CONSERVATION GENETICS OF ISLAND AND MAINLAND POPULATIONS OF THE SLEEPY LIZARD (*TRACHYDOSAURUS RUGOSUS* : GRAY)

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CERTIFICATE OF ORIGINALITY OF THESIS

This thesis is my original work and has not been submitted, in whole or in part, for a degree at any other university or college of advanced education. Nor does it contain, to the best of my knowledge and belief, any material published or written by another person, except as acknowledged in the text.

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ABSTRACT

The genetic consequences of isolation on wildlife populations in reserves is an important component of management strategies aimed at long term conservation. In this study, seven island populations of the sleepy lizard (*T.rugosus*) in South Australia were studied to establish the genetic effects of isolation. These effects were assessed by comparing genetic characteristics of the island populations with three adjacent mainland populations.

Genetic relationships among the populations were examined using mitochondrial DNA, allozyme electrophoresis and morphology. The mitochondrial DNA analysis showed congruence with geographic proximity suggesting a degree of endemism for the populations studied. However, departures from congruence with geographic proximity were observed using allozymes and morphology suggesting that evolutionary forces had caused divergence among populations in these characters. Changes in allele frequencies were observed among populations which appear to result largely from random genetic drift in the island populations. Alleles which were rare on the mainland were not present in the island populations. The divergence among populations was assessed using Wright's F statistics on allozyme data and discriminant function analysis of 20 morphological characters. Much greater divergence was observed among the island populations than among those on the mainland reinforcing the notion that evolutionary forces (e.g. genetic drift) were greater among the insular populations. The data also indicate that the between population component of diversity is more important among the island populations than it is among those on the mainland.

Levels of variation within the populations were measured using allozyme electrophoresis and average levels of developmental stability. Heterozygosity levels did not vary significantly among the populations but, two measures of developmental stability (fluctuating asymmetry and percent gross abnormalities) were significantly higher in three of the island populations. It is not possible to rule out environmental causes of the increased developmental instability, but it appears more likely that they are caused by genetic drift and inbreeding resulting from small population size. The inability of electrophoresis to detect these changes is probably a reflection of the proportionately small sample of the genome represented by the sample of allozymes used.

The results have several implications for conservation and wildlife management. They emphasise the importance of considering populations as the unit of conservation by demonstrating 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. These results also suggest that developmental stability may well be more useful in monitoring genetic changes in wildlife populations than the conventional method of allozyme electrophoresis.

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CHAPTER 1 GENERAL INTRODUCTION

1.1 CONSERVATION DEFINED

The term 'conservation' can be defined in a variety of ways. Camp (1973: p7) describes conservation as 'the maintenance of the productive capacity of our land and water resources and their development or improvement to meet future requirements' while Passmore (1974: p73), taking a similarly anthropocentric view, defines it as 'the saving of natural resources for later consumption'. Usher (1973) uses conservation in a less general sense by viewing it in terms of biological management, stressing the need to sustain production of natural resources in perpetuity. Within this definition, Usher identifies six categories of resource, namely agriculture, forestry, water, fisheries, recreation and wildlife. Usher (1986: p5) further refines his definition in relation to wildlife so that the conservation of wildlife enables 'the retention over time of a viable and hence self perpetuating, nature resource'. This approach to conservation, while still viewing wildlife as a human resource, emphasises the importance of maintaining wildlife in a viable long term manner.

The importance of a long-term approach to wildlife conservation was first discussed by Frankel (1974), who introduced the concept of the 'Time Scale of Concern'. Frankel argued that the time frame in which wildlife management was viewed would influence the priorities set for its conservation. He contended that nature conservation measures in their present form, although often supposedly aimed at the long term, would usually only be effective in the short term, and that conservation on an evolutionary time scale is the only meaningful biological perspective. In this context, the use of the word viable by Usher (1986) in defining wildlife conservation, must encompass the long-term ability of populations of organisms to adapt to environmental change.

Frankel and Soulé (1981: p4), in defining nature conservation, incorporated Frankel's (1974) idea of an evolutionary context. They described conservation as the 'policies and programs for the long-term retention of natural communities under conditions which provide the potential for continuing evolution'. It is this definition which has been adopted for this thesis. Viewing conservation on an evolutionary time scale forms the basic rationale for conservation genetics.

1.2 NATURE RESERVES AND THEIR CONSERVATION RATIONALE

The maintenance of biological diversity is a central theme in conservation literature (Frankel, 1970 a and b, 1974, 1977, 1982; Ehrlich, 1980; Soulé and Wilcox, 1980; Frankel and Soulé, 1981; Myers, 1986; Soulé, 1986). This has largely resulted from the realisation that the protection of wild plant and animal populations as genetic bases for potential food sources, medicinal uses and scientific studies may well be critical to the earth's future well-being (IUCN, 1980; Ehrlich and Ehrlich, 1982; Myers, 1984; Simberloff, 1986; Soulé *et al.,* 1986). There is little doubt that the extinction of many wildlife species is imminent unless preventative measures are forthcoming. Myers (1979), predicts that up to one third and possibly as many as one half of the earth's ten million species could be extinct by the middle of the twenty-first century and concluded that the conservation of populations of organisms to maintain biological diversity must receive urgent attention.

The rapid transformation in recent centuries from uninterrupted areas of natural habitat to isolated pockets (usually represented by nature reserves) surrounded by agricultural or urban land, has probably already precipitated the loss of wildlife species. If biological diversity is to be maintained, active policies for the management of flora and fauna retained within these reserves, will be necessary.

The management of natural areas requires an understanding of the effects that habitat fragmentation will have on the short and long term survival of the species and ecosystems held within them. Central to this, is understanding the mechanisms which may cause population extinction. Reserve area as a limit to population size, is seen as a major influence in the potential survival of many populations, particularly those species requiring large home ranges. Soulé (1980), argued that the areas that have been set aside in national parks and reserves are generally of insufficient size for the conservation of many species. Furthermore, park boundaries are often located along political rather than biological lines, and thus sometimes fail to accommodate the ecological processes necessary for the survival of species held within them (Newmark, 1985).

A lot of the debate in conservation biology has focused on the effects of fragmentation on natural habitat. Island biogeographical theory has provided much of the basis for predicting the effects of habitat segmentation on wildlife (Diamond, 1975; Wilson and Willis, 1975 Diamond and May, 1981; Willis, 1984). These theories have been developed to explain the empirical observation that the number of species present on an island increases with island area. One such theory, the 'dynamic equilibrium theory of island biogeography` developed by MacArthur and Wilson (1963, 1967), predicts that the number of species present on an island will be determined by a balance between the rate at which species extinction occurs on the island and the rate of species colonisation of the island. Initially, if it is cut off from the mainland, an island will have a higher number of species than it can accommodate causing a gradual process of extinction to follow until eventually, a new, lower equilibrium level is established. At this equilibrium level, the rate of species extinction will equal the rate of colonisation by the same or other species from nearby sources.

Integrated in the dynamic equilibrium theory of island biogeography, is the role of island area and distance from the nearest source of colonisation in determining the equilibrium level. Smaller islands are expected to have higher extinction rates owing to increased vulnerability to chance extinctions and because competitive exclusion could be expected to occur at a lower species number (MacArthur and Wilson, 1967). The distance from the nearest source of colonisation will influence the rate at which new species can colonise the island. The closer the island is to a colonisation source, the higher the equilibrium species number (MacArthur and Wilson, op. cit.). In addition, the differing abilities of organisms to disperse between isolates and their different area requirements will influence the degree of species loss and the type of species which colonise the island. For example, flying animals tend to disperse more effectively than non-flying animals, while many species are stopped by relatively narrow dispersal barriers (Diamond, 1975).

A second explanation for the observed species-area relationship, developed by Lack (1969; 1976), is that habitat diversity will determine the type and number of species which can exist on an island and that an island's area is simply a reflection of habitat diversity. Positive correlations between island area and habitat diversity have been observed many times. However, there have been occasions when species diversity on continental islands appears to be independent of habitat diversity (e.g. Abbott, 1978), suggesting that Lack's hypothesis is inadequate to explain some natural systems.

The potential application of island biogeographical theory to reserve design is clear. If area is critical in determining the number of species which can exist within a reserve, then maximising the area of each reserve will be paramount. However, if habitat diversity is critical, then maximising the variety of habitats within a reserve must be a major design criterion.

The application of island biogeographical theory to reserve design has been controversial for two main reasons. First, island biogeographical theories remain largely unsubstantiated under experimental conditions particularly with respect to the measurement of actual species turnover rates (L.E. Gilbert, 1980; Margules *et al.*, 1982; Burgman *et al.*, 1988). Second, the extrapolation of these theories to the suitability and management of habitat fragments is complicated by differences between surrounding habitats and their interactions with the insular communities (Janzen, 1983). For example, the supply of invasive species resulting in increased competition and predation and interactions with the surrounding agricultural land will influence the rate at which species are gained or lost from 'habitat' islands. The degree to which these factors influence species in a reserve will in turn be a function of the type of land which surrounds the reserve.

Another approach to the problems of reserve design, emphasising the role of individual species in determining the minimum reserve size, is being urged by some biologists (Jarvinen, 1982; Simberloff, 1986; Zimmerman and Bierregaard, 1986). This 'autecological' approach concentrates on species which are critical to the ecosystem being managed. Keystone species provide critical ecological links within natural systems and will have far greater effect on ecosystem stability when lost, than less central species (F.S. Gilbert, 1980). These species require consideration in any reserve design, as do those which may be extinction prone such as large vertebrates (Jarvinen, 1982). Clearly, the critical parameters involved in extinction dynamics must be defined if the management of wildlife in nature reserves is to be effective.

1.3 POPULATION FITNESS, GENETIC VARIATION AND DEVELOPMENTAL STABILITY

The maintenance of a population's long-term fitness (its ability to maintain viability and fecundity over time) is necessary for effective conservation. The maintenance of this fitness relies upon population size which affects the rate of inbreeding (Senner, 1980). In small populations, individuals may be mating at random but still with genetically related individuals causing an increase in the relative frequency of homozygotes in the population. In extreme cases, this can result in a reduction in average fitness levels (inbreeding depression) where characters which are closely linked with fitness (such as fertility and viability) are affected (Falconer, 1981).

Two explanations are generally invoked to account for the phenomenon of inbreeding depression. First, the increased frequency of homozygous recessive alleles causes average phenotypes to move away from dominant or overdominant phenotypes towards recessive traits. A shift towards the phenotypic expression of recessive alleles causes a decline in average fitness because disproportionate numbers of recessive alleles are deleterious. Typically, dominant traits are those which relate to viability and thus inbreeding may result in declines in fecundity, fertility, developmental rate and litter size (Frankel and Soulé, 1981).

Second, heterozygosity has been linked empirically to fitness (Soulé, 1980). That is, heterozygous individuals themselves appear to be fitter than individuals which are homozygous (a phenomenon known as heterosis). Consequently, a decline in the number of heterozygous individuals in a population will result in a net decrease in average fitness. The mechanisms underlying heterosis are unclear but it may result from increased biochemical versatility possessed by heterozygotes (Falconer, 1981). That is, two alleles at the same locus may be beneficial under different developmental conditions canalizing development towards an optimum phenotype. This versatility enables heterozygous individuals to cope with a more diverse range of developmental conditions than homozygotes and to better buffer themselves during development (Falconer, op. cit.). Consequently, individuals with reduced levels of heterozygosity will be less developmentally stable than heterozygous individuals and thus exhibit greater phenotypic variation (Eanes, 1978; Mitton, 1978).

Lerner (1954) postulated that heterosis would be important in 'developmental homeostasis` and predicted a genetic-phenetic relationship, where populations with higher levels of genetic variation, would have lower levels of morphological variation between individuals. This has been supported by several studies (e.g. Leary *et al.*, 1983; Danzman *et al.*, 1986) using heterozygosity as a measure of genetic variation. Moreover, Mitton and Grant (1984) by way of a literature review, found that approximately 70 to 80 percent of effects on growth and developmental stability can be attributed to heterozygosity *per se*. However, there remains some debate over the universality of this relationship (see in particular Zinc *et al.*, 1985). This topic is discussed further in section 5.1.

Clearly, although the exact reasons are not yet fully understood, the level of genetic variation in populations is important in determining population fitness. In the longer time scale, genetic variation is essential to a population's ability to adapt to changing environmental conditions (Beardmore, 1983). Thus, if populations in reserves are to be managed in perpetuity and their evolutionary potential maintained, then maintaining levels of genetic variation must be included in any wildlife management strategy.

1.4 FACTORS AFFECTING GENETIC VARIATION IN NATURAL POPULATIONS

Genetic variation in populations is influenced by mutation, migration, genetic drift and selection (Ayala and Kiger, 1984). Dobzansky (1951), Clarke (1979), Wills (1981) and others, have suggested that selection is important in maintaining levels of genetic polymorphism and heterozygosity in natural populations. Three fundamental mechanisms have been observed supporting this view.

First, the conferral of a fitness advantage upon an individual can result from the overdominance of the heterozygous form. The overdominance of heterozygotes at a locus, ensures the maintenance of more than one allele (Lewontin, 1974). Proven cases of such heterozygote advantage are however, rare although there are situations in which this is clearly the case (e.g. Allison, 1964 with sickle cell anaemia in humans).

Second, genetic diversity can be maintained in a population by frequency-dependent selection where ecological factors such as predation, parasitism and competition regulate the type and frequency of variants present in the population. For example, predators may take disproportionately large amounts of a common variant when faced with a choice and hence increase the proportion of rare variants (Clarke, 1979). Several instances of frequency-dependent selection maintaining polymorphisms have been observed including mimicry in the butterfly *Pseudacrea eurytes*. This has been shown to be frequency-dependent as a result of regulation by predation (Clarke and Sheppard, 1971).

Third, spatial and temporal variation in selective pressure among individuals within a population, can maintain genetic variation (Hedrick *et al.*, 1976). Variation in selective pressure may take the form of spatial heterogeneity in habitat or temporal selection pressures. For example, Nevo *et al.* (1984), found in a study of the literature, that organisms which were broad-niched, common and widespread were generally more polymorphic than those which were more specialised, geographically restricted and narrow niched. Moreover, many examples of spatial variation in selective pressure maintaining polymorphisms have been observed particularly in experimental or human affected natural situations. For example, polymorphism at the ADH locus in *Drosophila melanogaster* has been maintained by variation in substrate in the laboratory (Oakshott, 1979) while resistance to warfarin has resulted in polymorphism between treated and untreated populations of the Norwegian rat (Bishop, 1981).

Not all selection will promote variation. Quantitative characters will be influenced by selection which in some situations may result in reduced variation at loci affecting those traits. Mather (1953) lists three modes of selection which influence quantitative traits. These are 'stabilising' selection, which is selection against extreme values and towards an intermediate value, 'directional' selection where selection for one extreme is an advantage and 'disruptive' selection which is selection for extreme values and against intermediate values. The role of disruptive selection in natural populations is not well understood (Franklin, 1980), though laboratory evidence suggests that it could be important in maintaining genetic polymorphisms (Thoday, 1972; Mather, 1973). However, strong stabilising or directional selection can reduce genetic variation by the fixation of alleles affecting the mean of characters under selection (Falconer, 1981). Thus, selection for phenotypic intermediates will eliminate extreme deviants resulting in the fixation of alleles. This will occur provided there is not a strong requirement for developmental canalization (as might be expected in a fluctuating environment) which may assist in maintaining heterozygosity and thus counteract allelic fixation (Spiess, 1977). The extent of strong stabilising selection on polymorphisms in a population is unknown, but Franklin (1980) argues that although it can reduce genetic variation there is little evidence that weak stabilising or directional selection reduces additive variance to any great degree or at any great rate.

The importance of selection in maintaining genetic polymorphisms must be assessed in relation to other processes which affect alleles in populations. An alternative school of thought suggests that selection at the majority of loci is insignificant and effectively neutral with respect to fitness (Kimura, 1968). Under neutral conditions, the interaction between mutation rate and genetic drift becomes critical in generating polymorphisms. The neutralists claim that such an interaction can account for most polymorphisms observed in natural populations (Kimura, 1982).

Genetic drift is the change in gene frequency within a population caused by a sampling error from one generation to the next and will result in a reduction in genetic variation in the daughter generation. The smaller the sample size, the greater the loss of genetic variation. If the population experiences a bottleneck (a temporary decrease in numbers) or is founded with an initially low number of individuals (founder effect) then rare alleles may be lost.

Population size is critical in determining the level of genetic drift in a population (Frankel and Soulé, 1981) and consequently is critical in determining the relative effects of genetic drift and selection on levels of genetic variation in natural populations. For example, in a population which is large (say greater than 500), the effect of drift will be negligible if the selection coefficient is of a measurable intensity. However, in small populations (e.g. 100) genetic drift will counteract all but the strongest of selection forces (Frankel and Soulé, 1981). In natural populations where selection forces are found not to be strong, Franklin (1980), tentatively concludes that without inter-population gene flow, the level of genetic variance will be determined by the balance between genetic drift and mutation.

The influence of bottlenecks and founder effects in reducing levels of genetic variation needs to be considered in the management of populations. A bottleneck or small founding population does not necessarily mean a high loss of genetic variability. Success in retaining genetic variation in a population following a founding event depends upon several factors including the number of individuals in the founding population and their genetic constituency. Frankel and Soulé (1981) consider that unless the number of founders is in the order of two pairs or less, it is not the bottleneck *per se* that causes substantial reduction in genetic variation and they estimate that a founding number of two will contain 75 percent of the original population's genetic variability. Rather, it is the events which follow the bottleneck which will determine the degree of genetic variability retained in the population. If a population recovers from a bottleneck and expands rapidly, then genetic drift will be minimised and a large proportion of the initial genetic variability will be retained. However, if the population size remains small, then a gradual loss of genetic variability through drift will occur (Powell, 1983).

The genetic make-up of the founding individuals is also important in determining the effects of a bottleneck. If deleterious alleles concealed within heterozygotes were present in the initial founders, then mean fitness in the new population may be permanently reduced (Carson, 1983). Furthermore, the number and distribution of alleles in the founding population will affect the amount and type of variation which is lost. For example, an allelic distribution which has a few common alleles will probably be less affected by a bottleneck than a population with many rare alleles (Powell, 1983).

The rate of genetic drift in a population with no migration, depends on the size of the population, its mating structure, and the number and distribution of offspring (Frankel and Soulé, 1981). Thus, the population size to be considered is the "effective" population size (N_e) which takes these factors into account rather than the total number of breeding individuals. N_e will be affected by unequal sex ratios, non-random variation in progeny number, fluctuations in population size, departures from panmictic population structure and overlapping generations (Kimura and Crowe, 1964; Lande and Barrowclough, 1987). Franklin (1980) considers that without immigration, an effective population size of 50 is required to prevent inbreeding depression. However, to reach an equilibrium between genetic drift and mutation rates, he estimates that this number needs to be nearer 500. Low levels of migration greatly assist in the maintenance of genetic variability and Avery (1978) estimates that only one individual per generation is required to maintain equilibrium for neutral alleles.

In the design of nature reserves, effective population size, the presence and number of keystone species and species/area relations need to be recognised. Critical to this and to the management of wildlife within reserves, is some concept of the minimum number of individuals of any one species required to form a viable population. Attempts to define the minimum viable size for a managed population must consider the maintenance of a size sufficient to prevent genetic drift.

1.5 REVIEW OF PREVIOUS ISLAND STUDIES

The management of an insular population in any natural system relies on a thorough understanding of the factors involved in long-term population survival. Once the general principles are known, they must be applied individually to each complex ecological system under review. The complexities are best analysed by studying the simpler natural systems and applying the basic principles obtained from these to more diverse ecosystems.

Island systems have long been recognised as unique places for the study of organisms and their evolutionary and ecological adaptations. Generally, islands are relatively simple ecological systems and because of their easily definable boundaries, are possible to study without the complicating interactions of surrounding and overlapping ecosystems. Islands can be used to study the effects of long-term isolation of populations on their survivability. Information of this nature is essential if sound management decisions are to be made about nature reserves, many of which effectively, are now islands.

There have been many electrophoretic surveys of natural populations since the development of gel electrophoresis in the 1960's. These studies have examined a plethora of organisms (for reviews see Selander and Johnson, 1973; Powell, 1975; Nevo, 1978; Nevo *et al.*, 1984; Wayne *et al.*, 1986) for both population studies and systematic problems. However, few of these have assessed the temporal effects of isolation on the maintenance of genetic variation.

Several researchers (e.g. Gorman *et al.*, 1975, Berry 1986; Aquadro and Kilpatrick, 1981) have attempted intraspecific comparisons between island and mainland populations, examining levels of electrophoretic variation. However, except for of Soulé and Yang (1973), Patton *et al.* (1975), Schmitt and White (1979), Soulé (1979) and Schwaner (submitted), few studies have been extended to include morphological variation. Of these, only two (Soulé, 1979; Schwaner, submitted) have attempted to measure developmental stability as an assessment of the genetic effects of isolation.

A high degree of concordance between morphological and allozyme divergence was recorded among insular population of *Rattus fuscipes greyii* (Schmitt and White, 1979) and *Rattus rattus* (Patton *et al* 1975). Schmitt and White (op. cit.) also noted a measure of agreement between morphological and allozyme variation within populations with populations on smaller islands exhibiting less variation than those on larger ones.

Soulé and Yang (1973), compared mean heterozygosity levels of island populations of the side-blotched lizard *Uta stansburiana* and assessed the ability of 14 independent biogeographical and morphological variables to correlate with mean levels of heterozygosity using stepwise multiple regression analysis. The mean heterozygosity levels of the populations examined ranged from 1.4 to 11.1% and the variables used in the analysis included island area and elevation, distance from the nearest land mass, mean coefficient of variation, the number of confamilial species and a morphological assessment of phenotypic divergence. They found that mean heterozygosity had a strong positive correlation with the number of confamilial species. Soulé and Yang (op. cit.), suggested that this variable increased stabilising selection pressure on *U. stansburiana* on the islands. That is, the more lizard species on an island, the greater the pressure on the *U.stansburiana* to retain its original morphology, behaviour and habitat and thus the less directional selection acting on the population. This is supported by the measure of phenotypic divergence which Soulé and Yang used as an indicator of the directional selective pressure on each population. The closer the mean morphological values of the island population to those of the mainland population then the lower the directional selection pressure exerted since isolation.

Further, Soulé (1979) using the same electrophoretic data (cited above in Soulé and Yang, 1973) found a positive correlation between a measure of developmental stability (fluctuating asymmetry) and heterozygosity suggesting a genetic cause while Schwaner (submitted), using percentage of abnormalities as an index of developmental stability, found a similar correlation in island populations of the tiger snake (*Notechis scutatus-ater*).

1.6 PROJECT SCOPE AND SIGNIFICANCE

As described in section 1.5 very few studies have examined the relationship between heterozygosity and developmental stability using island and mainland populations of the same species. The elucidation of this relationship has important implications for conservation and the management of wildlife. If developmental stability represents an accurate reflection of the genetic status of faunal populations then its use as a monitoring technique could have wide application in wildlife management.

The sleepy lizard (*Trachydosaurus rugosus*) is a common and widespread reptile whose distribution was partially fragmented by rising sea levels 6,000 to 20,000 years before present (BP). Several populations of this lizard have been isolated on what are currently offshore islands while intact populations remain on adjacent mainland areas. The size of several of the islands upon which these animals live, suggests that the population sizes involved are small. This provides an ideal situation for studying the effects of isolation on a series of conspecific populations.

The objective of this project was to establish the genetic effects of isolation on insular populations of *T.rugosus* by making comparisons between island and mainland populations. Variation was assessed using mitochondrial DNA, allozyme electrophoresis and morphology. These parameters were used to assess the relationships of the populations to each other and to compare them to the known isolation histories of the islands. Levels of heterozygosity within each population were also measured using allozyme electrophoresis and compared with measures of developmental stability. The results are discussed with reference to their implication for conservation.

CHAPTER 2 SLEEPY LIZARD BIOLOGY AND STUDY SITE DESCRIPTION

2.1 REVIEW OF THE GENERAL ECOLOGY OF THE SLEEPY LIZARD

2.1.1 General biology

At the generic level, the taxonomic status of the sleepy lizard has been disputed by several workers and is still unresolved. Generally, the monotypic genus *Trachydosaurus* has been preferred, although Mitchell (1950), King (1973) and Hutchinson (1981), have suggested that the sleepy lizard be placed in the genus *Tiliqua* based on immunological, chromosomal and osteological evidence. Despite this, and because it is in general usage, the nomenclature *Trachydosaurus rugosus*, followed by Cogger *et al.* (1983), will be adopted in this thesis.

The sleepy lizard is a large, terrestrial lizard which is widely distributed over the southern half of the Australian continent inhabiting all states except the Northern Territory and Tasmania (Cogger, 1986). Its habitat varies considerably, ranging from desert and tussock grasslands to low shrubland and open forest (Cogger *et al.,* 1983). Sleepy lizards have short legs in relation to the rest of the body, rugose scales on the head and dorsal regions (Cogger, 1986), and may grow to a snout vent length of more than 300 mm (Bull, 1987). They are omnivorous, eating a wide range of flowers, snails, seedlings, fruit, green herbage, insects and carrion (Satrawaha and Bull, 1981; Dubas, 1987). Sleepy lizards are heliotherms, thermoregulating by behavioural means such as modification of activity patterns during different seasons and by accelerated breathing, panting, tongue movements and opening of the mouth (Warburg, 1965).

Sleepy lizards have short tails (usually 20 to 25% of snout vent length) which are used to store fat in the spring months (Bustard, 1970). To assist in maintaining water balance while coping with a diet of high salt intake, *T.rugosus* is capable of secreting concentrated brine through a salt gland located in the nasal passage (Braysher, 1971). In addition, sleepy lizards have a high tolerance to electrolytes in the plasma (Bentley, 1959) and exhibit a low evaporation rate (Warburg, 1965). These adaptations enable sleepy lizards to obtain sufficient moisture from food without requiring drinking water (Sharrad, 1979).

2.1.2 Distribution in South Australia

T.rugosus is distributed continuously throughout the southern regions of South Australia (see Fig. 2.1), in a variety of environmental conditions ranging from the cooler, wetter areas in the south east of the state (Thompson and Tyler, 1983) to the more arid regions such as Lake Eyre (Sharrad, 1979).

A variety of colour morphs showing geographic trends can be observed throughout the range of *T.rugosus* (Bustard, 1970). In South Australia, the southern lizards tend to be smaller and darker than their northern counterparts (Sharrad, 1979) although no quantitative assessment of this trend is available. The animals also exhibit sexual dimorphism in tail size (Cogger, 1986) however, this too is yet to be statistically substantiated in published works.

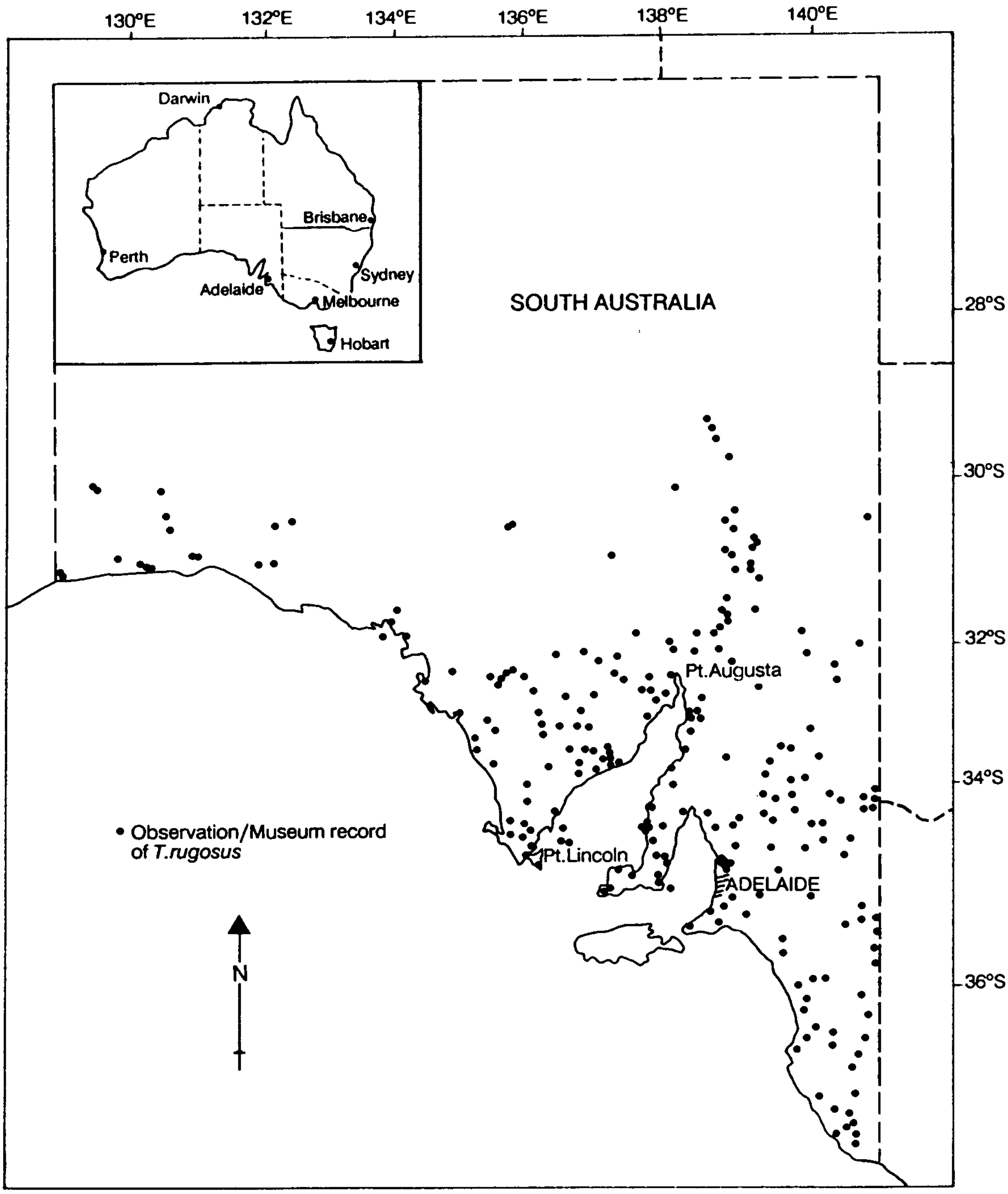


FIGURE 2.1 The distribution of *T.rugosus* in South Australia. Derived from voucher specimens at the South Australian Museum and the personal observations of R.Sharrad (in Sharrad, 1979).

2.1.3 Reproductive biology

Sleepy lizards are viviparous with one to three young born per female between the months of February and March (Bull, 1987). Maturity is reached within three to four years of birth with individuals living at least nine years and reproduction probably occurring annually. Mortality apparently peaks among juveniles and population size remains reasonably stable over time (Bull, op. cit.). Sex ratios have been found to be equal in population studies of the sleepy lizard (Dubas, 1987; Schwaner, 1988) and home ranges do not vary between the sexes (Dubas, op. cit.). Male and females pair during the mating season (late October to November) with pairing lasting an average of 13.9 days. The pairing is apparently non-random with a high degree of mate fidelity being maintained over consecutive years (Bull, 1988).

2.1.4 Home range and movement patterns

Sharrad (1979) investigated sleepy lizard activity in relation to their role as a major host of the reptile ticks *Aponomma hydrosauri* and *Ambolyomma limbatum* in a population at Mount Mary in South Australia. Seasonal variation in activity was marked, peaking in the spring months of late August to November when available food was maximal. Satrawaha (1980), Bull (1987) and Schwaner (1988) reported greatest activity in late September and October in populations of *T.rugosus* at Tickera and Wardang Island (Yorke Peninsula, South Australia) respectively. The lizards are least active in winter but do not hibernate and are often seen basking on warmer days. Activity in summer and autumn periods is variable and is possibly related to unseasonal rains or warmer weather (Sharrad, 1979).

T.rugosus maintains a well defined but overlapping home range (Dubas, 1987) though estimates of home range for sleepy lizards vary. Sharrad (1979) using radio transmitters obtained range sizes of 15,000 to 50,000 m^2 while home range sizes observed by Bull and Satrawaha (1981) from mark recapture data varied from 136 to 48,000 m^2 (average = 9,400 m^2). Similar results were also obtained in a study of a population of *T.rugosus* south of Perth W.A. where home ranges varied from 5,607 to 13,258 m^2 (average = 10,142 m^2) (Fergusson and Algar, 1986). Bull (1987) in eight years of mark recapture work reported generally small individual home ranges, though two distinct groups of mobility were observed within the population. Over 90 percent of the lizards recaptured during the eight years had moved less than 100m from their original site of capture. However, a small number of lizards had moved distances of up to 2 km. Some of these lizards were recaptured several times at the new site, indicating a new residence. The reason for this migration remained unclear as very few aggressive interactions between lizards were observed (Bull, op. cit.).

Temperature appears to influence the choice of refuge sites for sleepy lizards (Satrawaha, 1980). At low temperatures the lizards tend to choose grass clumps which provide shelter and protection and allow them to take advantage of rapidly increasing temperatures. Other refuge sites commonly used, include hollow logs, old tree stumps and bits of tin, with lizards tending to alternate between sites within their home range (Sharrad, 1979).

2.1.5 Population abundance

Bull (1987) used mark/recapture techniques to estimate the size of the Tickera population of sleepy lizards, including both the Peterson and Jolly-Seber measures of abundance. Seven annual population estimates from 1976 to 1982 showed no significant difference between the years. Bull concluded that the Peterson method was possibly the more reliable of the two measures and using this, estimated a density of around 2.7 lizards/ha.

2.2 DESCRIPTION OF STUDY SITES

2.2.1 Site location and Holocene history

The study area includes sixteen populations of the sleepy lizard (*T.rugosus*) (Fig. 2.2). Eight of these occur on continental islands off the coast of South Australia, with the remaining populations sampled from mainland localities. A full description of each of the main study sites, is given in section 2.2.2.

The past 125,000 years have seen several large changes in sea level owing to fluctuations in the pleistocene ice caps. The lowest levels were reached at 53,000 years BP and 20,000 years BP (Chappell, 1976) with subsequent rises in sea level. Various studies around Australia have been reasonably consistent, placing the termination of the final rise in sea level from 18,000 years BP to 6,500 ± 250 years BP with a subsequent standstill period (Thom and Roy, 1985). However, there has been some recent debate over fluctuations in sea level during the past 5,000 years. Thom and Roy (op. cit.) describe two

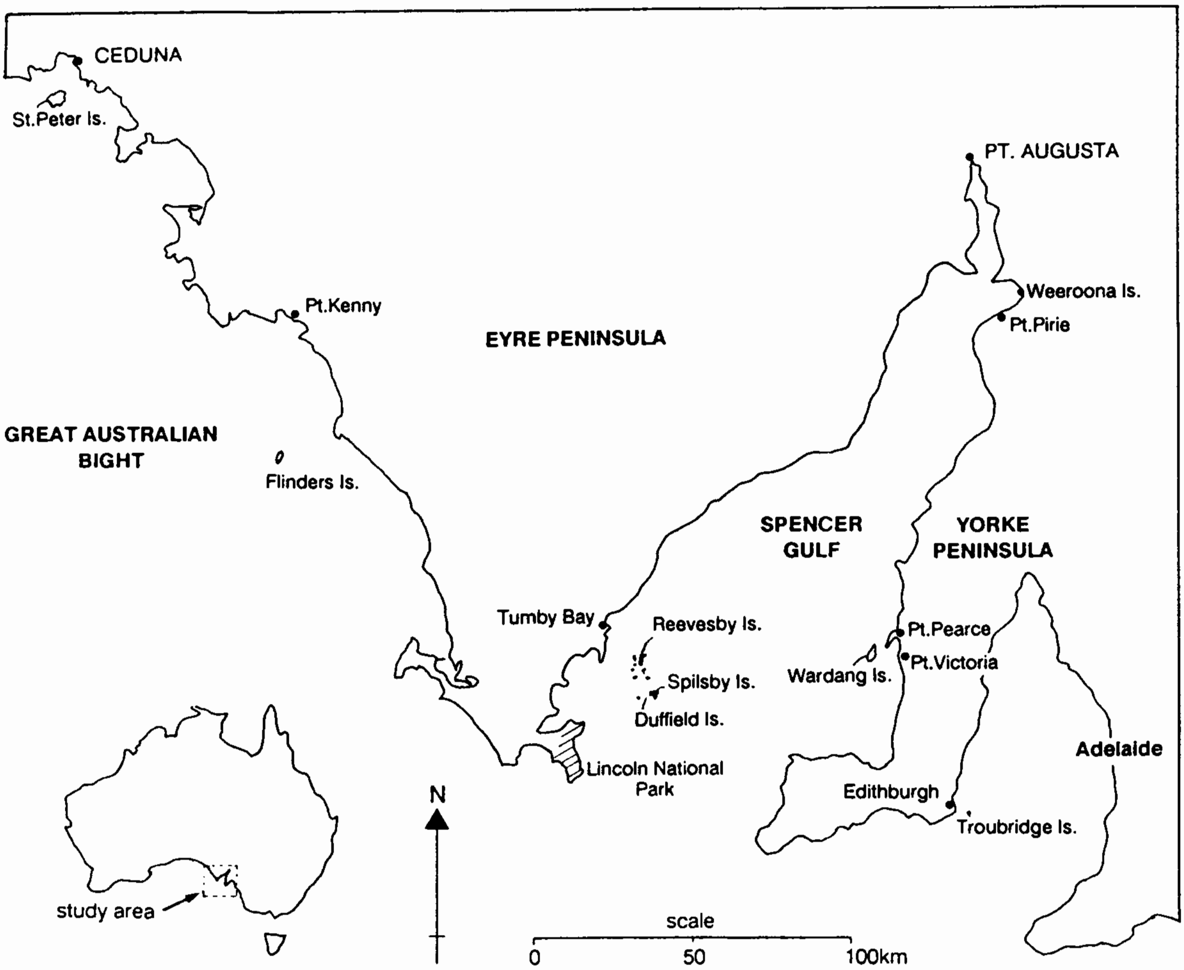


FIGURE 2.2 The location of all populations of *T.rugosus* sampled in this study.

major schools of thought. One of these, proposes a constant level which is indistinguishable to that of the present day. The other describes a period up to 5,000 years before present, where increases of 1 to 3m above the present level occurred followed by a general decline to current levels.

Fluctuations in sea level over this time period may well be significant only on a regional scale because of local features such as tectonic movement. Detailed studies of individual regions are required for local sea level histories to be accurately traced. Currently, little of the South Australian coast has been covered in detail (Belperio *et al.*, 1983). The only region of the state's coastline to be examined closely is Spencer Gulf where it appears that the region was inundated by Holocene seas approximately 8,000 years BP and that a level of between 2.5m and 3.8m (Burne, 1982; Hails *et al.*, 1983) above the present level was attained during the next 6,000 years. Subsequently, the sea level fell to its present level between 1740 and 1640 years ago. Unfortunately, extrapolation of these changes to other parts of the coast (including the islands of lower Spencer Gulf and the west coast of Eyre Peninsula) is of doubtful validity. For example, Hails *et al.* (1983) argue that the rise in sea level of the upper Spencer Gulf region could be attributed to hydrostatic upwarp because of its distance from the edge of the continental shelf edge relative to other parts of the coastline. Evidence suggests that local seismo - tectonism is the most likely cause of the subsequent fall in sea level (Belperio *et al.*, 1984; Hails *et al.*, 1983).

Mooney (unpub.) used sea level curves from Chappell (1976) and Chappell and Thom (1977) together with seabed topography to predict times of isolations for six of the islands involved in this study - St. Peter, Flinders, Spilsby, Duffield, Reevesby and Wardang islands. The areas and estimated times of isolation are given for each island in Table 2.1.

2.2.2 Site description

The eight islands surveyed in this study were those currently known to include populations of *T.rugosus* in South Australia and include St. Peter (32° 17'S 133° 34'E), Flinders (33° 43'S 134° 31'E), Spilsby (34° 40'S 136° 17'E), Duffield (34° 39'S 136° 19'E), Reevesby (34° 32'S 136° 17'E), Weeroona (33° 06'S 138° 02'E) Wardang (34° 30'S 137° 20'E) and Troubridge (35° 07'S X 137° 50'E). At least one other island not in S.A. (Rottnest Is., W.A.) is also known to have an endemic population of the sleepy lizard (Cogger, 1986) which has been given the sub-specific status of *T.rugosus konowi* (Cogger *et al.*, 1983). The three mainland areas chosen to represent non-fragmented populations were those as close as practicable to the islands in the study. However, no morphological comparisons were made with mainland areas adjacent to St. Peter, Flinders and Troubridge Islands because too few lizards were collected from St. Peter and Flinders Islands for rigorous morphological analyses, and the Troubridge Island population is introduced, with the origin and number of founders unknown.

The islands generally show a pattern of modification since European settlement, which sometimes is quite severe (pers. obs.). All islands were either inadequately surveyed or not surveyed at all before European use. Thus, survey information currently available, represents an incomplete description of the former environment of each

TABLE 2.1 Size and isolation times for each of the islands included in the study. All times of isolation are from Mooney (Unpub.). The area of Weeroona is unknown. BP, before present. * Separated from the mainland by mangrove swamp. ** Spilsby and Duffield were separated from Reevesby at approximately 7,700 years BP.

ISLAND	**SIZE (HA)**	**TIME OF ISOLATION FROM THE MAINLAND (Years BP)**	**TIME OF COMPLETE ISOLATION (Years BP)**
WARDANG	20,400[1]	6,000	6,000
ST PETER	4,028[2]	6,000$^+$	6,000
FLINDERS	2,642[3]	8,750	8,750
SPILSBY	425[2]	8,050**	6,000
REEVESBY	358[2]	8,050	6,000
WEEROONA	-	-	-*
DUFFIELD	8[2]	8,050**	6,000
TROUBRIDGE	8[4]	<150	<150

Source: [1] Stevenson (1985); [2] Robinson *et al.* (1985); [3] Hudson *et al.*, 1981; [4] Owers (1986).

island. A description of each island and the sampling undertaken is provided below.

St. Peter Island is the most westerly island examined. It has been farmed extensively this century but still retains a large proportion of native vegetation. In two trips (ca. 20 person days) in August and October (times of maximum activity) 1987, only eight animals were captured, suggesting a population of very low density. Anecdotal evidence from a former owner of the island (J.Nichols, pers. comm.) supports this conclusion, though the reasons for the low density are unclear.

Flinders Island is a large island which has been extensively cleared and farmed. The island was surveyed in June 1987 however, despite the existence of considerable amounts of surface rubbish (which is often used by *T.rugosus* for shelter in winter months) only three lizards were found in over eight person days. This island has been excluded from most analyses in this study.

