: StPtr, St Peter Is; TmyBy, Tumby Bay; Spsby, Spilsby I.; Dufld, Duffield I.; Rvsby, Reevesby I.; Werna, Weeroona I.; PtPre, Port Pirie; PtPce, Point Pearce; Wrdng, Wardang I.; Troub, Troubridge I. See Fig. 1 for the location of each population

Locus	Population									
	StPtr	TmyBy	Spsby	Dufld	Rvsby	Werna	PtPre	PtPce	Wrdng	Troub
Alb										
(N)	9	24	19	27	47	25	16	30	53	22
A	0·611	0·667	0·711	0·500	0·106	0·320	0·344	0·633	0·726	0·409
B	0·389	0·333	0·289	0·500	0·894	0·680	0·656	0·333	0·274	0·591
C	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·033	0·000	0·000
Tfn										
(N)	4	21	19	22	27	22	10	29	31	21
A	0·000	0·262	0·000	0·000	0·000	0·000	0·250	0·103	0·000	0·214
B	1·000	0·690	1·000	1·000	1·000	1·000	0·750	0·810	1·000	0·786
C	0·000	0·048	0·000	0·000	0·000	0·000	0·000	0·086	0·000	0·000
PepA2										
(N)	9	23	19	27	46	25	16	30	53	22
A	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·033	0·000	0·000
B	1·000	0·978	1·000	1·000	0·511	1·000	0·969	0·967	1·000	1·000
C	0·000	0·022	0·000	0·000	0·489	0·000	0·031	0·000	0·000	0·000
PepD										
(N)	9	23	19	27	46	25	16	28	53	21
A	1·000	0·804	1·000	1·000	0·815	0·720	0·969	0·839	0·821	0·762
B	0·000	0·174	0·000	0·000	0·185	0·280	0·031	0·161	0·179	0·238
C	0·000	0·022	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000
Gpi										
(N)	9	23	19	27	46	25	16	30	51	22
A	0·222	0·000	0·000	0·000	0·000	0·000	0·000	0·033	0·000	0·000
B	0·611	0·543	0·658	0·833	1·000	0·820	0·531	0·550	0·461	0·227
C	0·056	0·370	0·000	0·000	0·000	0·180	0·438	0·317	0·539	0·773
D	0·111	0·087	0·342	0·167	0·000	0·000	0·031	0·100	0·000	0·000
Pgi										
(N)	9	22	19	27	46	25	16	30	51	21
A	0·333	0·250	0·579	0·537	0·630	0·340	0·406	0·150	0·078	0·262
B	0·667	0·750	0·421	0·463	0·370	0·660	0·594	0·850	0·922	0·738
Ca										
(N)	9	21	18	26	46	25	16	30	50	21
A	0·333	0·476	0·250	0·385	0·337	0·620	0·750	0·500	0·510	0·310
B	0·667	0·524	0·750	0·615	0·663	0·380	0·250	0·500	0·490	0·690
Sod										
(N)	8	24	18	25	45	25	15	28	52	18
A	0·813	0·646	0·694	0·540	0·111	0·340	0·433	0·750	0·779	0·444
B	0·188	0·354	0·306	0·460	0·889	0·660	0·567	0·214	0·221	0·556
C	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·036	0·000	0·000

Cluster analysis using UPGMA based on Rogers' genetic distance measure produced several distinct clusters (Fig. 2). St Peter, Spilsby and Duffield formed one cluster, as did Tumby Bay, Point Pearce and Wardang, and Weeroona, Pt Pirie and Troubridge. Reevesby was a clear outlier, clustering from all other populations at a distance of 0·14. These relationships are confirmed using the Distance Wagner Procedure (Fig. 3).

