

Report to ACT Parks and Conservation Service, 1994

HABITAT SPECIALISATION AND THE ISOLATION OF REMNANT
POPULATIONS OF THE STRIPED LEGLESS LIZARD, *DELMA IMPAR*
(PYGOPODIDAE).

Helen Osmond

Division of Botany and Zoology
Australian National University

Abstract

The preferred habitat of *Delma impar*, lowland native grassland, has undergone extensive fragmentation this century through urban and agricultural development, and further disruption is inevitable. It is of interest to know whether the fragmentation of habitat effectively sub-divides populations of the lizard by obstructing migration, and gene flow, between remnant groups. Little is known about migratory behaviour in *D. impar*, primarily because it has proved difficult to track individuals. Dispersal ability may be minimal given that the species appears to be somewhat fossorial in habit, a lifestyle which is characterised by very restricted activity. An examination of the physiological qualities of fossorial lizards provides information on the physiological mechanisms that correspond with the restriction or enhancement of activity under given conditions. With support from information regarding the genetic relations of populations through which patterns of gene flow can be assessed, traits indicative of activity potential may indicate migrational abilities of *D. impar*.

Get info on animals

The thermal requirements of *D. impar* were assessed by determining temperature preferences of the species in a thermal gradient. Preferred temperatures averaged 26.0°C. The species' energy requirements, and the thermal dependence of physiological functioning were examined by determining standard rates of metabolism at 15, 20, and 30°C. Rates observed were similar to rates expected for the species considering body mass, temperature, and state of activity. Values of Q₁₀ for rates of metabolism were 1.74 between 15 and 20°C and 2.31 between 20 and 30°C. Allozyme electrophoresis was employed to detect the genetic differentiation and genetic variability of populations. Values of genetic similarity were high and within the range reported for conspecific populations of reptiles. Victorian populations were distinct from ACT populations, and genetic differentiation of lesser extent was observed among ACT populations. The physiological and genetic attributes of *D. impar* were examined in conjunction with temporal and spatial patterns of activity and temperature in the field, and were compared with those occurring in fossorial and surface active lizards. Temperature preferences, rates of metabolism and levels of genetic variation in the species are intermediate of those characteristic of fossorial species and surface active species.

The results indicate that *D. impar* is a moderately active species and has physiological adaptation to a cool and relatively homogeneous thermal regime. Geographically distinct populations of *D. impar* show slight genetic differentiation indicating that obstruction to gene flow occurs, although results suggest that, in general, gene flow has been largely effective in maintaining genetic similarities of populations.

It is concluded that *D. impar* is not operationally confined to the sheltered environment of the ground layer and is capable of considerable surface activity with which geographically distinct populations may be connected. Specific thermal requirements, however, indicate that activity of *D. impar* may be less efficient in areas that are exposed to wide fluctuations in temperature, and suggests that environments which lack suitably sheltered microhabitats may be involved in the obstruction of individual migration between populations.

Contents

Chapter 1.	Introduction	1
1.1	Habitat specialisation	1
1.2	Fossorial lizards.....	2
1.3	Physiology	5
1.4	Genetic variation.....	7
1.5	The pygopodid, <i>Delma impar</i>	10
Chapter 2.	Physiology	10
2.1	Introduction	11
2.2	Methods	15
2.3	Results.....	16
2.4	Discussion.....	24
Chapter 3.	Genetics	24
3.1	Introduction	27
3.2	Methods	29
3.3	Results.....	34
3.4	Discussion.....	38
Chapter 4.	General Discussion.....	39
Acknowledgements	40	
Appendix 1.....		
References.....		

Chapter 1. Introduction

1.1 Habitat specialisation

The geographical range of any species can be characterised in terms of climatic regime, substrate type and biotic community. Habitat specialists use only a small subset of the range of environmental components available. In contrast, habitat generalists use a wider range of resources and experience more diverse environmental conditions

The qualities of organisms that enable them to survive and reproduce in their home environments are inherited by their offspring. In this way, populations adapt to their surroundings. Because habitat specialists are typically suited to fewer environmental features relative to habitat generalists, the adaptations of habitat specialists are probably less of a compromise in performance between habitat types (Brown 1990). As such, habitat specialists can be species highly adapted to the particular conditions to which they are exposed. This specific adaptation may place constraints on times and places of activity in habitat specialists. For reasons of general biological concern, the particular qualities that limit activity in species, and through which activity capacities may be assessed are of interest. Such information is essential for an understanding of the effects that various forms of habitat modification have on the relations of populations and, ultimately, on population persistence (Soule 1976). In particular, fragmentation of habitat is a widespread occurrence and must act in the sub-division and isolation of populations of species with restricted migratory capacity.