The Sir Joseph Banks Group of islands consists of 17 islands (see Fig. 2.3) clustered in an area of 18 km by 22 km. The islands are generally low in topography, with a surface of kunker and sand overlaying a granitic base (Blissett and Warne, 1967). Three of these islands **Duffield, Spilsby** and **Reevesby** currently have populations of *T.rugosus*. However, Tubb (1938) reported populations of the lizard on Hareby, Kirkby, Langton and Winceby islands. In numerous survey trips to the Sir Joseph Banks Group between 1980 and 1987 by T. Schwaner (pers. comm.), no *T.rugosus* were observed from any of these islands indicating their almost certain extinction. All four islands have been

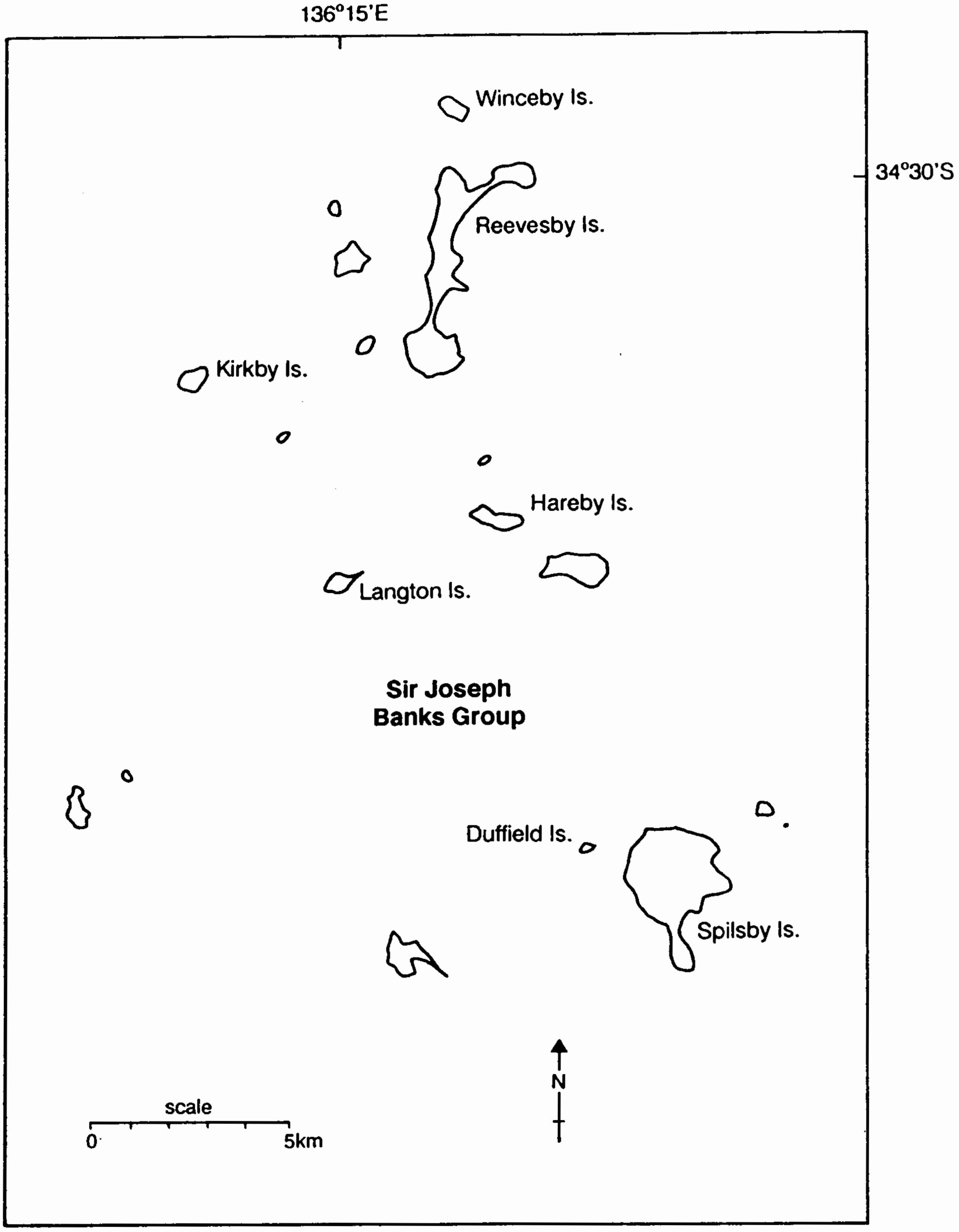


FIGURE 2.3 The Sir Joseph Banks Group of islands. The islands named are those reported to have populations of *T.rugosus* by Tubb (1938). Reevesby, Spilsby and Duffield are the only islands in the group to still retain these populations.

Both Reevesby and Spilsby Islands have been farmed extensively with large areas of land being cleared for pasture (Robinson *et al.*, unpub.). Spilsby Island had approximately 90 percent of its native vegetation cleared by 1910 (A.Scruby, pers. comm.) and has been heavily grazed by sheep and introduced rabbits for most of this century. In four trips to the island; June 1985, November 1986, August, 1987 and February 1988, totalling over 20 person days, only 24 animals were captured. Efforts were made to survey the entire island, but all captures were made at the northern end where the last remaining native vegetation exists. Clearly, population densities are very low on Spilsby. Anecdotal evidence from a former owner (A.Scruby, pers. comm.) suggests that this has been the case at least since the 1930's.

Duffield Island is only 500 m from Spilsby with a maximum sea depth between them of only four metres. It is sparsely vegetated but, though sometimes grazed in the early 1900's, was not grazed after 1930 (A. Scruby, pers. comm.) and has thus probably suffered little from human usage.

Reevesby Island is situated 11 km north of Spilsby and has also been farmed extensively. The island is now a conservation park and has not been grazed for at least 15 years. Consequences of human usage include the introduction of several weed species particularly boxthorn

(*Lycium ferossimum*) which is very common at the southern end of the island. In addition, animals such as cats and possibly goannas have been released on the island (Robinson *et al.*, 1985; Schwaner, 1985a). The island was never completely cleared and maintains a significant amount of original vegetation.

Owing to logistic problems involved in sampling the entire length of the island, the survey of Reevesby was confined to the southern and central sections of the island. Consequently, some variation through spatial constraints may not have been recorded from this population.

Wardang Island is the largest island studied. The island is highly modified, having been used since European settlement for sealing, pastoral activities, as an Aboriginal reserve, mining of calcareous sand, private tourist enterprises and as an outdoor education centre (Stevenson, 1985). Very little original vegetation remains and it is possible that tree species were removed from the island as early as the 1830's by sealers (Stevenson, op. cit.). The process of clearing continued throughout the 19th century as the island was leased for pastoral use and natural vegetation was replaced with sown pasture (Robinson *et al.*, unpub.).

Schwaner (1988) conducted a survey of sleepy lizards on Wardang Island in 1986/87, and it is from this study that population estimates for the island will be drawn (see section 2.3). The blood samples used in this study for the electrophoretic analysis were collected by T.Schwaner from several localities around the island. The morphological information was collected by myself in September, 1987 from a range of localities covering most of the island.

Weeroona Island is separated from the adjacent mainland by several hundred metres of mangrove swampland, 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 1940's by a narrow causeway which runs across the swamp. It is possible that there is some movement by *T.rugosus* between the island and mainland either via the causeway or overland at low tide. The island has been largely cleared for holiday housing, although some semi-natural vegetation remains on its southern side. There are significant areas of unused open grassland on Weeroona which probably provides good habitat for *T.rugosus*. Although no population estimates were possible for the island, sleepy lizards appeared to be numerous.

Troubridge Island is human-made, resulting from the entrapment of sand by a lighthouse placed on Troubridge Shoal in the 1850's. It is not certain when sleepy lizards were introduced, but it was probably this century as the island was nearly completely destroyed by an earthquake in 1904 (Owers, 1986). The lizards were thought to have been introduced by one of the lighthouse keepers as pets (R.Symons, pers. comm.) but the number of founders is unknown. At present, the island is about 8 ha in size, but lighthouse logs show that it is quite volatile in both size and shape, and was as small as 2 ha in the 1950's (R.Symons, pers. comm.).

Three mainland areas were sampled to represent non-fragmented populations and were chosen for their proximity to the islands studied. The mainland populations included Point Pearce (34^{o} 25'S 137^{o} 30'E), north of Port Pirie (33^{o} 07'S 138^{o} 03'E) and Tumby Bay (34^{o} 22'S X 136^{o} 06'E). The **Tumby Bay** population was sampled from an area on the outer perimeter of the Tumby Bay township. Samples were taken over a distance of approximately 4 km and the habitats sampled include the Tumby Bay Rubbish Tip situated on mudflats and a coastal dune system at the northern edge of the town. The population examined near Weeroona island (referred to as **Pt. Pirie** for convenience) was sampled by driving and walking along a narrow strip of vegetation adjacent to Weeroona island. The survey site has been extensively cleared but does not appear to be heavily grazed. The Point Pearce population was sampled by driving the roads on the peninsula adjacent to Wardang Island and examining piles of rubbish, bushes etc. for lizards.

2.3 FIELD METHODS

The animals used in the study were those sampled in the field and those available in the S.A. Museum collection. The majority of samples were taken from December 1986 to March 1988, although some museum specimens were collected as early as 1980. Given the high longevity of *T.rugosus*, its annual mode of reproduction and low numbers of young recruited each year (see section 2.1), it is unlikely that major changes in gene frequencies would have occurred over a period of less than two years. A survey system of random sampling of lizards along approximate transect lines was used for each island.

All animals captured in the field were sexed by the extrusion of a hemipenis (in males). The validity of this method was tested by sexing animals in the field and then examining those taken as vouchers again in the laboratory. The field sexing was 97% effective (n=31) and can thus be used with confidence.

Each animal was weighed and then measured in the field. Measurements of head scale characters were taken using Vernier dial callipers while snout vent length, tail length and hind limb length were obtained using a retractable tape measure. An index of melanism for each individual was obtained by estimating the percentage of area with melanistic pigment for both dorsal and ventral surfaces. Counts of small scale characters such as upper and lower palpebrals were obtained using a field magnifying glass. A list of all morphological measurements taken in this study and their abbreviations is given in Table 2.2 while diagrams of the different measurements are supplied in Fig. 2.4. Gross abnormalities such as missing toes, club feet and deformed head and labial scales were also noted for each individual.

Animals were bled using a direct heart puncture method, in which a fine tipped, 1 ml insulin syringe (with 5 to 10 microlitres of heparin) was used to pierce the soft skin under the left arm of the animal and enter the heart. A sample of 0.5 to 1.0 ml was extracted. No ill effects were observed using this method and animals bled in February 1985 on Duffield Island were recaptured 3 years later in February, 1988. The blood was frozen in liquid nitrogen within 30 to 60 seconds of extraction, and subsequently stored at $-100^{o}C$ at the South Australian Museum. Animals selected for release were marked individually by toe clipping, and then released at the point of capture.

2.4 POPULATION ESTIMATES FOR FOUR ISLAND POPULATIONS

It was possible to obtain estimates of population size for four island populations included in the study. No estimates were possible

TABLE 2.2 Morphological characters used in this study. Abbrev. = Abbreviations.

CHARACTERS	**ABBREV.**
Snout to vent length	SVL
Tail length	TL
Hind limb length	HLL
Snout length	SNL
Eye to nasal length	EN
Inter orbital width	IOW
Head width	HW
Rostral width	RW
Rostral height	RH
Eye diameter	Eye
Inter parietal width	IPW
Inter parietal length	IPL
Supra labials (left and right)	SL
Infra labials (left and right)	IL
Supra cillaries (left and right)	SC
Upper palpebrals (left and right)	UP
Lower palpebrals (Left and Right)	LP
Para vertebral scales	PV
Mid body scale rows	MSR
% Melanism on the dorsum	MELD
% Melanism on the venter	MELV

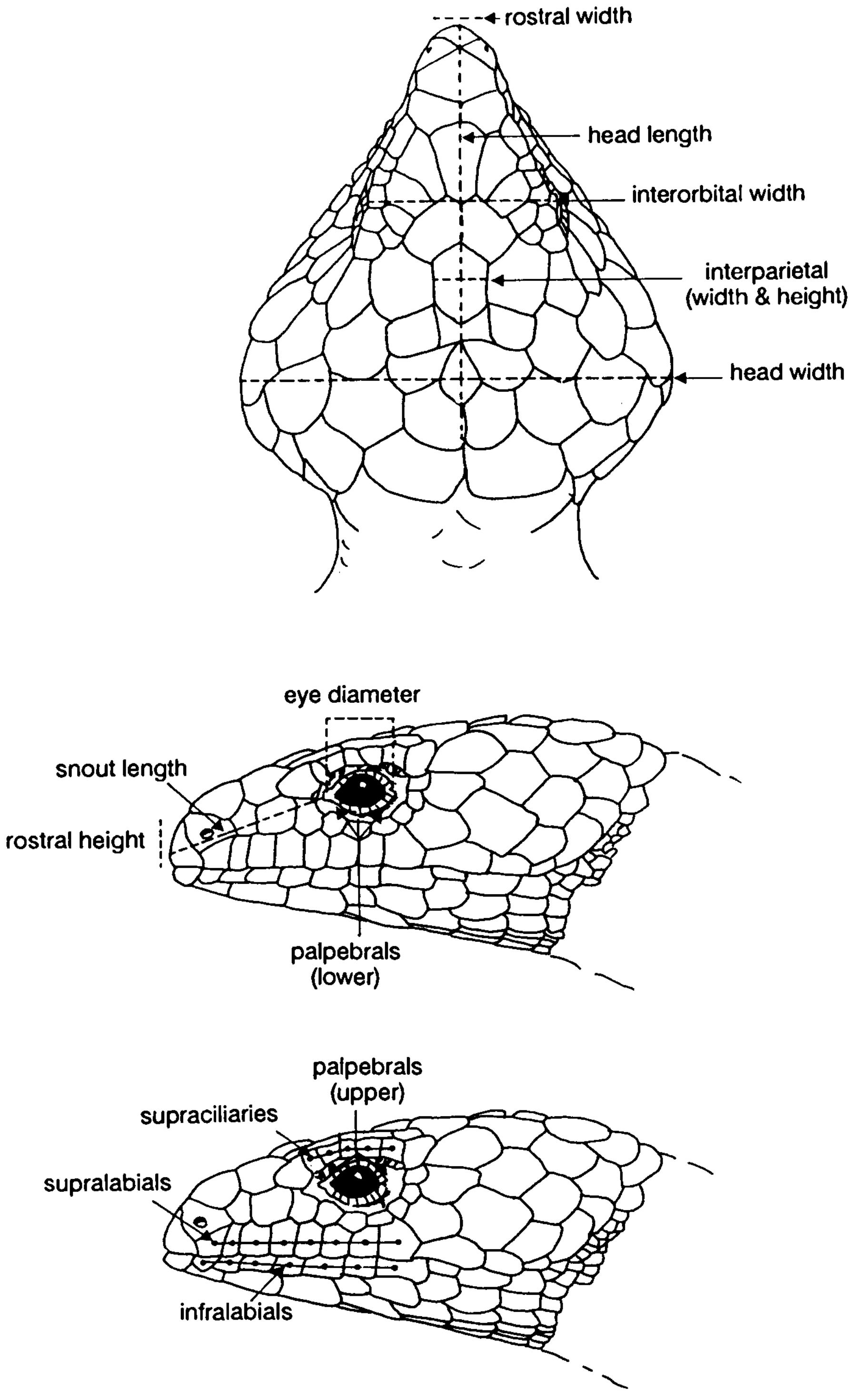


FIGURE 2.4 The head measurements and scale counts of *T.rugosus* used in this study.

for the mainland populations owing to sampling constraints. Estimates were obtained using mark/recapture techniques and varied with each island in the following manner.

Duffield Island was surveyed in February, 1985 and December, 1986 by T.Schwaner and me and again in February, 1988 (this study). Baileys Triple Catch (Caughley, 1980) was used to estimate population size using all three samples. Spilsby Island was surveyed in August 1987 and again in February, 1988 while Troubridge Island was surveyed in October, 1987 and March, 1988. Although sleepy lizards tend to bear their young in February - March (Bull, 1987), it is assumed that these surveys were between birth pulses. This assumption is supported by an almost complete lack of juveniles observed in both February and March surveys of Spilsby, Duffield, Reevesby and Troubridge Islands. On this basis, Peterson estimates (Caughley, 1980) were used to estimate population size for both Spilsby and Troubridge Islands.

The Wardang Island population was sampled by T.Schwaner at bi-monthly intervals from November 1986 to November 1987. In that time 340 individuals were marked in an area of approximately 100 hectares (Schwaner, 1988). Schwaner used the Jolly-Seber method to estimate population density for this area.

Total population estimates were obtained for each of these islands by multiplying the density estimates (per hectare) for each of the island areas sampled by the total area of the island. For Duffield and Troubridge Islands sampling covered the entire island so population estimates represent total numbers. However, only partial areas were surveyed on Spilsby and Wardang so estimates were based on the assumption that the sleepy lizards were evenly distributed across the island. This assumption may not be correct. Schwaner (1988) noted a clumped distribution of *T.rugosus* on Wardang correlating closely with areas of rubbish. The survey of Spilsby encompassed approximately 10% of the island which included most of the remaining native vegetation on the island. Efforts were made to survey other parts of the island but no sleepy lizards were observed and it is quite possible that the lizards are restricted to the remaining vegetated area. As a consequence, the total population estimates for these two islands can probably be considered to be maximum estimates.

CHAPTER 3. MITOCHONDRIAL DNA COMPARISONS AMONG POPULATIONS OF *T.RUGOSUS*

3.1 INTRODUCTION

3.1.1 General characteristics of mitochondrial DNA

Studies of the mitochondrion (a cytoplasmic organelle) have generally centred around its role in the generation of ATP. However, mitochondrial DNA (mtDNA) has also received considerable attention in recent years as a tool for both phylogenetic and population studies (see reviews by Avise *et al.*, 1987; Moritz *et al.*, 1987). MtDNA in animals is a small closed, circular molecule of double stranded DNA containing 37 genes which comprise 13 messenger RNA, 2 ribosomal RNA and 22 transfer RNA genes (Borst, 1972). These genes are tightly packed and contain little or no non-coding sequences between them (Attardi, 1985). The gene structure is highly conserved, maintaining a compact organisation of gene content which is apparently invariant among animals (Wallace, 1982). Animal mtDNA is also conservative in size (compared with fungi, plants and protists) although it does show some variation in length both among and within taxa (Attardi, 1985). The size of the mitochondrial genome in multicelled animals ranges from 14 kb to 26 kb (Sederoff, 1984; Moritz *et al.*, 1987) with differences generally confined to the displacement control region (D-Loop) of the genome (Moritz, op. cit.).

Mitochondrial DNA exhibits two key characteristics which make it particularly suitable for phylogenetic reconstruction and which differentiate it from techniques which use nuclear DNA or proteins coded for by nuclear DNA. First, with the possible exception of the 'D` Loop, mtDNA does not appear to undergo recombination (Wilson *et al.*, 1985) and so remains intact over generations. Second, the inheritance of mtDNA appears to be maternal (Hutchinson, 1974; Giles, 1980; Gyllensten *et al.*, 1985; Avise and Vryenhock, 1987). Although the exact mechanisms of this mode of inheritance are not yet fully understood, the transmission of mtDNA is apparently largely cytoplasmic. Thus, paternal mtDNA is either absent or occurs in very low frequencies and is swamped by the large amount of mtDNA inherited maternally (Avise and Vryenhock, 1987). These inheritance patterns permit the derivation of phylogeny which represent distinct maternal lineages (Avise and Lansman, 1983). Moreover, phylogenetic reconstruction involving allozymes or nuclear DNA, may be affected by genetic drift, altering allele frequencies within a population. The effects of genetic drift are likely to be particularly severe when comparisons are made between closely related species or conspecific populations where population related phenomena may overwhelm the phylogenetic information. Relationships between mtDNA molecules will not be affected by this process and in such situations, mtDNA can be particularly useful.

Polymorphism of mtDNA observed within natural populations is strongly affected by their finite population size. For example, Avise *et al.* (1984) demonstrated (using computer simulations) that stochastic extinction of mtDNA lineages will occur within a population, possibly leading to the extinction of all but one lineage in the population. They found that for a non-expanding population initiated by N females, all individual mtDNA in the population are likely to have descended from a single individual within 4N generations. The rate at which lineages are lost from a population relates directly to the demography of the females. In particular, high variance and/or low mean in progeny number will increase the extinction rate, as will static or declining population numbers. Thus, in small isolated populations which cannot expand, the random extinction of all but one mtDNA lineage could be expected over even a moderately short number of generations.

3.1.2 Evolutionary characteristics of mtDNA

Brown *et al.* (1979, 1982) examined the rate of evolution of mtDNA in primates by looking at the degree of sequence divergence among higher primates and plotting this against the known time of divergence obtained from the fossil record. It was found that mtDNA has an apparent rate of 0.02 substitutions per base pair per million years which is five to ten times that observed in nuclear DNA. Such a rapid rate of evolution if it is consistent among taxa, makes mtDNA particularly useful for the elucidation of phylogenies of conspecific populations or closely related species (Avise and Lansman, 1983; Lansman *et al.*, 1983; Moritz *et al.*, 1987). However, some caution must be used as differences among recently fractionated populations may well be the result of differences which arose before separation and may represent variation that was present in the ancestral stock (Lansman *et al.*, 1983).

The rapid rates of evolution observed in mtDNA are generally ascribed to one or both of two reasons. First, the mutation rate is higher because of inadequate replication and repair procedures of the mtDNA (Brown, 1983). This may be tolerated if animal cells rely on mitochondrial redundancy rather than DNA repair to retain viable mitochondria. Higher mutation rates may also be possible because of increased rates of replication when compared with nuclear DNA, permitting greater probabilities of error (Rabnowitz and Swift, 1970). Second, selective constraints upon products of the mtDNA may be lower than in nuclear DNA thus permitting more rapid fixation of mutations (Avise, 1986).

Brown *et al.* (1979) also noted that the rate of evolution of mtDNA in primates was not apparently linear over time and tended to decrease after an initial linear growth. This is believed to result from rates of evolution which are variable across sites. For example, transitions (A <-> G, C <-> T) have been found to outnumber transversions (A <-> C, A <-> T, G <-> C, G <-> T). That is, initially, transitions will probably occur at a constant rate. However, as the number of transitions increases then so does the probability of further substitutions at these sites back to the original state. The occurrence of multiple substitutions at the same site will distort the observed relationship between mtDNA molecules (Brown *et al.*, 1982). Variability in substitution rates across sites has also been observed in protein coding genes where substitution rates appear to be higher among silent sites than among those which code for amino acid replacement and in the displacement loop (Avise, 1986). The variation in substitution rate within the DNA suggests that some strong functional constraints do exist in sections of the mitochondrial genome.

3.1.3 Study aims

In this thesis, it is assumed that the isolation histories of the *T.rugosus* populations are synonymous with those of the islands upon which they are isolated. This may not be the case if humans act as modern day vectors of these animals. The null hypothesis to be tested with the mtDNA data is that there is no congruence between the relationship observed between homologous mtDNA genomes and geographic proximity. Such a finding would suggest a flaw in the assumption that the histories of the populations studied are synonymous with the geographic proximity and isolation histories of the island and mainland areas upon which they occur.

3.2 MATERIALS AND METHODS

3.2.1 Mitochondrial DNA extraction

The methods used for the extraction and restriction of mtDNA in this study were modified from Cathcart (1985) and all recipes for chemicals used in the laboratory are provided in Appendix 1. Purified mtDNA was obtained from *T.rugosus* in the following manner. Initially, liver from *T.rugosus* was frozen immediately after extraction in liquid nitrogen, and stored at -100°C. However, yields of mtDNA from this method were poor and a considerable improvement was obtained when the livers were frozen slowly. This entailed refrigeration of the liver on ice for approximately 30 minutes before freezing at -20° for 2 hours and finally at -80°C. Before the extraction of the mtDNA approximately 7g of tissue was thawed gently at - 20 ° C and then on ice to prevent the destruction of the mitochondrial organelle. The liver was macerated with a razor blade and homogenised with a teflon dounce in 5 ml of MIM (mitochondrial isolation medium). This solution was spun twice at 1,500g for 20 minutes to pellet out the nuclei and debris, and the mitochondrial fraction obtained by spinning the supernatant at 15,000g for a further 10 minutes. The supernatant from this spin was retained as the second fraction in addition to the main fraction obtained from the remaining solution.

The pellet from the main fraction was re-suspended in 5 ml of MIM and both fractions were spun for 10 minutes at 15,000g. The supernatants were discarded and the remaining fractions decanted and resuspended in 2 ml of SSC. 1.5 ml of lysing solution and 2 ml of phenol/0.1% 8-Hydroxylquinoline were added to this solution and incubated for 20 minutes at 46 - 47°C with mixing, before being cooled on ice and spun for 10 minutes at 1,500g. The supernatant was decanted into 10 ml tubes and one volume of chloroform/isoamylalcohol (24:1) was added. This solution was spun at 1500g for 7 minutes and the mtDNA precipitated by mixing 10 µl of 3M Na acetate with the supernatant (making the solution up to 10 ml with ethanol) and placing it in a -100°C freezer for 20 minutes. This solution was spun at 1,500g for 15 minutes and the resultant mtDNA pellet dried and then dissolved in 200 ml of TE buffer pH 8.0. The extracted mtDNA was subsequently cleaned by mixing equal volumes of dissolved mtDNA solution and phenol and centrifuging at 1500g. Precipitation of the mtDNA was then achieved in the manner described above.

3.2.2 Restriction of mitochondrial DNA

The restriction enzymes Hinf1 and Rsa1 were purchased from Boehringer Mannheim Biochemicals and EcoR1, HindIII, EcoRV, Bgl1, Pst1, Xho1 and Xba were purchased from Bethesda Research Laboratories. Digestion was achieved by mixing 4 ul of running buffer (10 * E buffer), 3 µl BSA and 10 ul of restriction enzyme with 25 µl of mtDNA solution in 2 ml Eppendorf tubes and incubating at 37° C for 5-6 hours (with the addition of a further 1 µl of enzyme added after 4 hrs). 2 µl of RNase was added 20 minutes before completion of the restriction and 4 µl of Stop DE added to each sample to end the reaction. The gel was constructed by pouring a 1.5% solution of agarose (3 g of agarose dissolved in 200 ml of 1 * E buffer) into a cassette (dimensions of gel 26cm * 16cm * 0.3cm) with the comb placed 3.5 cm from one end. Any bubbles were drawn up with a pipette and the gel allowed to set for one hour. Gels were run for approximately 13 - 14 hours at 1.54 volts/cm of gel.

The gels were stained with 15 ul of ethidium bromide (10 mg/ml) in 10 ml of 1*E buffer and covered for 20 minutes before being rinsed with distilled water for 30 minutes. Each gel was photographed using an orange filter with photos taken at 15, 30 and 60 seconds (F stop 11) before being hand scored under ultra-violet light and the distance of each band from the origin measured.

3.2.3 Data analysis

The size of the mtDNA of *T.rugosus* was estimated by using a single restriction of *T.rugosus* mtDNA calibrated with a Hind III restriction of Phage Lambda. Sizes of individual fragments were estimated by graphing the migration distance of Lambda (restricted by EcoR1 and EcoRV) calibrated track against the log of the size of the lambda fragments. A regression line was fitted to the graph and the size of the mtDNA fragments converted into kilobases using antilogs.

There are two major approaches to analysing restriction fragment data. The first is the quantitative formulation of a genetic distance between pairs of populations. Distances are based on estimates of the degree of divergence and are subsequently used to cluster the populations and derive genetic relationships. The second approach involves the derivation of relationships using the presence or absence of individual characters (fragments).

The quantitative method of converting fragment data to estimates of a distance reflecting nucleotide sequence divergence is an approach frequently used. The distance between two mitochondrial molecules is generally estimated from the proportion of restriction sites shared between the genomes. There are numerous methods for formulating these values (e.g. Upholt, 1977; Gotoh *et al.*, 1979; Nei and Li, 1979; Kaplan and Risko, 1981). However, the most widely used distance is that of Nei and Li (1979) who calculate the expected proportion of fragments that remain unchanged in both populations (F) using the formula

$$F = 2n_{xy}/(n_x + n_y)$$

where n_{xy} is the number of fragments shared by two populations (X and Y) and n_x and n_y are the total number of fragments in each population.

The closer the relationship between homologous molecules the more restriction sites or fragments they have in common. In the derivation of the expected proportion of fragments that remained unchanged between two populations, two primary assumptions are made: (1) that all nucleotides are distributed at random over the genome with a G+C content which remains constant, and (2) that nucleotide substitution occurs randomly across the genome and follows the Poisson process. Nei and Li (1979) note that the first assumption may not always be satisfied but, unless the non-random distribution is extreme, variation in G+C content is unlikely to affect such calculations.

The second assumption is more doubtful because, as discussed above, different substitution rates may apply to different parts of the molecule and when only a short divergence time is involved, chance alone may result in observed differences between molecules. Moreover, Tajima and Nei (1982) note that the variance of the proportion of ancestral sites is large when the number of restriction sites is small and therefore recommend that many different restriction enzymes be used. Nei and Li's (1979) formula relates to restriction enzymes which all recognise base sequences of a particular length (e.g. 6 base pairs). Combining data from enzymes which recognise sequences of different lengths may result in some bias because different types of enzymes (e.g. four or six base cutters) do exhibit differences in sequence divergence particularly with small numbers of shared fragments (Upholt, 1977). This relationship is non-linear but several authors have combined data derived from different base cutters (e.g. Kessler and Avise, 1984, 1985; Ashley and Wills, 1987, 1989) and weighted them according to the numbers of fragments produced by each type of restriction enzyme. Unfortunately, the methods used in weighting the data are not clear. Another problem with this formulation is that it breaks down if OTU's do not have fragments in common. If few fragments are involved, then it is possible that a change at a single restriction site can result in no common fragments between two OTU's. In this situation, the calculation of F values breaks down primarily because it gives equal weighting to the absence of a fragment as it does to its presence. Without restriction maps to determine the presence or absence of individual sites, OTU's which do not share fragments would have to be excluded from the analysis. In the present study, two OTU's (Ceduna and Pt. Kenny) have no fragments in common with any other OTU for the enzyme EcoRV, and would thus have to be left out of the analysis. To avoid this, and hence to maximise the information gained from mtDNA analysis, the calculation of genetic distances between OTU's has been obtained using the complement of Jaccard's index (Marczewski-Steinhaus distance, MS) (Orlóci, 1978) which uses the formula

$$MS = (b+c)/(a+b+c)$$

where a = number of fragments in common, b = number of fragments unique to OTU1, c = number of fragments unique to OTU2. This measure obtains the ratio of the number of unique fragments to the total number of fragments and has the advantage of not comparing fragments which are absent from either OTU. In order to account for differences in sequence divergence estimates between the 'six` and 'four` base enzymes the MS value was weighted. This was achieved by calculating the MS values separately for the two different types of base cutters used in the present study. The MS values are then added and averaged to obtain a single distance measure for each pair of OTU's. A heirachical cluster analysis was performed on the distance values using the unweighted pair-group method with arithmetic mean averaging (UPGMA) (Sneath and Sokal, 1973). The statistical computer package SAS (SAS Institute, 1987) was used to carry out the analysis.

The alternative approaches to phylogenetic reconstruction are those which are character based where phylogeny is inferred by determining the presences or absences of a range of characters. There are several ways of approaching this type of data but discussion will be confined to the two used in the present study.

Parsimony is one method commonly used to derive classifications, the most widely used of which is the Wagner parsimony method (Kluge and Farris, 1969). This method uses the presence or absence of discrete character states to determine the most parsimonious (smallest number of changes) evolutionary pathway necessary to define relationships between taxa (Felsenstein, 1983). The Wagner parsimony method is based on the assumption that character changes can occur in either direction. That is, each character can be either gained or lost from a taxa, therefore, it does not make assumptions on the ancestral or derived nature of different character states.

The presence or absence of particular fragment patterns in each population was recorded and a composite phenotype produced for each population. A Wagner analysis was carried out on this data using the statistical package PAUP (Swofford, 1985) and the most parsimonious relationships obtained using global branch swapping. Fragment patterns which were unique to a single population (autapomorphic) were excluded from the analysis because they are phyologenetically uninformative and populations with identical fragment patterns were combined to facilitate rapid analysis. Fifteen different phenotypes were observed (Fig. 3.1). Of these, eight (A, C, G, H, I, J, K, L, O) were autapomorphic and of the remaining seven, Weeroona, Pt. Pirie and Troubridge were identical for all fragment patterns. Thus, a data matrix of eight populations and six characters was analysed using the Wagner parsimony method. It was intended that a consensus tree would be produced from the most parsimonious trees and superimposed upon the geographic locality of the different populations (Avise *et al.*, 1979). However, a total of 292 trees of equal parsimony were produced and a consensus tree could not be obtained.

An Hennigian analysis (Hennig, 1966) was also performed using individual fragments to produce an unrooted network. This method assumes that character states are known to be either ancestral or derived and phylogeny can be inferred by grouping taxa with unique characters into monophyletic groups and superimposing all groupings upon each other. Problems may arise when two characters place the same taxa into different groups. The Hennigian solution to this dilemma is to re-examine and re-interpret the data until a resolution to the conflict is obtained (Felsenstein, 1982). Ancestral fragments were defined as those fragments shared by most populations and for which there was an alternate (derived) state common to the remaining populations. Only fragments restricted to two character states were considered, thus, Edithburgh which exhibited unique character states for two fragments could not be included in the analysis. All autapomorphies were excluded from the analysis and all identical morphs combined. This approach reduces the data set substantially and is therefore likely to provide only a broad outline of trends.

Both the Hennigian and Wagner parsimony methods require that changes in one character do not affect any other character. This requirement is violated for restriction fragment data because the gain or loss of a fragment will affect the presence of absence of at least one other fragment (Ovendon *et al.*, 1987). However, such occurrences can be considered to have a small effect on restriction fragment data because of the large number of fragments obtained per enzyme (Kessler and Avise, 1985). These types of analyses can also be confounded by the occurrence of non-homologous fragments with similar molecular weights. Restriction maps are necessary to ensure that this has not occurred.

The classifications of relationships among populations made in the present study, and the conclusions to be drawn from them are restricted in their scope for two reasons:

(1) line-up gels were not possible because of insufficient mtDNA so comparisons of fragment patterns among gels were made without the benefit of side by side comparisons. This increases the probability of mistakes in the scoring of non-homologous fragments as identical and of mis-identifying fragments exhibiting minor length variations;

(2) Insufficient mtDNA also meant that only three polymorphic restriction enzymes could be used in the analysis. Thus the resultant classifications must be viewed with appropriate caution when using such a small sample size.

3.3 RESULTS

A total of 19 animals comprising either one or two individuals from each of 11 populations (Ceduna, Pt. Kenny, St. Peter, Tumby Bay, Spilsby Is. Wardang, Pt. Pearce, Weeroona, Pt. Pirie, Edithburgh, Troubridge) were used in the analysis. Unfortunately, mtDNA could not be isolated from either Reevesby or Duffield. Not all individuals were run for each enzyme and the phenotypes produced from two populations (Pt. Kenny and Pt. Pirie) are composite phenotypes of the two individuals from those localities. No differences were observed within populations when both individuals from a population were restricted by the same enzyme. The animals used and the populations examined for each enzyme are given in Table 3.1.

Five restriction endonucleases were used in the analysis, of which three (Bgl1, GCCNNNN NGGC; Rsa1, GT AC; EcoRV, GTGCA G) exhibited polymorphisms among populations. A further three endonucleases (Xhol, Xba1, HpaII) were tested. Xhol failed to restrict in any samples while Xba1 and HpaII restricted spasmodically and generated many small bands which were difficult to resolve. These endonucleases have not been included in the data analysis. The total size of the mtDNA genome of *T.rugosus* was estimated to be 23 kb and 50 restriction sites representing 208 base pairs (or approximately 0.9% of the genome) were surveyed. Insufficient mtDNA was available to run lineup gels of the various restriction morphs so the data are presented only as restriction fragment maps (Fig 3.1, 3.2).

TABLE 3.1 The animals, their collection localities and the endonucleases used in the mtDNA analysis. * individual used in analysis; - not used in analysis; Reference Numbers (Ref. No.) are S.A. Museum collection numbers.

REF. NO.	LOCALITY	Pst 1	Hinf 1	Rsa 1	EcoRV	Bgl 1
R31472	CEDUNA	*	*	*	*	*
R31473	CEDUNA	*	-	-	-	-
R31560	PT. KENNY	*	-	-	-	*
R31561	PT. KENNY	*	-	*	*	-
R31477	ST. PETER	*	*	*	*	*
R31478	ST. PETER	*	*	-	*	-
R31558	TUMBY BAY	*	*	*	*	*
R31475	SPILSBY IS.	*	*	*	*	*
R31479	WARDANG IS.	*	*	*	*	*
R31587	PT. PEARCE	*	*	*	*	*
R31591	EDITHBURGH	*	*	*	*	*
R31418	TROUBRIDGE IS.	-	*	*	-	-
R31419	TROUBRIDGE IS.	*	*	*	*	*
R31618	WEEROONA IS.	-	*	*	*	-
R31619	WEEROONA IS.	*	*	*	*	*
R31620	PT. PIRIE	-	*	*	*	*
R31621	PT. PIRIE	*	*	*	*	-

NOTE: No individuals from Pt. Kenny were run for Hinf 1.

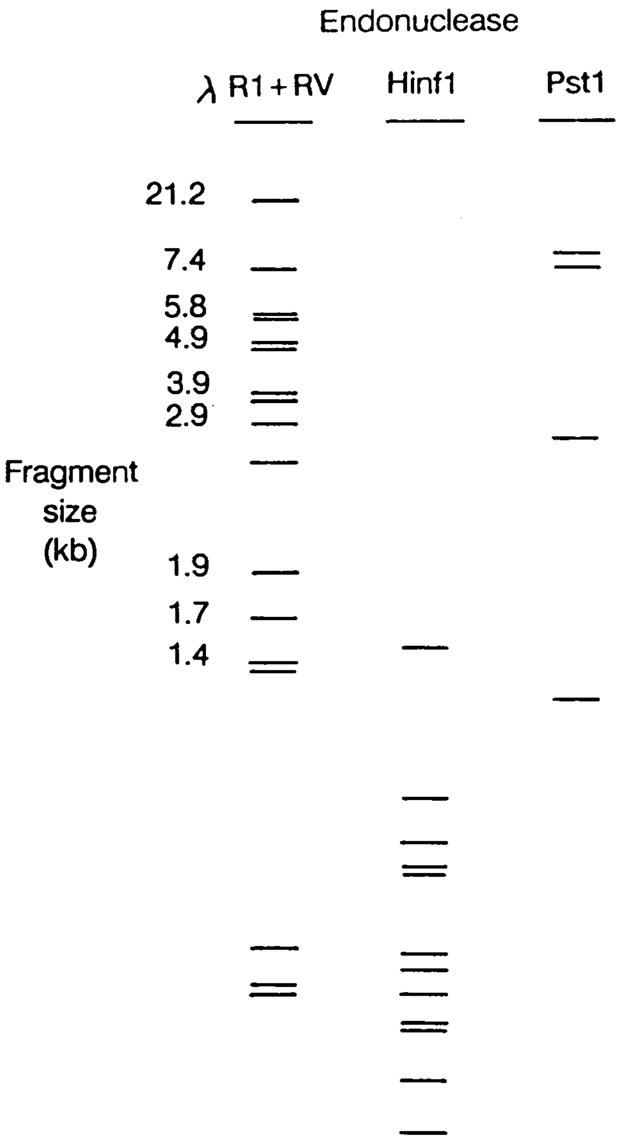


FIGURE 3.1 Restriction fragment patterns of *T.rugosus* mtDNA produced by Hinf1 and Pst1. These morphs were invariant among populations.

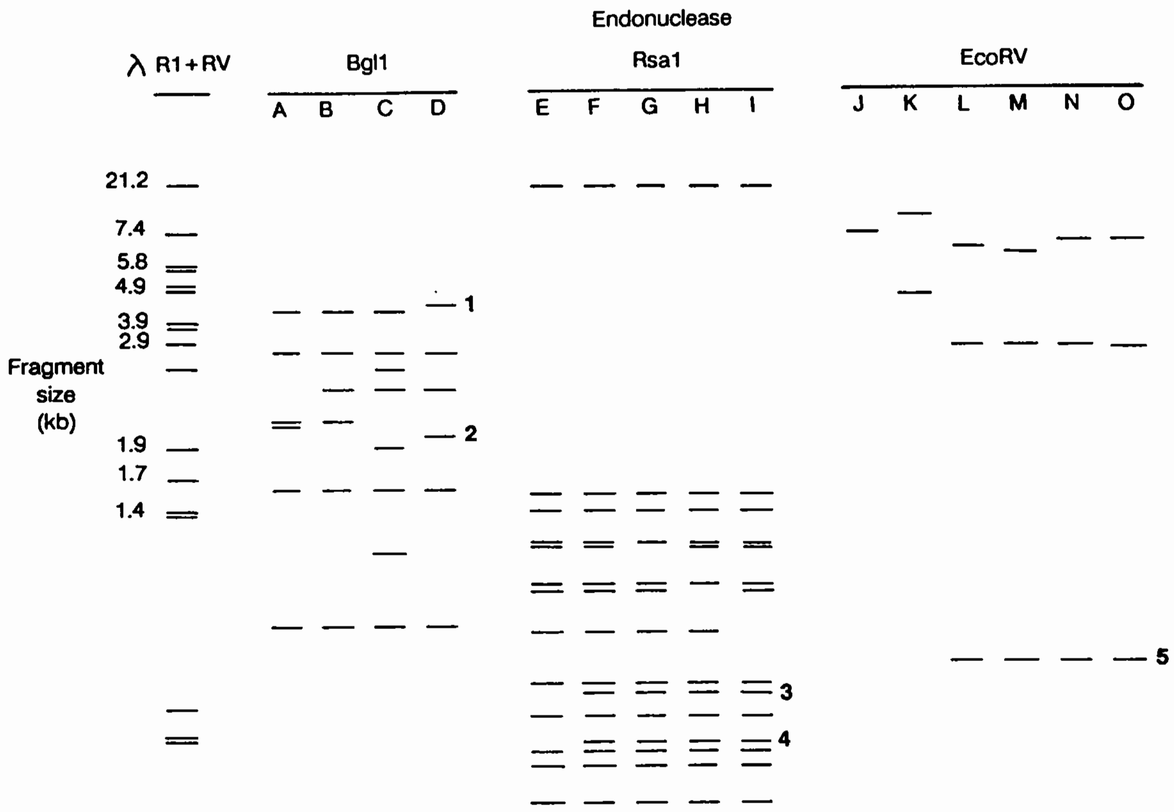


FIGURE 3.2 Restriction fragment patterns of *T.rugosus* mtDNA produced by the restriction enzymes Bgl1, Rsa1 and EcoRV. Each morph is denoted by a letter (A to O) and its distribution given in Figs. 3.3, 3.4 and 3.5. The numbers 1-5 refer to fragments which were used in the Hennigian analysis. (see Fig. 3.7).

Hinfl (G ANTC) and Pstl (GAT ATC) patterns were monomorphic for all populations, with Hinfl generating 12 observable fragments all less than 2 kb in size and totalling approximately 7 kb. This is considerably less than the total size of the genome and it is assumed that Hinfl (a five base cutter) has cut the DNA into many smaller fragments which were too small to be recorded. Pstl produced a three banded fragment pattern in all populations.

Bgll produced six fragments in four different morphs. Ceduna is quite distinct from all other populations with one fragment missing at approximately 2.6 kb and an additional fragment present at 2.16 kb. This indicates that at least one extra restriction site has been gained forming the extra fragment of 2.1 kb and one other which has not been observed. Most populations were of a single morph (morph 'B`) while Weeroona, Pt. Pirie and Troubridge formed one group with variation in two fragments of sizes 0.1 to 0.2 kb from the 'B` morph. Edithburgh also varied from the 'B` morph at two fragments with one being larger by approximately 0.3 kb and one smaller by approximately the same amount. Edithburgh also has an extra fragment of 1.0 kb which was not observed in any other population.

Rsal produced five morphs with differences between morphs arising from the loss or gain of fragments of a size less than 2.0 kb. Morph 'E` separates the three western populations of St. Peter, Ceduna and Pt. Kenny, while Tumby Bay, Weeroona, Pt. Pirie, Troubridge and Edithburgh are all grouped (morph 'F`). Spilsby, Wardang and Pt. Pearce differ by changes of one or two fragments.