There was some agreement between geographic proximity and the clusters formed by the UPGMA and Distance Wagner analyses. Populations from Duffield and Spilsby, that are only about 500 m apart were closely related. So were the populations from the mainland–island pairs of Pt Pearce and Wardang I., and Weeroona I. and Pt Pirie. However, the genetic similarity among populations from St Peter, Spilsby and Duffield, and among populations from Tumby Bay and Pt Pearce, could not have been predicted from geographic proximity. Nor was the high degree of divergence between Reevesby and nearest populations on Spilsby and Duffield Is consistent with the geographic distance between them. The similarity of populations from Tumby Bay to those of Pt Pearce and Wardang in both analyses

Table 3. Fisher's exact test for linkage disequilibrium between each pair of loci within each population of *T. rugosus*

Abbreviations for populations are as given in Table 2; —, test not applied

Locus	Population									
	StPtr	TmyBy	Splby	Dufld	Rvsby	Werna	PtPre	PtPce	Wrdng	Troub
Alb/Trf	—	0·608	—	—	—	—	0·619	0·813	—	0·546
Alb/PepA2	—	1·000	—	—	1·000	—	0·400	0·250	—	—
Alb/PepD	—	0·293	—	—	0·304	0·010	1·000	0·427	0·406	1·000
Alb/Gpi	1·000	0·913	1·000	0·669	—	0·794	0·634	0·192	0·864	0·422
Alb/Pgm	1·000	0·395	0·323	0·814	1·000	0·739	0·486	0·967	0·466	0·036*
Alb/Ca	0·071	0·042*	0·620	0·816	0·656	0·249	0·727	0·220	0·900	0·583
Alb/Sod	0·107	0·000***	0·557	0·000***	0·070	0·000***	0·008**	0·000***	0·000***	0·000***
Trf/PepA2	—	0·400	—	—	—	—	1·000	1·000	—	0·700
Trf/PepD	—	0·134	—	—	—	—	1·000	0·526	—	1·000
Trf/Gpi	—	0·569	—	—	—	—	0·548	0·257	—	1·000
Trf/Pgm	—	1·000	—	—	—	—	0·226	0·373	—	0·122
Trf/Ca	—	1·000	—	—	—	—	0·429	0·258	—	0·685
Trf/Sod	—	0·879	—	—	—	—	0·548	0·789	—	—
PepA2/PepD	—	1·000	—	—	0·547	—	1·000	1·000	—	—
PepA2/Gpi	—	0·682	—	—	—	—	0·333	1·000	—	—
PepA2/Pgm	—	0·476	—	—	0·666	—	1·000	0·256	—	—
PepA2/Ca	—	1·000	—	—	0·769	—	0·400	0·571	—	—
PepA2/Sod	—	1·000	—	—	0·536	—	1·000	1·000	—	—
PepD/Gpi	—	0·821	—	—	—	0·192	1·000	0·334	0·189	0·262
PepD/Pgm	—	0·659	—	—	0·272	0·335	1·000	0·244	1·000	1·000
PepD/Ca	—	0·413	—	—	0·538	0·492	1·000	0·035	0·842	0·757
PepD/Sod	—	1·000	—	—	0·429	0·120	1·000	0·277	0·465	0·850
Gpi/Pgm	0·036	0·881	0·914	0·319	—	0·113	0·530	0·742	0·528	0·605
Gpi/Ca	1·000	0·053	0·793	0·454	—	0·167	0·086	0·799	0·118	1·000
Gpi/Sod	0·857	0·766	1·000	0·428	—	1·000	0·052	0·142	0·980	0·579
Pgm/Ca	1·000	0·404	0·430	0·030*	0·725	0·353	0·820	0·938	0·890	0·636
Pgm/Sod	1·000	0·355	0·236	0·679	0·884	0·255	0·949	0·934	0·070	0·098
Ca/Sod	0·643	0·128	0·698	0·731	0·889	0·604	0·800	0·163	0·925	0·722

*$P<0·05$; **$P<0·01$; ***$P<0·001$.