1.2 Fossorial lizards

The soil layer is a very sheltered and stable environment. Temperature and humidity, factors which play important roles in the structuring of communities (Begon et al. 1990), fluctuate slowly and slightly within the soil. As such, the soil layer can be considered a relatively homogeneous environment, and fossorial lizards living in this environment would be considered habitat specialists. The environmental heterogeneity experienced by fossorial species appears to be further limited by the low activity levels they exhibit. Fossorial lizards are described as having low 'vagility' (Gorman et al. 1977). Vagility

refers to the mobility of an animal - how energetically active it is and the spatial extent of its activities. Fossorial lizards appear to expend little energy in activity and are localised in their daily movements (Miller 1944; Huey et al. 1974).

If functional qualities of fossorial lizards, such as rates of metabolism or thermal tolerance ranges, could be characterised and measured, they could provide a reference from which the activity potential of lizards whose activity patterns were largely unknown could be inferred. The fossorial habit in lizards is manifest in morphological, as well as behavioural attributes. Fossorial lizards are smooth and elongate forms, displaying reduced limbs and head width (Greer 1989). These are specialisations which enable them to burrow through soil efficiently. However, these morphological traits are exhibited by other species which are largely surface active and highly mobile, including many snakes, and so are relatively uninformative about the specific ecologies of species. Although little work has examined fossoriality in lizards, there is evidence to suggest that fossorial lizards are distinct from surface-dwelling species in a number of physiological and genetic properties.

1.3 Physiology

Activity in ectotherms is highly sensitive to environmental temperature, being minimal at lower temperatures and increasing, within biological limits, as temperatures increase. An ectotherm's immediate ability to perform important behaviours which enable it to survive and reproduce (e.g. foraging, social relations, predator avoidance) is, therefore, dependent on the thermal climate it experiences. Accordingly, physiological consequences of the thermal environment may be expected to be conspicuous in ectotherms, and to vary among species of different ecology or climatic association. In particular, the level of thermal dependence of activity in fossorial lizards may be relatively distinct because the range of temperatures within the soil strata is narrower than that associated with surface environments, such that fossorial lizards experience a relatively homogeneous thermal climate and are not likely to access temperatures as high as those experienced by their surface active counter-parts (Withers 1981; Patterson 1990). For the maintenance of comparable activity periods (Clark 1969), one might expect the thermal dependence of activity to vary between surface active and fossorial ectotherms.

It is the thermal correlates of activity which have been the focus of physiological analyses in this study.

Until fairly recently, most work on lizards has been concerned with diurnal, surface active species largely from warmer environments. Only during the last decade or so has it been possible to put together a substantial amount of data on lizards of varying habit in order to see what ecological trends occur. Variations in physiological attributes are apparent among ecological taxa. In particular, the temperatures in which species prefer to reside, and rates of metabolism during inactivity show considerable differences between species, and a consideration of these has permitted the generation of hypotheses concerning the adaptive properties of differences in physiological functioning.

1.3.1 Temperature preferences

Most lizards, when allowed to thermoregulate behaviourally, are able to maintain their body temperature at or near a level typical of their species, the so-called 'eccritic' or 'preferred' body temperature (Stewart 1965; Licht et al. 1966; Johnson 1977; Bennett and John-Alder 1986; Robertson and Weatherhead 1992). Fossorial lizards show preference for relatively low temperatures. The results of Licht et al. (1966), Spellerberg (1976) and Bury and Balgooyen (1976) provided early evidence of an ecological differentiation of temperature preference among surface and fossorial lizards. Withers (1981) demonstrated in his work on the Scincidae, a lizard family containing both surface-active and fossorial species, that the differences in temperature preference can be largely independent of phylogeny.