EcoRV produced six morphs. Ceduna is quite distinct with just a single fragment 9.3 kb in size. The small size of this fragment and the absence of any other can be explained in two ways. It is possible that the remainder of the molecule may have been cut in many places creating a large number of small fragments which could not be observed using ethidium bromide staining or, conversely, the mtDNA may have been cut into two fragments which are equivalent in size and consequently represented by one band. This could be resolved using a double digest of EcoRV with another endonuclease, allowing the determination of the amount of mtDNA which can be observed from a digest involving EcoRV. Morph 'O` grouped Wardang, Pt. Pearce, Weeroona, Pt. Pirie and Troubridge, St. Peter and Spilsby share morph 'M` while Ceduna, Tumby Bay and Edithburgh exhibited unique morphs.

The observed distribution of fragment patterns produced an array of combinations (see Fig. 3.3, 3.4, 3.5) with some consistent patterns of association evident. Edithburgh, Ceduna and the group of Weeroona, Pt. Pirie and Troubridge exhibit trends of divergence from the other populations. Weeroona, Pt. Pirie and Troubridge were identical across all three enzymes. This suggests that the Troubridge population (which was introduced) originated from the Port Pirie area rather than the adjacent mainland because the Edithburgh individual is quite different to these populations.

The cluster analysis (Fig. 3.6) demonstrates a clear dichotomy among the localities. Ceduna and Pt. Kenny form a group which is clearly different to all other populations. Generally, the clusters conform quite closely with geographic proximity. Weeroona is

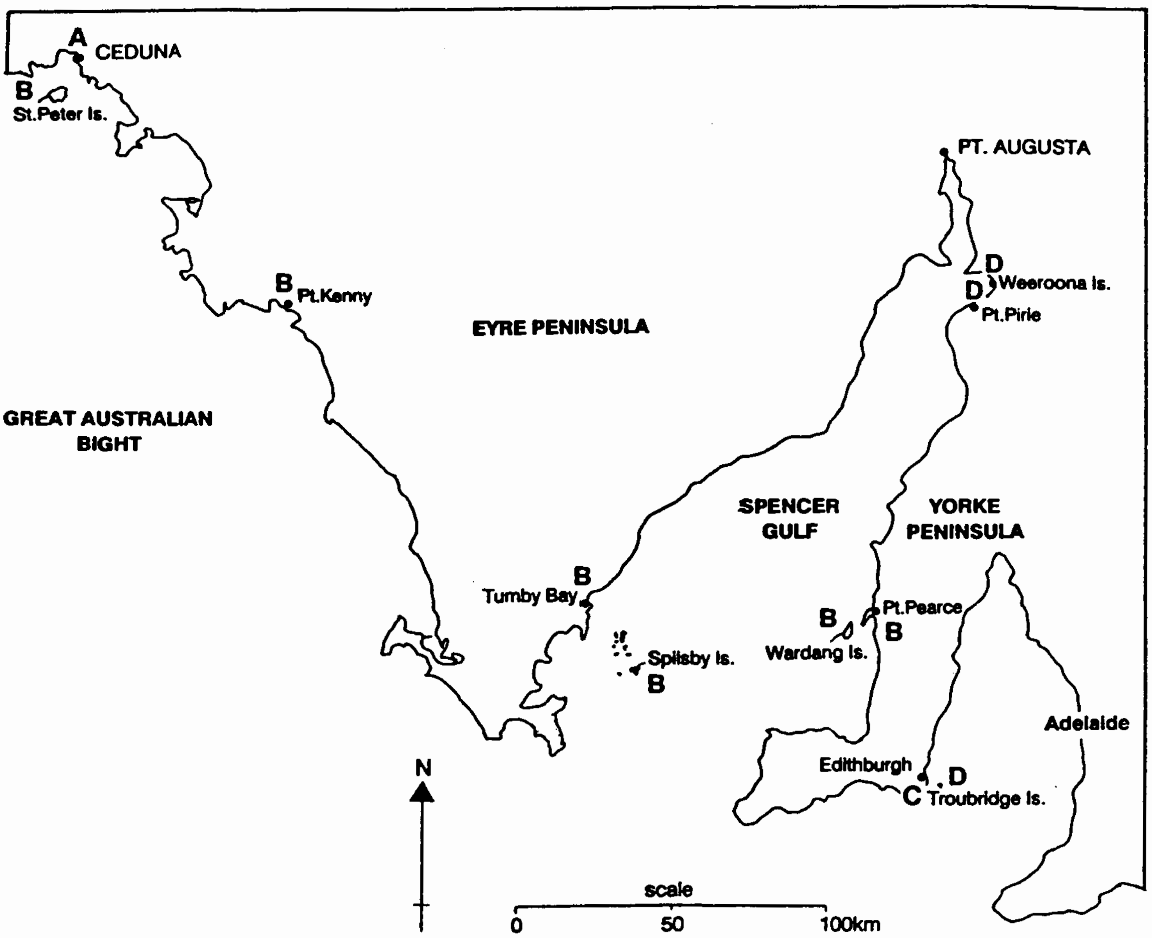


FIGURE 3.3 The distribution of mtDNA morphs obtained using the restriction enzyme Bgl1. Diagrams of each morph are provided in Fig. 3.2.

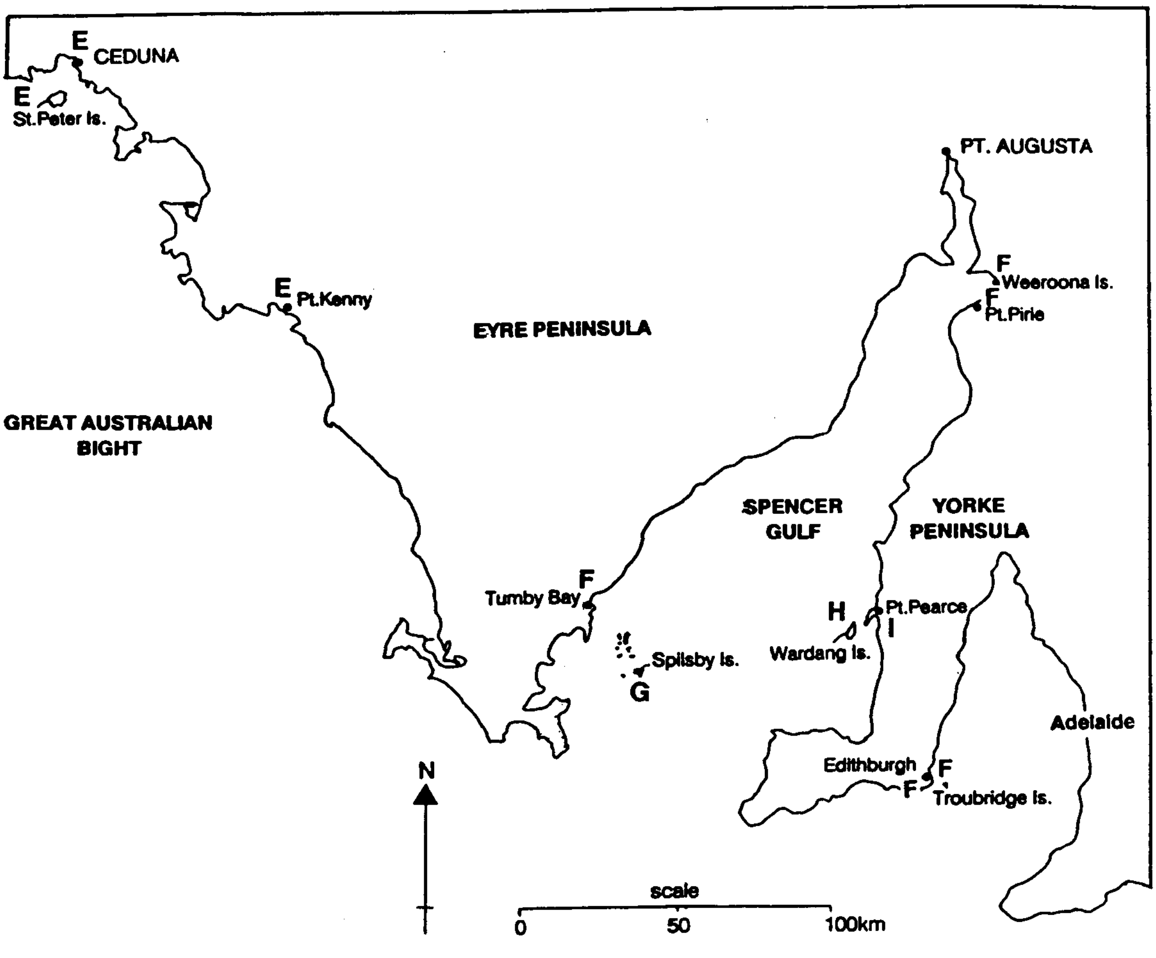


FIGURE 3.4 The distribution of MtDNA morphs obtained using the restriction enzyme Rsal. Diagrams of each morph are provided in Fig. 3.2.

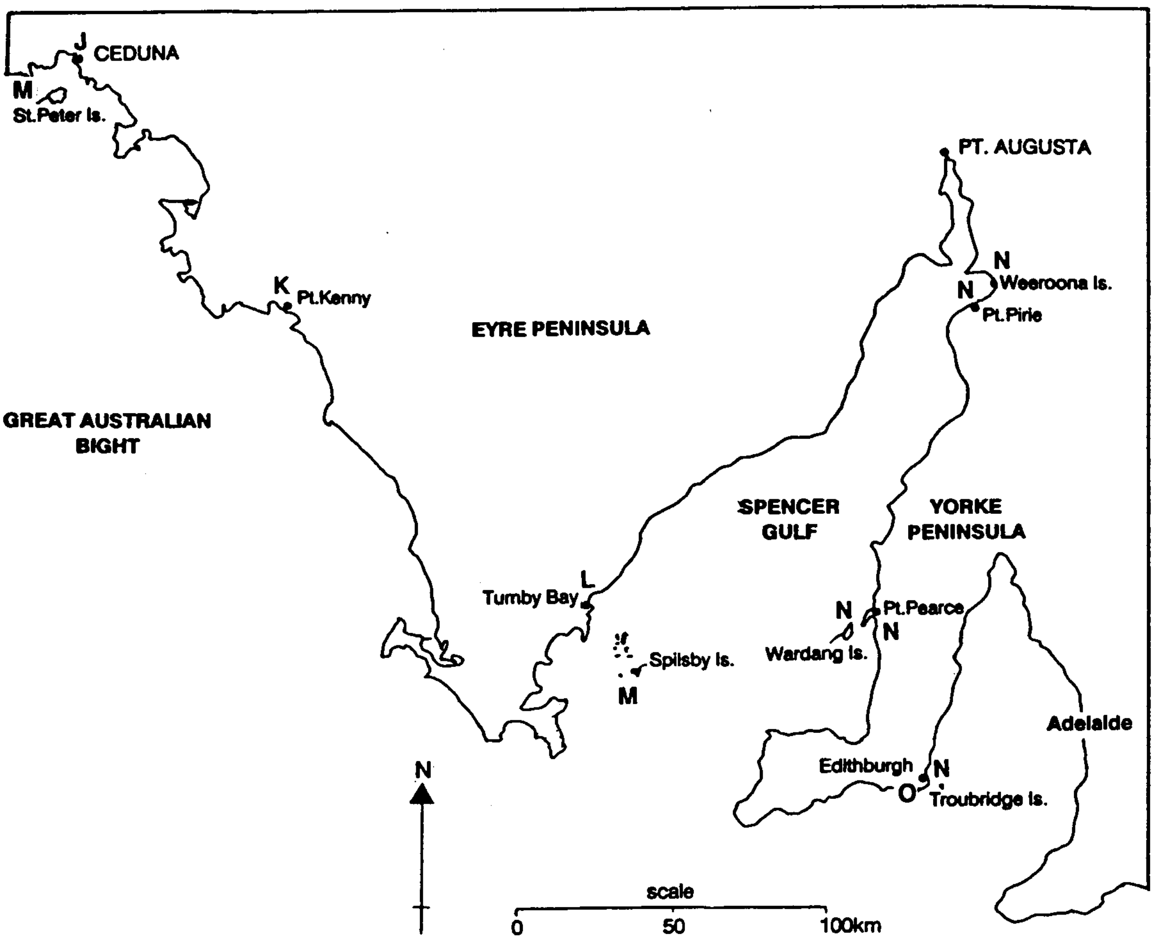


FIGURE 3.5 The distribution of mtDNA morphs obtained using the restriction enzyme EcoRV. Diagrams of each morph are provided in Fig. 3.2.

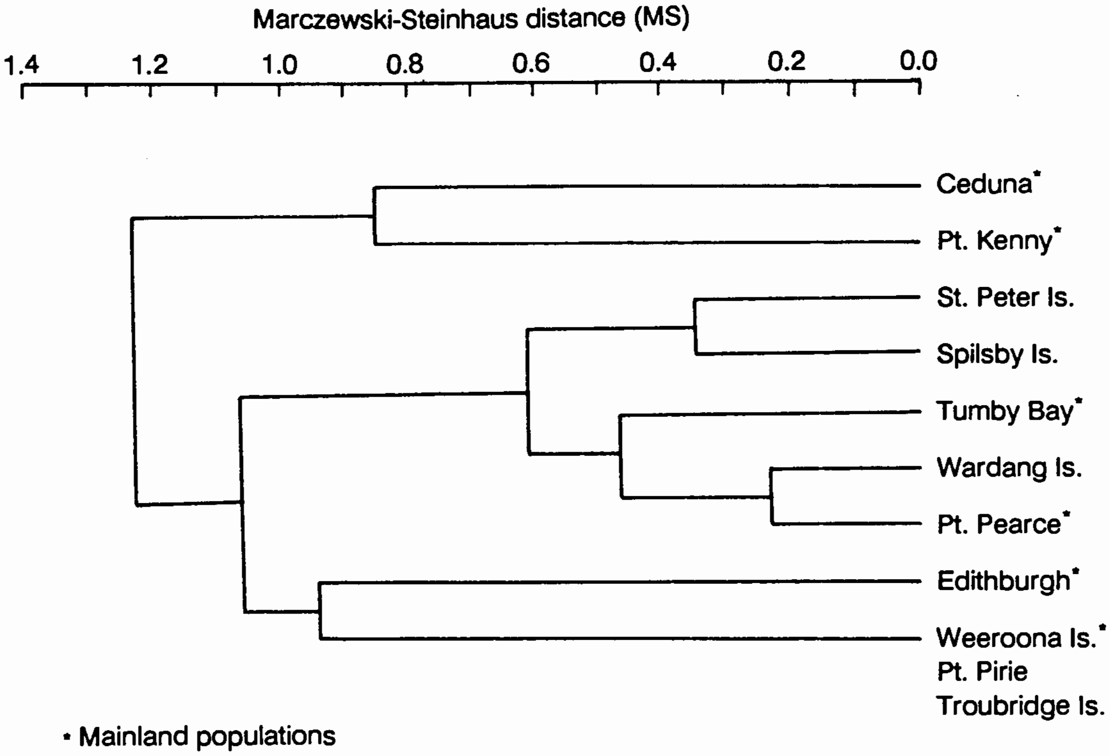


FIGURE 3.6 UPGMA cluster analysis of distance measures derived from mtDNA restriction fragment morphs among populations of *T.rugosus*.

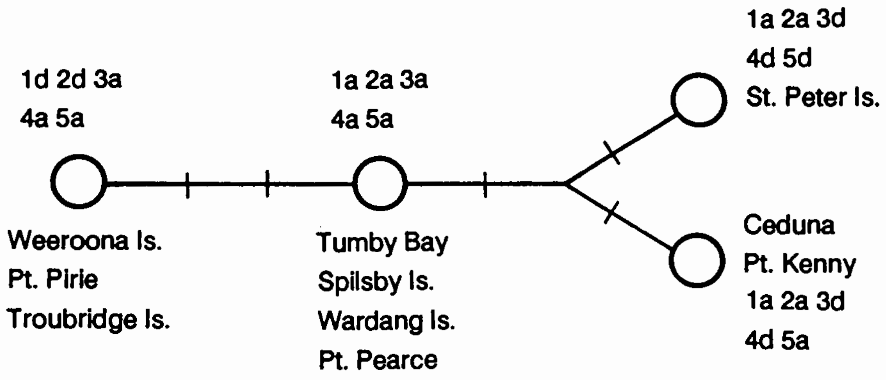


FIGURE 3.7 Hennigian analysis producing an unrooted classification using five fragments from the restriction analysis of mitochondrial DNA. The fragments used were numbered '1 - 5` (see Fig. 3.2). a = ancestral fragment (the most common fragment); d = derived fragment. Edithburgh has been excluded from the analysis.

identical to Pt. Pirie while Wardang clusters with Pt. Pearce indicating a close relationship between those two populations. The most incongruent clustering in this analysis is the grouping of St. Peter with those populations from the central Eyre and Yorke peninsulas. It could be expected from geographic proximity that St. Peter would cluster with the Western Eyre Peninsula populations of Ceduna and Pt. Kenny rather than those in the Eastern group. The sharing of the Rsal morph 'E` between these three populations suggests that there are perhaps more similarities between them than the cluster analysis reveals.

The Hennigian analysis (Fig. 3.7) using five fragments supports the broad trends evident in the cluster analysis. This classification divides the populations into four groups. The western populations (Ceduna, Pt. Kenny and St. Peter) are grouped together although St. Peter differs from the other two by one fragment. Tumby, Spilsby, Wardang and Pt. Pearce form a third group which is separate to the group of Weeroona, Pt. Pirie and Troubridge.

3.4 DISCUSSION

The examination of mtDNA in the present study shows a general level of congruence between the population and their geographic proximity to each other. Both the cluster and Hennigian analyses identify a major division between the eastern and western populations, although St Peter, which is situated on western Eyre Peninsula, clustered with those on the eastern side of Eyre Peninsula. The ambiguity observed in the relationship of the St.

Peter population could be explained in two ways. First, this population may be a relic of a lineage which spanned Eyre and Yorke Peninsula prior to fragmentation by rising sea levels. The differences between the current Ceduna population and St. Peter could be accounted for by the movement of northern or western lineages into western Eyre Peninsula in the last 6,000 years. This would leave the St. Peter population quite distinct compared with the adjacent mainland population of Ceduna. A second explanation might be that the St. Peter population has been recently introduced from eastern Eyre Peninsula. A more complete survey across Eyre Peninsula would be required to further elucidate this relationship.

As could be expected under conditions of gene flow between populations, Weeroona and Pt. Pirie which are separated only by mud flats at low tide and linked by a narrow causeway, showed no difference in any of the restriction fragment patterns. Troubridge did not vary from these two populations while varying distinctly from its adjacent mainland population of Edithburgh. This suggests that the origin of this 'introduced` island population is the Pt. Pirie area.

Differences were observed among mtDNA from all populations except (Weeroona, Pt. Pirie and Troubridge) which suggests that these populations are separate but enough similarities between them remain to indicate a recent (in evolutionary terms) separation between them. The general congruence of mtDNA populations with geographic proximity supports the assumption that the populations have been long term inhabitants of their current localities. Thus, it appears that the isolation histories of Spilsby and Wardang

Islands are consistent with those assumed from Holocene changes in sea level. However, these conclusions are constrained by two factors (given in section 3.3.2) and a more intensive examination would be required before hypotheses concerning the isolation histories of each of the insular populations could be developed. The mtDNA survey in the present study indicates that such an examination could be quite fruitful. In particular, the homogeneity observed in the fragments produced by Pst1 and Hinf1 enzymes suggests that there is some measure of conservatism in the mtDNA of *T.rugosus*, while the variation observed among fragments produced by three other enzymes indicates that there is sufficient variation to be useful. These characteristics should provide a good basis for phylogenetic studies among populations of the species.

A more systematic sampling across the range of *T.rugosus* would provide much important information about the historical origins of the various populations. For example, sampling *T.rugosus* to the west and north of Ceduna and in the central regions of Eyre Peninsula would help delineate an East-West dichotomy which is suggested by mtDNA analysis in this project. Also, the divergence of the Edithburgh and Pt. Pirie area mtDNA is quite marked and an intensive survey of Yorke Peninsula may assist in understanding the relationship between these populations and those in other areas of Yorke Peninsula.

An understanding of variation within populations would also provide a measure of the significance of the inter-population variation observed in this study. In the current project, comparisons were made between individual mtDNA molecules which assumes that mtDNA is homogeneous within the populations. When the populations to be compared are closely related, this assumption may not be satisfied (Nei and Li, 1979) and intra-population variation should be subtracted from the total inter-population difference. Thus, differences among the populations in the current study may be obscured by noise caused by intra-population variation. The sampling of several individuals from each population would alleviate this problem.

The variation in restriction fragment patterns observed in the present study could also form the basis for population studies of *T.rugosus*. Of particular relevance to conserving the island populations which were included in this study, is the examination of gene flow between populations. For example, an intensive survey of the mtDNA genomes of the Duffield and Spilsby Island populations could establish the relationship of these two populations. The allozyme data suggest that the two populations are closely related and it is quite possible that Duffield is a recent introduction from Spilsby. If this is the case then it is likely that these populations will share mtDNA morphs. This has important implications for the management of these populations because, if Spilsby is currently undergoing a genetic bottleneck as a result of human activities, then the introduction of individuals from Duffield which may have retained unique alleles, may assist in ameliorating the effects of genetic drift and allow the Spilsby populations to survive the bottleneck.

Finally, a more intensive survey of mtDNA variation within the insular populations could provide valuable information on historical aspects of each population particularly with respect to providing evidence for past bottlenecks. The maternal inheritance pattern of mtDNA means that it is more susceptible to loss of variation through bottlenecks than nuclear DNA and as such could be useful in interpreting the patterns observed in other genetic studies such as allozyme electrophoresis.

CHAPTER 4. ELECTROPHORETIC COMPARISONS WITHIN AND AMONG POPULATIONS

4.1 INTRODUCTION

The random fixation of alleles by genetic drift has been suggested as a potential mechanism of speciation particularly in small isolated populations (Mayr, 1963, 1970; Wright, 1970, 1978). More recently, attention has focused on the potential of this process to increase the vulnerability of small populations to extinction through the increase of homozygosity and the consequent lowering of fitness levels and a reduction in adaptability (Frankel and Soulé, 1981; Schoenwald-Cox *et al.*, 1983; Allendorf and Leary, 1986). Such a process has important implications for wildlife management (see Chapter 1.2) particularly in maintaining genetically viable populations in disjunct and often small habitat reserves. Moreover, the division of genetic variation among populations may also be important. For example, the genetic differentiation between populations of a species may assist in maintaining variation within that species, but in extreme cases where populations are very small and therefore subject to severe inbreeding, active inter-mixing of populations may be required (Templeton, 1986; Lande and Barrowclough, 1987). Isolation will also affect organisms with different life history strategies in different ways. The effects of such characteristics need to be understood if adequate protection for endangered populations is to be maintained (Lande, 1988).

The use of allozyme electrophoresis to estimate heterozygosity is widespread (Nevo *et al.*, 1984), predominantly because the technique is rapid and inexpensive compared with other measures (Chambers and Bayless, 1983; Lande and Barrowclough, 1987). In estimating heterozygosity three assumptions are made. The first is that the proteins examined are a random sample of all structural genes in the genome. Broadly speaking, this assumption is reasonable provided the sample is not weighted too heavily in favour of proteins with a particular enzymatic function and can be avoided by studying as wide a variety of enzymes as possible (Lewontin, 1974). This assumption relates directly to the second which is that the probability of polymorphism is equal at each locus. Levels of genetic variation have been shown to vary with protein type (Powell, 1975) indicating a functional link between loci and heterozygosity. Consequently, the possibility of bias is quite real, particularly in electrophoretic studies which emphasise proteins of a particular functional group.

The third assumption made is that variation in structural genes represents variation in all types of genes in the genome. This has generated some comment and requires discussion. The degree to which a small sample of loci accurately represents variation within the entire genome is a function of the ratio of loci sampled to the total number of loci in the genome and the variability among loci sampled (Chacraborty, 1981). Mitton and Pearce (1980) used simulations to demonstrate that even sampling small numbers of loci (e.g. 5 to 20), the correlation with actual heterozygosity was quite high, provided the total number of loci in the genome was small (e.g. 100). However, estimates of heterozygosity will decrease in reliability with increasing genome size and Chacraborty (1981) estimates that with true heterozygosity of 5% and a genome of 1000 loci, the correlation between the estimates and true value would be low. The significance of this becomes apparent when considering the human genome which is estimated to consist of between 50 and 100 thousand structural genes

(McKusick, 1976). A sample of 20 or even 100 loci is a very small proportion of a genome thus affecting the reliability of heterozygosity estimates (Chacraborty and Ryman, 1983).

Estimates of average heterozygosity will also be affected by the number of individuals sampled in the population. Nei and Roychoudury (1974), examined the effect of inter versus intra locus variance on the variance of average heterozygosity estimates and concluded that as inter-locus variance far exceeded intra-locus variance, the latter was relatively unimportant. As intra locus variance is caused entirely by sample size and gene frequencies, the number of individuals sampled is unimportant when compared with the number of loci sampled. Thus, as a general rule for estimating average heterozygosity, it is better to maximise the number of loci examined than the number of individuals per locus, unless inter locus variance is very small (Gorman and Renzi, 1979; Nei and Roychoudury, 1974). Nei (1978) suggests that a sample of greater than 50 loci is necessary to obtain a reliable estimate but points out that in practical terms this is often not possible. If many loci cannot be sampled, then a large number of individuals can still help reduce the standard error of the average heterozygosity estimates (Nei, 1978).

Despite the problems of obtaining reliable estimates of average heterozygosity using allozyme electrophoresis, many studies have used the technique to assess 'inter' and 'intra' population variation. Many of these studies have used small numbers of loci (mean = 23; Nevo *et al.*, 1984) which as discussed must reduce the accuracy of assessing genetic trends. One of the major reasons for the continued use of allozyme electrophoresis in such studies is the empirical observation that heterozygosity often correlates with various parameters of fitness (Mitton and Grant, 1984; Allendorf and Leary, 1986; Danzmann *et al.*, 1988).

In this chapter, eight island and eight mainland populations of the sleepy lizard (*Trachydosaurus rugosus*) are examined for genetic variation. The location and description of the study sites and the general biology of the sleepy lizard are given in Chapter 2. Gel electrophoresis is used to examine the levels of genetic variation and divergence among and within the populations.

4.2 MATERIALS AND METHODS

4.2.1 Data Collection

Blood samples for the electrophoretic analysis were collected from 285 sleepy lizards (*T. rugosus*) at eight mainland localities on Eyre Peninsula and Yorke Peninsula and eight adjacent continental islands. Sample sizes ranged from 2 to 53 individuals per population. Blood samples were used because they were easy to obtain without harming the animal. Because some of the populations examined were very small, it was not possible to take large numbers of individuals for liver and other tissue samples. This situation is analogous to many management situations involving rare or endangered species, but reduces the number of loci which can be sampled. The samples were frozen as whole blood because most samples were collected in the field where centrifugation was not possible.

Approximately 100 µl of each frozen sample was taken and allowed to thaw, mixed with two drops of lysing solution (see Appendix 2) and spun to remove insoluble debris. The supernatant was pipetted into capillary tubes, sealed and re-frozen. The samples were assayed for electrophoretic variation in sixteen systems, comprising fourteen 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), Umberlyferal Acetate Esterase (UAE) and two non-enzymatic proteins; Albumin (Alb) and Transferrin (Trf) providing seventeen informative loci. The systems examined and a full description of the running conditions for each are provided in Appendix 2. All proteins except Trf were assayed using cellogel (Chemetron, Milan) electrophoresis after Richardson *et al.* (1986) with some modifications in running times. Vertical Starch Gel electrophoresis (Harris and Hopkinson, 1976) was used to type Trf because its activity was low.

A draughtsmans pen was used to apply approximately 5 µl of sample to the cellogel which was then run at 200 volts for 1 to 3 hours. After staining, the gels were incubated at 37 ^{o} C and scored immediately. Gels were photocopied and the copies annotated to provide a permanent record. The vertical starch gels (Electrostarch, Madison, Wisconsin, lot 392, 9%) were loaded with 15 µl of sample, 8 µl of Tris-HCL buffer (pH8) and 2 µl of Fe^{59} (code IFS.1, Amersham) using a 50 µl syringe. Each gel was run for 18 hours at 200 volts. Gels were then sliced using a slicing wire, wrapped in Ceranwrap and placed against an x-ray film (Kodak XRP-5 100, Cat. 4079158) for 48 hours at

-100°C, before being developed and scored. Controls were included on each gel and appropriate lineup gels run for individual comparisons. Alleles were identified by alphabetic characters with the most anodal electromorph designated as 'A'.

4.2.2 Data analysis

All analyses of the electrophoretic data were carried out using the statistical packages BIOSYS-1 (Swofford and Selander, 1981) and SAS Version 6.03 (SAS Institute, 1987). The analysis was restricted to ten populations (seven island and three mainland) for which more than eight individuals were represented. All significance tests are at the 95% level unless otherwise stated.

Inheritance was not studied in the sleepy lizards, but phenotypic characteristics (i.e. quaternary structure and isozyme number) were compared with other electrophoretic surveys of reptiles to ensure that results were compatible. Linkage disequilibrium is rarely tested for in studies of this nature and the independence of loci is generally assumed. However, an apparent association was observed between two of the loci (Alb and SOD), so the non-association between all loci within each populations was tested. Expected frequencies less than one in one or more cells were often obtained, so Fisher's exact test was used in place of chi-squared contingency analysis.

The genetic relationships between the populations were examined in three ways. First, the distances between populations were determined using both Nei's unbiased genetic distance (Nei, 1978) and Rogers' genetic distance (Rogers, 1972) and a heirachical cluster analysis performed using the unweighted pair-group method with arithmetic mean averaging (UPGMA) (Sneath and Sokal, 1973). Second, chi-squared contingency analysis of allele frequencies were used to test for divergence between populations using the method of Workman and Niswander (1970). If expected frequencies were low, rare alleles were pooled.

Third, 'F' statistics (Wright, 1969, 1978) were calculated to determine the degree of differentiation occurring between populations. These statistics include F_{ST}, F_{IS} and F_{IT} where F_{ST} is the correlation between random gametes within populations relative to gametes over all populations, F_{IT} is the correlation between gametes that unite to produce the individuals, relative to the gametes of all the populations, and F_{IS} is the average over all populations of the correlation between uniting gametes relative to those of their own population (Wright, 1969). F_{ST} can be considered a measure of genetic differentiation between populations while 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 were calculated over all populations and for various sub-groups of populations. Island and mainland populations were examined both separately and together to determine the extent to which differentiation varied among these two groups. Separate analyses were run with specific single populations omitted sequentially to determine which populations if any, made major contributions to the overall genetic divergence.

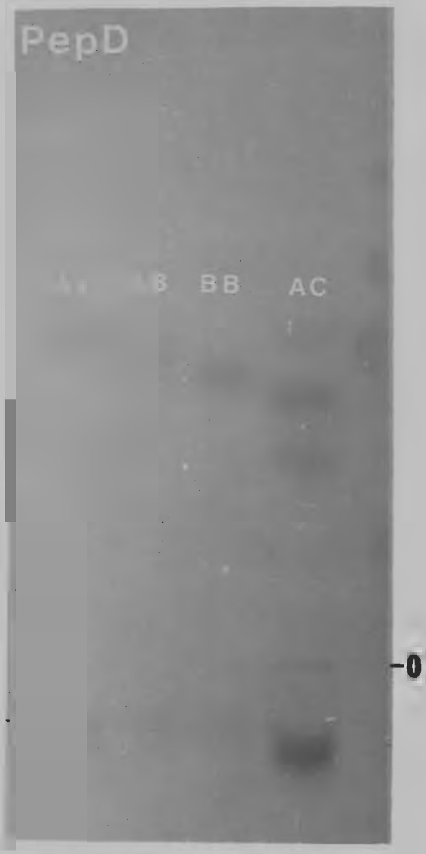
Average heterozygosity estimates were obtained for each population by direct count and from those expected under Hardy-Weinberg equilibrium, using Nei's unbiased estimate (Nei, 1978). Heterozygosity values were given an arcsine transformation (y'= arcsine y) and the significance of variation between populations tested using a one way ANOVA (Archie, 1985). 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 X 2 contingency table 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.

4.3 RESULTS

4.3.1 Locus description

Eight of the seventeen presumptive loci scored were found to be polymorphic in at least one population (see Plate 1). The number of loci observed (but not necessarily scored) per system and the quaternary structure of those loci examined were comparable with those generally found in vertebrates (Richardson *et al.*, 1986). Sample sizes per population ranged from 2 to 53 (mean=17.8) and the maximum number of alleles observed at any locus was four. Allele frequencies for all polymorphic loci are given in Table 4.1 and raw data are presented in Appendix 3.

PLATE 1 The alleles observed at six polymorphic loci. Genotypes are provided above each individual. Key to the abbreviations for each enzyme is provided in the text. A, the most anodal allele for each locus.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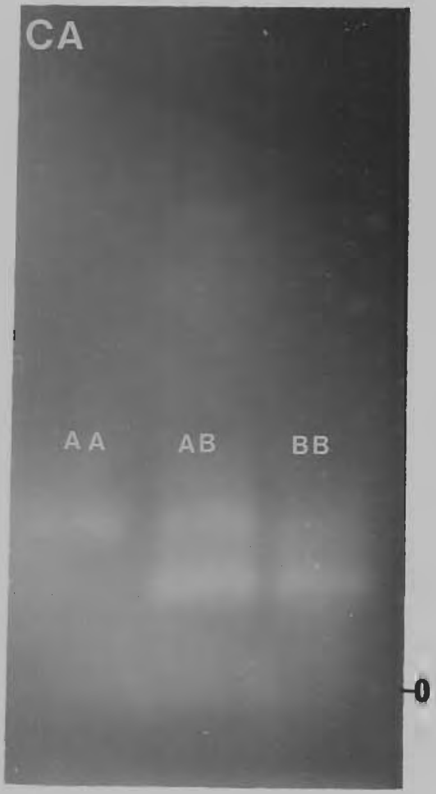
BB
AC
0


PepA2
AA
BB
BC
CC
0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AB
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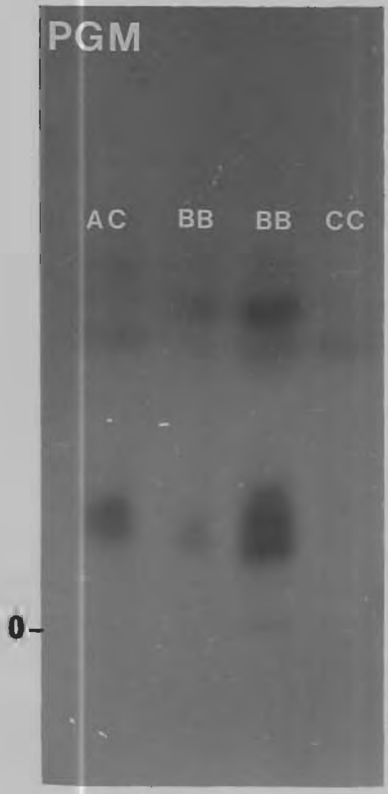
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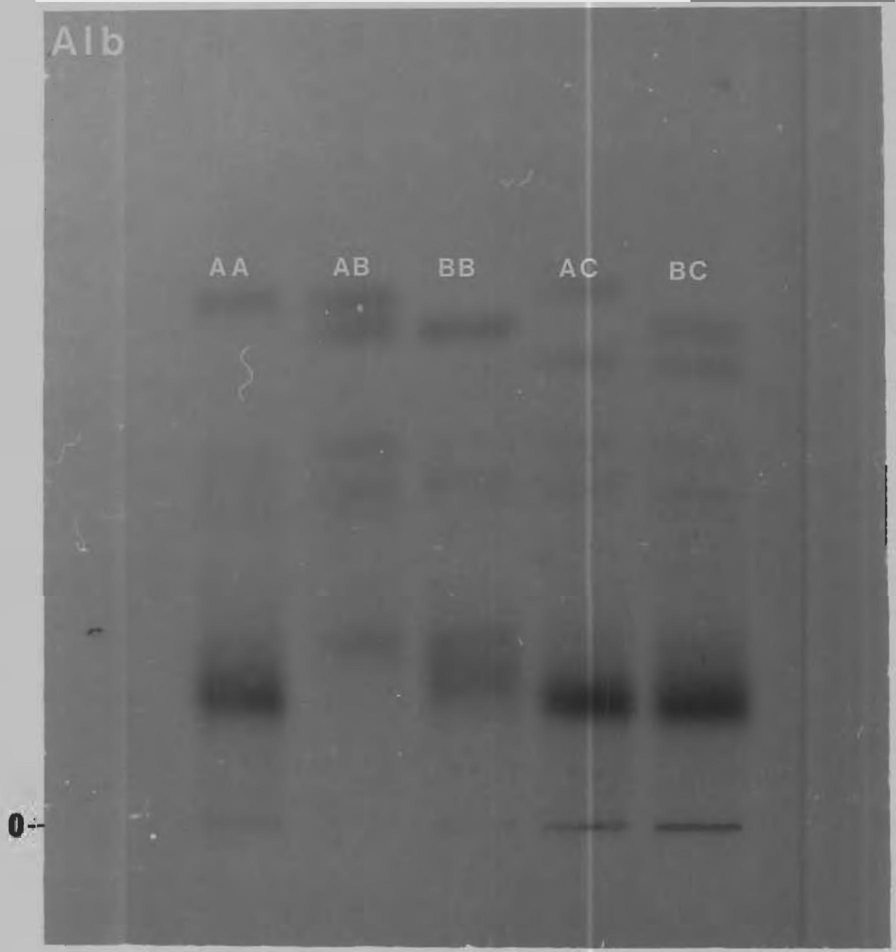
AC
BB
BB
CC
0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AB
BB
AC
BC
0

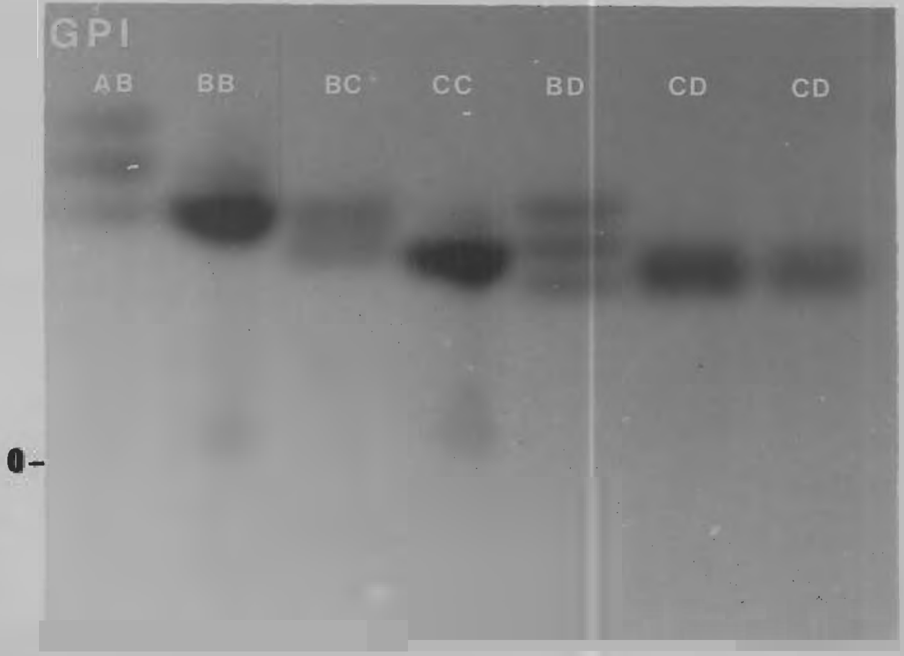
GPI
AB
BB
BC
CC
BD
CD
CD
0

TABLE 4.1 The allele frequencies for all populations at all polymorphic loci. Abbreviations: StPtr, St. Peter Island; Cedna, Ceduna; PtKny, Port Kenny; Flnds, Flinders Island; LcnNP, Lincoln National Park; TmyBy, Tumby Bay; Spsby, Spilsby Island; Dufld, Duffield Island; Rvsby, Reevesby Island; Werna, Weeroona Island; PtPre, Port Pirie; PtPce, Point Pearce; Wrdng, Wardang Island; PtVca, Port Victoria; Ethbg, Edithburg; Troub, Troubridge Island. See Figure 2.2 for the location of each population.

	POPULATION							
LOCUS	StPtr	Cedna	PtKny	Flnds	LcnNP	TmyBy	Spsby	Dufld
ALB-1								
(N)	9	3	2	3	5	24	19	27
A	0.611	0.500	0.250	0.500	0.400	0.667	0.711	0.500
B	0.389	0.500	0.750	0.500	0.600	0.333	0.289	0.500
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TFN-1								
(N)	4	1	2	3	5	21	19	22
A	0.000	0.000	0.000	0.000	0.400	0.262	0.000	0.000
B	1.000	1.000	1.000	1.000	0.600	0.690	1.000	1.000
C	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000
PEPA2								
(N)	9	3	2	3	5	23	19	27
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	0.800	0.978	1.000	1.000
C	0.000	0.000	0.000	0.000	0.200	0.022	0.000	0.000
PEPD1								
(N)	9	3	2	3	5	23	19	27
A	1.000	1.000	1.000	0.667	0.600	0.804	1.000	1.000
B	0.000	0.000	0.000	0.333	0.400	0.174	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000
GPI-1								
(N)	9	3	2	3	5	23	19	27
A	0.222	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.611	1.000	0.750	0.500	0.400	0.543	0.658	0.833
C	0.056	0.000	0.250	0.167	0.300	0.370	0.000	0.000
D	0.111	0.000	0.000	0.333	0.300	0.087	0.342	0.167
PGM-1								
(N)	9	3	2	3	5	22	19	27
A	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000
B	0.333	0.000	0.000	0.167	0.000	0.250	0.579	0.537
C	0.667	1.000	1.000	0.667	1.000	0.750	0.421	0.463
CA-1								
(N)	9	3	2	3	5	21	18	26
A	0.333	0.167	0.500	0.000	0.500	0.476	0.250	0.385
B	0.667	0.833	0.500	1.000	0.500	0.524	0.750	0.615
SOD-1								
(N)	8	3	2	3	5	24	18	25
A	0.813	0.667	0.500	0.167	0.400	0.646	0.694	0.540
B	0.188	0.333	0.500	0.833	0.600	0.354	0.306	0.460
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 4.1 (cont.)