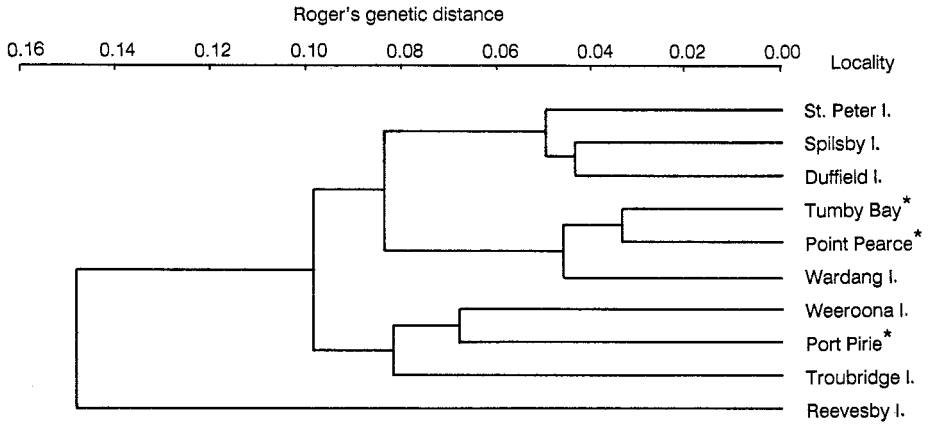

Fig. 2. UPGMA cluster analysis of Rogers (1972) genetic distance measures among island and mainland populations of *T. rugosus*.

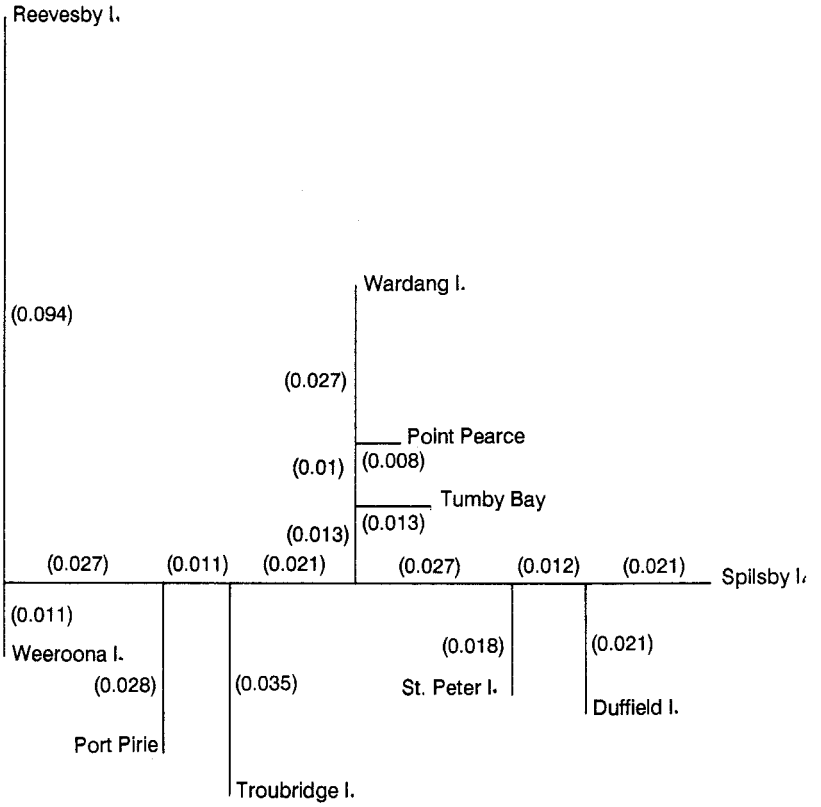

Fig. 3. Unrooted Wagner tree using Rogers genetic distance measure (Rogers 1972) among island and mainland populations of *T. rugosus*. Genetic distance in parentheses.

probably indicates that these populations were similar before isolation, and have undergone little divergence since.

The relative proportions of different alleles differed significantly among populations for all loci (chi-squared tests, $P<0{\cdot}001$, Table 4) indicating significant genetic heterogeneity among populations. This heterogeneity remained significant for island populations when mainland populations were removed from the analysis. The converse was not true: the three mainland populations showed no significant differences at four loci (*Trf*, *PepA2*,

Table 4. Chi-square analysis of allele frequencies at all polymorphic loci between populations of *T. rugosus*

Seven island populations (6 d.f.); three mainland populations (2 d.f.); and all ten populations combined (2 d.f.). Frequencies of rare alleles were pooled because expected frequencies were <1. NS, not significant