	POPULATION							
LOCUS	Rvsby	Werna	PtPre	PtPce	Wrdng	PtVca	Ethbg	Troub
ALB-1								
(N)	47	25	16	30	53	3	2	22
A	0.106	0.320	0.344	0.633	0.726	1.000	0.500	0.409
B	0.894	0.680	0.656	0.333	0.274	0.000	0.500	0.591
C	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
TRN-1								
(N)	27	22	10	29	31	3	2	21
A	0.000	0.000	0.250	0.103	0.000	0.167	0.000	0.214
B	1.000	1.000	0.750	0.810	1.000	0.833	1.000	0.786
C	0.000	0.000	0.000	0.086	0.000	0.000	0.000	0.000
PEPA2								
(N)	46	25	16	30	53	2	2	22
A	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
B	0.511	1.000	0.969	0.967	1.000	0.750	1.000	1.000
C	0.489	0.000	0.031	0.000	0.000	0.250	0.000	0.000
PEPD1								
(N)	46	25	16	28	53	3	2	21
A	0.815	0.720	0.969	0.839	0.821	0.667	1.000	0.762
B	0.185	0.280	0.031	0.161	0.179	0.333	0.000	0.238
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI-1								
(N)	46	25	16	30	51	2	2	22
A	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
B	1.000	0.820	0.531	0.550	0.461	0.250	0.750	0.227
C	0.000	0.180	0.438	0.317	0.539	0.750	0.250	0.773
D	0.000	0.000	0.031	0.100	0.000	0.000	0.000	0.000
PGM-1								
(N)	46	25	16	30	51	2	2	21
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.630	0.340	0.406	0.150	0.078	0.000	0.500	0.262
C	0.370	0.660	0.594	0.850	0.922	1.000	0.500	0.738
CA--1								
(N)	46	25	16	30	50	1	2	21
A	0.337	0.620	0.750	0.500	0.510	0.000	0.500	0.310
B	0.663	0.380	0.250	0.500	0.490	1.000	0.500	0.690
SOD-1								
(N)	45	25	15	28	52	1	1	18
A	0.111	0.340	0.433	0.750	0.779	1.000	1.000	0.444
B	0.889	0.660	0.567	0.214	0.221	0.000	0.000	0.556
C	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000

The allele frequencies consistently exhibit a pattern of reduced numbers of alleles in the insular populations in comparison with the adjacent mainland population. Alb exhibited three electromorphs, of which two ('A' and 'B') were common in all populations except Pt. Victoria. The rarity of 'B' in the latter population is almost certainly the result of a small sample size as the nearby Pt. Pearce population contained both 'A' and 'B' alleles in high frequencies. Neither of these alleles were consistently more common than the other among populations. For example, the 'B' allele had a frequency of 0.894 in the Reevesby Island population compared with a frequency of 0.289 in the Spilsby Island population. The 'C' allele was found in only one population (Pt. Pearce) at low frequencies (0.033).

Three alleles were observed at the Trf locus, but all islands except Troubridge (which has both 'A' and 'B' alleles) were fixed for the 'B' allele. This allele was also the most common allele in all mainland populations, possibly increasing its probability of fixation in the small isolated populations. The rare 'C' allele was found only at Tumby Bay and Pt. Pearce in low frequencies.

The PepA2 locus contained three alleles with the 'B allele being the most common. This allele was fixed on all islands except Reevesby where both the 'B'and 'C' alleles were present in about equal proportions. The 'C' allele was present in low frequencies at Tumby Bay, Pt. Pirie, and Pt. Victoria. The 'A' allele was extremely rare, found in only two individuals from Pt. Pearce.

Three electromorphs were observed at the PepD locus. The 'A' allele was the most common and was fixed on Spilsby, Duffield and

St.Peter island populations. The 'B' allele has been retained on Reevesby Island even though it is not present on the other Sir Joseph Banks Group islands (Spilsby and Duffield). The 'C' allele was found in only a single individual from Tumby Bay.

The four alleles at the GPI locus are found together in only the St. Peter and Pt. Pearce populations. The 'B' allele was found to be the most common allele in all populations except Wardang, Edithburgh and Troubridge. The 'A' allele is not present at Tumby Bay or on the three Sir Joseph Banks islands. Duffield and Spilsby have retained polymorphisms at this locus with 'B' and 'D' alleles but Reevesby is fixed for 'B'. Wardang, Weeroona and Troubridge have retained 'B and 'C' but not 'A' and 'D'.

Three alleles were observed at the PGM locus. The 'A allele was extremely rare and found in only one individual at Pt. Kenny. Both the 'B' and 'C' alleles were maintained in reasonably high frequencies in the St. Peter, Spilsby, Duffield, Reevesby, Weeroona, Pt. Pirie and Troubridge populations (i.e. frequency > 0.25) but in the Tumby Bay, Pt. Pearce and Wardang populations, the 'B' allele was in low frequencies.

Polymorphisms for both alleles found at the CA1 locus were maintained in all populations except Pt. Kenny and Pt. Victoria (fixed for the 'B' allele). The two alleles observed at the SOD1 locus were common in all populations except Pt. Victoria (fixed for 'A'), St. Peter (frequency 'B' = 0.188) and Reevesby (Frequency 'A' = 0.111). The 'C' allele was found in two individuals in only one population (Pt. Pearce).

4.3.2 Genetic relationships

A total of 180 Fisher's exact tests of association between loci were calculated (Table 4.2). Of these 13 (or 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 ($P < 0.01$). This suggests that these loci are linked and that the assumption of independence between them is questionable. Consequently, the locus SOD has been excluded from the analyses of genetic relationships and heterozygosity.

Cluster analysis using Rogers' and Nei's genetic distance measures (Fig. 4.1), produced several distinct clusters. St. Peter, Spilsby and Duffield form one cluster in both trees although the point of divergence between these three populations remains unresolved. Both measures group Tumby Bay, Point Pearce and Wardang in one cluster and Weeroona and Pt. Pirie in another. Reevesby is a clear outlier, clustering from all other populations at a distance of 0.14 (Rogers genetic distance). The point of divergence of the Troubridge population remains unresolved with the tree derived from Rogers Genetic Distance placing Troubridge closer to Weeroona and Pt. Pirie while that obtained using Nei's Genetic Distance places Troubridge closer to the cluster of Tumby Bay, Pt. Pearce and Wardang. This ambiguity probably reflects the recent isolation of this population from an unknown number of founders of an unknown population of origin.

There is some congruence apparent between geographic proximity and the clusters formed by the UPGMA analysis. For example, Duffield and Spilsby are closely related as are the geographic pairs of Point

TABLE 4.2 Fisher's exact test for linkage disequilibrium between each pair of loci within and each population. A key to the abbreviations for each population is provided in Table 4.1.

LOCUS	POPULATION									
	StPtr	Tmyby	Splby	Dufld	Rvsby	Werna	PtPre	PtPce	Wrdng	Troub
Alb/Transf	-	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***
Transf/PepA2	-	0.400	-	-	-	-	1.000	1.000	-	0.700
Transf/PepD	-	0.134	-	-	-	-	1.000	0.526	-	1.000
Transf/GPI	-	0.569	-	-	-	-	0.548	0.257	-	1.000
Transf/PGM	-	1.000	-	-	-	-	0.226	0.373	-	0.122
Transf/CA	-	1.000	-	-	-	-	0.429	0.258	-	0.685
Transf/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	-	-

TABLE 4.2 (Cont.) Fisher's exact test for linkage disequilibrium between each pair of loci within and each population. A key to the abbreviations for each population is provided in Table 4.1.

LOCUS	POPULATION									
	StPtr	Tmyby	Splby	Dufld	Rvsby	Werna	PtPre	PtPce	Wrdng	Troub
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

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

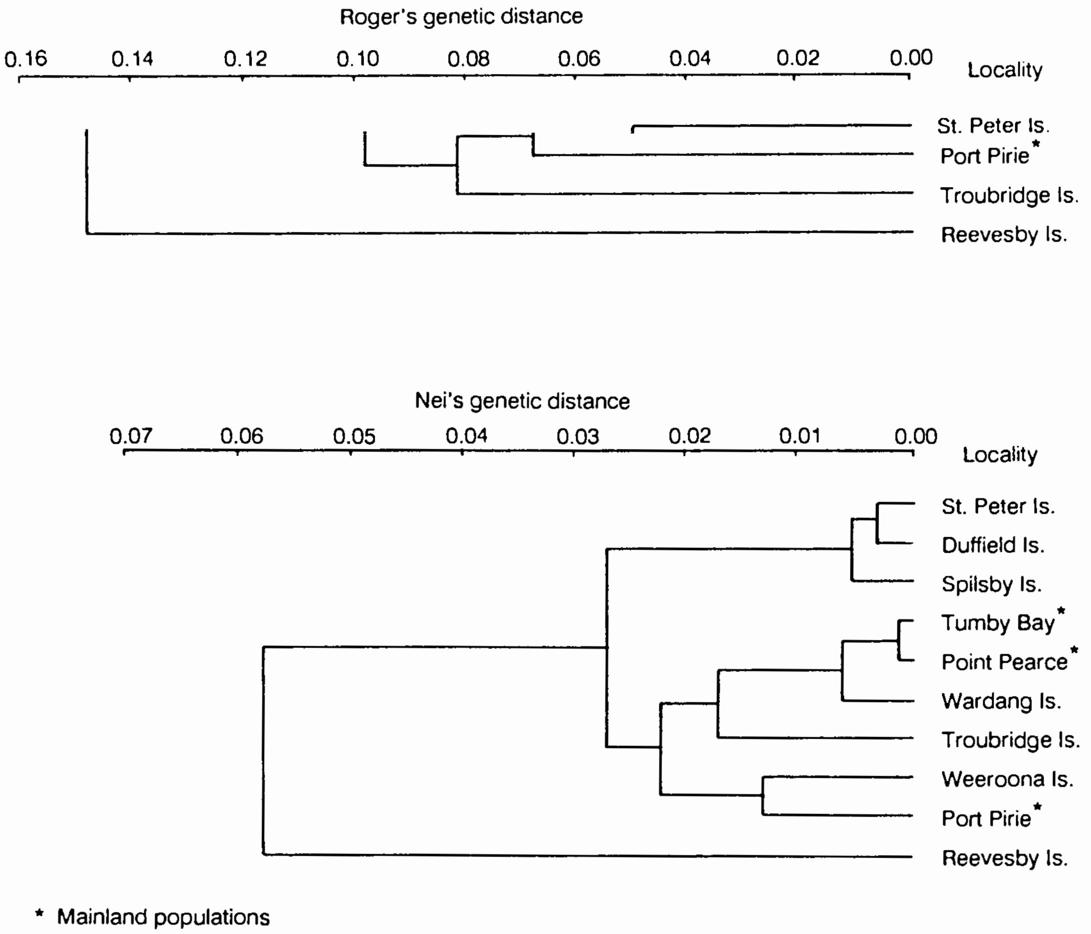

FIGURE 4.1 UPGMA cluster analysis of Rogers (1972) and Nei (1978) genetic distance measures among island and mainland populations of *T.rugosus.*

Pearce and Wardang and Weeroona and Pt. Pirie. However, the genetic closeness of St. Peter to Spilsby and Duffield and between Tumby Bay and Pt. Pearce is unexpected from geographic proximity as is the high degree of divergence between Reevesby and its nearest populations Spilsby and Duffield and between Tumby Bay and the three Sir Joseph Banks Group islands. However, the relationships among the populations observed using allozymes shows some similarities with those observed using mtDNA data (Chapter 3). A close relationship between St. Peter and Spilsby is suggested by both analyses, which indicates an eastern Eyre Peninsula origin for the St. Peter population. The closeness of Tumby Bay to Pt. Pearce and Wardang in both analyses indicates that these populations were similar prior to isolation and have undergone little divergence since fragmentation.

The relative proportions of different alleles differed significantly among populations for all loci (chi-squared tests, $p < 0.001$, Table 4.3) indicating significant genetic heterogeneity among populations. This heterogeneity remained significant for island populations when mainland populations were removed from the analysis (Table 4.3). However, when analysed separately, the three mainland populations showed no significant differences at four loci (Trf, PepA2, PepD, GPI), whereas the three remaining loci were significant. This indicates a greater divergence between the island populations than between those of the mainland.

The F statistics (Table 4.4) demonstrate substantial differences between individual loci. For example, PepA2 showed twice as much differentiation as the average over all loci among the island populations, principally because all islands were fixed for the 'B'

TABLE 4.3 Chi-Square analysis of allele frequencies at all polymorphic loci between the seven island (d.f.=6), three mainland populations (d.f.=2) and all ten populations combined (d.f.=2) of *T.rugosus*. Minimum sample size per population =8. Frequencies of rare alleles were pooled because expected frequencies were below 1. Abbreviations: d.f., degrees of freedom; p, probability of Type I Error; NS, Not Significant.

	ILD POPS		MLD POPS		ALL POPS	
LOCUS	CHI-SQUARE	P	CHI-SQUARE	P	CHI-SQUARE	P
Alb	93.604	<0.001	8.802	<0.05	108.129	<0.001
TRN	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 4.4 F statistics for the seven island and three mainland populations of T. rugosus for which sample sizes were greater than eight. The F statistics are those defined by Wright (1969) and locus abbreviations follow those of Richardson *et al* (1986).

	ISLANDS			MAINLAND			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

allele except for Reevesby Island. GPI also demonstrated high divergence in the island populations with all four alleles maintained over all populations but usually only two being maintained in any one population. The alleles maintained varied between populations but significantly, Reevesby Island was monomorphic at the GPI locus. In contrast, CA exhibited very low F_{ST} values with most populations maintaining both alleles. The mainland populations showed less variation between loci in F_{ST} values and mean F_{ST} values among the three mainland populations was very low ($F_{ST} = 0.046$) indicating low levels of differentiation.

The three mainland localities contribute little to overall genetic divergence among the populations. For example, the mean F_{ST} values over all ten populations is three times greater than the divergence observed between the three mainland populations and only slightly less than the levels observed between the island populations. When the three mainland populations are removed from the analysis the mean F_{ST} value is only marginally altered. To assess the contribution of each population to the mean F_{ST}, individual populations were systematically removed from the analysis and the remaining populations reanalysed. Generally, the removal of a single population from the analysis resulted in only minor differences in mean F_{ST}. The largest effect observed was when Reevesby Island was removed, indicating that this population is the most genetically divergent of all the populations (See also cluster analysis).

The mean F_{IS} values are lower among the islands than the mainland which suggests that inbreeding is more prevalent in the insular populations. However, few negative values were obtained which is consistent with the few significant departures from Hardy-Weinberg equilibrium observed within the populations (See section 4.3.2). The positive F_{IT} values are consistent with populations which form discrete demes suggesting that the populations examined form a set of systematic and real subdivisions. These values were higher among the islands emphasising the discrete nature of these populations.

4.3.3 Genetic variation within populations

The populations of *T.rugosus* examined in this study demonstrated considerable electrophoretic variation (Table 4.5) with observed levels of heterozygosity ranging from 10.5% on Wardang Island to 16.8% at Tumby Bay (mean = 12.58%). The value of 4.2 % observed on Flinders Island (not included in the analysis) is considerably less than the other populations and probably a result of the small sample size (n = 3). The observed levels of heterozygosity are within the range of values (0% to 30%, mean = 5.5%) recorded by Nevo *et al.* (1984) in a review of electrophoretic studies involving non-parthenogenic reptiles. However, they are considerably higher than those found in most electrophoretic studies of non-parthenogenic lizards. For example, Adest (1977) and Kim *et al.* (1978) found heterozygosity levels of 0 to 2.9% in species of fossorial lizards while Nevo (1981) found heterozygosity levels of up to 8.7% in species of Agamids. The three mainland populations exhibited higher levels of both observed and expected heterozygosity values than any of the island populations, however this trend was not significant ($F_{OBS} = 0.12$, $P > 0.1$; $F_{EXP} = 0.17$, $P > 0.1$).

TABLE 4.5 Mean genetic variability estimates for eleven populations at sixteen loci (Standard errors in parentheses).

				MEAN PERCENT HETEROZYGOSITY	
POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	DIRECT-COUNT	HDYWBG EXPECTED**
ST PETER IS	8.7	1.4	25.0	13.9	12.1
	(0.3)	(0.2)		(6.5)	(5.7)
FLINDERS IS	2.9	1.1	12.5	4.2	5.8
	(0.1)	(0.1)		(2.8)	(4.2)
TUMBY BAY	23.3	1.6	37.5	16.8	17.2
	(0.3)	(0.2)		(5.8)	(5.7)
SPILSBY IS	18.9	1.3	25.0	11.7	11.1
	(0.1)	(0.1)		(5.6)	(5.0)
DUFFIELD IS	26.6	1.3	25.0	11.5	11.1
	(0.3)	(0.1)		(5.3)	(5.1)
REEVESBY IS	45.4	1.3	31.3	12.1	12.0
	(1.2)	(0.1)		(5.0)	(4.9)
WEEROONA IS	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 IS	51.2	1.3	31.3	10.5	11.6
	(1.4)	(0.1)		(4.8)	(4.8)
TROUBRIDGE IS	21.8	1.4	37.5	12.0	15.0
	(0.1)	(0.1)		(4.7)	(5.1)

* 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)

The mean number of alleles per locus in the three mainland populations (mean = 1.6; range = 1.5 to 1.7) are higher than in any of the seven islands populations (mean = 1.33; range = 1.3 to 1.4). The percentage of polymorphic loci show a similar trend being consistently higher (mean percent_{ILD} = 30.233, mean percent_{MLD} = 39.6; 99% criterion) in the mainland populations.

Probability levels for departures from Hardy-Weinberg equilibrium are given in Table 4.6. Eleven of the 60 Fisher's exact tests, on eight loci departed significantly from Hardy-Weinberg equilibrium. St Peter, Duffield and Weeroona islands contained no departures from Hardy-Weinberg equilibrium while Tumby Bay, Pt. Pirie, Spilsby Island and Reevesby Island 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 islands 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 meta-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). That such a situation exists is supported by observations by Schwaner (op. cit.) who noted that the distribution of this population was extremely clumped around areas used as rubbish dumps.

Conversely, the entire area of Troubridge Island was sampled and the size of this population is known to be very small (see Table 4.7)

TABLE 4.6 Results of Fisher's exact tests for departures from Hardy-Weinberg equilibrium in ten populations of *T.rugosus* across all polymorphic loci. Abbreviations to the populations are given in Table 4.1. -, 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
Transf	-	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**

* $P < 0.05$; ** $0.05 < P < 0.01$

TABLE 4.7 Population estimates and the method of calculation for Duffield, Spilsby and Troubridge Islands. All calculations follow Caughley (1980). 1, first capture period; 2, second capture period; 3, third capture period; -, not surveyed. See text for the dates for each capture period.

ISLAND	METHOD OF ESTIMATION	NO. MARKED (TIME) 1	2	3	NO. RECAPT (TIME) 2	3	DENSITY ESTIMATE (/Ha)	POPULATION ESTIMATE (TOTAL)
DUFFIELD	BAILEYS T.C.	22	10	15	3	9	6.0	50 ± 27.4
SPILSBY	PETERSON	12	2	-	2	-	2.0	200 ± 63.0 *
TROUBRIDGE	PETERSON	19	13	-	4	-	6.9	53 ± 17.4
WARDANG	JOLLY SEBER	-	-	-	-	-	5.2	$10.6*10^4$ **

* See text for discussion of this estimate. ** Estimates for Wardang were obtained from Schwaner (1988). This estimate must be viewed as a maximum because it assumes an even distribution over the entire island. Schwaner (1988) noted that this assumption may not be correct as distribution of *T.rugosus* was found to be clumped around areas of rubbish.

and probably the result of the introduction of a few founding individuals. Departures from Hardy-Weinberg equilibrium would be expected under these circumstances resulting from the mating of close relatives. Two other loci (Alb, P = 0.071; CA, P = 0.115) also showed tendencies towards departures from Hardy-Weinberg equilibrium in this population.

4.4 DISCUSSION

The electrophoretic examination of island and mainland populations of *T.rugosus* demonstrates that little erosion of genetic variation has occurred within the insular populations. This is in contrast to studies of other vertebrates on South Australian islands by Schmitt (1978) on the bush rat (*Rattus fuscipes greyii*) and Schwaner and Adams (in prep.) on the tiger snake (*Notechis scutatus-ater*) which show large decreases in genetic variation, sometimes to the point of fixation at all loci examined.

Clearly none of the insular populations have experienced a severe bottleneck sufficient to induce large scale fixation of alleles. However, differences were observed in the level of allelic diversity and percent polymorphism between the island and mainland populations and significant divergence between the island populations was also observed, indicating that some genetic drift has occurred. As expected, the tendency appears to be towards a loss of rare alleles in the island populations, while fixation, if it has occurred, has involved those alleles which are most common in the mainland populations.

Four forces, selection, mutation, migration and genetic drift are involved in determining the levels of heterozygosity in natural populations. It is conceivable that extreme conditions on the islands have increased selective pressure on these populations which may have resulted in the maintenance of polymorphisms at the loci examined. However, the lack of consistent departures from Hardy-Weinberg equilibrium at any one locus suggest that selection has not been important in maintaining the observed polymorphisms.

No unique alleles were present in the island populations indicating that insufficient time has elapsed since isolation for new mutations to occur and become established in these populations. If all the alleles currently observed in the mainland populations have been lost in the island population through either founder effects or subsequent 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 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 to 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 several factors. The expected proportion of original heterozygosity remaining after a single generation bottleneck is $1 - 1/2N_e$ where N_e is the effective population size (Frankel and Soulé, 1981). Even the most severe case of 2 founders will retain a substantial proportion (75%) of total heterozygosity. Thus a bottleneck of one generation 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 such a bottleneck will have on allele diversity is more complicated and depends upon the number and frequency of alleles in the parent population (Allendorf, 1986). Allelic diversity is more likely to be affected when the loci involved have large numbers of unevenly distributed alleles (Sirkkomaa, 1983) with rare alleles being particularly susceptible to loss during a bottleneck (Allendorf, 1986). The island populations of *T.rugosus* have generally lost those alleles which on the mainland have lowest frequency and fixation has favoured those alleles which are in high frequencies on the mainland. This suggests that all the insular populations in this study have experienced a bottleneck at some stage during their period of isolation.

Several genetic and life history traits favour the retention of genetic variation in insular populations of *T.rugosus*. First, it is apparent from the mainland populations that this species is characterised by high levels of genetic variation so it can be assumed that founding populations were likely to have been correspondingly variable. Second, the lizards are long lived, thus minimising the number of generations since the time of isolation. Third, life history traits such as equal sex ratios and small fluctuations in population size which are important in maximising N_e (Lande and Barrowclough, 1987) and thus minimising genetic drift, appear to be characteristic of *T.rugosus* populations (see Chapt. 2.2). Such characteristics would favour the retention of most of the variation present in the parent population during and immediately following a founding event. However, the high level of mate fidelity and small home ranges observed in mainland populations (Bull, 1987; 88) if they are also characteristic of the island populations, would lower N_e by ensuring the non-random mating of individuals. Clearly, population size has been sufficiently large to maintain N_e at high enough levels to prevent severe genetic drift occurring.

Effective population sizes were not studied, but estimates of total population size are available for four islands (Table 4.7). Wardang Island has a population possibly in excess of 100,000 individuals which almost certainly represents an effective population size greater than the 500 necessary to prevent genetic drift (Franklin, 1980). In this case and if population numbers have remained relatively stable, it is likely that this population lost the majority of its allelic diversity through drift resulting from the initial founder event rather than a slow subsequent loss of alleles. Recovery of the present levels of variation through mutation is unlikely given the relatively short isolation time and low numbers of generations (in comparison to Nei *et al.* (1975) estimate of 10^5 to 10^7 generations). The lack of variation 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 time the Troubridge population has been able to retain levels of heterozygosity and polymorphism consistent with a mainland population but has not maintained allelic diversity. If this population is unable to expand to greater than N_e = 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 Island currently has an extremely small population (see Table 4.7) and island size and isolation time suggests that this has been the case for 6,000 years. Without gene flow and strong selection pressure, such a situation should have resulted in extreme drift and subsequent fixation at most loci. The fact that this has not occurred and that nearby Spilsby Island is genetically similar, suggests two possible explanations. The first is that the Duffield population has recently been introduced from Spilsby Island and insufficient time has elapsed since this introduction to enable significant differentiation to occur. Second, it is conceivable that sufficient gene flow exists between these two populations to have enabled the maintenance of alleles in both populations. Evidence from modelled populations (e.g. Boeklen and Bell, 1986) suggests that even very low rates of gene flow can maintain allelic diversity and heterozygosity between two populations. Experimental evidence involving flotation of *Anolis sagrei* in salt water has demonstrated that some flotation without the aid of rafts is possible among lizards (Schoener and Schoener, 1984). So the possibility of gene flow between Spilsby and Duffield cannot be ruled out. However, with the absence of the necessary historical data it is not possible to distinguish between these two hypotheses.

The relatively low levels of genetic differentiation among the mainland populations compared with differentiation among the island populations demonstrates the effect that isolation can have on an organism such as the sleepy lizard. The F statistics and genetic relationships are indicative of the role of isolation in the divergence of the sub-populations of *T.rugosus*. Low F_{ST} values between the mainland populations compared with those of the islands suggest that sufficient drift (probably as a result of the initial founder event) has occurred in these populations to enable significant divergence among the island populations.

CHAPTER 5. MORPHOLOGICAL COMPARISONS AMONG AND WITHIN POPULATIONS

5.1 INTRODUCTION

Many studies have been made on trends of divergence in morphology among insular populations and extreme morphological variation between insular populations has often been observed. For example, a wide variety of bill shapes and sizes probably originating from a single species were observed in Darwin's finches among islands of the Galapagos Archipelago (Lack, 1969). Many other cases of morphological divergence within island systems have been recorded including the *Partula* snails of the Society Islands (Clarke and Murray, 1969; 1971), rice rats on the Galapagos (Williamson, 1981) and among plants of the genus *Hemizonia* on Californian islands (Carlquist, 1965).

The occurrence of insular divergence is also well documented in reptiles. For example, insular populations of reptiles often exhibit extreme variation in body size (Case, 1978). Kramer (1951) recorded reduced hind limb and tail lengths in island populations of *Lacerta serpa* while divergence has been observed among *Anolis* lizards in the West Indies Archipelago (Williams, 1969; 1972) and among insular populations of *Uta stansburiana* in the Gulf of California (Soulé, 1966). Insular divergence has also been observed among land tortoises of the Galapagos Islands (Williamson, 1981) and among tiger snakes on offshore islands of Southern Australia (Schwaner 1985b; Schwaner and Sarre, 1988).

Islands have several characteristics which encourage morphological divergence. First, the formation of archipelagos by rising sea levels or volcanic activity can create conditions of allopatry among conspecific populations. The formation of allopatry is generally regarded as critical to the speciation process (Mayr, 1970; but see also White, 1978; Bush and Howard, 1986) thus the first step towards speciation is already present in island populations.

Second, novel selection pressures are often present on islands favouring the evolution of unique character expressions. Species composition is usually impoverished in insular populations compared with mainland areas (MacArthur and Wilson, 1967) leading to different selection pressures than in mainland communities. For example, predators are often absent from islands (Williamson, 1981) permitting the evolution of traits such as flightlessness in birds (Carlquist, 1965). Moreover, fewer species generally mean that interspecific competition on islands is reduced, creating situations in which those species present are able to increase in density (e.g. among insular populations of the Chuckawalla; Case, 1982) to levels which are higher than would be possible in mainland populations. This can often result in altered conspecific interactions such as reduced aggressiveness and smaller or overlapping territories (Stamps and Buechner, 1985). These unique island characteristics result in niche shifts for species contained within them which is often reflected in morphological or ecological change.

Third, genetic drift can result in divergence among insular populations. As described in section 1.4, the rate of genetic drift is directly affected by effective population size and is likely to be of particular significance in small insular populations. Genetic drift resulting from small effective population sizes, population bottlenecks or founder events can have a profound effect on the genetic constituency of a population. Genetic drift may cause random changes in allele frequencies or result in reduced heterozygosity or inbreeding. Both processes can influence microevolutionary processes and lead to differences in morphology, life history and development (Templeton, 1980). In particular, the founder effect has been implicated in the large diversity of *Drosophila spp.* in the Hawaiian islands (Carson and Templeton, 1984) possibly resulting from the sequential colonisation by a small number of founders on newly formed islands in the group.

The separation of the effects of genetic drift and those of selection presents a difficult problem in studies of genetic and morphological divergence on islands (Arthur, 1984). Unlike variation involving random surveys of allozymes, variation in morphology is usually polygenic in origin so separation of the environmental and genetic components of phenotypic variation becomes difficult. Furthermore, Lewontin (1984) has contended that even if the loci examined do influence the quantitative trait being measured, the probability of measuring the correlation is questionable. Possibly as a result of these difficulties, conflicting results have been obtained from attempts to correlate genetic divergence with morphological divergence (e.g. Turner, 1974; Coyne *et al.*, 1983; Patton, 1984).

In this chapter, morphological variation among populations of *Trachydosaurus rugosus* is measured with the aim of assessing the general patterns of divergence between the island and mainland groups. The degree to which morphological patterns are reflected in the genetic analysis (Chapter 4) will also be discussed. Morphological variation within individuals (measuring developmental stability) are also compared, with the aim of establishing indices of inbreeding depression independent of the allozyme data obtained in chapter 4. The rationale for adopting this approach is discussed below.

As discussed in section 1.3, fitness generally declines with increasing inbreeding in normally outbreeding species. The role of heterozygosity *per se* in such phenomena and in the fitness of non inbreeding populations is also poorly understood. Lerner (1954) argued that heterozygosity was important, attributing the ability of individuals to buffer themselves during development (developmental homeostasis) to their level of heterozygosity. He proposed that this mechanism was the result of the canalization of the developmental process ensuring that the phenotypic expression is close to optimum in response to natural selection. Accordingly, the more heterozygous loci an individual has, the more phenotypically normal (i.e. the nearer the selected optimum) it will be.

A predicted consequence of developmental homeostasis is that individuals which live in ecologically peripheral or marginal areas or which are under recently altered conditions of selection will be more prone to developmental instability than individuals under stable environmental conditions. Moreover, the more heterozygous an individual is then the less likely it is to be affected by environmental stress (Thoday, 1956; Levin, 1970). These effects have been observed under laboratory conditions (Dobzansky and Levene, 1956; Bradley, 1980) and in the field (Jogoe and Haines, 1985). Lerner (1954) (and others e.g. Mayr, 1970; Mather, 1973) argued that because developmental homeostasis results from a balance between interacting genes, any process which disrupts this balance (such as hybridisation, strong selection pressures or inbreeding) will also influence developmental stability. Thus, developmental instability associated with inbreeding could result from the exposure of deleterious recessive alleles, disruption of previously 'coadapted' genotypes or decreased heterozygosity. The extent to which the effects of inbreeding can be attributed to these three mechanisms is unknown.

Several workers have examined the relationship between heterozygosity and components of fitness by laboratory experiments examining viability of heterozygote and homozygote chromosomal variants of *Drosophila* in varied environments (e.g. Dobzansky and Levene, 1956) or by looking for correlations between components of fitness and levels of protein heterozygosity. The relationship between heterozygosity and fitness has been measured using viability (Watt, 1977; Samollow and Soulé, 1983), fertility (Smith *et al.*, 1975), growth (Singh and Zouros, 1978; Ledig *et al.*, 1983) and oxygen consumption (Koehn and Shumway, 1982; Danzmann *et al.*, 1988). Generally a positive relationship between these parameters and allozyme heterozygosity has been observed so it appears that whatever the mechanism, heterozygosity is important in maintaining fitness.

Given the general correlation between heterozygosity and fitness, it could be expected that if developmental stability reflects heterozygosity then it will also be related to fitness. Few studies have examined the link between developmental stability and fitness, but developmental stability has been linked to heterozygosity, and thus can be indirectly linked to fitness. There are two general approaches to measuring the relationship between heterozygosity and developmental stability. The first involves comparisons of the phenotypic variance among individuals which are heterozygous (e.g. at a single locus) with those which are homozygous or by comparing mean levels of morphological and genetic variation among populations. Negative associations have been observed (Eanes, 1978; Mitton, 1978; Fleischer *et al.*, 1983), but this tendency is by no means universal (Handford, 1978; McAndrew *et al.*, 1982; Zinc *et al.*, 1985).

The second approach is to measure developmental stability and heterozygosity within the individual and either compare heterozygous and homozygous individuals or compare average levels of heterozygosity and developmental stability among populations (Soulé, 1967).

One measure of developmental stability which has received particular attention is fluctuating asymmetry (FA) (Mather, 1953; Van Valen, 1962). FA can be defined as the degree of random difference between left and right sides in bilateral structures. It is different to both directional asymmetry (a consistent bias towards one side of a bilateral character) and antisymmetry (a platykurtic or bimodal distribution of one side minus the other around a mean of zero) (Palmer and Strobeck, 1986). FA has been observed to be correlated with protein heterozygosity (Bruckner, 1976; Soulé, 1979; Vrijenhoek and Lerman, 1982; Biémont, 1983; Leary, Allendorf and Knudsen, 1985; Leary, Allendorf, Knudsen and Thorgaard, 1985) either as population averages or at the level of the individual although there have been exceptions (e.g. Jackson, 1973; Bradley, 1980).

It is likely, then, that indices of developmental stability could provide a measure of the level of heterozygosity which will in turn be affected by the level of inbreeding and environmental effects. Consequently, they can provide important information on processes occurring within populations.

In the present study, the use of FA as an indicator of inbreeding depression in populations of *T.rugosus* is examined. Severe disruption of developmental processes could be expected to result in morphological abnormalities and in this study, the frequency of gross abnormalities was also measured for each population as an alternative measure of developmental stability. The results of these analyses are discussed in terms of their implications for the fitness of the various populations. Comparisons are also made between the estimates of inbreeding obtained from FA and abnormality counts and those expected from the electrophoretic estimates of heterozygosity obtained in Section 4.3.

5.2 MATERIALS AND METHODS

5.2.1 Data collection

A total of 375 live and museum specimens of *T.rugosus* were examined from 35 different localities on Eyre Peninsula, Yorke Peninsula and adjacent offshore islands in South Australia for 29 metric and meristic characters. The sampling methods and measurements taken are described in Chapter 2.3.

5.2.2 Data analysis: sexual dimorphism and variation among populations

T.rugosus is sexually dimorphic (Cogger, 1986), so it was necessary to quantify differences between the sexes in the populations examined and determine which characters contribute to any observed dimorphism before undertaking comparisons among populations.

The multivariate technique of stepwise canonical discriminant function analysis was used to assess the level of sexual dimorphism in *T.rugosus* and the level of difference between populations. This analysis establishes a subset of measurement variables that best discriminates among the values of a categorical variable (i.e. sex or population). When performed in a stepwise fashion, a variable is tested for its ability to discriminate between sexes (or populations) over and above the discrimination already achieved by variables that have been previously tested and found to be significant. Only variables that were significant at the 0.05 level contribute to the final discriminant function. The functions were then tested for their ability to predict the sex or population of individuals for which this information was available (by resubstitution). Low error rates suggest that there are significant morphological differences among the sexes or populations. All populations were pooled for the analysis of sexual dimorphism and the variable population was included in the discriminant function to determine if this affected the discriminatory power of the derived model.

For the analysis of population differences the ten populations which were used in the electrophoretic analysis (St. Peter, Tumby, Spilsby, Duffield, Reevesby, Wardang, Pt. Pearce, Weeroona, Pt. Pirie and Troubridge; see chapter 4) were included. Separate analyses were also conducted for the island and mainland populations and for subsets of adjacent island and mainland populations. These analyses were restricted to males only to avoid complications from differences between the sexes and because males represented the largest sample size from several localities from which only small total sample sizes were available.

Snout vent length (SVL) was forced into all discriminant function analyses to eliminate the effect of variation in size among individuals before testing subsequent variables for discrimination. The right-hand side measurements of bilateral characters SNL, EN, SL, IL, SC, UP and LP (see Table 2.2 for the key to character abbreviations) were excluded from the analysis to avoid duplication and the HLL measures were excluded because the measurement of this character from museum specimens was not considered accurate. Thus, a total of 20 characters were used in the multivariate analyses. Extreme values in the counts of meristic variables were considered to be the result of developmental abnormalities (e.g. fused or fragmented labial scales) and were not included in the analysis.

To further examine the morphological relationships among the ten populations, a heirachical cluster analysis was performed using the unweighted pair-group method with arithmetic mean averaging (UPGMA) (Sneath and Sokal, 1973). The analysis used mean population values for the 20 metric and meristic characters included in the discriminant function analysis.

For all multivariate analyses, only mature individuals were used. Bamford (1980), examined two populations of *T.rugosus* in Western Australia and noted that the lizards achieved sexual maturity (determined by the presence of mature gonads) towards the end of their third year at a SVL of approximately 250mm. These rates of growth were also found by Bull (1987) using weight, but were not equated with SVL. In the present study, seven apparent reproductive pairs were captured and nine females in the S.A. Museum collection were found to be gravid. The SVL's of these lizards were examined and found to have a minimum of 250mm (mean = 277.35 ± 15.84, n = 23). Thus, a snout-vent length of 250 mm or greater was adopted as the criterion for determining sexual maturity.

5.2.3 Data analysis: variation within populations

To obtain a measure of the relative levels of mean developmental stability within each population, two independent sets of data were collected. The first is average levels of fluctuating asymmetry and the second the incidence of gross abnormalities.

An estimate of fluctuating asymmetry (FA) was obtained by counting the right and left hand sides of bilateral characters and subtracting one from the other. Meristic characters were used because the error involved in counting such characters was considered to be less than those obtained through the measurement of metric bilateral characters in the field. This may decrease the sensitivity of FA as a measure of developmental stability (Swain, 1987), but in the absence of skeletal material to enable accurate laboratory measurement, such parameters were considered unreliable. The population FA parameter was derived using the formula (Palmer and Strobeck, 1986)

$$(L - R)^2/N$$

where L = left-hand side count; R = right-hand side count; N = sample size. This formula was used because of its sensitivity (Palmer and Strobeck, 1986), provided three conditions are met. These are: (1) that FA values are independent of character size, (2) directional asymmetry (a consistent bias towards one side of a bilateral character) and (3) anti-symmetry (a platykurtic or bimodal distribution of R-L around a mean of zero) are absent. To determine if FA varied with character size within each population, the mean character size [(R+L)/2] was plotted against asymmetry (R-L) and examined for evidence of trends. No clear trends were apparent so it is assumed that FA in the five variables used is independent of character size. Anti-symmetry and directional asymmetry were assessed visually by plotting the frequency of signed differences between left and right-hand side counts for all characters in each population. With very few exceptions, no tendencies towards skewness, platykurtic or bimodal distributions were observed. Therefore, it is assumed that these factors are not significant and can be ignored in this analysis.

The meristic characters SL, IL, SC, UP, and LP were used in this analysis because these characters are independent of body size. Sexual dimorphism in FA within each population was tested using non-parametric Wilcoxon tests of the unsigned differences between left and right hand side measurements. These were found to be non-significant in all populations for all five variables so the analysis was carried out on pooled samples of males and females. Differences among populations in FA values were assessed using F_{max} tests (Sokal and Rohlf, 1981) for each character. Those that showed significant differences were then tested for concordance with each other using Friedmans method for randomised blocks (Sokal and Rohlf, 1981).

An index of abnormalities was obtained by calculating the percentage of individuals within each population which exhibited some form of gross abnormality. Abnormalities were categorised as gross if individuals exhibited severely fused labial or head scales, more than one missing toe, deformed feet or the loss of ear openings (see Plate 2). From these data, the frequency of abnormalities within each population was calculated and the 'actual' counts compared using a Fisher's exact test. When this analysis was computationally untenable (i.e. when considering > 2 populations) a chi-squared contingency analysis was used.

St. Peter was omitted from both the FA and percent gross abnormalities because of small sample size. All analyses of the morphological data were carried out using SAS version 6.03 (SAS Institute, 1987).

5.3 RESULTS

5.3.1 Sexual dimorphism and variation among populations

Mean values for each variable used in the multivariate analyses are given in Tables 5.1 and 5.2 and the raw morphological data provided in Appendices 4 and 5.

PLATE 2 (A - D) Gross abnormalities observed in populations of *T.rugosus*. A, club foot; B, missing fingers; C, missing ear openings; D, fragmented supra labial scales.

A

B

C

D

TABLE 5.1 The mean values for all metric variables used in the discriminant function analyses for differences between sexes and among populations. Standard errors are given in parentheses. A key to the variable abbrieviations is given in Table 2.1. Only animals with SVL > 250mm were used in the analysis. $\bar{N}$ mean sample size.

LOCALITY	SEX	$\bar{N}$	SVL	TL	HL	IO	HW	RW
ALL	M	152	281.76 (1.27)	68.12 (0.59)	53.14 (0.33)	25.03 (0.17)	54.31 (0.37)	9.11 (0.09)
	F	131	284.46 (1.47)	65.58 (0.66)	48.20 (0.31)	23.60 (0.15)	50.46 (0.35)	8.42 (0.07)
ST PETER	M	3.0	296.00 (9.54)	67.00 (1.53)	53.87 (2.21)	24.40 (0.86)	57.20 (2.41)	8.63 (0.47)
DUFFIELD	M	16.7	291.18 (3.64)	70.18 (2.08)	52.61 (1.04)	23.27 (0.81)	53.32 (0.94)	8.88 (0.36)
SPILSBY	M	8.4	310.22 (2.70)	73.78 (1.98)	55.18 (0.54)	26.77 (0.31)	57.26 (0.91)	9.99 (0.14)
REEVESBY	M	20.8	278.81 (2.94)	68.52 (1.16)	54.39 (0.52)	26.04 (0.22)	53.92 (0.60)	10.20 (0.16)
TUMBY BAY	M	9.8	272.20 (4.72)	70.00 (2.62)	50.26 (1.12)	23.88 (0.49)	52.58 (1.10)	8.60 (0.19)
WEERNA	M	13.6	275.15 (2.09)	64.00 (1.47)	50.68 (0.58)	25.34 (0.22)	52.98 (0.51)	8.82 (0.15)
PT PIRIE	M	11.0	291.09 (2.13)	69.64 (1.27)	55.12 (0.81)	26.95 (0.37)	57.26 (0.84)	9.19 (0.19)
PT PEARCE	M	13.7	281.79 (3.72)	70.77 (1.75)	52.19 (0.90)	23.97 (0.37)	53.19 (0.90)	8.05 (0.28)
WARDANG	M	27.8	272.32 (2.85)	69.75 (1.35)	50.06 (0.73)	23.80 (0.45)	50.54 (0.74)	8.61 (0.14)
TRBRDGE	M	11.8	268.50 (2.82)	61.08 (1.40)	52.23 (0.73)	25.27 (0.54)	53.83 (0.78)	8.58 (0.19)

TABLE 5.1 (Cont.)

LOCALITY	SEX	RH	EYE	IPW	IPL	SNLL	ENL
ALL	M	5.61 (0.06)	7.71 (0.07)	7.11 (0.08)	9.71 (0.10)	21.21 (0.12)	13.79 (0.09)
	F	5.17 (0.05)	7.39 (0.07)	6.84 (0.08)	9.25 (0.11)	19.45 (0.12)	12.60 (0.09)
ST PETER	M	6.10 (0.40)	8.20 (0.95)	7.73 (0.77)	10.57 (0.33)	21.05 (0.85)	13.96 (0.41)
DUFFIELD	M	5.47 (0.19)	7.08 (0.17)	6.39 (0.22)	8.67 (0.21)	20.59 (0.29)	13.22 (0.20)
SPILSBY	M	6.02 (0.11)	7.06 (0.21)	6.83 (0.21)	10.09 (0.26)	21.81 (0.27)	13.71 (0.32)
REEVESBY	M	6.16 (0.12)	7.59 (0.19)	6.50 (0.15)	9.20 (0.20)	22.98 (0.22)	14.79 (0.17)
TUMBY BAY	M	5.03 (0.15)	7.40 (0.29)	7.10 (0.27)	9.67 (0.34)	19.95 (0.35)	12.92 (0.29)
WEERNA	M	5.52 (0.15)	7.88 (0.22)	6.71 (0.41)	9.59 (0.59)	20.70 (0.27)	13.29 (0.16)
PT PIRIE	M	5.40 (0.16)	8.65 (0.27)	7.40 (0.28)	10.69 (0.41)	21.95 (0.26)	14.19 (0.21)
PT PEARCE	M	5.55 (0.32)	7.93 (0.22)	7.31 (0.27)	10.22 (0.27)	20.36 (0.25)	13.56 (0.26)
WARDANG	M	5.25 (0.11)	7.32 (0.15)	7.40) (0.15)	9.39 (0.23)	20.15 (0.28)	13.07 (0.18)
TRBRDGE	M	5.31 (0.20)	7.88 (0.31)	6.86) (0.21)	9.43 (0.33)	20.60 (0.23)	13.35 (0.17)

TABLE 5.2 The mean values for all meristic variables and percent melanis the discriminant function analyses for differences between sexes populations. Standard errors are given in parentheses. A key to the abbrieviations is given in Table 2.1. Only animals with SVL > 250mm wer the analysis.

LOCALITY	SEX		SLL		ILL		SCL		
ALL	M	160.6	10.27	(0.07)	10.19	(0.07)	3.61	(0.07)	10.
	F	155.3	10.10	(0.08)	10.06	(0.08)	3.64	(0.06)	10.
ST. PETER	M	3.0	10.00	(0.00)	10.33	(0.33)	3.67	(0.33)	12.
DUFFIELD	M	16.2	10.31	(0.21)	10.00	(0.17)	3.40	(0.25)	10.
SPILSBY	M	9.0	10.44	(0.18)	10.33	(0.24)	3.89	(0.35)	10.
REEVESBY	M	21.0	10.67	(0.26)	10.52	(0.21)	3.67	(0.29)	11.
TUMBY BAY	M	10.0	9.90	(0.23)	10.00	(0.21)	3.40	(0.22)	10.
WEEROONA	M	13.0	10.23	(0.20)	10.46	(0.24)	4.08	(0.18)	9.
PT. PIRIE	M	11.0	10.18	(0.18)	10.18	(0.18)	3.64	(0.15)	10.
PT PEARCE	M	14.0	10.21	(0.30)	10.43	(0.20)	3.71	(0.13)	10.
WARDANG	M	27.1	10.14	(0.16)	9.96	(0.17)	3.56	(0.11)	10.
TROUBDGE	M	11.7	10.42	(0.40)	9.75	(0.22)	3.18	(0.35)	9.

(TABLE 5.2 Cont.)

LOCALITY	SEX	LPL		PV		MSR		MELD		MELV	
ALL	M	10.37	(0.11)	22.64	(0.19)	25.50	(0.18)	57.11	(2.50)	56.77	(1.51)
	F	10.22	(0.14)	22.75	(0.22)	25.69	(0.19)	67.35	(2.52)	60.07	(1.28)
ST. PETER	M	10.00	(0.58)	23.00	(0.00)	29.67	(1.86)	43.33	(6.33)	45.00	(7.64)
DUFFIELD	M	10.40	(0.38)	25.06	(0.28)	27.41	(0.38)	93.06	(1.86)	55.59	(3.18)
SPILSBY	M	10.56	(0.34)	26.11	(0.35)	27.67	(0.44)	96.33	(0.97)	87.78	(2.65)
REEVESBY	M	10.90	(0.36)	25.52	(0.31)	26.86	(0.27)	81.14	(4.21)	69.76	(1.52)
TUMBY BAY	M	10.80	(0.39)	21.30	(0.73)	24.90	(0.53)	41.90	(10.56)	39.50	(3.53)
WEEROONA	M	9.15	(0.41)	20.77	(0.39)	26.69	(0.47)	50.38	(4.95)	64.23	(3.92)
PT. PIRIE	M	10.00	(0.33)	21.73	(0.45)	25.45	(0.45)	44.45	(10.25)	63.64	(7.23)
PT PEARCE	M	10.43	(0.31)	22.07	(0.46)	23.36	(0.57)	36.43	(7.59)	51.79	(4.68)
WARDANG	M	10.38	(0.21)	21.41	(0.27)	23.70	(0.37)	57.04	(4.91)	52.59	(3.09)
TROUBDGE	M	9.90	(0.74)	19.92	(0.26)	23.83	(0.51)	32.83	(8.02)	45.83	(3.36)

T.rugosus is sexually dimorphic. With the forced inclusion of SVL (which demonstrated non-significant differences between the sexes) in the initial step to correct for body size, eight characters (HL, IOW, HW, RH, EYE, IPW, IPL and SNLL) each could, taken alone, significantly discriminate between the sexes. All of these parameters are related to head shape. They also correlate closely with HL such that when HL was included in the equation, several of these characters become non-significant as they could not contribute further to discrimination. Stepwise discriminate function analysis (forward inclusion) included the variable TL but reduced the overall number of variables in the discriminant function equation to four (SVL, HL, TL and RH; see Table 5.3). These variables remained unaltered when locality was forced into the equation suggesting that HL, TL and RH will discriminate among the sexes irrespective of population. The error count estimates for sex gives a classification error rate of 13.75% (Table 5.4) which is perhaps too high for practical use in sex allocation but nevertheless demonstrates a degree of sexual dimorphism in *T.rugosus* resulting mainly from differences in head shape and tail length.

Stepwise discriminant function analysis of ten populations derived ten discriminatory characters (PV, SNLL, MSR, IPW, MELV, MELD, UPL, HW, and IOW: Table 5.5) after the forced inclusion of SVL. The ten populations can be discriminated with an error rate of 28.32% (Table 5.6) indicating that despite its statistical significance, the discrimination was poor. Of the ten populations, three islands (Spilsby, Duffield and Reevesby) exhibit error rates of less than 10% indicating that they are morphologically quite distinct from the remaining populations. The variables PV, MSR, and percent melanism (MELD and MELV) are clearly significant in separating the three Sir

TABLE 5.3 Summary of the stepwise discriminant function analysis among sexes without locality.

Step	Variable Entered	Variable Removed	Number In	Partial R^2	F Statistic	Prob < F
0			1	*	*	*
1	HL		2	0.4434	182.420	0.0001
2	TL		3	0.0618	15.021	0.0001
3	RH		4	0.0186	4.312	0.0001

TABLE 5.4 Error count estimates (percentage) for allocation to each of the sexes. Includes all populations and individuals over 250 mm SVL.

	MALES	FEMALES	TOTAL
RATE	19.61	7.86	13.73
N	153	140	293

TABLE 5.5 Summary of the stepwise discriminant function analysis among all populations. SVL was included in the initial equation. All animals used with SVL>250 mm.

Step	Variable	Number	Partial R^2	F Statistic	Prob > F
0		1	-	-	-
1	PV	2	0.6521	20.199	0.0001
2	SNLL	3	0.6059	16.400	0.0001
3	MSR	4	0.3795	6.456	0.0001
4	IPW	5	0.2781	4.024	0.0002
5	MELV	6	0.2736	3.891	0.0003
6	MELD	7	0.2593	3.578	0.0007
7	SCL	8	0.2635	3.618	0.0007
8	HW	9	0.2486	3.309	0.0016
9	IO	10	0.1911	2.337	0.0206

TABLE 5.6 The error count estimates (percentage) for allocation to each population by discriminant function analysis, using morphological characters. Estimates are for males only. Separate analyses are included for three groups: all populations combined, island populations only, mainland populations only and for adjacent island and mainland groups. See table 4.1 for a key to the populations abbreviations.

	POPULATION										
	StPtr	Dufld	Splby	Rvsby	TmyBy	Werna	PtPre	PtPce	Wrdng	Troub	TOTAL
SAMPLE SIZE	3	11	8	21	10	12	11	14	26	11	139
ALL POPULATIONS	33.33	6.67	0.00	4.76	4.00	16.67	27.27	50.00	50.00	54.55	28.32
ISLAND POPULATIONS	33.33	2.00	0.00	0.00	-	16.67	-	-	19.23	9.09	14.05
ISLAND[1] POPULATIONS	33.33	0.00	0.00	0.00	-	0.00	-	-	7.69	-	6.84
MAINLAND POPULATIONS	-	-	-	-	30.00	-	18.18	42.86	-	-	30.35
SIR JOSEPH BANKS GROUP + TUMBY BAY[2]	-	0.00 0.00	0.00 0.00	0.00 0.00	10.00 0.00	- -	- -	- -	- -	- -	2.50 0.00

[1] Island populations run without Troubridge Island. [2] PV included in the discriminant function.

Joseph Banks Group islands from all other populations as they are consistently higher in these populations than any other (Table 5.2). The inclusion of characters such as SNLL, IPW, UPL, HW and IOW in the discriminant function suggests that there is also some variation in head-shape among the populations.

The apparent differentiation is particularly clear when the island and mainland populations are analysed separately. Stepwise discriminant function analysis among the seven islands selected eight characters (PV, SNLL, MSR, UPL, IPW, MELV, MELD, and SCL; Table 5.7) for the discriminant function model. The error rate for the linear discriminant functions obtained from these variables was 14.05% (Table 5.6) which is approximately half that obtained for all populations together. The Sir Joseph Banks islands and Weeroona exhibit distinct differences with zero error rates and the exclusion of Troubridge from the analysis reduces the total error rate to 6.84% (Table 5.6). By comparison, separate analyses of the three mainland populations revealed poor discriminatory power (Error rate = 30.35, Table 5.6) with only one variable (IOW) differentiating significantly among these populations. This indicates that the island populations have undergone greater divergence among themselves than have those on the mainland.

Separate analyses of islands with their adjacent mainland populations reveals generally low error rates with Tumby Bay and the adjacent Sir Joseph Banks Group (SJBG) producing the lowest error rate (2.5%; Table 5.6). This error rate was reduced to zero when PV was forced into the discriminant function. PV was forced in because it was found to be highly discriminatory among all populations and because it was highly significant ($F=14.98$, Prob $F < 0.001$) in the initial

TABLE 5.7 Summary of the stepwise discriminant function analysis among the island populations. SVL was included in the initial equation. Only animals with SVL>250mm were used.

Step	Variable	Number In	Partial R^2	F Statistic	Prob > F
0	SVL	1	*	*	*
1	PV	2	0.7418	36.202	0.0001
2	SNLL	3	0.6909	27.713	0.0001
3	MSR	4	0.4178	8.753	0.0001
4	SCL	5	0.3539	6.573	0.0001
5	IPW	6	0.2557	4.053	0.0031
6	MELV	7	0.2427	3.718	0.0055
7	MELD	8	0.2273	3.353	0.0101
8	SOL	9	0.1967	2.742	0.0276

discriminant step. Thus, its omission from the final model is probably an anomaly resulting from high correlations with other less interpretable variables.

Average linkage cluster analysis of males from the seven island and three mainland populations of *T.rugosus* produced two distinct clusters (Fig. 5.1) at a distance level of 1.32. This separates the Sir Joseph Banks Group of islands (Duffield, Reevesby and Spilsby) from all other populations and supports the discriminant function analysis by indicating the presence of a distinct set of morphological characteristics within this group. The inclusion of all remaining populations in the second cluster suggests that a large amount of morphological overlap exists among these populations. This is supported by the poor geographic relationship of the populations within the three clusters designated to this group. For example, St. Peter and Pt. Pirie which are located at opposite ends of the study area form a separate cluster while Wardang and Weeroona cluster with each other rather than with their adjacent mainland populations. In addition, Duffield and Reevesby cluster separately from Spilsby despite the latter's close geographic proximity to Duffield.

5.3.2 Variation within populations

Levels of FA were consistently higher in the two smallest islands (Duffield and Troubridge) and Reevesby Island than the other populations. These differences were significant for three characters (SL, $F_{max} = 3.859$; SC, $F_{max} = 4.133$; LP, $F_{max} = 3.277$; $P < 0.05$ all comparisons; see Fig. 5.2) which were significantly correlated with each other ($\chi^2 = 17.956$, $P < 0.05$, d.f. = 8, Friedmans method for

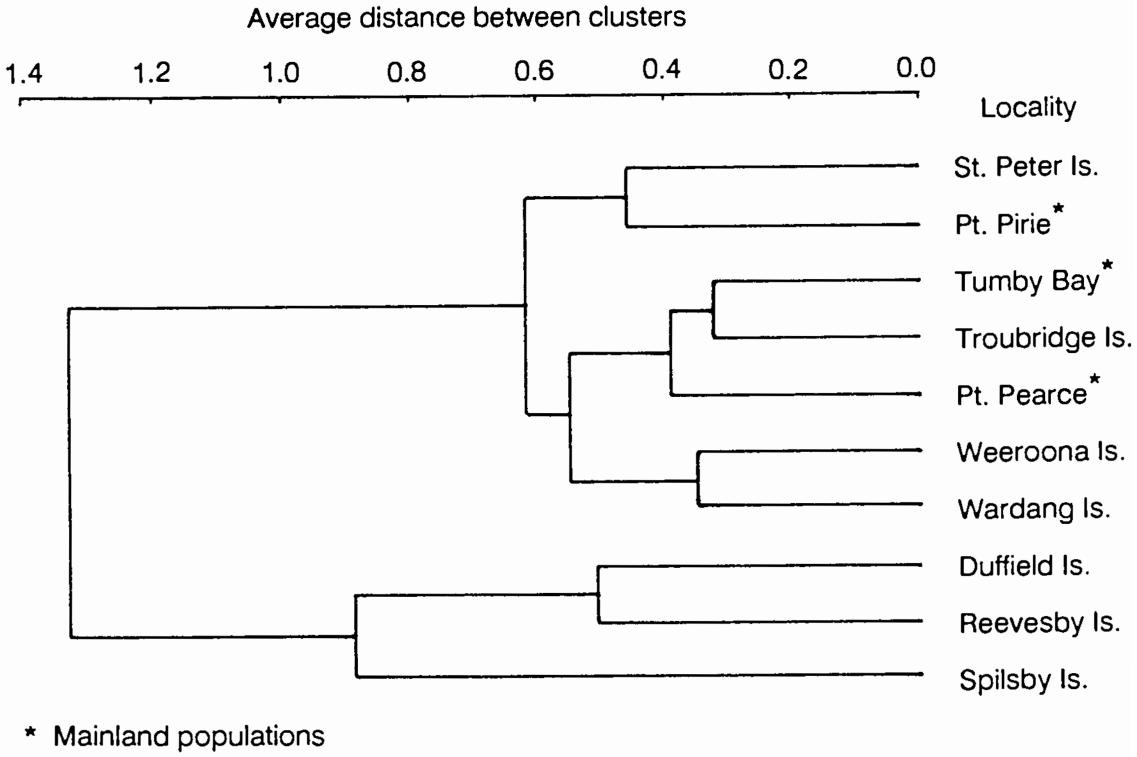

FIGURE 5.1 UPGMA cluster analysis of mean values of 20 morphological characters among island and mainland populations of *T.rugosus*.

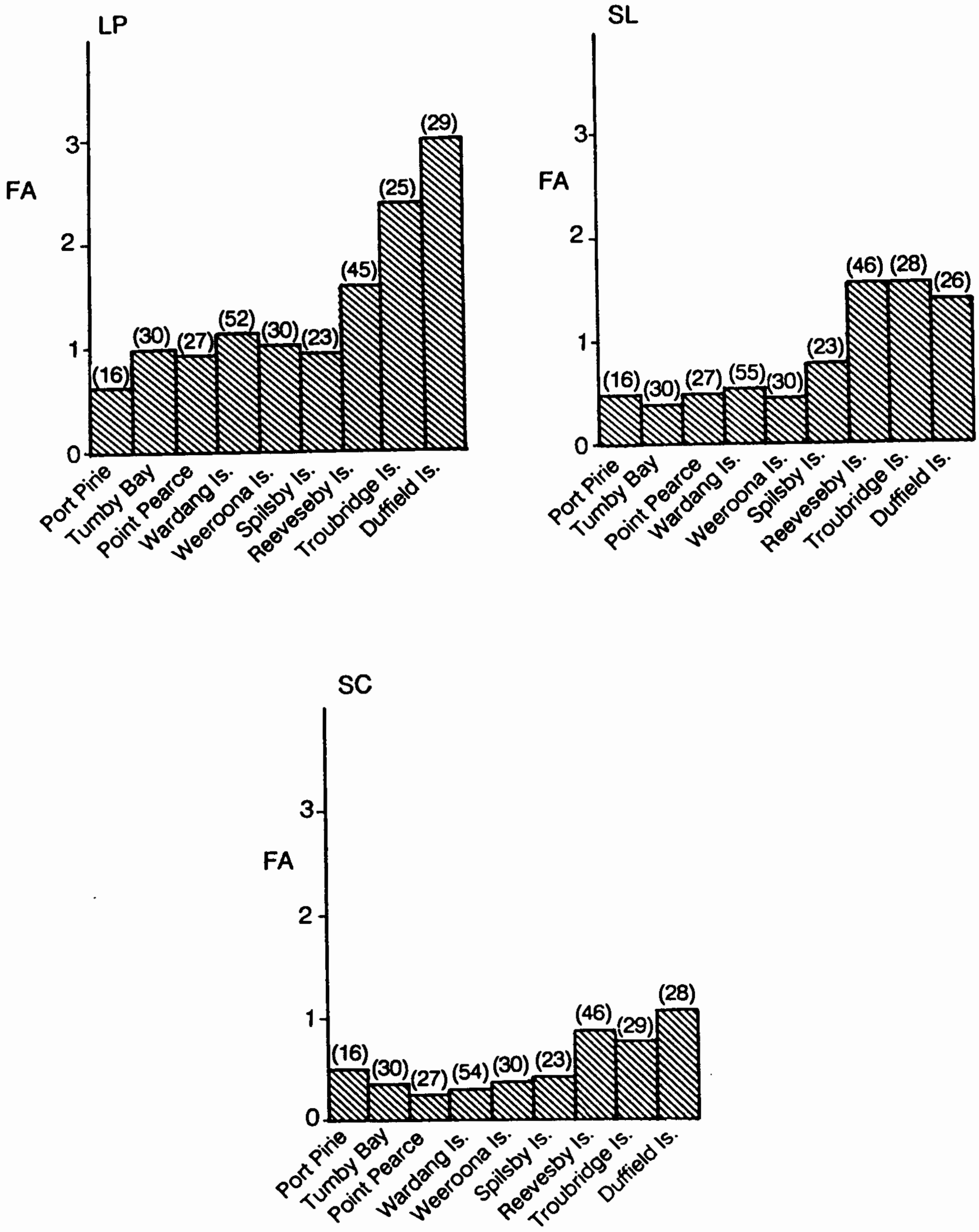

FIGURE 5.2 Levels of fluctuating asymmetry in nine populations of *T.rugosus* for the three meristic characters found to vary significantly among populations. See text for formula used to calculate these values. Sample sizes are in parentheses.

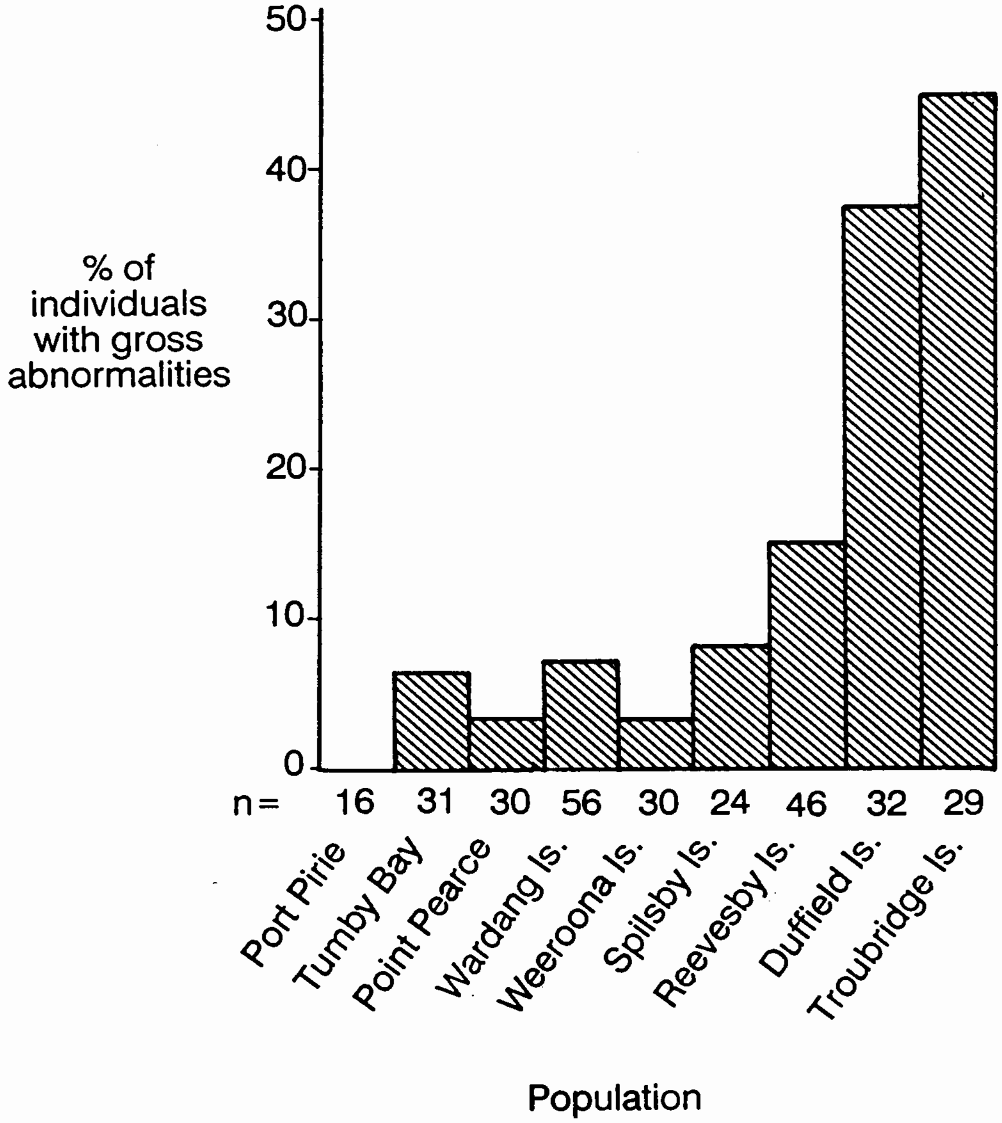

FIGURE 5.3 Frequency of gross abnormalities within nine populations of *T.rugosus*. See text for description of gross abnormalities. Male and female were pooled for these values because no significant differences were observed between them ($\chi^2 = 3.376$, $0.05 < p < 0.1$).

randomised blocks). The frequencies of abnormalities also varied significantly among populations ($\chi^2 = 52.086$, $P < 0.001$, d.f. = 9) with the two smallest islands (Duffield and Troubridge) containing the highest levels of abnormalities (Fig 5.3). These two populations, when separated from the remaining eight populations and re-analysed were not significantly different ($\chi^2 = 0.3382$, $P = 0.610$, 2-tailed Fisher's exact test). The remaining eight populations did not differ significantly from each other ($\chi^2 = 7.948$, $P = 0.337$, d.f. = 7) although Reevesby showed a tendency towards higher frequencies.

Both mean FA and frequencies of abnormalities suggest that levels of developmental stability are significantly lower in the two small insular populations (Duffield and Troubridge) and the larger island Reevesby than any of the other large islands or mainland populations.

5.4 DISCUSSION

The morphological analysis of *T.rugosus* populations identifies two important trends. First, the morphological divergence among populations suggests that divergence has occurred among the ten populations examined. This divergence is most marked among the insular populations. Second, developmental stability varies significantly among the populations suggesting that inbreeding depression or extreme environmental stress is present in some populations. These results generally do not support the conclusions derived from the genetic data described in Chapter 4 although there are some areas in which similar trends are evident. The trends in morphology are discussed below and compared with the electrophoretic data where appropriate.

The morphological divergence among populations, while not concordant with the electrophoretic assessment of the populations, does show similar trends in divergence. Divergence is greater among the island populations than among those on the mainland using both morphological and genetic measures. This concurs with trends usually observed among insular fauna and is generally characteristic of island biotas. However, relationships derived from the genetic and morphological data show very few similarities. For example, a distinct cluster of the three SJBG islands is obtained using morphological characters (Fig. 5.1) whereas both Nei and Rogers genetic distance measures cluster Duffield and Spilsby closer to all other populations than to Reevesby (Fig. 4.2). Moreover, within the distinct cluster formed by the three SJBG islands, Duffield is closer to Reevesby than it is to Spilsby. While there is some congruence between the mtDNA classification, electrophoretic classification and geographic proximity, only the SJBG islands exhibit such trends for morphology.

The lack of congruence between electrophoretic and morphological data was not totally unexpected. Several authors (e.g. Patton, 1984) have obtained conflicting results between the two measures. This variation probably results from differences in selection pressures exerted on individual proteins as opposed to those exerted on polygenic morphological characters (Chambers and Bayless, 1983).

The morphological similarity between the three SJBG islands suggests three alternative but not mutually exclusive explanations. First, it is conceivable that before isolation by rising sea levels, the SJBG populations were part of a single population which had already diverged significantly from other *T.rugosus* populations in other areas. The subsequent fragmentation of these populations, although resulting in divergence among them, has maintained several characteristics which group the Sir Joseph Banks Group as a distinct cluster. Second, there is a possibility that sufficient gene flow exists between these three islands to enable divergent genetic characteristics to spread between the populations. Presumably, gene flow is insufficient to prevent some divergence occurring. Third, selection pressures on these populations may be similar, resulting in the convergence of morphological characteristics. It is not possible to distinguish between hypotheses 'one' and 'three' but consideration of the electrophoretic data (Section 4.3) tends to rule out the second explanation because Reevesby is distinct from either Spilsby or Duffield indicating that little or no gene flow occurs between Reevesby and the other two islands.

If differentiation had occurred prior to isolation then it could be expected that the population closest to the SJBG (Tumby Bay) would be morphologically similar to them. This is not the case. However, the SJBG were separated from the mainland at 8,050 BP but not from each other until 7,700 BP (Mooney, unpub.) Consequently, they had over 300 years of isolation from the mainland but not from each other in which to develop distinct local morphological characteristics. Under this scenario, the subsequent isolation from each other has produced morphological change sufficient to discriminate between the three islands.

Convergent evolution also cannot be dismissed as a contributor to the pattern of differentiation particularly with respect to melanism. Melanistic tendencies have been observed in other insular reptile populations (Mertens, 1934, 1961 and Radovanovic, 1956 cited in Carlquist, 1974; Gibson and Falls, 1979; Schwaner, in press), although the exact reasons for this trend are unknown. Carlquist (1974) suggested that insular melanism may be related to sea salts in the diet or to the screening of radiation. Gibson and Falls (1979) found that melanistic forms of the Garter snake had a thermal advantage over non-melanistic forms and were able to maintain higher body temperatures in the colder part of the active season because of greater reflectance from non-melanistic surfaces.

Gibson and Falls (op. cit.) also found an improved ability in the melanistic form of the garter snake to maintain cooler temperatures in warmer times of the year. They concluded that this was possibly because of an improved ability to minimise the vascular transport of heat to the core in the melanistic form which allows more heat to be lost at the animals' periphery. Whatever the reason, it is quite possible that the melanism observed in the Sir Joseph Banks Group of islands is adaptive and that this adaptation relates in some way to their insularisation. The lack of melanism observed on the remaining four islands may be related to other aspects such as island size or degree and time of isolation which have negated the pressures towards melanism present on the smaller and more isolated SJBG islands.

The two measures of developmental stability (FA and percent gross abnormalities) show clear and significant differences among populations. Duffield and Troubridge exhibited significantly higher levels of percent gross abnormalities than all other populations while the same two populations and Reevesby showed consistently higher levels of FA for three characters.

The observed differences in developmental stability contrast markedly with the heterozygosity estimates which were not significantly different among all populations (see section 4.3). There are two alternative explanations for this observation. First, it is possible that some environmental effect peculiar to these islands is responsible for the observed developmental instability. This could take the form of insufficient good quality habitat available to allow females to thermoregulate efficiently while gravid, or some form of dietary deficiency. Morphological abnormalities resulting from variation in incubation temperature have been observed in reptiles (Fox *et al.*, 1961; Bustard, 1969; Vinegar, 1974; Osgood, 1978) with effects ranging from tail abnormalities to split or fused scales and reductions in numbers of ventral, subcaudal and labial scales. However, it is unlikely that major climatic effects (e.g. changes in latitude) are important in affecting incubation temperatures as Spilsby and Duffield are only approximately 500m apart and dense vegetation or penguin burrows (i.e. habitat suitable for good thermoregulation) are available on Troubridge, Reevesby and Duffield. Furthermore, the type of abnormalities are often similar within populations suggesting a genetic component in these traits. For example, Duffield had 7 out of 32 individuals with at least one missing ear opening. This particular abnormality was not observed in any other individual (n = 343) examined in this study. Troubridge animals also exhibited almost exclusively, abnormalities comprising deformed head and lip scales. It seems unlikely that an environmental effect would cause such specific defects so consistently within a population. Thus, although it is possible that the developmental instability observed could be environmentally induced, such effects are more likely to have a genetic base.

The second possible explanation is that the high levels of abnormalities are manifestations of inbreeding depression. This could result from the expression of deleterious alleles previously present in the population but masked by dominant alleles, the disruption of coadapted genotypes by inbreeding, or the effects of heterozygosity *per se* (see section 5.1 for a discussion of these phenomena). As discussed in Chapter 4, no statistically significant differences in levels of average heterozygosity among the populations were observed in the present study although levels tended to be reduced in the insular populations. Despite the error involved in such heterozygosity estimates (see section 4.1), it is clear that the large differences among populations in FA and % gross abnormalities are not reflected in heterozygosity levels. Thus, reduced levels of heterozygosity *per se* are unlikely to be the prime cause of the observed developmental instability. Therefore, the likeliest explanations for the levels of developmental instability observed in the three populations is inbreeding depression caused by either the exposure of deleterious recessive alleles or genetic disruption of coadapted genotypes through inbreeding. This may not show up in heterozygosity because Troubridge and Duffield are probably recent introductions so erosion of genetic variation probably has not had sufficient time to occur and be detected by electrophoresis. Reevesby is a larger island and is unlikely to have been a recent introduction. The population does not have the high frequency of abnormalities of the two smallest islands, but FA is detecting some inbreeding effects which are not yet serious enough to manifest themselves in gross abnormalities.

It is interesting to speculate on the loss of ear openings in the Duffield population. Carson and Templeton (1984) argue that speciation can occur when coadapted gene complexes are disrupted through founder events. Disruption of the old coadapted gene complexes could result from increased homozygosity in peripheral populations or genetic drift in outcrossed and polymorphic populations. The disorganisation of the genome, under relaxed ecological and selective conditions, may allow recombination to occur producing novel characters which are normally selected against. The loss of ear openings of a substantial proportion of the Duffield population could well be an example of such an occurrence. There are no known predators on Duffield (Goannas are present on at least four of the other islands: Spilsby, Reevesby, St. Peter and Wardang), so the selective pressure against impaired hearing is probably absent in this population.

While the consistency of the abnormality type (Duffield and Troubridge) suggests that the exposure of deleterious recessive alleles may be responsible for the observed gross abnormalities, it must be stressed that genetic disruption can also produce consistent characters. For example, Watson *et al.* (1971) describe hybridisation experiments between two species of frog (*Litoria spp.*) which produced consistent and specific abnormalities primarily relating to failure to form normal eyes. Thus, it is not possible to rule out either mechanism as the prime cause of the observed developmental instability.

The extent to which FA and percent gross abnormalities are genetically influenced remains problematical. However, it does seem likely that they are important, particularly on the smallest islands of Troubridge and Duffield. This hypothesis could be tested by laboratory based breeding experiments or the transplantation of lizards from other populations to sites where lizards exhibit significant developmental instability.

One further point to be made here is that whatever is responsible for the developmental instability observed in three of the insular populations, it is severe enough to cause gross abnormalities. Thus, it is not possible to separate the phenomenon of FA from this severe developmental disruption. However, FA did reveal significant tendencies in developmental instability within the Reevesby population which were not significant using gross abnormalities. This is important because it suggests that FA is more sensitive to developmental perturbations than the measure of gross abnormalities. Thus, FA is more likely to be able to detect changes in developmental stability before major genetic disruption or severe environmental stress causes fitness problems within a population.

CHAPTER 6 SUMMARY AND CONCLUSIONS

6.1 OVERVIEW OF RESULTS

The morphological and electrophoretic examination of island and mainland populations of *T.rugosus* illustrates several characteristics of insular populations which are important for wildlife management. They demonstrate that divergence, whether genetic (such as the changes observed in allele frequencies) or morphological, is significantly pronounced in the insular populations suggesting that evolutionary forces are more extreme in these environments.

However, the congruence between the two data sets does not extend to a classification of the relationships between the different populations. One clear example is the Sir Joseph Banks Group of islands which are grouped together morphologically but not electrophoretically. This emphasises the difference between evolutionary rates and direction at the morphological level compared with those at the level of structural genes. The classification produced by these approaches was also unable to correlate accurately with classifications based on geographic proximity, although of the two, genetic distance derived from allozymes exhibited greater congruence with geographic proximity. A third measure of variation using restriction fragment comparisons of mtDNA, was able to produce a classification generally congruent with geographical location, and it is possible that a more intensive survey using this technique could provide a much more informative estimate of phylogeny.

Heterozygosity levels within the island populations were not significantly lower than those of the mainland populations although reductions in both allelic diversity and percent polymorphism were observed within the island populations. Similar surveys done on *Rattus fuscipes* and *Notechis scutatus-ater* on comparable islands off the coast of South Australia showed large losses of allozyme variation often to the point of fixation at all loci.

Clearly, some characteristic(s) of *T.rugosus* have enabled the insular populations to maintain genetic variation. It is likely that although sleepy lizards maintain small home ranges and exhibit high mate fidelity (characteristics which will reduce effective population size) features such as long life span, stable population size and equal sex ratios, combined with large original levels of variation in the founding populations, have assisted the island populations to retain a large portion of their genetic variation. Moreover, a survey of electrophoretic variation in three mainland populations of *T.rugosus*, reveals that the most common alleles found on the mainland are those that have survived in the island populations. The most alleles found at any single locus in mainland populations was four. Generally, the polymorphic loci had two common alleles with the remaining alleles found only in very low frequencies. Such a distribution of alleles has favoured the retention of variation in the form of heterozygosity rather than allelic diversity. Thus, although heterozygosity has been maintained, some diversity and hence adaptability, has been lost from the insular populations.

The high levels of genetic variation observed in the insular populations using allozyme electrophoresis is supported in part by low levels of developmental instability (measured by fluctuating asymmetry and percent gross abnormalities) found in most of the island populations. The exceptions to this were the three smallest islands Troubridge, Duffield and Reevesby which exhibited high levels of both measures of developmental stability. This suggests that these three populations are either suffering from inbreeding depression which is not being detected by allozyme electrophoresis, or some form of environmental stress unique to those three islands.

The small size of the Troubridge population suggests that it could be undergoing some form of inbreeding. However, this has not been translated into changes in heterozygosity, possibly because insufficient time has elapsed since the population was introduced to the island, to allow detectable changes in heterozygosity to occur. A recent origin (or at least substantial gene flow between Duffield and Spilsby) is inferred from the genetic closeness of Duffield and Spilsby and suggests that Duffield Island has a similar history to that of the Troubridge population. Therefore, it appears likely that the significant levels of developmental instability observed in these populations results from inbreeding, causing the exposure of deleterious recessive alleles or the disruption of coadapted gene complexes.

The developmental instability observed in the Reevesby population is more subtle. It is not manifested significantly in high levels of gross abnormalities but exhibits substantial levels of average fluctuating asymmetry. Thus, it appears that this population has been large enough to maintain sufficient levels of variation to prevent change from being detected by allozyme electrophoresis, but is still suffering some developmental problems.

It is not possible to rule out environmental effects in any of these three populations, but the occurrence of decreased developmental stability in the three smallest islands under similar climatic conditions suggests a population effect. Moreover, it is likely that polygenic morphological characters such as scale counts, will be more likely to detect the effects of inbreeding than allozyme electrophoresis which is sampling only a few genes.

6.2 IMPLICATIONS FOR CONSERVATION

Four important conservation principles affecting intra-specific conservation strategies are illustrated in this study. The first is the importance of considering populations as the unit of 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* revealed in the present study, has implications for any 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.

Second, these findings emphasise the relative importance of the role of insular populations in phenotypic divergence. Both the electrophoretic and morphological data show that divergence is greatest among the insular populations. This has implications for populations which have become isolated owing to human activities and to those currently isolated on islands. The results emphasise the importance of island populations in the speciation process and that speciation is a population phenomenon. Strategies of species conservation must look towards conserving populations, because each population is potentially a new species.

Third, Wayne *et al.* (1986) and others have suggested that electrophoretic and morphological variation will be useful to zoo managers as tools for genetic monitoring of captive animals. Presumably, this could also apply to wild populations. In the present study, allozyme heterozygosity did not vary significantly among the populations but developmental stability did. Clearly, FA and percent abnormalities are monitoring something which is not being detected by allozyme electrophoresis. Although environmental influences cannot be ruled out, it is likely that inbreeding is implicated. Thus, it appears that both FA and percent gross abnormalities are more sensitive to variation in fitness compared with variation in allozymes.

Fourth, the potential role of demographic and genetic characteristics of a species in its ability to retain genetic variation within populations is emphasised. In the current study, *T.rugosus* was found to have maintained high levels of heterozygosity despite isolation for over six thousand years whereas two other vertebrates isolated on the same or similar islands showed significant reductions in levels of heterozygosity. Thus, it is important to realise that different organisms and different populations of organisms, will vary in their susceptibilities to the genetic effects of isolation. If effective management strategies involving genetic considerations are to be made, then these aspects need to be considered.

6.3 SUGGESTIONS FOR FURTHER RESEARCH

There are many theoretical and practical aspects of conservation genetics which require investigation. To list them all is beyond the scope of this section. However, the present study does suggest several important areas of enquiry.

Although long generation time prohibits the extensive use of *T.rugosus* for experimental manipulation, several potential extensions of the current study can be suggested. Developmental stability has been shown to vary among populations, indicating real differences. Using developmental stability as a genetic monitor has application in the management of small natural populations and requires further investigation. One way to test whether the high levels of FA and gross abnormalities on Duffield, Troubridge and Reevesby are environmentally or genetically induced would be to transplant mainland animals on to the islands using enclosures to keep them separate from the indigenous populations. The effect of the environment on developmental asymmetry could then be measured by comparing progeny of both populations on each island.

Elucidation of the relationships between the populations included in this study would possibly shed more light on these questions. In particular, an analysis of mtDNA in the current study revealed variation which could be potentially useful for delineating the relationships among the population with more precision. Thus, an intensive survey of mtDNA within the islands could provide important information on the likely isolation histories of the various islands. For example, this analysis could reveal evidence of population bottlenecks in recent history or gene flow between populations. They could also help in establishing the origins of populations on Troubridge and Duffield which could shed light on the likely role of genetics in affecting levels of abnormalities and fluctuating asymmetry.

In the more general area of conservation genetics, the presumed relationship between genetic drift and effective population size has been derived almost entirely from laboratory populations of *Drosophila*. There is an urgent need for both field and laboratory based experimental studies to explore this relationship in other organisms. Laboratory experiments will necessarily be restricted to species which are small and breed rapidly, but there is scope in field based studies of insular populations to examine organisms which are not so conducive to laboratory conditions. Habitat patches which have been isolated by human activities and for which there are known isolation histories, provide good opportunities for study in this area. Such studies need to quantify effective population size by detailed ecological analysis and compare changes in genetic variation for different effective population sizes. Comparative studies of two or more species may also shed light on the demographic features of species which are important in retaining genetic variation.

REFERENCES

Abbott, I. (1978) Factors determining the number of land bird species on islands around south-western Australia. *Oecologia*. **33**: 221-223.

Adest, G.A. (1977) Genetic relationships in the genus *Uma* (Iguanidae). *Copeia* (1): 47-52.

Allendorf, F.W. (1986) Genetic Drift and the loss of alleles versus heterozygosity. *Zoo Biol*. 5: 181-190.

Allendorf, F.W. and Leary, R.F. (1986) Heterozygosity and fitness in natural populations of animals. In M.E. Soulé (ed.): *Conservation Biology: The science of scarcity and diversity*. Sinauer Assoc. Inc., Sunderland, Massachusetts: pp 57-76.

Allison, A.C. (1964) Polymorphism and natural selection in human populations. *Cold Spr. Harbr. Symp. Quant. Biol*. 29: 139-149.

Aquadro, C.F. and Kilpatrick, C.W. (1981) Morphological and biochemical variation and differentiation in insular and mainland deer mice (*Peromyscus maniculatus*). In M.H. Smith and J. Joule (eds): Mammalian Population Genetics. Univ. Georgia Press, Athens: pp. 214-230.

Archie, J.W. (1985) Statistical analysis of heterozygosity data: Independent sample comparisons. *Evolution* 39: 623-637.

Arthur, W. (1984) *Mechanisms of morphological evolution: a combined genetic developmental and ecological approach.* John Wiley and Sons: Chichester.

Ashley, M. and Wills, C. (1987) Analysis of mitochondrial DNA polymorphisms among channel island deer mice. *Evolution* **41** (4) 854-863.

Ashley, M. and Wills, C. (1989) Mitochondrial DNA and allozyme divergence patterns are correlated among island deer mice. *Evolution* 43 (3) 646-650.

Attardi, G. (1985) Animal mitochondrial DNA: An extreme example of genetic economy. *Internatl. Rev. cytol.* 93: 93-145.

Avery, P.J. (1978) The effects of finite population size on models of linked overdominant loci. *Genet. Res. Camb.* 31: 239-254.

Avise, J.C. (1986) Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. Roy. Soc. Lond. B.* 312: 325-342.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. and Saunders, N.C. (1987) Intraspecific Phylogeography : The mitochondrial DNA bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.* 18: 489-522.

Avise, J.C., Lansman, R.A. and Shade, R.D. (1979) The use of restriction endonucleases to measure mitochondrial sequence relatedness in natural populations I. Population Structure and evolution in the genus *Peromyscus*. *Genetics* 92: 279-295.

Avise, J.C. and Lansman, R.A. (1983) Polymorphism of mitochondrial DNA in populations of higher animals. In M. Nei and R.K. Koehn (eds): *Evolution of genes and proteins*. Sinauer and Assoc., Sunderland, Massachusetts pp. 147-64.

Avise, J.C., Neigel, J.E. and Arnold, J. (1984) Demographic influences on mitochondrial DNA lineage survivorship. *J.Mol.Evol.* 20: 99-105.

Avise, J.C. and Vrijenhoek, R.C. (1987) Mode of inheritance and variation of mitochondrial DNA in hybridogenetic fishes of the Genus *Poeciliopsis*. *Mol. Biol. Evol.* 4 (5): 514-525.

Ayala, F.J. and Kiger, J.A. (1984) *Modern Genetics*. Benjamin/Cummings Publ. Co., Menlo Park, California.

Bamford, M.J. (1980) *Aspects of the population biology of the bobtail* *skink Tiliqua rugosa* (Gray). Unpub. Hons. Thesis. Murdoch Univ. W.A..

Beardmore, J.A. (1983) Extinction, survival and genetic variation. In C.M. Schoenwald-Cox, S.M.Chambers, B. MacBryde, L. Thomas (eds): *Genetics and conservation - A reference for managing wild animal and plant populations.* Benjamin/Cummings Publ. Co., Menlo Park, California: pp. 125-151.

Belperio, A.P., Hails, J.R. and Gostin, V.A. (1983) A review of holocene sea levels in South Australia. In D. Hopley (ed): *Australian sea levels in the last 15,000 years. : A review.* James Cook Univ. of Northern Queensland, Dept. of Geography: Monograph Series Occasional paper, No.3: pp. 37-47.

Belperio, A.P., Smith, B.W., Polach, H., Nittrover, C.A., DeMaster, D.J., Hails, J.R. and Gostin, V.A. (1984) Chronological studies of the quaternary marine sediments of northern Spencer Gulf, S.A. *Marine Geol.* 61: 265-296.

Bently, P.J. (1959) Studies on the water and electrolyte metabolism of the lizard *Trachydosaurus rugosus* (Gray). *J. Phys.* 145: 37-47.

Berry, R.J. (1986) Genetics of insular populations of mammals, with particular reference to differentiation and founder effects in British small mammals. *Biol. J. Linn. Soc.* 28: 205-230.