Locus	Island populations		Mainland populations		All populations	
	Chi-square	*P*	Chi-square	*P*	Chi-square	*P*
Alb	93·604	<0·001	8·802	<0·05	108·129	<0·001
Tfn	55·275	<0·001	1·764	NS	69·079	<0·001
PepA2	170·743	<0·001	0·132	NS	214·336	<0·001
PepD	30·180	<0·001	4·475	NS	34·899	<0·001
Gpi	121·686	<0·001	0·288	NS	123·938	<0·001
Pgm	78·784	<0·001	7·476	<0·05	96·828	<0·001
Ca	21·360	<0·005	6·706	<0·05	35·474	<0·001

Table 5. *F* statistics for the seven island and three mainland populations of *T. rugosus*

The *F* statistics are those defined by Wright (1969), and locus abbreviations follow those of Richardson *et al.* (1986)

Locus	Island populations			Mainland populations			All populations		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Alb	0·009	0·180	0·173	0·026	0·112	0·088	0·015	0·163	0·151
Trf	0·576	0·656	0·189	0·450	0·022	0·469	0·469	0·553	0·155
PepA2	0·000	0·450	0·451	0·367	0·372	0·008	0·092	0·443	0·387
PepD	0·206	0·299	0·117	0·031	0·071	0·041	0·150	0·229	0·094
Gpi	0·043	0·287	0·316	0·177	0·182	0·006	0·046	0·265	0·229
Pgm	0·105	0·231	0·140	−0·053	0·007	0·057	0·061	0·184	0·131
Ca	−0·159	−0·090	0·060	−0·093	−0·029	0·063	−0·141	−0·041	0·087
Mean	0·015	0·197	0·185	0·097	0·138	0·046	0·044	0·194	0·157

PepD, *Gpi*). This indicates a greater divergence among island populations than among those of the mainland.

The F statistics (Table 5) demonstrate substantial differences between individual loci. For example, *PepA2* showed twice as much differentiation as the average over-all loci among the island populations. *Gpi* also demonstrated high divergence in the island populations, with all four alleles maintained over all populations, but usually only two maintained in any one population. In contrast, *Ca* exhibited very low F_{ST} values, most populations maintaining both alleles. The mainland populations showed less variation between loci in F_{ST} values, and the mean F_{ST} value for the three mainland populations was very low ($F_{ST}=0{\cdot}046$) indicating low levels of differentiation.

The three mainland localities contribute little to total genetic divergence among the populations. For example, the mean F_{ST} value over all ten populations is three times greater than the divergence observed among the three mainland populations, and only slightly less than the levels observed among the island populations.

The mean F_{IS} values are positive for the island and mainland populations, indicating slight heterozygote deficiencies in both groups. However, most F_{IS} values observed were close to zero, which is consistent with the few significant departures from Hardy–Weinberg equilibrium (see below) observed within the populations. The preponderance of positive F_{IT} values obtained is consistent with populations that are discrete, suggesting that the populations examined form a set of real subdivisions. These values were higher among the islands, a reflection of the discrete nature of these populations.

Genetic Variation within Populations

The populations of *T. rugosus* examined in the present study demonstrated considerable electrophoretic variation (Table 6), with observed levels of heterozygosity ranging from 10·5% on Wardang I. to 16·8% at Tumby Bay (mean, 12·58%). Observed levels of heterozygosity are within the range of values (0–30%; mean, 5·5%) recorded by Nevo

Table 6. Mean genetic variability estimates for ten populations of *T. rugosus* at sixteen loci

A locus is considered polymorphic if the frequency of the most common allele does not exceed 0·95. Calculated using Nei's formula for small sample sizes (Nei, 1978). Values in parentheses are standard errors