Biémont, C. (1983) Homeostasis, enzymatic heterozygosity and inbreeding depression in natural populations of *Drosophila melanogaster. Genetics* 61: 179-189.

Bishop, J.A. (1981) A neodarwinian approach to resistance : Examples from mammals. In J.A. Bishop and L.M. Cook (eds): *Genetic consequences of man-made change.* Academic Press, New York: pp. 27-51.

Blissett, A.H. and Warne, A.H. (1967) Reconnaissance for scheelite deposits in the Sir Joseph Banks Group of Islands. *Min. Rev.* 122: 84-98.

Boecklen, W.J. and Bell, G.W. (1986) Consequences of faunal collapse and genetic drift to the design of nature reserves. In D.A. Saunders, G.W. Arnold, A.A. Burbidge and A.J.M. Hopkins (eds): *Nature conservation: The role of remnants of native vegetation.* Surrey Beatty and Sons Pty. Ltd., Chipping Norton, N.S.W.: pp 141-149.

Borst, P. (1972) Mitochondrial Nucleic Acids. *Ann. Rev. Biochem.*41: 333-376.

Bradley, B.P. (1980) Developmental stability of *Drosophila melanogaster* under artificial and natural selection in constant and fluctuating environments. *Genetics* 95: 1033-1042.

Braysher, M. (1971) The structure and function of the nasal salt gland from the Australian sleepy lizard *Trachydosaurus rugosus*: family Scincidae. *Physiol. Zool.* 44: 129-136.

Brown, W.M. (1983) Evolution of animal mitochondrial DNA. In M. Nei and R.K. Koehn (eds): *Evolution of genes and proteins.* Sinauer and Assoc., Sunderland, Massachusetts: pp. 62-88.

Brown, W.M., George, M. and Wilson, A.C. (1979) Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. USA.* 76 (4): 1967-1971.

Brown, W.M., Prager, E.M., Wang, A. and Wilson, A.C. (1982) Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *J. Mol. Evol.* 18: 225-239.

Bruckner, D. (1976) The influence of genetic variability on wing symmetry in Honey bees (*Apis Mellifera*). *Evolution* 30: 100-108.

Bull, C.M. (1987) A population study of the viviparous Australian lizard *Trachydosaurus rugosus* (Scincidae). *Copeia* (3): 749-757.

Bull, M. (1988) Mate fidelity in an Australian lizard *Trachydosaurus rugosus*. *Behav. Ecol. Soc. Biol.* 23: 45-49.

Bull, C.M. and Satrawaha, R. (1981) Dispersal and social organisation in *Trachydosaurus rugosus*. In C.B. Banks and A.A. Martin (eds): *Proc. Melb. Herp. Symp.*. Zool. Board Vic., Blackburn, Vic.: p. 24.

Burgman, M.A., Akcakaya, H.R. and Loew, S.S. (1988) The use of extinction models for species conservation. *Biol. Cons.* 43: 9-25.

Burne, R.V. (1982) Relative fall of holocene sea level and coastal progrodation, north-eastern Spencer Gulf, South Australia. B.M.R. *J. Aust. Geol. Geophys.* 7: 35-45.

Bush, G.L. and Howard, D.J. (1986) Allopatric and non-allopatric speciation; assumptions and evidence. In S. Karlin and E. Nevo (eds): *Evolutionary processes and theory*. Academic Press inc., New York: pp. 411-438.

Bustard, H.R. (1969) Temperature and water tolerances of incubating crocodile eggs. *Br. J. Herpet.* 1971 (4): 198-200.

Bustard, R. (1970) *Australian lizards.* Collins, Sydney and London.

Camp, H.W. (1973) Modern methods and devices available to decision-makers in planning land use. In A.B. Costin and R.H. Groves (eds): *Nature conservation in the Pacific.* ANU Press, Canberra.

Carlquist, S. (1965) *Island life.* Natural History Press, New York.

Carlquist, S. (1974) *Island biology.* Columbia Univ., New York.

Carson, H.L. (1983) The genetics of the founder effect. In C.M. Schoenwald-Cox, S.M. Chambers, B. MacBryde, and L. Thomas (eds): *Genetics and conservation: A reference for managing wild animal and plant populations.* Benjamin/Cummings Publ. Co., Menlo Park, California: pp. 189-200.

Carson, H.L. and Templeton, A.R. (1984) Genetic revolutions in relation to speciation phenomena: the founding of new populations. *Ann. Rev. Ecol. Syst.* 15: 97-131.

Case, T.J. (1978) A general explanation for insular body size trends in terrestrial vertebrates. *Ecology* 59: 1-18.

Case, T.J. (1982) Ecology and evolution of the insular gigantic chuckawallas, *Sauromalus hispidus* and *Sauromalus varius.* In G.M. Burghart and A.S. Rand (eds): *Iguanas of the world.* Noyes Publ., Park Ridge, New Jersey: pp. 182-212.

Cathcart, C.A. (1985) *Mitochondrial DNA analysis in related species of Petrogale (Rock-Wallabies).* Unpub. Masters Thesis, Macquarie Univ..

Caughly, G. (1980) *Analysis of vertebrate populations.* Wiley and Sons, Chichester.

Chacraborty, R. (1981) The distribution of the number of loci in an individual in natural populations. *Genetics* 98: 461-466.

Chakraborty, R. and Ryman, N. (1983) Relationship of mean and variance of genotypic values with heterozygosity per individual in a natural population. *Genetics* 103: 149-152.

Chambers, S.M. and Bayless, J.W. (1983) Systematics, conservation and the measurement of genetic diversity. In C.M. Schoenwald-Cox, S.M.Chambers, B. MacBryde, L. Thomas (eds): *Genetics and conservation - A reference for managing wild animal and plant populations.* Benjamin/Cummings Publ. Co., Menlo Park, California: pp. 349-363.

Chappell, J. (1976) Aspects of late Quaternary palaegeography of the Australian-East Indonesian Region. In R.L. Kirk and A.G. Thorne (eds): *The origin of the Australians.* Aust. Inst. Ab. Stud., Canberra: pp. 11-22.

Chappell, J. and Thom, B.G. (1977) Sea levels and coasts. In J. Allen and R. Jones (eds): *Sunda and Sahul: Prehistoric studies in South East Asia, Melanesia and Australia.* Academic Press, London: pp. 275-91.

References

Clarke, B. (1979) The evolution of genetic diversity. *Proc. Roy. Soc. Lond. B.* 205 (4): 453-474.

Clarke, B. and Murray, J. (1969) Ecological genetics and speciation in land snails of the genus *Partula*. *Biol. J. Linn. Soc.* 1: 31-42.#

Clarke, B. and Murray, J. (1971) Polymorphism in a polynesian land snail *Partula suturalis vexillum*. In R. Creed (ed): *Ecological genetics and evolution.* Blackwell Scientific Publ., Oxford and Edinburgh: pp. 51-64.

Clarke, C.A. and Sheppard, P.M. (1971) Further studies on the mimetic butterfly *Papillo memnon* L. *Phil. Trans. R. Soc. Lond. B.* 263: 35-70.

Cogger, H.G. (1986) *Reptiles and amphibians of Australia.* Reed, Forest, N.S.W.

Cogger, H.G., Cameron, E.E. and Cogger, H.M. (1983) *Zoological catalogue of Australia 1. Amphibia and reptilia.* Aust. Govt. Publ. Serv., Canberra.

Coyne, J.A., Bundgaard, J. and Prout, T. (1983) Geographic variation of tolerance to environmental stress in *Drosophila Pseudoobscura*. *Amer. Nat.* 122 (4): 474-488.

Danzmann, R.G., Ferguson, M.M. and Allendorf, F.W. (1988) Heterozygosity and components of fitness in a strain of Rainbow trout. *Biol. J. Linn. Soc.* 33: 285-304.

Danzmann, R.G., Ferguson, M.M., Allendorf, F.W. and Knudsen, K.L. (1986) Heterozygosity and developmental rate in a strain of rainbow trout (*Salmo gairdner*). *Evolution* 40 (1): 86-93.

Diamond, J.M. (1975) The island dilemma: Lessons of modern biogeographic studies for the design of nature reserves. *Biol. Cons.* 7: 129-146.

Diamond, J.M. and May, R.M. (1981) Island biogeography and the design of nature reserves. In R.M. May (ed): *Theoretical ecology: principles and applications*. Blackwell Sci. Pub., Oxford: pp. 228-252.

Dobzansky, T. (1951) *Genetics and the origin of species*. Columbia Univ. Press, New York.

Dobzansky, T. and Levene, H. (1955) Genetics of natural populations. XXIV. Developmental homeostasis in natural populations of *Drosophila pseudoobscura*. *Genetics* 40: 797-808.

Dubas, G. (1987) *Biotic determinants of home range size of the scincid lizard Trachydosaurus rugosus (Gray)*. Unpub. Ph.D. thesis, Flinders Univ., S.A..

Eanes, W.F. (1978) Morphological variance and enzyme heterozygosity in the monarch butterfly. *Nature* 276: 263-264.

Ehrlich, P. and Ehrlich, A. (1982) *Extinction: The causes and consequences of disappearance of species*. St. Edmundsburry Press, Suffolk.

Ehrlich, P.R. (1980) The strategy of conservation, 1980-2000. In M.E. Soulé and B.A. Wilcox (eds): *Conservation biology - An evolutionary - ecological perspective*. Sinauer Assoc., Sunderland, Massachusetts: pp. 329-344.

Falconer, D.S. (1981) *Introduction to quantitative genetics*. Longman, London.

Felsenstein, J. (1982) Numerical methods for inferring evolutionary trees. *Q. Rev. Biol.* 57 (4) 379-404.

Felsenstein, J. (1983) Parsimony in systematics: biological and statistical issues. *Ann. Rev. Ecol. Syst.* 14: 313-33.

Ferguson, B. and Algar, D. (1986) Home range and activity patterns of pregnant female skinks, *Tiliqua rugosa*. *Aust. Wild. Res.* 13: 287-94.

References

Fleischer, R.C., Johnston, R.F. and Klitz, W.J. (1983) Allozyme heterozygosity and morphological variation in house sparrows. *Nature* **304**: 628-629.

Fox, W., Gordon, C. and Fox, M.H. (1961) Morphological effects of low temperatures during the embryonic development of the garter snake, *Thamnophis elegans*. *Zoologica* **46**: 57-71.

Frankel, O.H. (1970a) Genetic conservation in perspective. In O.H. Frankel and E. Bennett (eds): *Genetic resources in plants - their exploration and conservation. I.B.P. Handbook No.11.* Blackwell Sci. Pub., Oxford: pp. 1-4.

Frankel, O.H. (1970b) Variation - the essence of life. *Proc. Linn. Soc. N.S.W.* 95: 158-69.

Frankel, O.H. (1974) Genetic conservation: Our evolutionary responsibility. *Genetics* 78: 53-65.

Frankel, O.H. (1977) Natural variation and its conservation. In A. Muhammed, R. Aksel, and R.C. Von Borstel (eds): *Genetic diversity in plants.* Plenum press, New York: pp. 21-44.

Frankel, O.H. (1982) The role of conservation genetics in the conservation of rare species. In R.M. Groves and W.D.L. Ride (eds): *Species at risk*. Aust. Acad. Sci., Canberra: pp. 159-162.

Frankel, O.H. and Soulé, M.E. (1981) *Conservation and evolution.* Cambridge Univ. Press, Cambridge.

Franklin, I.R. (1980) Evolutionary change in small populations. In M.E. Soulé and B.A. Wilcox (eds): *Conservation Biology : An evolutionary - ecological perspective.* Sinauer Assoc., Sunderland, Massachusetts: pp. 135-150.

Gibson, A.R. and Falls, J.B. (1979) Thermal biology of the common garter snake *Thamophis sirtalis* (L.) II. The effects of melanism. *Oecologia* **43**: 99-109.

Gilbert, F.S. (1980) The equilibrium theory of island biogeography : fact or fiction. *J. Biogeogr.* 7: 209-235.

Gilbert, L.E. (1980) Food web organisation and conservation of neotropical diversity. In M.E. Soulé and B.A. Wilcox (eds): *Conservation biology: An evolutionary - ecological perspective.* Sinauer Assoc., Sunderland, Massachusetts: pp. 11-34.

Giles, R.E., Blanc, H., Cann, H.M. and Wallace, D.C. (1980) Maternal inheritance of human mitochondrial DNA. *Proc. Natl. Acad. Sci. USA.* 77 (11): 6715-6719.

Gorman, G.C. and Renzi, J. (1979) Genetic distance and heterozygosity in electrophoretic studies: effects of sample size. *Copeia* (2): 242-249.

Gorman, G.C., Soulé, M.E., Yang, S.Y. and Nevo, E. (1975) Evolutionary genetics of insular adriatic lizards. *Evolution* 29 (1): 52-71.

Gotoh, O., Hayashi, J., Yonekawa, H. and Tagashira, Y. (1979) An improved method for estimating sequence divergence between related DNA's from changes in restriction endonuclease cleavage sites. *J. Mol. Evol.* 14: 301-10.

Gyllensten, U., Wharton, D. and Wilson, A.C. (1985) Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. *J. Heredity* 76: 321-24.

Hails, J.R., Belperio, A.P. and Gostin, V.A. (1983) Holocene sea levels of upper Spencer Gulf, South Australia. In D. Hopley (ed): *Australian sea levels in the last 15,000 years: A review - Monograph series occasional paper No. 3.* James Cook Univ. of Northern Queensland, Dept. of Geography: pp. 48-53.

Hamrick, J.L. (1983) The distribution of genetic variation within and among natural plant populations. In C.M. Schoenwald-Cox, L. Thomas, S.M. Chambers, and B. MacBryde (eds): *Genetics and conservation. A reference for managing wild animal and plant populations.* Benjamin/Cummings Publ. Co., Menlo Park, California: pp. 335-348.

Handford, P. (1978) Heterozygosity at enzyme loci and morphological variation. *Nature* 286: 261-62.

Harris, H. and Hopkinson, D.A. (1976) *Handbook of enzyme electrophoresis in Human genetics.* North - Holland Pub. Co., Amsterdam.

Hedrick, P.W., Ginevan, M.E. and Ewing, E.P. (1976) Genetic polymorphism in heterogeneous environments. *Ann. Rev. Ecol. Syst.* 7: 1-32.

Hennig, W. (1966) *Phylogenetic systematics.* Univ. Illinois Press, Urbana.

Hudson, P., Mirtschin, P. and Garrett, C. (1981) Notes on Flinders Island (South Australia). Its reptiles and birds. *Sth. Aust. Nat.* 56 (2): 21-31.

Hutchinson, C.A. III., Newbold, J.E., Potter, S.S. and Edgell, M.H. (1974) Maternal inheritance of mammalian mtDNA. *Nature* 251: 536-538.

Hutchinson, M.N. (1981) The systematic relationships of the genera *Egernia* and *Tiliqua* (Lacertilia: Scincidae). A review and immunological reassessment. In C.B. Banks and A.A. Martin (eds): *Proc. Melb. Herp. Symp.,* Zool. Board of Vic., Blackburn, Vic.: pp. 176-193.

IUCN (1980) *World conservation strategy.* Internatl. Union for the Conservation of Nature and natural resources. U.N. Envt. prog., World Wildlife Fund, Gland, Switzerland.

Jackson, J.F. (1973) A search for the population asymmetry parameter. *Syst. Zool.* 22: 166-170.

Janzen, D. (1983) No park is an island: Increase in interference from outside as park size decreases. *Oikos* 41: 402-410.

References

Jarvinen, O. (1982) Conservation of endangered plant populations: single large or several small reserves. *Oikos* 38: 301-304.

Jogoe, C.H. and Haines, T.A. (1985) Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. *Can. J. Zool.* 63: 130-138.

Kaplan, N. and Risko, K. (1981) An improved method for estimating sequence divergence of DNA using restriction endonuclease mappings. *J. Mol. Evol.* 13: 295-304.

Kessler, L.G. and Avise, J.C. (1984) Systematic relationships among waterfowl (Anatidae) inferred from restriction endonuclease analysis of mitochondrial DNA. *Syst. Zool.* 33 (4): 370-380.

Kessler, L.G. and Avise, J.C. (1985) A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. *Mol. Biol. Evol.* 2 (2): 109-25.

Kim, Y.J., Gorman, G.C. and Huey, R.B. (1978) Genetic variation and differentiation in two species of the fossorial African skink *Typhlosaurus* (Sauria: Scincidae). *Herpetologica* 34: 192-194.

Kimura, M. (1968) Evolutionary rate at the molecular level. *Nature* 217: 624-26.

Kimura, M. (1982) The neutral theory as a basis for understanding the mechanism of evolution and variation at the molecular level. In M. Kimura (ed): *Molecular evolution protein polymorphism and the neutral theory*. Japan Sci. Soc. Press, Tokyo: pp. 3-58.

Kimura, M. and Crow, J.F. (1964) The number of alleles that can be maintained in a finite population. *Genetics* 49: 725-38.

King, M. (1973) Karyotypic studies of some Australian scincidae (Reptilia). *Aust. J. Zool.* 21: 21-32.

Kluge, A.G. and Farris, J.S. (1969) Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18: 1-32.

Koehn, R.K. and Shumway, S.R. (1982) A genetic/physiological explanation for differential growth rate among individuals of the American Oyster *Crassostrea virginica* (Gmelin). *Marine Biol. Letters* 3: 35-42.

Kramer, G. (1951) Body proportions of mainland and island lizards. *Evolution* 5: 193-206.

Lack, D. (1969) Subspecies and sympatry in Darwin's finches. *Evolution* 23: 252-63.

Lack, D. (1976) *Island biology illustrated by the land birds of Jamaica*. Blackwell Sci. Pub., Oxford.

Lande, R. (1988) Genetics and demography in biological conservation. *Science* 241: 1455-1460.

Lande, R. and Barrowclough, G.F. (1987) Effective population size, genetic variation, and their use in population management. In M.E. Soulé (ed): *Viable populations for conservation.* Cambridge Univ. Press, Cambridge: pp. 87-123.

Lansman, R.A., Avise, J.C., Aquadro, C.F., Shapira, J.F. and Daniel, S.W. (1983) Extensive genetic variation in mitochondrial DNA among geographic populations of the deer mouse *Peromyscus maniculatus*. *Evolution* 37 (1): 1-16.

Leary, R.F., Allendorf, F.W. and Knudsen, K.L. (1985) Inheritance of meristic variation and the evolution of developmental stability in rainbow trout. *Evolution* 39 (2): 308-314.

Leary, R.F., Allendorf, F.W., Knudsen, K.L. and Thorgaard, G.H. (1985) Heterozygosity and developmental stability in gynogenetic diploid and triploid rainbow trout. *Heredity* 54: 219-225.

Leary, R.F., Allendorf, F.W. and Knudson, K.L. (1983) Developmental stability and enzyme heterozygosity in Rainbow trout. *Nature* 30: 71-72.

Ledig, F.T., Guries, R.P. and Bonefield, B.A. (1983) The relation of growth to heterozygosity in pitch pine. *Evolution* **37**: 1227-1238.

Lerner, I.M. (1954) *Genetic homeostasis.* Oliver and Boyd, Edinburgh and London.

Levin, D.A. (1970) Developmental instability and evolution in peripheral isolates. *Amer. Nat.* **104** (938): 343-353.

Lewontin, R.C. (1974) *The genetic basis of evolutionary change.* Columbia Univ. Press, New York.

Lewontin, R.C. (1984) Detecting population differences in quantitative characters as opposed to gene frequencies. *Amer. Nat.* **123** (1): 115-24.

MacArthur, R.H. and Wilson, E.O. (1963) An equilibrium theory of insular zoogeography. *Evolution* **17**: 373-87.

MacArthur, R.H. and Wilson, E.O. (1967) The theory of island biogeography. Princeton Univ. Press, Princeton.

Margules, C.R., Higgs, A.J. and Rafe, R.W. (1982) Modern biogeographic theory: Are there any lessons for nature reserve design? *Biol. Cons.* 24: 115-128.

Mather, K. (1953) The genetical structure of populations. *Symp. Soc. Exp. Biol.* **7**: 61-95.

Mather, K. (1973) *Genetical structure of populations.* Chapman and Hall, London.

Mayr, E. (1963) *Animals, species and evolution.* Belknap Press, Harvard Univ. Press, Cambridge, Massachusetts.

Mayr, E. (1970) *Populations, species and evolution.* Belknap Press, Harvard Univ. Press, Cambridge, Massachusetts.

McAndrew, B.J., Ward, R.D. and Beardmore, J.A. (1982) Lack of relationship between morphological variance and enzyme heterozygosity in the Plaice *Pleuronectes platessa*. *Heredity* 48: 117-125.

McKusick, V.A. (1976) *Mendelian inheritance in man*. Johns Hopkins Univ. Press, Baltimore.

Mitchell, F.J. (1950) The scincid genera *Egernia* and *Tiliqua* (Lacertilia). *Rec. S.A. Museum* 9: 275-308.

Mitton, J.B. (1978) Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations. *Nature* 243: 661-662.

Mitton, J.B. and Grant, M.C. (1984) Associations among protein heterozygosity, growth rate and developmental homeostasis. *Ann. Rev. Ecol. Syst.* 15: 479-499.

Mitton, J.B. and Pierce, B.A. (1980) The distribution of individual heterozygosity in natural populations. *Genetics* 95: 1043-1054.

Mooney, P.A. (unpub.). In A.C. Robinson, P.D. Canty, P.A. Mooney, and P.M. Rudduck (eds): South Australia's offshore islands. Special Publication, NPWS, Dept. for Envt. and Planning, S.A..

Moritz, C., Dowling, T.E. and Brown, W.M. (1987) Evolution of animal mitochondrial DNA : Relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.* 18: 269-92.

Myers, N. (1979) *The sinking ark*. Pergamon Press, New York and Oxford.

Myers, N. (1984) Genetic resources in jeopardy. *AMBIO* 13: 171-174.

Myers, N. (1986) Tropical deforestation and a mega-extinction spasm. In M.E. Soulé (ed): *Conservation Biology - The science of scarcity and diversity*. Sinauer Assoc. Inc., Sunderland Massachusetts: pp 394-409.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Nei, M. and Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76 (10): 5269-5273.

Nei, M., Maruyama, T. and Chakraborty, R. (1975) The bottleneck effect and genetic variability in populations. *Evolution* 29 (1): 1-10.

Nei, M. and Roychoudury, A.K. (1974) Sampling variances of heterozygosity and genetic distance. *Genetics* 76: 379-399.

Nevo, E. (1978) Genetic variation in natural populations : patterns and theory. *Theor. Pop. Biol.* 13: 121-77.

Nevo, E. (1981) Genetic variation and climatic selection in the lizard *Agama stellio* in Israel and Sinai. *Theor. Appl. Genet.* 60: 360-380.

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

Newmark, W.D. (1985) Legal and biotic boundaries of western North American national parks: A problem of congruence. *Biol. Cons.* 33: 197-208.

Oakshott, J.G. (1979) Selection affecting enzyme polymorphisms in laboratory populations of *Drosophila melanogaster. Oecologia* 43: 341-54.

Orlóci, L. (1978) *Multivariate analysis in vegetation research.* W. Junk, The Hague.

Osgood, D.W. (1978) Effects of temperature on the development of meristic characters in *Natrix fasciata. Copeia* (1): 33-47.

Ovendon, J.R., Mackinlay, A.G. and Crozier, R.H. (1987) Systematics and mitochondrial genome. Evolution of Australian Rosellas (Aves: Platycercidae). *Mol. Biol. Evol.* **4** (5): 526-543.

Owers, R.T. (1986) A visitor management plan for Troubridge Island Conservation Park. Unpub..

Palmer, A.R. and Strobeck, C. (1986) Fluctuating asymmetry measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.* **17**: 391-421.

Passmore, J. (1974) *Man's responsibility for nature.* Duckworth, London.

Patton, J.L. (1984) Genetical processes in the Galapagos. *Biol. J. Linn. Soc.* 21: 97-111.

Patton, J.L., Yang, S.Y. and Myers, P. (1975) Genetic and morphologic divergence among introduced rat populations (*Rattus rattus*) of the Galapagos Archipelago, Ecuador. *Syst. Zool.* 24: 296-310.

Powell, J.R. (1975) Protein variation in natural populations of animals. *Evol. Biol.* 8: 79-119.

Powell, J.R. (1983) Molecular approaches to studying founder effects. In C.M. Schoenwald-Cox, S.M. Chambers, B. MacBryde, and L. Thomas (eds): *Genetics and conservation: A reference for managing wild animal and plant populations.* Benjamin/Cummings Publ. Co., Menlo Park, California: pp. 229-240.

Rabinowitz, M. and Swift, H. (1970) Mitochondrial nucleic acids and their relation to the biogenesis of mitochondria. *Physiol. Rev.* 50: 376-427.

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

Robinson, A.C., Canty, P.D., Mooney, P.A. and Rudduck, P.M. (Unpub.)

South Australian offshore islands. Special publ., NPWS Dept. Envt. & Planning.

Robinson, A.C., Mirtschin, P.J., Copley, P.D., Canty, P.D. and Jenkins, R.B. (1985) The Reevesby Island Goanna - A problem in conservation management. *Sth. Aust. Nat.* 59 (4): 56-62.

Rogers, J.S. (1972) Measures of genetic similarity and genetic distance. *Studies in Genetics, Univ. Texas Publ.* 7213: 145-153.

Samollow, P.B. and Soulé, M.E. (1983) A case of stress-related heterozygote superiority in nature. *Evolution* 37: 646-649.

SAS Institute, (1987) *SAS/Stat guide for personal computers version 6.* Cary N.C. U.S.A..

Satrawaha, R. (1980) *Ecology and activity patterns of the lizard Trachydosaurus rugosus.* Unpub. Masters Thesis, Flinders Univ..

Satrawaha, R. and Bull, C.M. (1981) The area occupied by an omnivorous lizard, *Trachydosaurus rugosus. Aust. Wildl. Res.* 8: 435-42.

Schmitt, L.H. (1978) Genetic variation in isolated populations of the Australian Bush-rat, *Rattus fuscipes. Evolution* 32: 1-14.

Schmitt, L.H. and White, R.J. (1979) A comparison of metric and protein variation in the Australian bush rat, *Rattus fuscipes greyii. Aust. J. Zool.* 27: 547-559.

Schoener, A. and Schoener, T.W. (1984) Experiments on dispersal: Short-term flotation of insular anoles, with a review of similar abilities in other terrestrial animals. *Oecologia* 63: 289-294.

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

Schwaner, T.D. (1985a) The eradication of a native animal from an island reserve - a critical assessment of the Reevesby Island goanna case history. *Sth. Aust. Nat.* 59 (4): 52-56.

Schwaner, T.D. (1985b) Population structure of black tiger snakes *Notechis ater niger*, on offshore islands of South Australia. In G. Grigg, R. Shine, and H. Ehmann (eds): *Biology of Australasian frogs and reptiles.* Surrey Beatty & Sons Pty. Ltd., Chipping Norton: pp. 35-46.

Schwaner, T.D. (1988) An inventory of reptiles on Wardang Island, South Australia, with recommendations for their conservation and management. Unpub. Report to ANPWS.

Schwaner, T.D. (in press) A field study of thermoregulation in black tiger snakes (*Notechis ater niger* : Elapidae) on the Franklin Islands, South Australia. *Herpetologica.*

Schwaner, T.D. (submitted) Geographic variation in scale and skeletal anomalies in insular populations of tiger snakes (*Notechis scutatus-ater* complex: Elapidae).

Schwaner, T.D. and Adams, M. (in prep.) Genetic variation in mainland and island populations of tiger snakes (*Notechis scutatus-ater* complex: Elapidae) in southern Australia.

Schwaner, T.D. and Sarre, S.D. (1988) Body size of tiger snakes in southern Australia, with particular reference to *Notechis-ater serventyi* (Elapidae) on Chappell Island. *J. Herpet.* 22 (1): 24-33.

Sederoff, R.R. (1984) Structural variation in mitochondrial DNA. *Adv. Genet.* 22: 1-108.

Selander, R.K. and Johnson, W.E. (1973) Genetic variation among vertebrate species. *Ann. Rev. Ecol. Syst.* 4: 73-91.

Senner, J.W. (1980) Inbreeding depression and the survival of zoo populations. In M.E. Soulé and B.A. Wilcox (eds): *Conservation Biology : An evolutionary - ecological perspective.* Sinauer Assoc. Inc., Sunderland, Massachusetts: pp. 209-224.

Sharrad, R.D. (1979) *Studies of factors which determine the distributions of three species of S.A. reptile tick.* : Unpub. Ph.D. thesis, Univ. of Adelaide, S.A..

Simberloff, D. (1986) Are we on the verge of a mass extinction in tropical rain forests. In D.K. Elliott (ed): *Dynamics of extinction.* John Wiley and Sons, New York: pp. 165-180.

Singh, S.M. and Zouros, E. (1978) Genetic variation associated with growth rate in the American oyster (*Crassostra virginica*). *Evolution* 32: 342-353.

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

Smith, M.H., Garten, Jr. C.T. and Ramsay, P.R. (1975) Genetic heterozygosity and population dynamics in small mammals. In C.L. Markert (ed): *Isozymes IV, Genetics and evolution.* Academic Press, New York: pp. 85-102.

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

Sokal, R.R. and Rohlf, F.J. (1981) *Biometry.* Freeman and Co. San Francisco.

Soulé, M.E. (1966) Trends in the insular radiation of a lizard. *Amer. Nat.* 100: 47-64.

Soulé, M.E. (1967) Phenetics of Natural Populations.II. Asymmetry and evolution in a lizard. *Amer. Nat.* 101 (918): 141-160.

Soulé, M.E. (1979) Heterozygosity and developmental stability : another look. *Evolution* 33 (1): 396-401.

Soulé, M.E. (1980) Thresholds for survival : maintaining fitness and evolutionary potential. In M.E. Soulé and B.A. Wilcox (eds): *Conservation Biology : An evolutionary - ecological perspective.*

Sinauer Assoc., Sunderland, Mass: pp. 135-150.

Soulé, M.E. (1986) Conservation and the "real world". In M.E. Soulé (ed): *Conservation biology. The science of scarcity and diversity.* Sinauer Assoc. inc., Sunderland, Massachusetts: pp. 1-12.

Soulé, M.E., Gilpin, M., Conway, W. and Foose, T. (1986) The millennium ark: How long a voyage, how many staterooms, how many passengers? *Zoo Biol.* 5: 101-113.

Soulé, M.E. and Wilcox, B.A. (1980) *Conservation Biology: An evolutionary - ecological perspective.* Sinauer Assoc. inc., Sunderland, Massachusetts.

Soulé, M. and Yang, S.Y. (1973) Genetic variation in side-blotched lizards on islands in the Gulf of California. *Evolution* 27: 593-600.

Spiess, E.B. (1977) *Genes in populations.* John Wiley and Sons, New York.

Stamps, J.A. and Buechner, M. (1985) The territorial defence hypothesis and the ecology of insular vertebrates. *Quart. Rev. Biol.* 60 (2): 155-181.

Stevenson, P.M. (1985) Draft report on the conservation status of Wardang Island. A.N.P.W.S..

Swain, D.P. (1987) A problem with the use of meristic characters to estimate developmental stability. *Amer. Nat.* 129 (5): 761-768.

Swofford, D.L. (1985) *PAUP. Phylogenetic analysis using parsimony. Version 2.4.* Illinois natural History Survey, Champaign, Illinois.

Swofford, D.L. and Selander, R.B. (1981) BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72:** 281-283.

Tajima, F. and Nei, M. (1982) Biases of the estimates of DNA divergence obtained by the restriction enzyme technique. *J. Mol. Evol.* 18: 115-120.

Templeton, A.R. (1980) The theory of speciation via the founder principle. *Genetics* **94:** 1011-1038.

Templeton, A.R. (1986) Coadaptation and outbreeding depression. In M.E. Soulé (ed): *Conservation Biology. The science of scarcity and diversity.* Sinauer Associates, Sunderland, Massachusetts: pp. 105-116.

Thoday, J.M. (1956) Balance, heterozygosity and developmental stability. *Cold. Spr. Harbr. Symp. Quant. Biol.* **21:** 318-326.

Thoday, J.M. (1972) Disruptive selection. *Proc. Roy. Soc. Lond. B.* **182:** 109-143.

Thom, B.G. and Roy, P.S. (1985) Relative sea levels and coastal sedimentation in south-east Australia in the Holocene. *J. Sed. Pet.* 55 (2): 257-264.

Thompson, M.B. and Tyler, M.J. (1983) Reptiles and Amphibians. In M. J. Tyler, C.R. Twidale, J.K. Ling, and J.W. Holmes (eds): *Natural history of the South East.* Roy. Soc. S.A. Inc.: pp. 151-56.

Tubb, J.A. (1938) The Sir Joseph Banks Islands. 2.Reptilia, Part 1. General. *Proc. R. Soc. Vic.* 50: 383-393.

Turner, B.J. (1974) Genetic divergence of Death Valley pupfish species : biochemical versus morphological evidence. *Evolution* **28:** 281-94.

Upholt, W.B. (1977) Estimation of DNA sequence divergence from comparison of restriction endonuclease digest. *Nuc. Acids. Res.* **4:** 1257-1265.

Usher, M.B. (1973) *Biological management and conservation.* Chapman and Hall, London.

Usher, M.B. (1986) Wildlife conservation evaluation: attributes, criteria and values. In M.B. Usher (ed): *Wildlife conservation evaluation.* Chapman and Hall, London: pp. 3-44.

Van Valen, L. (1962) A study of fluctuating asymmetry. *Evolution* 16: 125-42.

Vinegar, A. (1974) Evolutionary implications of temperature induced anomalies of development in snake embryos. *Herpetologica* 30 (1): 72-74.

Vrijenhoek, R.C. and Lerman, S. (1982) Heterozygosity and developmental stability under sexual and asexual breeding systems. *Evolution* 36: 768-776.

Wallace, D.C. (1982) Structure and evolution of organelle genomes. *Microbiol. Rev.* 46: 208-40.

Warburg, M.R. (1965) The influence of ambient temperature and humidity on the body temperature and water conservation from two Australian lizards. *Aust. J. Zool.* 13: 331-350.

Watson, G.F., Loftus-Hills, J.J. and Littlejohn, M.J. (1971) The *Litoria ewingi* complex (Anura: Hylidae) in south-eastern Australia. I. A new species from Victoria. *Aust. J. Zool.* 19: 401-416.

Watt, W.B. (1977) Adaptation at specific loci I. Natural selection on phosphoglucose isomerase of Colials butterflies: biochemical and population aspects. *Genetics* 87: 177-194.

Wayne, R.K., Forman, L., Newman, A.K., Simonson, J.M. and O'Brien, S.J. (1986) Genetic monitors of zoo populations morphological and electrophoretic assays. *Zoo Biol.* 5 (2): 215-232.

White, M.J.D. (1978) *Modes of speciation.* Freeman & Co., San Francisco.

Williams, E.E. (1969) The ecology of colonisation as seen in the zoogeography of anoline lizards on small islands. *Q. Rev. Biol.* **44**: 345-89.

Williams, E.E. (1972) The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. *Evol. Biol.* 6: 47-89.

Williamson, M.H. (1981) *Island populations.* Oxford Univ. Press, Oxford.

Willis, E.O. (1984) Conservation, subdivision of reserves and the anti-dismemberment hypothesis. *Oikos* 42 (3): 396-98.

Wills, C. (1981) *Genetic variability.* Clarendon, Oxford.

Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G. and Palumbi, S.R. (1985) Mitochondrial DNA and two perspective on evolutionary genetics. *Biol. J. Linn. Soc.* 26: 375-400.

Wilson, E.O. and Willis, E.O. (1975) Applied biogeography. In M.L. Cody and J.M. Diamond (eds): *Ecology and evolution of communities.* Belknap Press of Harvard University Press, Cambridge, Massachusetts: pp. 522-534.

Workman, P.L. and Niswander, J.D. (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Human Genet.* 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.* Univ. Chicago Press. Chicago.

Wright, S. (1970) Random drift and the shifting balance theory of evolution. In K. Kojima (ed): Mathematical topics in population genetics. Springer-Verlag, Berlin: pp. 1-31.

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

Zimmerman, B.L. and Bierrgaard, R.O. (1986) Relevance of the equilibrium theory of island biogeography and species-area relations to conservation with a case from Amazonia. *J. Biogeogr.* 13: 133-143.

Zinc, R.M., Smith, M.F. and Patton, J.L. (1985) Associations between heterozygosity and morphological variance. *J. Heredity* **76**: 415-420.

APPENDIX 1

Recipes for all solutions used in the mitochondrial DNA analyses.

Mitochondrial Isolation Medium

0.25M Sucrose
0.001 Na_2EDTA
0.02 HEPES
0.05 mg/ml BSA
pH = 7.4 - 8.0 with KOH
* Add BSA fresh.

SSC Solution

0.15M NaCl
0.015 NaCitrate
pH 7.0 (HCl)

Lysis Solution

0.1M Na2EDTA
adjust to pH 8.0 with NaOH
Then add;
2% SDS
0.5M NaPerchlorate
0.15M NaCl

Standard Buffer

0.01M Tris
0.001M EDTA

T.S. Buffer

0.10M NaCl
0.01m Tris
0.002M EDTA
pH 7.8

BSA

5% Sterile Stock Solution
1/50 dilution for 0.1% BSA
400ul SSH_2O

Restriction Enzyme Stop Solution

5g Sucrose
2.5mls Na_2EDTA
5mls 10*E Buffer
2.5mls H_2O + Bromophenol Blue

Electrophoresis Buffer

0.04M Tris
0.03M $NaH^2PO_4.2H_2O$
0.001M $Na_2EDTA.2H_2O$

APPENDIX 2

The running conditions, buffers and grinding solutions for each of the allozymes examined in the electrophoretic analysis.

ALLOZYME	E.C.NO.	BUFFER *	RUN TIME (hours)	NO. OF LOCI SCORED	QUATERNARY STRUCTURE
Albumin (Alb)	-	D	1	1	Monomeric
Carbonic anhydrase (CA)	4.2.1.1	A	3	1	Monomeric
Glucose-6-Phosphate dehydrogenase (G6PD)	1.1.1.49	B	1.5	1	Unknown
Glucose phosphate isomerase (GPI)	5.3.1.9	C	3	1	Dimeric
Isocitrate dehydrogenase (IDH)	1.1.1.42	A	2	1	Unknown
Lactate dehydrogenase (LDH)	1.1.1.27	A	1	1	Unknown
Malate dehydrogenase (MDH)	1.1.1.37	A	2	1	Unknown
Mannose -Phosphate Isomerase (MPI)	1.1.1.40	B	1	1	Unknown
Peptidase A (Pep A)	3.4.11	A	1	2	Monomeric
Peptidase B (Pep B)		A	1	1	Unknown
Peptidase D (Pep D)	3.4.13.9	A	1	1	Dimeric
6Phosphate Glucose dehydrogenase (6PGD)	1.1.1.44	B	2	1	Unknown

APPENDIX 2 (cont.)

ALLOZYME	E.C.NO.	BUFFER	RUN TIME (hours)	NO. OF LOCI SCORED	QUATERNARY STRUCTURE
Phosphoglucomutase (PGM)	2.7.5.1	C	2	1	Monomeric
Superoxide dismutase (SOD)	1.15.1.1	B	1	1	Monomeric
Umbelliferal acetate esterase (UAE)	3.4.13.9	C	3	1	Unknown
Transferrin (Transf)		E	18	1	Monomeric

*BUFFERS USED IN ELECTROPHORESIS:

A......0.01M Citrate-Phosphate, pH 6.4
B......0.02M Phosphate, pH 7.0
C...... Tris-Malate, pH 7.8
D...... Barbitone, pH 8.6
E......0.26M NaBorate, pH 8.6

RECIPE FOR THE LYSING SOLUTION

100 ml H_2O
10 mg NADP
100 ul B-Mercapthoethanol

APPENDIX 3

The electrophoretic data obtained for 16 populations of *T.rugosus* examined for the following 17 loci: ALB CA1 GPI1 G6PD1 IDH1 LDH1 MDH1 MPI1 PEPA1 PEPA2 PEPB1 PEPD1 6PGD1 PGM1 SOD1 TRF UAE1.