Population	Mean sample size per locus	Mean No. of alleles per locus	Percentage of loci polymorphic	Mean percentage heterozygosity	
				Direct-count	Hardy–Weinberg expected
St Peter I.	8·7	1·4	25·0	13·9	12·1
	(0·3)	(0·2)		(6·5)	(5·7)
Tumby Bay	23·3	1·6	37·5	16·8	17·2
	(0·3)	(0·2)		(5·8)	(5·7)
Spilsby I.	18·9	1·3	25·0	11·7	11·1
	(0·1)	(0·1)		(5·6)	(5·0)
Duffield I.	26·6	1·3	25·0	11·5	11·1
	(0·3)	(0·1)		(5·3)	(5·1)
Reevesby I.	45·4	1·3	31·3	12·1	12·0
	(1·2)	(0·1)		(5·0)	(4·9)
Weeroona I.	24·8	1·3	31·3	11·7	13·1
	(0·2)	(0·1)		(4·7)	(5·1)
Port Pirie	15·6	1·5	31·3	12·3	15·1
	(0·4)	(0·2)		(4·8)	(5·4)
Point Pearce	29·8	1·7	37·5	13·3	15·8
	(0·1)	(0·2)		(4·9)	(5·5)
Wardang I.	51·2	1·3	31·3	10·5	11·6
	(1·4)	(0·1)		(4·8)	(4·8)
Troubridge I.	21·8	1·4	37·5	12·0	15·0
	(0·1)	(0·1)		(4·7)	(5·1)

Table 7. Results of Fisher's exact tests for departures from Hardy–Weinberg equilibrium in ten populations of *T. rugosus* across all polymorphic loci

Abbreviations for populations are as given in Table 2; —, test not applied

Locus	Population									
	StPtr	TmyBy	Splby	Dufld	Rvsby	Werna	PtPre	PtPce	Wrdng	Troub
Alb	1·000	1·000	0·253	0·440	0·414	0·353	0·591	0·128	0·171	0·071
Trf	—	0·050*	—	—	—	0·046*	—	1·000	—	0·019*
PepA2	—	1·000	—	—	1·000	—	1·000	0·017*	—	—
PepD	—	0·544	—	—	0·631	—	1·000	0·113	0·045*	0·046*
Gpi	0·462	0·401	0·345	1·000	—	0·558	0·153	0·481	0·403	1·000
Pgm	0·457	0·269	0·017*	1·000	0·536	0·075	1·000	0·504	0·020*	0·578
Ca	0·457	0·206	0·524	0·221	1·000	0·082	1·000	0·723	1·000	0·115
Sod	1·000	0·391	1·000	0·688	0·005**	1·000	0·294	0·033*	0·009**	0·001**

*$P<0·05$; **$0·05<P<0·01$.

et al. (1984) in a review of electrophoretic studies involving non-parthenogenic reptiles. The three mainland populations exhibited higher levels of both observed and expected heterozygosity than did any of the island populations, but this trend was not significant ($F_{obs}=0\cdot12$, $P>0\cdot1$; $F_{exp}=0\cdot17$, $P>0\cdot1$).

The number of alleles per locus in the three mainland populations (mean, 1·6; range, 1·5–1·7) was greater than in any of the seven island populations (mean, 1·33; range, 1·3–1·4). This measure is very dependent on sample sizes, which vary in the present study. However, mean sample size is higher in the island populations (mean sample size for island populations, 27·4; mean sample size for mainland populations, 22·4), which will increase the probability of finding extra alleles in the island populations. The percentage of polymorphic loci showed a similar trend, being consistently greater in the mainland populations than on the islands (island mean, 30·233%; mainland mean, 39·6%; 99% criterion).

Probability levels for departures from Hardy–Weinberg equilibrium are given in Table 7. Eleven of the 60 Fisher's exact tests on eight loci departed significantly from Hardy–Weinberg equilibrium. Pt Pirie and St Peter, Duffield and Weeroona Is contained no departures from Hardy–Weinberg equilibrium whereas Tumby Bay, Spilsby and Reevesby showed heterozygote deficiencies at one locus. One locus (*Sod*) departed significantly from Hardy–Weinberg equilibrium in four populations (Reevesby, Wardang, Pt Pearce and Troubridge). Wardang and Troubridge Is exhibited significant heterozygote deficiencies at three loci, suggesting that one or more of the assumptions necessary to maintain Hardy–Weinberg equilibrium are violated in these populations. However, the Wardang population was sampled from several localities (Schwaner 1988) so if the population consists of a series of sub-populations rather than a single panmictic unit, departures from Hardy–Weinberg equilibrium may be expected because of the Wahlund effect (Wright 1965). Schwaner (1988) observed that the distribution of this population was clumped around rubbish dumps, which is consistent with the hypothesis of a series of sub-populations.