ST PETER ISLAND

1477	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
1478	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
1994	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	--	AA
0003	AB	AB	AA	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0004	AA	BB	AA	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0006	AA	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	--	AA
0007	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	--	AA
0008	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0009	AA	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA

FLINDERS ISLAND

0001	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0002	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	--	AA
0003	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA

CEDUNA

1472	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
1473	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA

PT. KENNY

0008	BB	BB	CD	AA	AA	AA	AA	AA	AA	BB	AA	AC	AA	BB	AA	BB	AA
0009	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	BB	BB	AA
0010	AA	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA

LINCOLN NP

8610	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	AB	AA
0483	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	BB	BB	AA
0484	AA	BB	DD	AA	AA	AA	AA	AA	AA	CC	AA	CC	AA	AA	AA	AA	AA
0485	BB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA
0486	BB	AB	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	AB	AA

TUMBY BAY

8402	AA	AB	BC	AA	AA	AA	AA	AA	AA	BC	AA	CC	AA	AA	AA	AB	AA
8403	AA	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AB	BB	AA
8404	BB	AA	BD	AA	AA	AA	AA	AA	AA	BB	AA	--	AA	AA	AC	BB	AA
8406	AB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0481	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA
0482	AB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	--	AA
0001	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AB	AA	AA
0002	AA	--	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AB	BB	AA
0003	AB	--	--	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AB	AA	AA
1555	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AB	AA	AA
1556	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
1557	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	AB	AA
1558	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AB	BC	AA
1559	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0009	BB	--	BB	AA	AA	AA	AA	AA	AA	BB	AA	--	AA	BB	AA	BC	AA
0010	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0011	AB	AB	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	AB	AA
0012	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0013	AA	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA

0014	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AB	--	AA
0015	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0016	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0017	AA	AB	BB	AA	AA	AA	AA	AA	AA	--	AA	CC	AA	AA	--	AA	AA
0018	AA	AA	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	--	AA

SPILSBY ISLAND

0100	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0200	AA	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0300	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0400	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0500	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0600	AB	BB	DD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
1474	AB	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
1475	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	BB	AA
0039	AA	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0040	AB	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0002	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0003	AA	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0005	AA	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0006	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0007	AA	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0008	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0009	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0011	AA		BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0013	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA

DUFFIELD ISLAND

2771	AA	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0002	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0005	AA	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0009	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0010	BB	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	--	AA
0015	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0017	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	--	AA
0019	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	--	AA
0020	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	--	AA
0021	AB	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0022	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0023	AB	BB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0024	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0025	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	--	AA
0026	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0027	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	BB	AA	BB	AA
0028	BB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	BB	AA	BB	AA
0029	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0030	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0031	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0032	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0033	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0034	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0035	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0036	AB	AB	BD	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	BB	AA
0037	AA	--	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	--	AA	BB	AA
0038	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA

REEVESBY ISLAND

8591	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	--	AB	BB	AA

8592	BB	--	--	AA	AA	AA	AA	AA	AA	--	AA	--	AA	--	--	BB	AA
8593	BB	BB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BB	AA	BB	AA	BB	AA
8594	AB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	AB	AA	BB	AA
1482	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AA	BB	AA
0001	AB	BB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BC	AA	AB	AA	BB	AA
0002	BB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AA	BB	AA
0003	BB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	BB	BB	AA
0004	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AB	BB	AA
0005	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AB	BB	AA
0006	BB	AB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BC	AA	BB	AB	BB	AA
0007	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AB	BB	AA
0008	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0009	BB	AB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BC	AA	AB	AB	BB	AA
0010	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AB	BB	AA
0011	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0012	BB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AA	BB	AA
0013	BB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	CC	AA	BB	AA	BB	AA
0014	BB	AA	BB	AA	AA	AA	AA	AA	AA	CC	AA	BB	AA	BB	AB	BB	AA
0015	BB	AA	BB	AA	AA	AA	AA	AA	AA	CC	AA	BC	AA	BB	AA	--	AA
0016	BB	AA	BB	AA	AA	AA	AA	AA	AA	BC	AA	CC	AA	BB	AA	--	AA
0017	BB	AA	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AB	--	AA
0018	BB	AA	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AB	--	AA
0019	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AB	--	AA
0020	AB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AA	--	AA
0021	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	BB	AA	--	AA
0022	BB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AA	--	AA
0023	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	BB	AA	--	AA
0024	BB	AB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BB	AA	BB	AA	--	AA
0025	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AA	--	AA
0026	BB	AB	BB	AA	AA	AA	AA	AA	AA	CC	AA	CC	AA	BB	AA	--	AA
0027	BB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AA	--	AA
0028	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AA	--	AA
0031	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0032	AB	BB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BB	AA	BB	AA	BB	AA
0033	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AA	BB	AA
0034	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	CC	AA	BB	AA	BB	AA
0035	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BB	AA	BB	AA	BB	AA
0036	BB	BB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BC	AA	BB	AA	BB	AA
0037	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	AA	AA	BB	AA
0038	AB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	CC	AA	BB	AA	--	AA
0041	BB	AB	BB	AA	AA	AA	AA	AA	AA	CC	AA	BB	AA	BB	AA	BB	AA
0042	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	--	AA
0043	AA	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AB	--	AA
0045	AB	AB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	AA	AA	--	AA
0046	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	BB	--	AA
0047	BB	BB	BB	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AB	--	AA

WEEROONA ISLAND

0001	BB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA
0002	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	BB	BB	AA
0003	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0004	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	BB	BB	AA
0005	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0006	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AB	BB	AA
0007	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0008	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0009	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA

0010	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AB	BB	AA
0011	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA
0012	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0013	BB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA
0014	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0015	BB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	--	AA
0016	BB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AB	BB	AA
0017	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AB	--	AA
0018	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0019	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0020	BB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA
0021	BB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	--	AA
0022	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0023	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AB	BB	AA
0024	BB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	BB	AA	BB	AA
0025	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA

NORTH OF PORT PIRIE

1620	BB	AB	CC	AA	AA	AA	AA	AA	AA	BC	AA	BC	AA	BB	AA	BB	AA
1621	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AB	BB	AA
1623	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0004	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	--	AA
0005	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	--	AA
0006	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	--	AA
0007	BB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	BB	AA	--	AA
0008	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0009	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	AB	AA
0010	BB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	BB	AA
0011	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0012	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0013	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	AA	AA
0014	BB	BB	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	AA	AA
0015	AB	AA	CC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	--	AA
0016	AB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA

POINT PEARCE

0001	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	--	BB	AA
0002	AB	BB	AB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	--	BB	BB	AA
0003	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	--	BB	AA
0004	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	CC	AA
0005	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	BB	AA
0006	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	BB	AA
0007	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	AB	AA
0008	BB	AA	BC	AA	AA	AA	AA	AA	AA	AA	AA	BC	AA	AA	AA	BB	AA
0009	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BC	AA
0010	BC	AB	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BC	AA	--	AA
0011	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0012	AA	AA	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	AB	AA
0013	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	BB	AA
0014	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0015	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BC	AA
0016	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AA	BB	AA
0017	AB	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BC	AA
0018	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0019	BB	AA	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0020	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	AB	AA
0021	BB	AB	AB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0022	AA	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AB	BB	AA

0390	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	BB	AB	AA
0391	AB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0392	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0393	AB	BB	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0394	AC	AA	BD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AC	AB	AB	AA
0395	BB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	AB	AA
0396	AA	AB	CD	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0397	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA

WARDANG ISLAND

0001	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0002	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	--	AA
0003	AA	AA	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0004	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0005	AB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0006	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	--	AA
0007	AA	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0008	AA	AA	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0009	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AB	AB	--	AA
0010	AB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	--	AA
0011	BB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	--	AA
0012	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0013	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	--	AA
0014	AA	--	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	BB	AA
0015	AA	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0016	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	BB	AA
0017	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	BB	AA
0018	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	BB	BB	AA
0019	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0020	AA	--	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0021	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0022	AB	--	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	--	AA
0023	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0024	AB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0025	AA	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0026	AA	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0027	AA	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AB	--	AA
0028	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	BB	--	AA
0029	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0030	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0031	AA	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0032	AA	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0033	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0034	BB	AA	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0035	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0036	AB	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AB	--	AA
0037	AB	AA	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0038	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0040	BB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	BB	BB	AA
0041	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	BB	BB	AA
0042	AB	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0043	BB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0044	AA	AA	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0054	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0055	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AB	AA	BB	AA
0056	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0057	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0058	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA

1594	BB	BB	--	AA	AA	AA	AA	AA	AA	BB	AA	--	AA	BB	AA	BB	AA
1595	AA	AB	--	AA	AA	AA	AA	AA	AA	BB	AA	--	AA	--	AB	BB	AA
1596	BB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0388	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
0389	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	--	AA

PORT VICTORIA

0010	AA	--	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	BB	AA
0011	AA	--	--	AA	AA	AA	AA	AA	AA	--	AA	--	AA	--	AA	BB	AA
0012	AA	BB	CC	AA	AA	AA	AA	AA	AA	BC	AA	CC	AA	--	BB	AB	AA

EDITHBURGH

0015	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	--	AA	BB	AA
0016	AB	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA

TROUBRIDGE ISLAND

0001	BB	--	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	BB	BB	AA
0002	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AB	BB	AA
0003	AA	AB	BB	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	BB	BB	AA
0006	AA	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AA	BB	AA
0819	AA	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	AA	--	AA
1418	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	AB	AA
1419	AA	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	--	AA	BB	AA
0007	AA	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	--	AA	AA	AA	AB	AA
0008	BB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	AA	AA
0009	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	AB	AA
0010	BB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0011	AB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AB	AA	BB	AA
0012	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	AA	BB	AA
0013	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0014	BB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AA	BB	AA
0015	AB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	AA	BB	BB	AA
0016	AB	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	AA	AA	BB	AA
0017	BB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	BB	--	BB	AA
0018	AA	BB	CC	AA	AA	AA	AA	AA	AA	BB	AA	BC	AA	AA	AB	AA	AA
0019	AB	AB	BC	AA	AA	AA	AA	AA	AA	BB	AA	BB	AA	--	AA	BB	AA
0020	AB	BB	BC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	--	AB	AA	AA
0021	BB	AB	CC	AA	AA	AA	AA	AA	AA	BB	AA	CC	AA	BB	AB	BB	AA

APPENDIX 4

The metric data from all *T.rugosus* examined in this study. The characters measured (in order of collumn) are locality of collection, Identification Number, Sex: 1=male, 2=female, 3=juvenile; SVL, TL, HL, IOW, HW, RW, RH, EYE, IPW, IPL, HLLL, SNLL. The identification number refers either to the museum number or to the field number; All measurements are in millimetres; key to the character abbreviations are given in Table 2.2.

St. Peter	R21944	2	298	51	52.1	25.4	52.5	8.6	5.9	8.5	7.7	9.6	.	20.00	13.60
	R31477	2	305	62	51.9	22.0	55.5	8.5	5.7	7.3	5.5	11.0	59.0	20.20	.
	R31478	2	295	63	53.3	25.0	57.4	8.4	5.7	8.3	8.1	11.1	64.0	22.40	.
	ST-P03	2	299	66	50.4	24.8	52.1	8.3	5.4	8.0	7.8	10.9	62.5	20.20	13.60
	ST-P04	1	313	69	57.0	25.5	61.6	9.2	6.5	9.9	9.2	11.2	67.5	22.20	14.25
	ST-P05	.	280	.	.	.	.	.	.	.	.	.	.	.	.
	ST-P06	1	280	64	55.0	25.0	56.7	9.0	6.5	8.1	7.4	10.4	60.0	21.55	14.50
	ST-P07	1	295	68	49.6	22.7	53.3	7.7	5.3	6.6	6.6	10.1	64.0	19.40	13.15
	ST-P08	2	266	62	44.8	22.0	49.6	7.5	5.2	6.5	7.6	8.5	60.0	18.80	12.70
	ST-P09	2	317	65	50.8	23.9	53.7	7.9	5.1	7.9	8.1	9.6	64.5	20.40	13.15
Ceduna	R31472	2	297	67	48.1	27.1	56.6	8.7	5.7	6.7	6.3	9.4	54.5	19.60	13.40
	R31473	2	288	60	49.8	25.2	62.3	8.8	5.2	7.6	5.9	10.2	54.5	18.75	13.30
Streaky By	R16933	1	145	30	30.0	13.0	33.5	5.4	3.0	.	4.6	6.8	.	15.20	.
Flinders	R31301	1	273	60	48.2	23.2	46.7	8.3	5.3	.	6.5	8.1	46.0	20.00	13.45
	R31302	2	292	48	47.7	22.2	47.9	8.1	5.5	.	6.5	9.5	45.0	19.35	13.25
	R31303	1	221	50	39.3	19.8	40.8	6.3	4.8	.	5.8	7.2	41.5	15.70	10.85
	R17275	2	295	70	51.7	24.6	50.8	9.0	6.4	8.0	7.8	9.3	.	20.30	.
Minnipa	R28378	1	295	73	61.5	28.0	62.0	12.5	6.3	9.4	8.5	11.9	.	22.85	15.25
	R28379	1	275	73	60.0	26.5	61.5	9.7	6.4	8.5	6.9	10.8	.	23.15	15.10
	R28384	1	301	69	56.7	25.1	55.7	10.0	5.5	7.8	9.0	11.3	.	22.75	15.00
	R28404	3	175	40	61.6	28.1	64.9	9.8	6.2	8.5	8.0	12.2	.	22.85	15.45
	R28405	1	315	81	64.5	27.8	66.1	10.5	6.9	8.5	9.2	10.5	.	24.35	16.40
	R28406	1	285	67	61.3	28.1	65.0	10.1	6.1	7.6	7.3	11.9	.	22.60	15.55
	R28407	1	309	63	64.0	25.6	61.2	12.8	6.4	7.3	6.8	8.8	.	23.70	15.65
	R28470	1	277	46	61.9	28.2	64.2	10.8	7.0	8.4	9.1	13.2	.	24.55	15.85
	R28476	2	235	53	44.4	20.6	46.5	7.1	4.6	7.1	6.0	7.2	.	18.15	12.40
	R28507	1	274	60	62.1	27.1	67.8	9.6	6.7	7.8	8.4	12.7	.	23.55	15.50
	R28593	1	293	60	55.5	25.9	58.1	9.3	5.4	8.3	7.8	11.0	.	21.35	15.10
	R28594	1	301	57	55.8	26.2	64.3	9.1	6.0	7.8	8.4	10.3	.	22.20	14.60
	R28595	2	302	63	54.4	26.1	59.1	9.8	6.2	8.4	8.5	11.0	.	22.00	15.50
	R28602	1	280	58	60.3	30.2	63.5	10.4	5.9	9.3	7.5	8.4	.	22.60	14.65
	R28603	3	186	42	36.1	17.3	38.5	5.9	2.9	6.7	6.0	7.1	.	14.80	10.25
	R28604	1	300	68	61.8	26.2	64.7	10.3	6.2	9.4	.	.	.	23.00	15.40
	R28605	.	260	43	38.5	19.3	42.1	6.4	3.8	6.7	6.1	9.2	.	15.95	10.65
	R29206	1	271	62	55.3	24.4	58.4	7.9	5.7	8.9	8.2	8.7	.	22.25	14.70
Lincoln NP	R30485	2	275	44	47.2	24.5	50.9	8.7	4.9	7.9	8.1	11.2	.	18.00	11.90
	R30486	1	275	72	55.0	25.3	54.8	9.0	5.6	8.8	6.6	9.6	.	21.85	14.45
Pt Lincoln	R4043	1	261	74	51.9	25.3	50.7	8.2	5.5	7.8	7.6	9.4	.	19.95	13.25
Duffield	R22771	2	291	65	50.3	21.7	47.8	8.0	5.2	7.2	5.7	7.5	.	19.40	12.65
	R30468	1	295	64	56.9	26.3	55.7	10.8	6.5	7.5	7.0	9.8	.	21.50	14.15
	R30469	1	300	64	57.2	27.5	53.0	9.3	6.0	7.5	.	.	.	21.80	14.60
	R30470	1	250	56	44.4	21.9	46.7	6.8	4.9	6.6	6.3	7.7	.	17.85	11.75
	R30471	2	283	58	48.9	21.7	48.6	8.4	5.1	7.1	.	.	.	19.35	12.95
	R30472	1	286	58	54.6	22.7	53.3	10.2	5.9	6.6	6.9	10.0	.	20.65	13.55
	R30473	1	302	70	55.6	21.8	51.5	10.8	5.5	6.6	7.8	8.5	.	21.05	13.40
	R30474	1	300	60	51.3	20.8	51.8	8.3	4.9	6.1	5.7	8.4	.	19.60	12.75
	DUFF38	3	161	42	30.0	15.3	32.9	5.5	3.1	4.6	5.0	6.0	33.0	12.25	7.95
	DUFF35	1	286	72	49.3	24.2	52.6	8.2	5.1	5.9	5.0	8.9	57.0	20.45	13.05

	DUFF39	2	230	58	37.8	19.1	39.6	6.6	3.7	4.9	6.1	7.4	45.0	15.75	10.30
	DUFF12	2	285	72	48.1	23.3	51.2	8.7	4.6	6.5	6.2	10.7	54.0	19.10	11.15
	DUFF33	2	303	75	50.4	21.4	51.2	8.4	5.0	8.0	7.0	9.3	55.5	19.80	12.05
	DUFF21	1	276	70	50.4	12.9	48.6	8.7	5.0	7.4	5.2	8.3	58.0	20.10	12.80
	DUFF40	1	302	80	56.1	23.6	56.7	9.0	5.8	8.4	7.7	9.2	63.0	21.80	13.70
	DUFF24	1	265	60	43.3	22.5	48.6	4.6	7.8	7.8	6.0	7.8	54.0	18.40	11.40
	DUFF41	1	297	79	53.5	25.4	55.5	8.5	5.2	7.8	.	.	59.0	20.50	13.35
	DUFF42	2	220	64	40.7	20.1	43.2	3.8	5.8	4.8	6.9	8.0	43.5	16.15	10.40
	DUFF14	1	302	80	53.0	21.3	49.8	9.4	4.9	6.9	6.9	8.2	61.5	20.15	12.75
	DUFF43	2	306	65	49.9	23.9	52.3	8.2	4.9	5.9	6.7	9.9	55.5	20.30	13.00
	DUFF44	2	281	75	47.6	23.9	54.9	7.8	5.0	6.4	6.9	8.4	53.0	20.20	12.85
	DUFF45	2	289	72	46.6	24.0	52.0	7.9	3.8	7.4	6.8	8.7	55.0	19.30	12.10
	DUFF46	1	240	63	41.3	21.4	43.9	6.6	3.8	6.7	4.9	7.4	48.0	16.90	11.05
	DUFF47	2	292	80	47.1	23.2	50.2	8.4	4.9	5.4	6.2	8.5	55.5	19.55	12.45
	DUFF48	2	289	79	50.1	22.9	50.8	9.1	5.1	6.4	7.1	9.6	52.0	19.70	12.40
	DUFF49	2	278	72	48.3	22.2	50.8	8.5	4.8	6.8	6.8	7.8	54.0	19.10	11.60
	DUFF52	1	296	71	47.8	24.1	50.3	8.7	5.1	6.1	5.5	8.0	59.0	20.30	12.70
	DUFF23	2	285	70	48.8	23.5	52.5	8.7	5.5	7.7	6.4	8.7	52.0	18.50	11.35
	DUFF50	1	290	79	53.4	25.8	56.0	9.0	4.6	7.1	6.0	7.7	60.5	20.70	13.15
	DUFF02	1	302	83	54.8	22.8	57.2	9.7	5.5	7.1	6.9	8.7	60.0	20.95	13.40
	DUFF51	1	306	75	56.6	27.1	59.9	9.3	5.6	7.8	6.7	10.1	58.0	22.20	14.35
	DUFF19	1	295	72	56.2	24.9	59.2	9.6	4.7	7.2	6.3	8.8	61.5	22.05	13.85
Spilsby	R30475	2	281	66	51.6	24.1	51.8	9.6	5.3	6.9	7.7	10.3	51.0	21.05	13.80
	R30476	2	318	76	54.4	25.6	55.6	10.2	6.1	7.4	6.3	8.4	55.0	21.05	12.75
	R30477	2	308	62	54.0	24.6	54.4	9.5	5.7	8.0	7.5	9.8	52.0	21.05	13.25
	R30478	1	297	68	55.3	27.6	53.4	9.9	5.9	6.9	6.3	11.4	54.0	21.85	14.45
	R30479	2	292	72	49.6	23.9	52.0	9.8	5.8	7.1	6.6	9.0	52.0	19.65	12.80
	R30480	2	332	68	55.7	26.0	53.7	9.8	5.4	6.3	7.0	8.5	56.0	20.85	14.00
	SPLY0A	2	309	75	50.5	25.5	45.7	7.4	5.3	7.5	5.9	7.3	55.0	18.60	.
	SPLY01	1	311	74	52.9	25.5	54.1	9.6	5.5	7.1	6.9	9.0	58.0	20.15	.
	SPLY02	1	307	68	53.2	26.0	58.0	9.5	6.1	7.9	6.9	10.3	59.0	21.20	.
	SPLY0B	2	312	76	50.8	26.7	55.5	9.4	6.0	7.4	6.3	9.0	53.0	20.35	.
	SPLY03	1	299	72	55.0	26.5	57.6	9.5	5.6	7.0	7.5	10.4	58.0	21.65	.
	SPLY04	3	194	41	31.1	15.7	31.5	5.0	3.0	4.9	4.9	6.4	34.5	12.90	.
	SPLY05	1	315	79	54.0	26.5	54.0	10.1	6.2	7.8	7.1	10.3	62.0	21.65	.
	SPLY06	2	288	68	47.6	24.6	52.3	9.2	5.3	6.2	5.9	8.9	56.0	19.15	.
	SPLY07	2	308	80	52.0	27.1	52.7	9.6	5.7	7.5	5.8	9.9	56.0	19.85	.
	SPLY08	1	320	73	55.5	28.1	59.0	10.0	6.3	5.7	7.5	9.4	61.5	22.50	.
	SPLY09	2	296	73	49.6	24.5	52.9	9.2	5.3	6.0	7.8	10.3	53.0	20.50	.
	SPLY10	1	309	71	57.5	25.8	60.7	10.4	6.0	6.7	.	.	60.0	21.95	13.35
	SPLY11	1	318	72	56.0	27.8	60.0	10.2	6.1	7.3	6.6	9.7	58.0	22.90	13.05
	R31474	1	316	87	57.2	27.1	58.5	10.7	6.5	7.1	5.8	10.2	61.0	22.45	14.00
	SPLY13	2	304	75	51.2	24.8	52.4	8.7	5.1	6.6	6.0	8.5	54.0	20.70	.
	R31475	2	307	85	50.8	24.7	49.9	8.5	5.0	6.6	6.8	9.4	55.5	20.05	12.85
	SPLY16	2	293	75	48.5	22.7	49.3	7.9	5.5	7.6	5.5	8.6	55.0	19.55	11.65
	SPLY17	2	304	83	51.7	23.1	53.2	8.8	5.6	6.8	7.1	8.8	55.5	20.40	12.30
Reevesby	R18591	2	273	54	49.4	26.6	50.9	9.6	6.1	7.1	6.8	8.5	.	20.65	13.65
	R18592	2	270	46	48.6	24.8	49.3	9.2	5.2	6.7	6.3	7.2	.	19.20	12.85
	R18593	1	270	59	54.2	25.5	54.1	11.3	6.8	7.9	6.2	9.5	.	22.50	14.05
	R18594	2	263	54	46.9	24.7	47.4	9.1	5.0	6.0	6.5	8.6	.	19.75	12.20
	R30584	1	253	69	53.4	26.0	52.0	10.1	5.9	6.0	6.6	9.2	.	21.75	13.90
	R30585	1	265	67	53.2	26.1	52.4	.	.	8.0	4.9	9.0	.	21.55	14.75
	R30586	2	237	51	43.4	20.9	44.3	7.6	4.9	6.7	6.0	6.7	.	18.85	12.50
	R30587	2	243	54	43.7	20.2	43.9	7.7	5.0	5.9	6.7	7.1	.	18.95	13.00
	R30588	1	290	70	57.1	28.1	51.6	10.6	7.1	7.3	6.7	8.1	.	24.45	15.90
	R30589	1	262	67	49.2	24.0	48.3	8.2	5.4	7.6	7.5	9.2	.	21.60	14.60
	R30591	1	275	63	54.0	26.2	52.3	9.7	6.1	7.6	7.3	8.4	.	22.90	15.85
	R30592	1	284	70	57.1	27.5	55.7	9.9	6.3	8.2	7.9	8.4	.	23.50	16.45

	R30593	2	268	57	50.5	24.4	52.9	8.8	5.6	7.4	6.5	6.8	.	21.20	14.30
	R31554	2	294	64	56.5	26.3	55.4	7.6	5.8	8.7	7.2	9.2	59.0	24.15	.
	R31482	2	305	64	50.3	26.2	51.6	9.9	5.1	7.0	6.6	7.7	56.0	21.10	13.60
	R30590	1	265	72	53.1	26.2	51.8	10.7	6.0	6.3	7.0	8.6	.	22.95	14.50
	REEV48	2	282	69	50.1	24.7	53.1	8.3	5.1	8.2	6.9	9.0	56.5	21.80	14.20
	REEV50	2	289	66	49.3	23.9	54.5	9.2	5.5	8.2	7.3	9.3	55.0	21.80	13.40
	REEV49	2	295	68	48.8	25.7	54.6	9.4	5.7	6.9	7.1	8.3	57.5	22.00	13.55
	REEV51	1	291	74	55.7	27.1	53.8	10.2	6.1	8.5	6.8	10.1	63.0	23.65	14.15
	REEV52	2	302	70	49.6	24.8	51.2	9.3	5.4	7.8	7.8	7.9	58.0	20.90	13.60
	REEV53	2	293	62	51.1	24.9	50.3	9.2	5.9	7.1	7.6	10.2	60.0	21.55	13.50
	REEV54	2	304	76	51.5	24.4	57.2	9.6	5.6	7.0	6.5	9.0	59.5	22.45	13.70
	REEV55	2	299	69	49.1	24.0	55.6	10.0	6.6	7.3	7.7	11.4	58.5	21.80	14.15
	REEV56	1	277	74	56.0	25.8	56.0	10.3	6.3	8.4	6.4	9.3	61.0	23.15	14.50
	REEV57	1	286	74	55.2	25.8	56.6	9.8	5.7	8.1	5.5	10.4	60.5	23.55	15.10
	REEV58	2	295	77	50.2	24.8	53.2	9.1	5.8	7.9	7.4	9.1	57.0	21.25	13.55
	REEV59	1	297	75	56.1	26.5	50.4	10.1	6.1	6.6	6.8	8.6	63.5	23.45	14.85
	REEV60	2	259	58	44.9	22.0	45.0	9.2	5.8	6.9	5.4	6.7	51.5	19.80	12.65
	REEV61	2	225	56	39.7	19.1	43.3	6.8	4.2	5.9	5.4	8.9	47.0	17.30	11.20
	REEV62	1	257	66	49.5	24.6	50.2	9.1	5.3	6.9	6.5	7.6	57.5	21.45	14.30
	REEV63	2	297	77	54.4	27.4	60.5	10.7	7.0	9.3	6.7	9.8	61.5	23.00	14.80
	REEV64	1	287	72	55.0	27.0	57.7	11.5	6.5	8.6	6.3	10.1	66.0	24.35	15.65
	REEV65	1	300	77	59.1	25.9	55.5	10.1	6.5	7.7	6.4	10.9	62.0	24.90	15.75
	REEV66	1	285	65	52.3	26.0	57.0	10.1	5.6	6.9	6.8	11.2	59.0	22.20	13.90
	REEV67	1	272	71	56.5	24.7	55.6	10.4	6.2	8.5	5.8	8.3	64.0	23.20	14.30
	REEV68	2	294	62	47.3	24.8	50.4	9.3	5.9	7.1	6.3	9.3	57.0	21.00	13.45
	REEV69	1	279	65	54.0	25.1	55.7	10.1	6.0	6.7	6.6	8.6	59.5	22.75	14.70
	REEV70	2	268	67	44.9	21.4	47.2	8.4	5.0	7.0	5.8	8.2	55.0	19.90	13.00
	REEV71	3	181	47	33.6	17.9	37.6	5.6	3.3	6.2	4.4	6.8	40.5	14.95	9.70
	REEV72	1	242	61	44.7	20.6	48.7	7.5	4.4	7.2	5.7	7.5	57.0	19.55	12.85
	REEV73	1	295	58	54.6	26.6	59.1	10.9	6.8	9.3	6.6	8.8	59.5	24.10	15.35
	REEV74	1	291	61	54.5	26.8	52.8	10.6	7.1	6.8	6.2	9.3	60.5	22.60	14.45
	REEV75	2	293	67	49.0	24.8	54.7	9.6	5.7	7.9	7.3	9.3	58.5	21.30	14.00
	REEV76	2	295	70	50.1	24.9	52.9	9.2	5.3	7.5	6.5	10.6	58.0	21.60	13.95
	REEV77	1	274	70	52.3	25.3	53.7	10.3	5.4	7.5	5.7	9.7	61.5	21.95	13.65
	REEV78	2	314	73	49.6	25.8	57.9	9.9	6.0	7.9	7.7	10.9	56.0	22.50	14.15
Tumby Bay	R18402	1	240	62	48.7	24.6	48.1	8.4	5.6	8.5	7.6	10.4	.	18.95	13.30
	R18403	1	257	55	50.3	22.9	49.9	8.5	4.2	7.0	5.7	7.2	.	18.65	12.60
	R18404	1	273	65	53.4	26.9	53.8	9.3	5.7	8.1	8.6	10.3	.	20.75	14.00
	R18405	2	243	56	50.7	24.1	50.4	9.1	4.8	7.3	6.4	10.0	.	19.20	12.25
	R18406	2	246	64	44.8	22.8	47.4	7.0	4.5	7.2	8.0	9.2	.	16.70	10.95
	R30481	2	277	64	48.0	22.9	50.6	8.7	5.8	6.7	7.2	9.7	.	19.00	12.45
	R30482	2	270	60	46.6	21.4	46.8	7.1	4.3	6.7	7.3	9.6	.	18.00	11.90
	TB01	1	244	61	43.9	22.7	47.3	7.4	4.6	5.5	6.4	10.0	52.0	18.55	.
	TB02	1	239	61	42.4	20.5	39.7	7.4	4.0	5.8	5.0	8.4	49.0	16.75	.
	TB03	2	270	70	50.2	23.8	46.7	9.6	5.0	8.9	6.2	11.2	58.5	20.20	.
	R31555	2	243	64	43.1	21.2	47.3	6.5	4.0	5.6	6.1	8.4	51.0	17.60	.
	R31556	2	293	74	51.6	24.8	49.9	9.2	4.7	7.9	9.5	9.7	54.5	19.35	.
	R31557	1	281	76	53.4	25.6	54.8	9.1	5.5	7.4	7.1	9.6	54.0	20.80	.
	R31558	1	248	63	43.8	21.4	44.1	7.0	4.4	7.5	6.1	9.6	50.0	17.05	.
	R31559	2	262	62	44.4	22.9	51.1	7.4	4.2	7.2	6.5	8.4	50.0	17.60	.
	TB09	1	308	87	54.3	24.8	56.4	8.0	5.0	7.1	6.8	9.5	57.5	20.90	.
	TB10	2	260	62	41.3	19.6	43.4	7.3	4.5	7.5	6.2	9.2	50.5	16.25	.
	TB11	2	290	75	44.4	22.0	48.0	6.9	4.3	7.8	6.9	9.4	53.0	18.45	12.60
	TB12	1	272	70	53.0	24.5	55.3	8.6	4.8	8.4	8.1	10.9	56.0	21.00	14.00
	TB13	2	290	69	45.9	22.2	44.9	7.1	4.1	6.8	6.4	9.5	62.0	18.20	11.75
	TB14	1	257	66	47.6	21.9	51.1	8.7	4.8	7.6	7.3	10.0	59.5	19.65	12.80
	TB15	1	268	67	46.8	22.6	49.7	8.3	4.6	6.7	7.2	9.1	58.5	19.05	12.35
	TB16	2	282	68	43.2	21.3	49.0	7.7	4.9	6.8	6.8	9.4	49.5	17.35	11.50

	TB17	1	260	69	43.4	22.9	45.4	7.9	4.8	8.2	7.4	9.9	54.0	17.90	11.65
	TB18	2	282	73	48.1	22.9	49.4	8.3	4.9	7.8	8.1	10.1	54.5	19.15	12.80
	TB19	3	192	50	34.3	18.3	41.2	5.9	3.7	6.1	5.7	6.9	42.5	14.15	9.10
	TB20	2	245	65	44.3	22.2	47.9	7.7	4.9	6.5	5.9	9.6	53.5	18.60	12.45
	TB21	1	276	74	48.8	23.8	55.8	9.7	5.5	8.1	6.5	10.9	59.0	20.95	13.35
	TB22	2	263	57	44.9	20.4	43.7	7.4	4.8	5.8	6.0	9.4	52.5	17.70	11.20
	TB23	2	260	75	42.8	20.5	49.5	7.7	4.5	6.7	6.5	8.4	53.5	17.60	11.40
	TB24	1	270	71	51.6	22.9	53.6	7.9	5.4	5.4	6.3	9.3	58.0	19.85	12.60
Iron Knob	R28392	1	240	53	46.2	22.5	51.0	7.6	5.1	7.8	7.1	10.6	.	18.25	12.50
	R28395	2	220	47	37.8	20.0	40.0	6.7	4.3	6.2	5.4	5.2	.	15.35	10.45
	R28396	1	245	65	58.1	26.7	63.5	9.7	5.6	8.3	6.8	11.2	.	23.20	15.30
	R28589	2	305	60	54.7	24.9	59.3	10.0	5.9	8.1	9.1	10.5	.	21.60	15.15
	R28590	2	316	69	56.0	27.6	61.3	10.0	6.7	9.0	7.8	12.2	.	21.05	14.85
	R28591	2	315	62	66.2	24.8	58.2	9.3	5.8	7.8	7.8	12.6	.	21.00	14.50
	R28592	1	292	57	63.0	27.0	64.2	10.9	6.7	9.1	8.4	10.6	.	23.85	15.30
Tulka	R18610	2	239	52	41.5	19.5	46.1	6.7	4.3	7.0	6.2	10.0	.	15.90	11.15
Weeroona	WERA01	2	286	77	48.7	24.1	50.7	8.2	4.7	9.2	6.7	10.3	58.0	19.35	12.45
	WERA02	1	275	69	48.9	25.3	52.0	9.4	5.4	8.1	4.2	6.7	58.0	20.70	13.60
	WERA03	2	291	69	48.8	22.8	50.1	.	5.7	7.9	6.8	10.0	58.5	20.40	13.15
	WERA04	1	270	.	48.4	25.1	53.9	8.0	4.9	6.8	8.3	8.1	55.5	19.85	12.85
	WERA05	2	259	59	42.6	22.3	43.2	7.0	4.7	6.5	5.6	6.9	50.0	18.10	11.50
	WERA06	2	257	60	42.9	22.0	49.9	8.5	6.0	7.5	4.4	6.7	53.5	17.80	11.70
	WERA07	1	268	63	48.0	24.4	49.3	8.3	5.2	8.5	6.7	11.2	58.5	19.00	12.10
	WERA08	2	226	59	39.5	20.4	44.4	6.8	4.3	6.7	5.2	8.4	43.5	16.00	10.15
	WERA09	2	253	61	42.2	21.6	49.3	6.7	3.9	6.5	8.0	7.9	50.5	17.70	11.80
	WERA10	1	286	.	50.7	24.1	55.8	9.2	5.5	7.3	4.8	5.8	60.5	20.75	12.90
	WERA11	2	288	.	47.8	26.6	54.7	9.1	5.0	8.6	5.8	11.2	58.5	19.85	12.70
	WERA12	2	319	75	51.8	25.2	55.9	9.0	5.5	7.9	6.1	10.6	57.0	20.35	13.55
	R31619	2	265	61	45.4	22.7	49.6	7.3	5.1	7.4	6.6	9.8	48.0	18.60	12.40
	R31618	2	278	68	45.1	22.9	50.4	7.5	5.4	7.8	.	.	53.0	17.80	11.40
	R31622	1	276	62	49.4	25.6	52.2	8.8	5.7	8.0	.	.	56.0	20.75	13.15
	WERA16	2	303	77	48.5	25.2	53.2	7.7	4.8	7.8	6.5	11.4	58.0	19.95	12.75
	WERA17	2	238	63	39.0	21.3	47.8	6.7	4.3	6.0	.	.	48.0	16.45	10.20
	WERA18	1	262	57	51.0	26.2	54.5	9.3	6.3	7.6	5.6	7.9	56.5	20.80	13.35
	WERA19	1	270	.	50.1	25.3	52.1	8.3	5.9	7.7	7.9	11.4	59.5	19.80	13.05
	WERA20	2	290	66	48.9	25.4	52.5	9.3	5.9	8.5	.	.	55.0	20.30	13.40
	WERA21	2	299	.	50.4	23.9	49.9	9.2	5.4	8.5	7.0	8.8	55.5	20.30	12.65
	WERA22	1	282	66	54.4	25.8	50.6	9.1	4.9	6.6	7.1	11.0	60.0	21.75	14.20
	WERA23	2	292	71	52.7	26.3	57.7	7.5	4.4	7.7	.	.	55.5	19.40	12.75
	WERA24	1	273	.	50.7	26.8	53.6	8.7	5.6	9.4	7.0	9.8	62.0	20.00	12.90
	WERA25	2	300	70	48.4	25.0	54.3	8.5	5.7	7.8	7.1	8.4	57.5	19.70	12.80
	WERA26	1	286	72	51.8	24.7	53.6	8.0	4.5	7.5	5.5	10.4	58.0	20.65	13.75
	WERA27	1	273	62	52.8	26.3	55.4	9.7	6.2	8.8	8.6	11.8	57.5	22.70	14.15
	WERA28	2	281	68	51.1	25.2	56.9	8.9	4.4	8.3	5.4	7.3	56.5	21.05	13.50
	WERA29	1	271	63	48.6	24.8	51.9	8.7	5.7	7.8	8.1	9.3	58.0	20.50	13.00
	WERA30	1	285	62	54.0	25.0	53.9	9.2	6.0	8.4	6.7	11.7	58.5	21.85	13.75
Port Pirie	R31620	2	305	61	50.6	25.1	53.7	8.0	4.8	8.7	8.5	13.0	58.0	20.40	13.40
	R31623	2	265	62	46.3	24.2	46.6	8.1	4.8	8.5	5.8	9.9	56.0	18.95	12.45
	R31621	1	279	70	52.6	26.3	55.0	8.5	5.8	8.4	5.7	9.0	60.5	21.25	13.30
	NRWE08	2	215	56	33.7	18.5	43.8	6.1	3.3	6.2	5.9	8.4	40.5	14.50	9.65
	NRWE09	2	291	62	49.6	25.6	52.4	9.1	4.6	7.8	7.2	9.1	55.0	19.45	12.90
	NRWE10	1	289	72	55.9	28.5	56.9	9.4	5.8	10.8	6.6	11.4	60.0	21.20	13.30
	NRWE11	1	294	70	52.3	27.4	53.3	8.8	4.8	8.0	7.8	8.6	60.0	21.25	13.95
	NRWE12	1	304	73	57.5	27.0	54.6	9.7	5.3	9.8	6.6	9.6	60.5	22.55	14.60
	NRWE13	1	297	64	57.6	24.7	57.2	9.3	6.0	8.4	7.8	10.5	59.0	21.95	14.40
	NRWE14	1	287	72	60.1	28.6	62.5	9.0	5.2	8.9	9.1	11.1	62.5	23.00	14.95
	NRWE15	1	287	69	53.8	27.1	56.2	8.6	4.5	8.3	8.1	11.5	63.0	22.40	14.45
	NRWE16	1	288	65	56.9	27.3	61.1	10.5	6.2	7.8	8.0	12.1	58.5	23.45	15.25

	NRWE06	1	290	71	54.6	26.3	55.7	9.3	5.2	8.4	7.3	12.8	60.5	21.85	14.75
	NRWE05	1	300	63	53.6	28.0	58.7	9.7	5.7	8.5	7.5	11.6	60.5	22.10	13.90
	NRWE04	1	287	77	51.4	25.3	58.7	8.3	4.9	7.8	6.9	9.4	60.0	20.50	13.25
	NRWE07	2	299	60	48.7	24.3	51.5	8.1	5.2	7.5	7.9	11.4	59.5	19.70	13.05
Pt. Pearce	YP01	3	181	44	30.0	16.0	13.7	6.7	3.7	6.3	5.0	8.1	35.5	13.00	8.30
	YP02	3	181	54	30.1	14.8	31.8	5.7	3.5	5.0	5.2	6.4	37.5	12.55	7.90
	YP03	1	305	75	51.1	24.4	51.1	8.2	5.2	8.2	9.3	10.1	61.5	20.55	13.25
	YP04	2	294	70	47.5	24.7	49.1	8.8	4.3	8.1	7.6	9.3	59.5	18.20	11.75
	YP05	1	303	75	53.1	24.6	51.3	8.8	5.0	8.5	7.1	10.1	60.5	20.45	13.10
	YP06	1	270	81	48.4	24.2	47.3	7.8	4.3	8.5	6.5	10.6	58.0	19.45	12.90
	R31587	2	277	64	46.3	22.5	48.4	8.1	4.7	6.7	6.3	8.9	54.5	19.10	12.95
	R31588	2	290	70	46.8	25.0	50.4	7.9	5.2	7.8	7.4	10.0	54.0	18.95	12.50
	R30391	2	265	53	46.9	22.6	49.4	7.7	5.1	7.4	7.8	8.3	53.0	18.85	12.80
	R30392	2	279	49	49.8	25.4	50.5	8.4	4.9	8.4	7.2	10.6	51.0	19.90	13.75
	R30390	1	263	66	55.8	24.6	55.6	8.7	5.0	6.6	7.9	10.4	56.0	22.20	14.80
	R30393	1	293	.	56.1	24.1	57.8	8.7	5.7	8.4	8.5	10.7	56.0	21.90	15.95
	R30394	1	275	63	51.9	22.1	55.3	8.0	5.7	7.4	6.5	9.2	53.0	19.70	13.40
	R30395	1	268	63	52.2	21.5	57.2	.	.	8.1	7.9	12.7	50.0	20.20	13.50
	R30396	2	275	59	48.8	22.9	49.3	7.8	4.7	7.4	5.7	8.0	48.0	19.60	12.95
	R30397	1	263	63	49.7	22.5	50.8	5.2	9.1	7.4	6.2	8.8	56.0	20.00	14.15
	PTP09	2	276	71	46.7	24.7	49.4	7.1	4.9	7.9	6.1	9.2	56.0	19.15	12.25
	PTP10	1	278	82	49.5	24.2	50.9	8.2	4.8	7.6	7.1	9.5	60.0	19.80	12.95
	PTP11	1	294	71	59.2	24.5	52.8	7.1	4.7	8.4	6.1	9.4	60.0	19.40	12.65
	PTP12	3	183	51	32.5	16.1	36.7	5.6	3.4	6.1	4.5	6.1	36.5	13.45	8.80
	PTP13	1	289	69	49.1	23.9	53.7	8.7	5.3	7.3	5.9	.	56.0	19.75	12.85
	PTP14	2	280	60	44.5	22.5	48.5	8.8	5.1	7.8	6.0	7.1	56.0	19.65	12.65
	PTP15	2	289	64	45.4	21.3	47.8	8.7	5.0	6.5	7.0	9.8	51.0	17.85	11.20
	PTP16	2	278	63	59.5	22.9	51.2	8.1	4.9	7.5	7.9	9.3	55.5	19.20	12.00
	PTP17	2	283	75	48.8	21.9	49.6	8.6	5.1	7.7	8.0	10.0	55.0	19.70	12.50
	PTP18	2	257	53	41.6	21.5	44.1	7.0	5.6	6.2	5.5	9.0	49.5	17.65	11.05
	PTP19	2	287	72	46.2	23.6	49.5	8.3	5.0	6.9	7.1	8.8	57.0	18.40	12.45
	PTP20	1	289	70	55.1	26.5	53.8	8.5	5.8	8.8	8.5	10.2	62.0	21.50	14.35
	PTP21	1	280	72	51.9	25.9	58.3	8.9	5.8	9.3	7.4	10.6	59.5	20.90	13.60
	PTP22	1	275	70	47.6	22.6	48.7	7.8	5.7	6.5	7.4	10.6	57.0	19.30	12.45
Wardang	WRD011	1	303	70	59.0	27.2	60.8	10.5	5.9	8.8	9.4	10.8	71.0	23.20	14.45
	WRD212	1	302	76	54.8	25.9	56.2	9.7	5.8	8.0	7.3	13.4	64.5	21.85	14.30
	WRD213	1	255	64	43.9	22.3	45.9	8.1	4.9	7.1	7.4	8.9	55.0	18.35	11.65
	WRD214	1	252	57	45.6	23.1	48.6	8.1	4.8	8.0	7.3	8.5	54.5	18.40	11.60
	WRD215	3	172	47	30.5	15.2	34.1	6.3	3.2	4.9	4.5	8.5	34.5	12.70	8.60
	WRD216	2	285	76	44.5	21.4	44.4	8.4	4.7	8.0	8.4	8.3	51.5	17.60	11.10
	WRD217	2	270	68	47.1	22.5	45.6	7.9	5.2	7.1	6.8	9.4	58.0	18.95	11.90
	WRD218	2	231	63	40.6	20.3	41.9	6.7	4.3	7.1	6.2	7.5	51.0	16.25	10.60
	WRD219	1	266	63	47.4	22.9	46.4	8.9	4.8	8.0	7.4	10.3	57.0	19.65	13.10
	WRD220	2	278	70	44.3	21.6	46.5	7.9	4.9	7.2	7.0	7.2	54.0	17.60	11.45
	WRD221	1	270	61	50.7	24.6	51.4	8.6	5.6	7.4	7.6	8.6	55.0	21.05	13.30
	WRD???	1	263	80	49.6	23.5	49.4	9.0	5.1	6.4	7.0	8.4	58.0	19.55	12.85
	WRD222	2	269	71	44.9	21.2	45.4	7.1	4.1	6.9	7.4	9.6	55.5	18.70	13.00
	WRD094	2	250	62	42.5	20.8	44.7	6.8	4.6	6.2	5.8	8.8	55.5	17.00	11.70
	WRD135	2	234	57	38.5	19.2	39.7	5.8	3.2	5.8	6.4	8.2	49.0	15.45	10.00
	WRD223	2	288	68	48.6	25.0	48.3	8.0	4.4	7.8	7.3	8.4	55.5	19.50	13.00
	WRD099	1	290	77	53.2	26.5	55.3	10.0	6.7	8.1	8.5	10.4	63.0	23.05	14.70
	WRD224	2	304	72	48.1	24.0	51.0	9.7	5.6	8.4	6.9	9.8	59.5	20.20	12.55
	WRD225	1	287	71	54.1	25.8	54.8	8.5	5.2	7.9	8.2	11.3	63.5	21.05	13.25
	WRD226	1	267	81	49.7	14.1	51.3	8.8	5.6	7.5	7.3	9.7	56.0	20.00	12.75
	WRD160	1	300	73	55.3	27.0	56.0	9.3	6.2	6.6	7.5	10.2	65.0	22.35	14.15
	WRD227	.	.	.	36.2	18.3	41.1	6.8	3.6	5.1	6.3	6.6	.	14.80	9.30
	WRD178	2	245	66	40.6	20.5	39.7	8.1	4.4	4.5	7.4	9.4	50.5	16.90	11.15
	WRD109	2	284	66	47.9	22.5	48.0	8.2	5.5	7.9	8.2	8.9	53.5	19.60	12.70