Conversely, the entire area of Troubridge I. was sampled. The population on this island is small (Table 1) and probably originated from the introduction of a few individuals. Departures from Hardy–Weinberg equilibrium caused by the mating of close relatives would be expected under these circumstances. Two other loci (*Alb*, $P=0\cdot071$; *Ca*, $P=0\cdot115$) also showed tendencies towards departure from Hardy–Weinberg equilibrium in this population.

Discussion

The electrophoretic examination of island and mainland populations of *T. rugosus* demonstrates that there has been little erosion of heterozygosity within the insular populations. This is in contrast to studies of other vertebrates on South Australian islands such as studies of the bush rat, *Rattus fuscipes greyii* (Schmitt 1978), and the tiger snake, *Notechis scutatus-ater* (T. D. Schwaner & M. Adams, unpublished data), which show large decreases in genetic variation, sometimes to the point of fixation at all loci examined. However, differences were observed in the level of allelic diversity and per cent polymorphism between the island and mainland populations, suggesting that some genetic drift has occurred. The tendency appears to be towards a loss of rare alleles in the island populations. Fixation, if it has occurred, has involved those alleles that are most common in the mainland populations.

Selection, mutation, migration and genetic drift are involved in determining the levels of heterozygosity in natural populations. It is conceivable that conditions on the islands have increased selective pressure on these populations, counteracting the effects of drift, and resulting in the maintenance of polymorphisms at the loci examined. However, the lack of consistent departures from Hardy–Weinberg equilibrium at individual loci does not support this hypothesis.

No unique alleles were present in the island populations, indicating that insufficient time has elapsed since isolation for new mutations to become established in these populations. If the alleles currently observed in the mainland populations that are absent from the island populations have been lost through genetic drift, then the establishment of new alleles becomes a function of the mutation rate and population size. Lande (1988) suggests that, for a single neutral locus, effective population sizes (N_e) of greater than 10^5 individuals may

be required to maintain substantial levels of heterozygosity by mutation, and the subsequent recovery of variation following a severe bottleneck would require 10^5–10^7 generations (Nei *et al.* 1975).

Without selection, mutation or immigration, the extent to which an isolated population will retain genetic variation will be affected by effective population size. However, a bottleneck of one generation, even a severe one, will have a small effect on heterozygosity, provided the population grows to a substantial size within a small number of generations (Nei *et al.* 1975). The effect that a bottleneck will have on allele diversity depends upon the number and frequency of alleles in the parent population (Allendorf 1986), and is more likely to be affected when the loci involved have large numbers of unevenly distributed alleles (Sirkkomaa 1983), rare alleles being particularly susceptible to loss during a bottleneck (Allendorf 1986). The island populations of *T. rugosus* have generally lost those alleles that on the mainland have lowest frequency, and fixation has favoured those alleles that are in high frequencies on the mainland. This suggests that all the insular populations in the present study have experienced periods of small population size during their period of isolation.

It is likely that, although sleepy lizards maintain small home ranges and exhibit high mate fidelity (characteristics that will reduce effective population size), features such as long life span, stable population size and equal sex ratio, combined with large original levels of variation in the founding populations, have resulted in the island populations retaining a large portion of their genetic variation. Moreover, the allele distribution at polymorphic loci (two common alleles with the remaining alleles found only in very low frequencies) has favoured the retention of variation in the form of heterozygosity rather than allelic diversity.

The lack of significant differences in genetic variation between the small and large islands suggests that effective population sizes have been sufficient in all populations to prevent large losses of heterozygosity. The retention of genetic variation in the Troubridge population helps to reinforce this conclusion because, over a short period of isolation, the Troubridge population has retained levels of heterozygosity and polymorphism consistent with a mainland population but has not maintained allelic diversity. If this population is unable to expand in size to an N_e of greater than 500, then an erosion of heterozygosity can also be expected.

There are some inconsistencies between the observed levels of genetic variation within the island populations and population size. For example, Duffield I. currently has a small population and island size, and isolation time suggests that this has been the case for at least 6000 years. Without gene flow and strong selection pressure, extreme genetic drift and the subsequent fixation at most loci would be expected. That this has not occurred and that the nearby Spilsby I. population is genetically similar, suggests two possible explanations. The first is that the Duffield population has recently been introduced from Spilsby I., and insufficient time has elapsed since this introduction to enable significant differentiation. Second, there may be sufficient gene flow between these two populations to enable maintenance of alleles in both populations. Evidence from modelled populations (e.g. Boeklen & Bell 1986) suggests that even very low rates of gene flow can maintain allelic diversity and heterozygosity between two populations. Experimental evidence involving the floating of the lizard *Anolis sagrei* in salt water has demonstrated that some floatation without the aid of rafts is possible among lizards (Schoener & Schoener 1984). So the possibility of gene flow between Spilsby and Duffield cannot be ruled out. However, in the absence of the necessary historical data it is not possible to distinguish between these two hypotheses.

Significant differences in divergence among island and among mainland groups were observed. F_{ST} statistic demonstrates clearly that the insular populations have diverged at a greater rate than the three mainland populations. It is likely that genetic drift that has reduced allelic diversity in the island populations has also caused differentiation among them.

Both the reduced allelic diversity and the increased divergence observed in the island populations have implications for conservation. Heterozygosity levels do not appear to have been reduced significantly in the island populations, which suggests that average fitness levels

within those populations have not been greatly affected by isolation. However, reductions in genetic diversity within the island populations will affect the ability of those populations to adapt to environmental changes. Thus, although the probability of short-term survival may not have been greatly affected by genetic drift in the island populations, the evolutionary potential of those population may have been eroded.

The findings in the present study also illustrate the importance of considering individual populations as the unit of evolution and hence conservation. If the maximisation of intra-specific variation is accepted as an important criterion in conservation, then the substantial differentiation observed among the island populations of *T. rugosus* in the present study has implications for strategies involving the conservation of this species. That is, if decisions are to be made about the suitability of populations of a species for conservation, then a desirable strategy would be to maximise variation within that species. For *T. rugosus*, if the populations examined in the present study were the only populations in existence, then a priority listing for conservation value based on maximising diversity could be produced. The data also show that divergence is greatest among the insular populations. This has implications for populations that have become isolated as a result of human activities and for those currently isolated on islands. Strategies of species conservation must look towards conserving populations, because each population is potentially a new species.

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References

Allendorf, F. W., 1986. Genetic Drift and the loss of alleles versus heterozygosity. *Zoo Biology* **5**, 181–90.

Archie, J. W., 1985. Statistical analysis of heterozygosity data: independent sample comparisons. *Evolution* **39**, 623–37.

Boecklen, W. J. & G. W. Bell, 1986. Consequences of faunal collapse and genetic drift to the design of nature reserves. Pp. 141–9 *in*: 'Nature conservation: The role of remnants of native vegetation'. Eds D. A. Saunders, G. W. Arnold, A. A. Burbidge & A. J. M. Hopkins. (Surrey Beatty and Sons Pty Ltd: Chipping Norton, NSW.)

Bull, C. M., 1987. A population study of the viviparous Australian lizard *Trachydosaurus rugosus* (Scincidae). *Copeia* **1987**, 749–57.

Bull, C. M., 1988. Mate fidelity in an Australian lizard *Trachydosaurus rugosus*. *Behavioral Ecology & Sociobiology* **23**, 45–9.

Caughley, G., 1980. 'Analysis of vertebrate populations.' (Wiley & Sons: Chichester.)

Cogger, H. G., 1986. 'Reptiles and amphibians of Australia.' (Reed: Forest, NSW.)

Danzmann, R. G., M. M. Ferguson & F. W. Allendorf, 1988. Heterozygosity and components of fitness in a strain of Rainbow trout. *Biological Journal of the Linnaean Society* **33**, 285–304.

Dubas, G., 1987. Biotic determinants of home range size of the scincid lizard *Trachydosaurus rugosus* (Gray). Ph.D. Thesis, Flinders University, South Australia.