	WRD122	1	225	60	40.4	20.2	46.3	6.6	4.0	6.8	5.3	7.2	48.0	16.35	10.95
	WRD228	2	266	69	44.2	21.4	46.6	7.0	4.1	6.6	5.9	8.4	54.0	17.55	11.05
	WRD229	1	277	79	50.1	24.1	50.0	8.7	5.5	7.2	8.1	9.5	56.5	19.90	12.95
	WRD031	1	269	66	51.9	24.1	51.5	8.3	5.1	6.9	7.9	8.6	62.5	20.25	13.10
	WRD032	2	270	68	46.9	23.1	43.7	8.4	4.8	5.9	6.7	8.2	55.0	18.65	11.90
	WRD232	1	264	78	48.7	24.2	49.5	7.9	4.5	7.2	7.5	8.4	57.0	19.50	12.25
	WRD231	1	280	79	51.2	23.9	46.2	9.1	4.8	7.9	6.3	9.7	60.0	20.45	13.10
	WRD230	1	266	67	50.9	24.2	50.7	8.7	5.2	8.1	8.0	8.1	58.0	20.30	12.85
	WRD233	2	267	67	45.7	22.2	44.9	8.4	4.9	6.7	5.4	8.4	56.5	18.50	11.55
	WRD234	1	254	66	41.8	21.5	43.7	6.8	4.7	5.7	6.0	7.9	51.5	17.10	11.00
	WRD113	1	251	71	45.1	22.5	46.3	7.8	4.9	5.9	6.2	8.3	55.0	19.00	12.15
	WRD235	1	264	66	46.1	23.1	44.6	7.7	4.8	5.7	7.4	8.9	55.5	18.85	12.35
	WRD123	1	279	73	48.6	24.7	51.9	8.4	5.5	7.4	8.7	10.0	57.5	20.55	13.30
	WRD251	1	293	70	54.4	25.6	50.4	8.7	5.7	8.2	6.8	10.3	63.0	21.85	14.40
	WRD252	3	169	43	30.0	16.3	34.4	5.6	3.3	5.8	5.4	5.7	36.0	12.35	8.05
	WRD253	1	280	72	51.6	24.8	55.6	8.5	5.3	6.8	7.0	8.8	60.5	20.90	14.15
	WRD254	2	259	55	43.2	21.5	43.5	7.8	4.4	8.4	8.5	11.3	48.0	16.50	10.85
	WRD255	2	265	58	45.0	20.1	46.7	8.2	4.6	7.4	6.4	9.3	54.0	18.15	11.50
	WRD256	2	277	56	46.8	23.2	51.4	8.5	5.6	6.7	7.0	9.3	54.0	18.70	11.30
	WRD194	1	269	62	52.1	23.4	49.5	8.2	4.5	7.9	7.8	9.8	60.0	19.55	12.65
	WRD248	2	238	69	41.2	20.8	45.2	6.8	4.4	6.0	6.6	7.8	50.0	17.00	11.00
	WRD249	1	252	73	45.3	22.1	48.5	7.9	4.3	7.3	7.1	8.0	55.0	18.05	11.75
	WRD250	2	280	70	49.1	23.5	50.2	8.9	5.2	8.5	7.2	7.3	56.0	20.20	12.50
	R31594	2	270	61	46.6	23.0	44.5	8.0	4.6	7.8	8.3	10.1	.	18.10	12.25
	R31595	2	256	68	42.1	21.8	44.1	7.5	5.0	6.8	7.1	9.1	51.0	17.85	11.60
	R31596	1	266	65	50.3	23.0	50.4	8.9	5.4	7.1	6.2	8.7	.	20.10	12.60
	WRD283	2	266	58	47.2	23.1	43.8	8.5	4.8	6.0	7.4	9.9	50.5	19.15	12.30
	R31037	2	258	48	46.5	21.9	46.4	7.6	4.4	6.7	5.8	8.1	.	16.50	10.15
	R31038	2	275	55	48.4	24.5	48.6	7.9	5.1	7.1	6.9	11.3	.	18.35	12.65
	R31479	1	273	58	49.6	23.8	49.4	.	.	6.8	6.7	8.8	.	19.30	14.25
	R31480	1	271	77	47.4	23.4	48.2	8.4	5.2	7.1	6.5	8.8	56.0	18.90	13.30
	R31481	2	297	62	39.9	19.5	38.3	7.2	4.4	6.6	6.0	8.3	53.0	15.40	10.40
	R30388	1	262	58	53.3	25.0	52.6	9.0	5.8	8.0	8.1	9.9	53.0	21.25	13.60
	R30389	2	250	52	42.6	20.2	42.5	7.1	4.6	7.1	4.7	5.7	49.0	17.35	11.55
Pt Vict	R1393A	2	150	30	31.1	15.5	31.6	5.3	3.6	6.1	5.4	7.0	.	13.30	9.40
	R1393B	2	132	24	28.7	12.9	24.1	5.0	3.7	6.1	4.5	5.0	.	11.80	8.50
	R1393C	1	145	25	28.5	14.4	27.8	5.7	3.2	5.5	4.6	6.0	.	12.20	8.45
	R1393D	1	125	23	29.3	12.8	23.8	5.7	3.0	5.6	5.3	6.3	.	11.85	8.65
	R1393E	2	145	21	29.6	15.1	27.8	5.7	3.6	5.5	4.9	6.8	.	13.15	8.95
	R1393F	1	150	30	31.7	13.1	26.0	6.2	3.5	5.7	5.6	6.0	.	13.70	9.10
	R1393G	2	143	26	29.5	14.7	29.5	5.5	3.6	6.1	4.9	6.4	.	12.05	8.25
	R1393H	1	144	29	30.3	14.2	25.4	4.8	3.7	5.3	5.3	6.8	.	13.25	9.15
	R1393I	1	139	24	28.6	14.5	29.0	5.3	3.2	4.8	5.0	6.9	.	12.15	8.85
	R31577	1	270	67	.	.	.	.	.	.	.	.	.	.	.
	R31592	2	272	64	46.8	24.9	52.8	7.9	4.7	8.9	7.5	9.2	53.5	18.45	11.50
	R31593	1	297	75	56.2	26.7	52.8	10.1	5.4	8.9	7.8	10.8	63.0	22.00	14.80
Maitland	R1256	1	228	48	39.3	22.3	46.7	7.5	4.7	6.6	7.0	8.9	.	16.90	.
Minlaton	R31589	1	223	54	40.5	21.7	45.7	6.8	4.2	7.0	8.5	7.8	46.5	17.30	11.50
Innes NP	R16972	1	218	58	39.1	10.7	10.9	6.5	4.7	6.6	5.7	8.2	.	15.55	10.55
Edithburg	R31578	2	303	72	.	.	.	.	.	.	.	.	.	.	.
	R31590	1	281	71	51.8	22.9	50.7	9.1	5.4	7.4	6.9	9.8	55.0	20.25	12.90
	R31591	1	279	72	51.8	24.4	52.2	9.1	4.8	7.9	7.5	9.1	55.5	20.10	12.60
Troubridge	R31419	2	261	60	42.5	22.2	48.2	6.5	5.8	8.0	6.8	8.7	44.5	17.25	12.50
	R31418	1	258	60	51.8	22.7	51.3	9.6	5.5	6.7	6.7	8.4	52.0	21.15	13.95
	R20819	2	228	47	42.1	21.0	41.9	6.6	4.6	6.8	5.5	8.5	.	17.05	11.55
	TROU07	2	278	58	44.8	22.8	48.3	8.4	4.6	7.5	5.8	7.7	48.0	19.05	12.50
	TROU08	1	284	71	55.1	25.5	55.9	7.5	.	8.2	7.2	8.3	62.5	20.05	13.15
	TROU09	2	270	65	47.7	22.9	47.8	8.2	4.3	7.3	6.2	7.7	50.5	18.90	12.40

	TROU01	1	270	61	56.7	26.7	57.6	9.5	6.0	9.2	7.0	9.7	60.5	22.00	14.15
	TROU10	1	257	61	51.2	24.3	49.4	8.6	5.8	7.7	7.8	9.3	57.5	20.30	13.40
	TROU02	1	281	67	53.7	27.7	53.4	9.2	4.1	8.9	8.0	10.7	57.5	20.95	13.40
	TROU11	1	260	58	50.9	25.4	52.8	8.5	4.8	8.3	6.0	9.9	54.5	20.10	13.15
	TROU12	2	245	57	43.1	21.8	46.5	7.0	5.2	7.9	6.1	9.1	50.5	17.85	11.70
	TROU06	2	240	49	41.9	21.8	45.5	7.0	4.4	6.4	5.3	8.0	46.0	17.45	11.40
	TROU13	1	240	52	46.3	23.4	47.3	6.2	4.2	7.5	6.7	8.0	54.5	18.30	11.40
	TROU14	1	268	56	53.4	25.6	56.3	7.9	4.7	8.2	6.8	8.5	56.5	21.55	13.80
	TROU15	1	267	52	49.7	27.6	56.1	8.4	6.1	7.7	5.7	11.7	53.0	20.50	13.00
	TROU16	2	244	58	43.8	22.5	46.0	7.7	4.7	8.1	6.7	8.4	47.0	18.20	11.55
	TROU18	1	278	61	53.7	25.8	53.8	9.1	5.6	8.2	6.4	9.2	55.0	20.95	13.75
	TROU17	2	240	64	42.1	23.1	48.3	6.7	4.5	8.4	6.2	8.5	48.5	17.40	11.30
	TROU19	2	278	60	46.7	24.4	52.4	8.4	4.9	8.2	5.4	9.4	55.5	19.50	12.30
	TROU04	2	264	60	43.1	24.4	49.3	5.8	5.9	7.4	6.3	8.2	48.5	17.05	12.15
	TROU20	2	250	57	43.7	21.0	47.1	7.4	4.6	7.1	6.1	10.0	49.0	18.20	11.55
	TROU21	1	240	60	47.2	23.1	49.3	7.8	5.2	7.9	6.7	9.1	55.5	18.50	11.50
	TROU30	2	217	53	34.0	17.8	41.0	5.4	3.9	5.4	5.0	8.8	41.5	14.20	9.50
	TROU22	2	265	61	46.9	23.1	50.1	8.3	4.8	7.8	5.7	8.6	51.0	18.65	12.15
	TROU23	1	273	63	51.2	24.8	53.5	8.0	5.7	6.5	.	.	56.0	20.25	13.30
	TROU24	2	260	67	43.6	21.0	45.8	7.7	5.6	7.7	7.0	9.3	47.0	18.20	11.85
	TROU25	1	272	62	52.2	25.9	56.3	8.6	5.5	9.1	6.9	9.9	54.0	20.35	13.40
	TROU26	3	174	39	29.8	15.7	32.6	5.6	3.0	4.8	3.5	5.7	37.0	13.65	9.35
	TROU27	2	237	55	39.6	19.4	42.0	6.7	4.2	5.3	5.6	8.4	42.0	17.00	11.50
	TROU28	1	254	61	47.1	21.2	49.6	8.0	4.6	5.8	7.0	8.1	55.0	19.00	11.80
	TROU29	2	258	60	44.1	22.1	44.8	7.9	4.8	5.2	6.3	9.2	48.0	18.70	11.90
Stansbury	R12054	1	242	.	45.4	23.7	45.4	7.0	5.5	7.2	7.0	9.2	.	18.40	12.05
Pine Point	R12050	1	280	56	49.3	25.3	52.5	9.3	7.5	8.5	7.0	10.2	.	20.55	13.55
Minlacowie	R12051	2	265	59	43.2	22.3	48.6	7.7	4.4	6.8	6.7	9.3	.	18.15	11.90
Laura	R15434	2	164	34	31.1	15.2	33.7	6.0	3.7	5.8	5.3	6.0	.	12.75	8.65
Kimba area	R20797	.	235	37	39.8	18.7	42.9	6.4	3.8	6.6	5.6	7.0	.	14.90	10.15
Whyalla	R27311	1	295	56	60.3	27.3	67.7	11.3	5.8	8.1	7.8	10.8	.	24.15	16.45
Kimba area	R27315	1	280	50	60.3	26.1	58.2	10.7	6.1	8.4	7.5	11.2	.	23.50	15.40
Cleve	R20587	.	263	58	56.8	25.7	56.0	9.7	4.7	8.2	6.7	10.3	.	21.15	13.95
Wanna	R30483	2	278	58	46.6	24.4	49.9	8.6	5.4	7.5	7.5	8.8	.	18.70	12.60
	R30484	2	285	59	47.8	23.1	49.5	8.7	6.2	6.6	6.9	10.5	.	18.65	12.15
Goose Is	G01	2	322	64	50.5	24.0	52.0	8.6	5.7	8.1	8.5	10.5	50.0	19.95	.
Colona Stn	R26361	1	290	71	58.4	25.0	59.8	7.3	5.6	8.2	10.2	10.5	.	22.50	15.90

APPENDIX 5

Raw data for the meristic characters for all *T.rugosus* included in the analyses. Data included (in order of collumn) are locality of collection, collection number (either the S.A. Museum register number or the field number), SLL, SLR, ILL, ILR, SCL, SCR, UPL, UPR, LPL, LPR, MELD, MELV. See Table 2.2 for the key to character abbreviations.

St. Peter	R21944	9	10	11	11	4	5	10	9	11	9	26	29	40	50
	R31477	10	10	10	11	3	3	11	11	10	8	24	26	98	40
	R31478	10	11	11	12	3	4	10	8	10	9	21	28	98	40
	ST-P03	11	10	10	10	4	4	12	13	11	11	19	26	30	30
	ST-P04	10	10	11	12	3	3	14	11	10	10	23	31	50	50
	ST-P05	10	12	10	11	3	3	.	.	.	.	23	29	20	50
	ST-P06	10	10	10	10	4	4	11	10	11	12	23	26	40	55
	ST-P07	10	11	10	12	4	4	11	12	9	10	23	32	40	30
	ST-P08	10	10	9	10	3	4	10	10	11	11	22	29	40	30
	ST-P09	9	9	9	9	4	4	12	12	10	11	23	29	20	40
Ceduna	R31472	9	9	9	9	3	3	10	9	8	8	21	28	95	70
	R31473	10	10	11	10	4	3	9	8	10	9	21	23	98	60
Streaky	R16933	9	8	10	11	.	.	.	.	.	.	21	.	10	10
Flinders	R31301	10	9	10	9	4	4	9	9	11	10	21	24	95	40
	R31302	9	9	8	10	5	4	11	11	9	9	22	24	90	30
	R31303	11	11	11	11	4	4	9	9	9	9	23	24	95	20
	R17275	10	10	10	10	4	4	11	11	10	11	21	26	90	50
Minnipa	R28378	11	12	10	10	4	4	11	11	11	10	24	24	20	60
	R28379	10	10	10	9	4	4	11	12	12	11	22	28	80	70
	R28384	9	10	9	9	3	3	10	10	10	10	22	23	10	40
	R28404	11	11	11	10	3	4	11	11	10	10	22	27	60	40
	R28405	11	11	11	11	4	4	10	11	12	12	22	24	10	50
	R28406	10	12	11	10	3	4	10	10	.	.	23	25	50	60
	R28407	12	13	10	10	.	4	12	10	10	9	23	27	70	80
	R28470	9	10	11	11	3	3	10	11	10	11	20	25	30	50
	R28476	10	10	10	10	3	3	11	12	12	12	21	27	30	70
	R28507	10	11	10	11	3	3	10	11	10	11	22	25	10	90
	R28593	10	11	9	9	4	3	8	9	9	9	20	25	10	30
	R28594	11	10	10	10	4	4	8	11	10	10	19	25	40	80
	R28595	10	10	12	11	4	4	11	11	10	10	23	25	20	70
	R28602	10	10	11	10	4	4	12	10	11	11	22	28	30	75
	R28603	10	10	9	9	4	4	11	12	12	12	20	28	5	5
	R28604	10	10	9	10	2	3	10	10	9	12	21	25	30	10
	R28605	10	9	10	10	3	3	10	10	11	11	20	24	20	40
	R29206	11	11	10	10	4	4	12	12	13	11	25	27	20	10
Lincoln NP	R30485	10	10	10	10	4	4	13	12	11	11	23	24	95	60
	R30486	10	11	12	11	4	4	11	11	11	11	24	23	5	30
Pt Lincoln	R4043	12	10	12	11	4	4	11	10	11	12	22	25	40	40
Duffield	R22771	10	12	11	12	4	5	13	12	10	10	27	26	99	80
	R30468	9	10	10	11	4	4	10	10	11	10	24	27	98	50
	R30469	10	10	11	10	4	4	10	11	10	11	25	25	98	60
	R30470	10	10	9	10	4	4	10	10	11	10	26	26	70	70
	R30471	10	11	11	11	3	.	11	11	12	8	27	28	99	50
	R30472	11	11	9	11	4	4	11	8	8	11	26	27	95	40
	R30473	.	.	10	11	1	3	10	6	7	9	26	26	99	60
	R30474	.	.	11	10	.	.	2	8	.	.	26	29	80	60
	DUFF38	9	9	11	10	4	4	9	9	10	11	27	29	95	80
	DUFF35	10	10	10	11	3	3	10	10	.	11	24	28	99	75
	DUFF39	10	10	10	10	4	3	10	10	11	10	24	29	90	60
	DUFF12	13	10	10	11	3	3	12	11	10	10	24	28	95	75

	DUFF33	9	.	10	.	4	2	11	12	11	7	26	26	99	65
	DUFF21	10	11	10	10	3	4	10	10	10	9	23	26	95	50
	DUFF40	10	10	10	10	3	4	11	9	12	12	24	27	95	30
	DUFF24	10	10	9	10	4	4	10	10	12	11	26	27	99	80
	DUFF41	10	9	9	9	5	3	11	11	11	11	26	29	99	50
	DUFF42	10	10	11	10	4	4	9	12	11	11	27	26	95	75
	DUFF14	.	.	10	10	.	.	11	11	11	11	24	28	90	45
	DUFF43	10	11	11	10	4	5	10	12	11	11	22	28	95	50
	DUFF44	11	10	10	10	4	2	11	11	12	12	24	30	99	60
	DUFF45	11	10	10	10	2	2	9	10	10	10	25	29	80	60
	DUFF46	6	12	10	10	3	4	12	12	12	12	26	25	80	70
	DUFF47	10	10	10	11	3	3	12	11	11	7	23	27	90	70
	DUFF48	12	9	10	11	4	3	13	12	12	11	24	29	90	50
	DUFF49	10	10	10	10	2	4	11	12	12	10	23	30	95	40
	DUFF52	10	10	10	10	4	4	11	10	9	7	25	26	90	50
	DUFF23	11	12	11	10	4	4	10	10	12	13	26	29	95	80
	DUFF50	11	12	11	11	3	2	12	9	12	13	27	30	90	40
	DUFF02	12	10	10	10	4	4	13	10	10	13	24	27	95	60
	DUFF51	11	11	10	10	2	3	10	12	11	11	26	27	95	60
	DUFF19	.	10	11	12	3	4	13	11	11	10	24	31	95	65
Spilsby	R30475	11	10	10	9	4	4	13	12	10	11	25	26	90	60
	R30476	10	11	10	10	4	4	12	12	11	11	25	27	90	70
	R30477	11	11	10	10	4	4	13	11	12	11	28	24	90	70
	R30478	10	10	11	10	4	4	10	11	12	13	26	29	95	90
	R30479	10	11	9	10	4	3	11	11	12	13	26	26	90	95
	R30480	11	11	10	10	4	4	11	11	10	11	27	25	99	90
	SPLY0A	11	12	12	11	4	4	10	10	9	8	27	26	99	90
	SPLY01	11	11	11	11	4	4	12	9	9	10	27	26	99	90
	SPLY02	11	10	10	11	3	3	11	11	10	10	27	27	95	95
	SPLY0B	11	12	10	10	3	4	9	11	11	12	28	27	95	70
	SPLY03	11	10	10	10	4	4	10	9	12	11	26	29	98	90
	SPLY04	11	11	10	10	4	4	12	9	9	9	27	30	90	60
	SPLY05	10	10	11	10	4	4	12	11	10	11	26	29	95	90
	SPLY06	11	11	10	10	4	4	10	10	11	11	27	28	95	70
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	SPLY08	10	10	10	10	4	4	11	10	10	11	27	29	98	80
	SPLY09	10	10	9	9	4	4	9	10	11	12	29	28	20	70
	SPLY10	11	9	11	11	2	4	10	12	10	9	24	26	99	95
	SPLY11	10	10	9	9	4	4	10	10	11	11	27	27	90	90
	R31474	10	9	10	10	6	4	8	10	11	10	25	27	98	70
	SPLY13	11	10	10	10	4	4	11	11	11	10	25	31	95	70
	R31475	10	10	10	11	4	4	11	11	11	10	26	28	95	60
	SPLY16	11	10	10	11	4	4	12	13	13	12	25	26	90	90
	SPLY17	12	10	11	12	4	4	12	13	12	14	27	29	90	65
Reevesby	R18591	8	11	10	10	3	4	10	10	10	9	24	26	99	75
	R18592	8	9	8	9	5	4	11	11	9	10	27	25	99	75
	R18593	9	9	9	9	4	5	11	12	9	10	24	24	95	75
	R18594	11	12	10	11	5	4	12	11	10	10	28	26	95	60
	R30584	11	11	9	10	5	4	11	13	12	10	25	25	95	60
	R30585	8	10	9	8	5	4	12	13	10	11	28	28	95	60
	R30586	12	12	10	12	5	6	13	11	12	11	27	26	60	70
	R30587	10	9	10	11	4	4	12	11	11	11	25	26	60	70
	R30588	11	10	11	11	5	5	12	11	8	11	26	27	95	70
	R30589	10	9	10	9	4	4	12	12	12	11	27	26	60	80
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	R30593	11	12	11	11	5	4	11	11	10	10	25	27	95	70
	R31554	12	12	11	12	4	5	11	10	12	10	26	27	95	70

	R31482	10	11	10	11	4	4	.	.	.	.	26	24	95	60
	R30590	11	12	11	10	7	6	12	11	11	10	27	27	99	65
	REEV48	11	10	11	11	3	4	11	11	11	11	25	27	90	40
	REEV50	11	11	10	10	4	4	12	11	10	10	29	29	95	60
	REEV49	10	11	11	11	2	4	11	11	8	10	28	28	90	75
	REEV51	10	13	11	11	2	2	8	8	7	7	24	27	95	60
	REEV52	10	10	11	11	4	4	11	10	10	11	26	28	60	50
	REEV53	11	10	12	12	5	5	11	12	9	11	26	23	90	70
	REEV54	11	10	11	12	2	1	11	12	10	7	25	27	95	75
	REEV55	11	10	11	10	1	1	11	12	8	10	24	27	95	65
	REEV56	11	12	12	12	3	2	11	13	11	11	27	28	95	70
	REEV57	11	11	10	11	4	4	10	10	9	9	25	27	20	70
	REEV58	11	10	10	11	4	3	12	12	12	12	26	26	95	65
	REEV59	12	11	12	12	2	1	12	11	11	10	26	26	95	75
	REEV60	10	10	11	12	4	4	10	9	9	11	25	30	95	60
	REEV61	10	11	10	10	4	4	11	10	13	12	25	29	40	75
	REEV62	11	12	10	10	4	4	11	12	10	10	26	27	70	60
	REEV63	11	11	11	12	4	4	10	11	11	11	24	27	90	75
	REEV64	9	12	11	11	2	3	13	13	12	11	25	26	70	70
	REEV65	10	11	11	11	4	1	13	12	13	12	26	29	80	75
	REEV66	11	12	12	11	3	3	11	12	12	11	24	26	80	70
	REEV67	10	11	10	11	3	1	12	12	13	11	26	29	80	75
	REEV68	10	9	10	10	4	4	12	13	11	11	25	25	90	60
	REEV69	11	11	10	10	2	1	14	13	12	13	23	27	60	75
	REEV70	10	10	10	11	4	4	13	11	11	11	24	29	20	80
	REEV71	11	12	12	12	5	5	12	11	12	12	23	28	20	50
	REEV72	11	10	12	11	4	4	12	10	10	11	25	25	20	65
	REEV73	10	11	10	10	2	4	11	11	10	10	24	26	95	80
	REEV74	13	10	10	10	3	3	13	13	13	10	27	28	90	80
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	REEV76	10	12	11	12	4	4	12	12	11	11	24	27	20	70
	REEV77	13	14	12	11	4	3	14	13	12	13	26	28	95	70
	REEV78	11	11	9	11	4	4	12	12	11	12	27	26	50	60
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	R18403	9	10	10	10	4	4	8	11	10	9	22	24	30	40
	R18404	9	9	10	11	3	3	12	14	11	13	24	24	60	50
	R18405	10	11	10	9	3	3	11	10	10	10	21	22	40	30
	R18406	10	10	9	11	3	3	11	12	10	11	22	24	40	60
	R30481	9	10	10	11	4	5	11	10	9	10	21	28	60	70
	R30482	9	9	9	9	3	3	10	9	10	10	21	24	30	70
	TB01	10	11	10	11	4	4	9	8	8	9	20	26	95	50
	TB02	11	11	11	10	4	3	9	10	8	9	22	26	90	40
	TB03	9	10	11	11	3	3	10	10	11	10	22	26	99	50
	R31555	10	10	10	9	5	4	10	10	10	10	22	27	95	30
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	R31557	10	10	10	11	4	3	10	11	13	13	23	23	99	40
	R31558	11	11	10	10	4	4	10	10	12	12	23	24	95	40
	R31559	10	10	10	10	3	4	11	11	9	10	23	23	99	80
	TB09	10	9	10	10	3	3	9	9	10	10	23	26	90	60
	TB10	10	10	12	12	4	5	11	10	11	11	22	22	95	70
	TB11	9	10	11	11	3	3	9	10	9	11	22	25	10	60
	TB12	10	11	11	10	4	3	11	10	12	12	18	26	10	50
	TB13	9	9	10	10	3	3	10	11	12	12	21	25	40	55
	TB14	11	11	9	9	2	3	10	10	10	10	18	27	30	40
	TB15	11	11	11	10	3	4	10	10	10	11	20	23	40	30
	TB16	10	9	10	10	3	4	9	9	10	11	19	29	80	70
	TB17	10	10	10	10	4	4	11	9	11	11	23	27	10	25
	TB18	9	9	10	10	4	4	11	11	11	10	19	21	40	40

	TB19	11	11	10	10	4	4	10	11	11	11	20	25	50	70
	TB20	9	9	9	9	3	3	10	9	9	9	22	24	70	70
	TB21	10	10	10	10	4	4	10	11	12	13	19	26	0	30
	TB22	9	10	10	11	3	3	9	9	10	10	21	26	95	50
	TB23	10	10	12	12	4	4	12	12	11	10	23	27	60	40
	TB24	9	9	9	11	3	3	10	11	9	9	23	23	50	30
Iron Knob	R28392	10	10	10	9	3	3	10	10	10	10	22	26	95	60
	R28395	10	10	11	9	4	4	9	9	10	9	24	29	60	60
	R28396	11	11	10	11	4	4	10	10	10	11	20	26	10	40
	R28589	11	11	11	11	4	4	10	11	11	11	24	26	10	60
	R28590	10	10	10	11	3	3	11	10	10	11	24	30	50	70
	R28591	10	11	11	11	4	4	11	11	10	10	22	27	10	60
	R28592	9	9	10	10	4	3	11	10	11	11	20	24	50	60
Tulka	R18610	10	9	9	9	3	3	9	10	9	10	23	26	60	70
Weeroona	WERA01	9	10	11	12	5	4	10	10	10	11	18	27	60	50
	WERA02	10	10	11	11	4	4	9	9	7	7	23	25	30	50
	WERA03	9	9	10	10	4	5	12	11	11	10	21	28	50	50
	WERA04	10	11	11	13	5	5	9	9	9	9	23	27	40	40
	WERA05	10	10	10	10	3	3	9	9	9	10	20	22	30	50
	WERA06	11	11	12	11	4	4	9	11	10	9	22	30	80	70
	WERA07	11	11	10	11	4	4	8	9	10	10	19	26	30	70
	WERA08	11	10	7	8	3	5	11	12	10	11	21	24	30	40
	WERA09	9	10	10	10	5	4	11	10	11	10	21	27	30	40
	WERA10	11	10	10	9	4	4	8	8	7	9	22	28	50	50
	WERA11	12	12	11	11	4	4	9	10	9	9	22	28	30	60
	WERA12	11	11	9	10	4	5	10	10	10	10	21	24	70	80
	R31619	9	9	10	10	4	4	11	11	10	11	21	26	50	70
	R31618	10	10	11	10	4	4	9	10	10	11	22	26	70	80
	R31622	10	9	10	11	5	5	11	10	8	10	21	26	95	80
	WERA16	10	9	10	10	4	4	10	8	10	12	21	27	70	60
	WERA17	10	11	11	11	4	4	10	11	11	10	22	26	20	80
	WERA18	11	10	10	12	4	4	9	8	8	9	19	26	40	60
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	WERA21	9	11	10	11	3	3	9	9	11	10	20	26	70	60
	WERA22	9	9	10	10	4	4	9	9	9	8	21	28	60	90
	WERA23	10	10	10	10	4	4	11	9	9	10	20	23	20	20
	WERA24	10	10	9	10	4	4	10	11	12	12	19	26	40	60
	WERA25	11	11	10	9	4	4	9	11	10	11	21	24	60	80
	WERA26	10	10	11	12	3	2	9	9	9	9	21	25	50	60
	WERA27	9	9	10	10	4	4	10	11	9	9	20	26	50	80
	WERA28	11	11	12	12	4	4	11	11	9	10	20	27	80	60
	WERA29	11	11	12	11	4	4	10	11	11	11	22	31	60	75
	WERA30	11	11	10	10	3	4	9	10	10	10	20	28	70	60
Port Pirie	R31620	10	9	10	11	4	4	10	9	10	10	20	24	40	60
	R31623	10	10	9	9	3	4	11	10	10	11	18	25	80	60
	R31621	9	9	9	9	4	4	10	9	9	10	19	25	90	95
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	NRWE09	9	9	10	11	3	3	10	10	10	10	20	23	99	75
	NRWE10	10	11	11	11	4	4	11	11	10	10	21	24	30	80
	NRWE11	10	10	10	10	4	4	11	11	10	10	23	26	50	90
	NRWE12	10	11	10	10	3	4	11	11	12	11	23	25	20	20
	NRWE13	10	11	10	10	3	4	10	10	9	9	24	27	99	75
	NRWE14	11	10	10	11	4	3	10	10	11	12	22	28	40	50
	NRWE15	11	11	11	12	4	4	9	11	9	10	22	25	40	40
	NRWE16	10	10	10	9	4	4	10	10	9	9	21	26	10	80
	NRWE06	10	9	11	11	4	3	12	11	11	12	23	27	10	40
	NRWE05	11	11	10	10	3	4	11	11	11	12	20	24	10	55

APPENDIX 5.

	NRWE04	10	9	10	11	3	4	9	10	9	10	21	23	90	75
	NRWE07	11	10	11	11	3	4	6	11	10	9	23	25	30	60
Pt. Pearce	YP01	11	11	10	10	4	4	12	9	11	10	21	24	80	60
	YP02	9	10	11	10	4	4	9	9	9	8	23	20	80	50
	YP03	11	11	11	11	4	3	11	10	10	11	24	23	40	50
	YP04	10	10	9	8	5	5	9	9	6	8	22	24	99	90
	YP05	10	10	10	11	3	3	8	10	11	11	21	21	95	80
	YP06	10	9	11	11	4	4	12	12	11	11	22	24	40	50
	R31587	10	10	10	10	3	3	9	9	10	9	20	25	50	60
	R31588	10	11	10	11	3	4	10	9	10	10	22	22	40	70
	R30391	9	9	9	9	3	3	10	9	7	7	23	25	30	60
	R30392	10	10	11	11	4	4	12	11	11	11	22	27	30	60
	R30390	10	10	10	10	4	4	9	10	10	10	20	26	10	40
	R30393	9	9	11	13	4	4	9	7	9	10	20	21	30	60
	R30394	13	13	12	10	4	3	12	11	10	12	21	21	60	70
	R30395	11	12	10	11	4	4	11	11	12	13	22	20	10	75
	R30396	10	10	11	11	3	3	9	10	11	10	26	27	20	70
	R30397	9	10	10	9	3	3	9	9	9	9	21	25	10	60
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	PTP10	9	10	10	11	4	3	10	10	10	10	21	23	30	30
	PTP11	10	10	11	10	3	4	12	11	13	13	25	25	20	30
	PTP12	9	9	10	10	3	4	12	12	11	12	21	23	20	30
	PTP13	11	10	10	10	4	4	12	13	11	12	25	25	95	70
	PTP14	10	11	10	10	4	4	13	13	10	11	23	23	70	40
	PTP15	10	9	9	9	3	3	10	10	10	9	19	24	30	60
	PTP16	10	10	9	11	4	4	10	9	9	10	20	28	70	70
	PTP17	10	9	10	10	4	4	10	10	12	12	21	25	30	60
	PTP18	9	9	10	9	4	3	10	9	9	10	22	25	80	50
	PTP19	10	10	10	9	3	3	9	10	10	9	20	22	50	30
	PTP20	10	10	9	9	4	4	9	8	9	9	21	22	30	40
	PTP21	9	10	10	10	4	4	9	9	10	12	22	24	20	30
	PTP22	11	11	11	9	3	4	13	10	11	12	24	27	20	40
Wardang	WRD011	11	11	10	10	3	3	9	10	10	10	21	22	40	50
	WRD212	10	9	9	10	4	3	12	11	10	10	21	23	40	40
	WRD213	10	9	10	9	4	4	11	10	9	8	21	23	60	60
	WRD214	10	11	10	10	4	4	11	12	10	10	22	26	80	20
	WRD215	10	10	9	9	3	4	13	10	10	10	22	26	30	60
	WRD216	10	10	10	10	4	4	12	11	11	11	21	24	50	60
	WRD217	11	10	9	9	3	3	10	10	9	10	21	24	50	50
	WRD218	11	9	9	11	3	4	12	11	10	11	21	26	40	50
	WRD219	11	12	9	11	4	4	8	9	11	11	23	24	60	50
	WRD220	9	9	9	10	3	3	11	11	11	11	21	23	70	80
	WRD221	9	8	12	11	3	4	11	11	10	10	24	28	90	70
	WRD???	10	11	10	10	4	4	11	12	10	11	21	23	20	20
	WRD222	9	9	9	9	3	3	9	9	10	10	22	23	30	50
	WRD094	10	10	9	12	4	3	9	9	10	9	21	21	80	60
	WRD135	10	10	10	10	3	3	11	11	10	11	22	25	40	70
	WRD223	10	10	11	10	4	4	11	10	9	10	21	24	50	40
	WRD099	9	10	10	11	4	3	11	11	9	9	20	24	50	50
	WRD224	11	11	11	9	3	4	9	10	9	9	20	25	90	70
	WRD225	10	10	11	11	4	5	11	10	11	10	21	23	60	70
	WRD226	11	10	10	11	4	4	10	11	10	9	22	22	80	80
	WRD160	10	10	9	9	3	3	9	10	9	11	21	22	60	60
	WRD227	9	9	9	9	4	4	11	12	12	13	21	27	50	50
	WRD178	9	9	9	10	3	3	12	10	11	9	20	24	90	50
	WRD109	11	10	10	10	3	3	11	10	11	11	20	24	40	70
	WRD122	10	10	11	10	4	3	11	10	10	11	21	25	60	60
	WRD228	10	9	11	11	4	4	10	11	12	12	21	21	30	70

	WRD229	10	10	10	10	3	4	10	11	11	11	21	26	90	50
	WRD031	11	11	11	11	4	4	10	9	10	10	19	22	20	40
	WRD032	9	9	9	10	4	4	10	9	10	8	20	23	95	50
	WRD232	11	11	10	10	3	4	11	12	11	11	21	24	40	50
	WRD231	9	9	10	10	3	3	12	10	12	11	21	24	90	60
	WRD230	11	10	11	12	5	4	11	11	11	11	23	23	95	80
	WRD233	10	10	9	10	3	3	11	12	10	12	20	21	40	60
	WRD234	10	9	8	9	3	3	10	10	9	10	21	22	60	50
	WRD113	10	9	10	10	3	4	9	9	10	11	24	25	20	50
	WRD235	10	10	10	9	4	4	10	9	9	10	22	22	50	40
	WRD123	8	8	9	9	3	4	10	11	10	11	20	22	60	70
	WRD251	10	10	9	10	3	3	11	10	11	11	21	24	80	50
	WRD252	9	8	9	9	3	3	10	10	11	10	19	25	20	40
	WRD253	10	11	10	10	3	3	10	9	10	10	20	22	60	70
	WRD254	10	11	11	9	3	3	12	11	13	13	23	22	20	60
	WRD255	10	11	9	10	3	3	9	11	10	11	21	24	80	35
	WRD256	10	10	11	11	4	4	10	9	6	8	22	23	90	25
	WRD194	10	9	9	9	3	2	11	11	12	11	21	21	40	40
	WRD248	10	10	10	10	3	3	10	9	10	7	19	21	80	20
	WRD249	10	10	11	11	4	4	12	12	10	10	22	29	70	70
	WRD250	10	9	8	9	4	4	10	9	10	11	20	23	90	50
	R31594	9	9	9	10	3	4	9	11	12	11	19	21	98	80
	R31595	12	12	11	10	4	4	13	12	11	12	24	26	60	30
	R31596	11	10	11	12	4	4	12	11	13	12	19	25	20	30
	WRD283	10	9	10	10	3	3	10	9	9	8	20	24	95	40
	R30388	9	9	9	9	3	3	11	11	12	12	21	24	10	40
	R30389	9	10	11	11	4	3	12	13	13	13	22	26	30	50
	R31037	9	10	9	9	4	4	9	10	10	10	18	25	70	60
	R31038	9	10	11	11	4	4	12	15	16	13	26	25	90	50
	R31479	11	11	10	9	.	.	.	.	.	.	.	.	.	.
	R31480	12	11	11	11	4	4	.	.	.	.	25	25	95	60
	R31481	11	11	10	10	4	3	.	.	.	.	23	24	50	60
Pt Vict	R1393A	12	12	11	11	3	3	12	10	12	11	21	.	.	.
	R1393B	9	9	9	9	3	3	.	10	.	.	24	.	.	.
	R1393C	10	10	8	10	4	4	11	13	10	11	24	.	.	.
	R1393D	10	10	10	9	3	4	12	12	12	12	22	.	.	.
	R1393E	9	10	9	10	4	4	11	11	10	10	23	.	.	.
	R1393F	10	10	11	11	4	4	10	11	12	11	19	.	.	.
	R1393G	10	11	10	11	5	5	12	13	12	12	20	28	.	.
	R1393H	10	9	10	10	4	4	11	10	10	11	23	.	.	.
	R1393I	11	11	9	10	3	3	11	12	12	11	21	.	.	.
	R31577	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	R31592	10	11	10	10	4	4	10	10	10	11	20	25	50	60
	R31593	11	11	11	12	4	4	10	12	12	12	22	26	40	60
Maitland	R1256	11	11	11	11	4	4	11	13	14	12	21	26	30	50
Minlaton	R31589	10	9	10	11	4	4	10	11	11	11	21	27	90	40
Innes NP	R16972	10	9	10	11	4	4	12	12	12	11	23	25	40	60
Edithburg	R31578	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	R31590	10	11	12	12	3	3	10	11	10	12	22	24	40	40
	R31591	9	10	10	11	5	5	11	11	11	11	20	26	50	35
Troubridge	R31419	9	9	9	9	3	3	8	10	8	7	22	24	99	60
	R31418	8	9	10	9	4	4	7	5	4	6	21	24	99	50
	R20819	9	9	9	8	3	3	9	8	8	9	19	21	20	40
	TROU07	10	10	8	8	2	2	10	10	10	9	21	25	20	75
	TROU08	10	11	9	9	4	3	10	11	11	11	19	24	40	50
	TROU09	9	10	8	9	4	3	10	10	5	9	18	23	80	70
	TROU01	13	11	10	10	4	3	11	10	11	9	19	22	15	40
	TROU10	11	8	10	9	2	3	10	9	9	8	20	24	70	40

	TROU02	11	.	11	12	4	4	10	9	9	10	20	22	20	30
	TROU11	9	9	9	9	4	3	11	11	10	11	20	22	30	40
	TROU12	10	11	9	9	3	3	10	10	10	11	20	22	30	40
	TROU06	9	11	10	9	5	4	8	10	.	.	19	27	20	40
	TROU13	11	11	10	11	4	4	12	12	12	11	18	25	20	50
	TROU14	12	10	9	9	4	4	10	11	10	.	22	27	10	50
	TROU15	10	8	9	9	2	3	11	11	12	11	20	22	40	40
	TROU16	10	9	10	10	2	1	11	11	12	9	22	23	30	50
	TROU18	10	10	9	10	.	.	11	9	.	.	19	23	0	50
	TROU17	11	.	10	10	2	3	10	10	10	11	18	21	30	30
	TROU19	9	11	8	8	3	4	9	10	9	10	22	23	60	40
	TROU04	9	10	8	9	3	3	10	8	.	.	21	25	20	60
	TROU20	11	11	10	10	2	4	10	10	9	8	19	24	10	40
	TROU21	10	9	10	10	5	5	11	11	10	11	20	24	10	30
	TROU30	10	10	8	10	4	3	12	10	10	9	19	25	30	30
	TROU22	11	11	8	9	4	3	7	8	1	1	20	24	5	30
	TROU23	11	10	11	11	1	2	9	12	12	10	20	24	30	70
	TROU24	9	11	9	8	4	2	11	10	9	10	20	24	0	50
	TROU25	11	11	10	10	4	4	11	11	11	13	19	26	30	60
	TROU26	11	11	9	9	4	4	11	10	10	10	18	26	10	40
	TROU27	10	9	8	9	5	4	8	8	10	10	22	25	10	30
	TROU28	9	9	10	10	2	2	7	5	.	9	20	26	10	30
	TROU29	10	9	9	9	3	3	9	9	8	10	20	26	10	60
Stansbury	R12054	10	10	9	9	4	4	10	10	8	9	22	23	30	40
Pine Point	R12050	11	10	9	9	3	3	10	10	10	10	23	25	20	50
Minlacowie	R12051	11	11	9	9	3	4	10	10	11	10	23	25	60	40
Laura	R15434	9	10	10	10	4	4	10	10	10	11	23	25	10	50
Kimba area	R20797	10	10	10	10	3	3	11	8	11	11	19	26	10	0
Whyalla	R27311	9	10	8	9	3	3	10	9	10	10	25	26	95	75
Kimba area	R27315	11	10	12	11	3	4	10	11	12	11	23	26	10	5
Cleve	R20587	10	11	10	11	4	4	10	11	10	10	21	25	50	50
Wanna	R30483	10	9	10	9	4	5	12	12	11	11	23	22	95	40
	R30484	10	10	10	10	4	3	12	12	13	12	24	28	70	45
Goose Is	G01	10	10	9	9	4	4	10	10	12	12	22	26	98	95
Colona Stn	R26361	9	10	9	10	4	4	10	9	10	10	24	28	50	40