Farris, J. S., 1972. Estimating phylogenetic trees from distance matrices. *American Naturalist* **106**, 645–68.

Frankel, O. H. & M. E. Soulé, 1981. 'Conservation and evolution.' (Cambridge University Press: Cambridge.)

Hamrick, J. L., 1983. The distribution of genetic variation within and among natural plant populations. Pp. 335–48 *in*: 'Genetics and conservation—A reference for managing wild animal and plant populations'. Eds C. M. Schoenwald-Cox, S. M. Chambers, B. MacBryde & L. Thomas. (Benjamin/Cummings Publ. Co.: Menlo Park, California.)

Harris, H. & D. A. Hopkinson, 1976. Handbook of enzyme electrophoresis in Human genetics.' (North-Holland Publ. Co.: Amsterdam.)

Lande, R., 1988. Genetics and demography in biological conservation. *Science* **241**, 1455–60.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–90.

Nei, M., T. Maruyama & R. Chakraborty, 1975. The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10.

Nevo, E., A. Beiles & R. Ben-Schlomo, 1984. The evolutionary significance of genetic diversity: Ecological, demographic and life history correlates. Pp. 13–213 *in*: 'Evolutionary dynamics of genetic diversity—Lecture notes in biomathematics'. Ed. G. S. Mani. (Springer-Verlag: Berlin.)

Richardson, B. J., P. R. Baverstock & M. Adams, 1986. 'Allozyme electrophoresis. A handbook for animal systematics and population studies.' (Academic Press: Sydney.)

Robinson, A. C., P. D. Canty, P. A. Mooney & P. M. Rudduck, in press. 'South Australian offshore islands.' (Government Printer: Adelaide.)

Robinson, A. C., P. J. Mirtschin, P. D. Copley, P. D. Canty & R. B. Jenkins, 1985. 'The Reevesby Island Goanna—A problem in conservation management.' *South Australian Naturalist* **59**(4), 56–62.

Rogers, J. S., 1972. Measures of genetic similarity and genetic distance. Studies in Genetics, University of Texas Publication No. 7213, 145–53.

SAS Institute, 1987. 'SAS/Stat guide for personal computers version 6.' (Cary N.C., USA.)

Schmitt, L. H., 1978. Genetic variation in isolated populations of the Australian bush-rat, *Rattus fuscipes*. *Evolution* **32**, 1–14.

Schoener, A. & T. W. Schoener, 1984. Experiments on dispersal: short-term flotation of insular anoles, with a review of similar abilities in other terrestrial animals. *Oecologia* **63**, 289–94.

Schoenwald-Cox, C. M., S. M. Chambers, B. MacBryde & L. Thomas (Eds), 1983. 'Genetics and conservation—A reference for managing wild animal and plant populations.' (Benjamin/Cummings Publ. Co.: Menlo Park, California.)

Schwaner, T. D., 1988. An inventory of reptiles on Wardang Island, South Australia, with recommendations for their conservation and management. Report to Australian National Parks and Wildlife Service.

Sirkkomaa, S., 1983. Calculations on the decrease of genetic variation due to the founder effect. *Hereditas* **99**, 11–20.

Sneath, P. H. & R. R. Sokal, 1973. 'Numerical Taxonomy: the principles and practice of numerical classification.' (Freeman & Co.: San Francisco.)

Swofford, D. L., 1981. On the utility of the distance Wagner procedure. Pp. 25–43 *in*: 'Proceedings of the first Willi Hennig Society'. Eds V. A. Funk & D. R. Brooks. (New York Botanical Gardens: New York.)

Swofford, D. L. & R. B. Selander, 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**, 281–3.

Workman, P. L. & J. D. Niswander, 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal Human Genetics* **22**, 24–49.

Wright, S., 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **9**, 395–420.

Wright, S., 1969. 'Evolution and the genetics of populations, Vol. 2. The theory of gene frequencies.' (University of Chicago Press: Chicago.)

Wright, S., 1978. 'Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations.' (University Chicago Press: Chicago.)

Zar, J. H., 1984. 'Biostatistical Analysis.' 2nd Edn. (Prentice Hall: New Jersey.)

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