In general, there is a trend for reptiles inhabiting cold environments to prefer relatively low temperatures. Nocturnal, aquatic, cryptozoic and fossorial species all tend to prefer relatively low temperatures compared with diurnal, terrestrial or arboreal species (Licht et al. 1966; Spellerberg 1976; Withers 1981; Bennett and John-Alder 1986; Hailey and Davies 1986; Arad et al. 1989; review in Greer 1989). Moreover, seasonal adjustments in temperature preference can occur, such that temperature preference increases as temperatures increase (van Damme et al. 1986). Thus, the temperature preferences of species seem matched to the environmental thermal regimes to which species are exposed.

The preferred temperature range of a species seems to be the range of temperatures adopted by individuals in the field during their active period (Licht et al. 1966; Bennett and John-Alder 1986; Patterson 1990; Huey 1991; Rosen 1991), and seems to specify temperatures that maximise physiological performance (Bennett 1980; Huey 1982). The effectiveness of digestion, reproduction and other important activities is presumed to depend greatly on the maintenance of preferred body temperatures (Spellerberg 1976;

Huey 1991). Thus, an examination of the temperature preferences of species provides information from which the temperatures required by a species for efficient operating may be inferred.

1.3.2 Metabolism during inactivity

Metabolism is the processing of energy for activity within an individual. When ectotherms are inactive, metabolism is maintained at a standard rate. The standard rate is largely determined by temperature and body mass (Bennett and Dawson 1976; Andrews and Pough 1985) - as temperature and body mass increase, so does standard metabolic rate. Bennett and Dawson (1976) and Kamel and Gatten (1983) noted that a number of fossorial lizards had standard rates of metabolism slower than expected considering body mass and temperature. Andrews and Pough (1985) found this pattern to be reasonably widespread among squamatic reptiles and one that may lack strong phylogenetic association. Furthermore, Withers (1981) noted that fossorial species may show a reduced thermal dependence of metabolism during activity. A similar pattern has been noted in the standard rates of metabolism of lizard species inhabiting cold environments, at low temperatures (Aleksuik 1971; Al-Sadoon and Spellerberg 1985; Al-Sadoon and Abdo 1989). The possession of a thermally independent metabolic rate would free an organism from metabolic fluctuations with minor changes in body temperature. This would be useful in ectotherms where variation in rates of metabolism were undesirable e.g. where activity occurs at temperatures near thermal limits of physiological responsiveness. The reduced thermal dependence of metabolic rate has been interpreted as a method by which activity levels could be maintained as temperatures cool (Withers 1981).

Thus, an analysis of metabolic rate during inactivity over a range of temperatures enables changes in metabolic costs and capacities that occur with variations in temperature to be determined, and shows how different environments can restrict activity in species in different ways and incur different energetic costs.

1.4 Genetic variation

Every individual has its own genetic identity, and all of the genetic resources present within individuals of a population constitute the genetic variation contained within that population. If individuals from different populations are able to migrate between groups

and interbreed, the genetic resources are spread amongst populations, and the genetic diversity present within those populations may be expected to be similar. Thus the estimation of the genetic similarities of populations can provide information on the migrational abilities of individuals. However, genetic variation is acquired and lost in numerous ways and these must all be considered if the genetic relations of populations are to be understood. Furthermore, because different methods used in the assessment of levels of genetic variation differ in the information they provide, effective assessment of genetic diversity depends on use of appropriate techniques and a consideration of their limitations.

1.4.1 Origin and maintenance of genetic variation

Genetic variation between individuals is derived from errors in the replication of DNA (Futuyma 1986). Genetic variation can also be acquired by populations through the introduction of foreign genes by individuals through sexual reproduction.

Genetic mutations are maintained where they are reproduced and inherited. Mutations are unlikely to persist where they reduce the survivorship or reproductive success of the organism in which they occur. Mutations are also lost through 'genetic drift', where, by chance, they are not passed on from parent to offspring. Populations can diverge, therefore, where different genes are of differential advantage to individuals according to the environment in which they occur, and where populations lose different mutations through genetic drift (Selander and Johnson 1973; Spieth 1974; Allendorf and Phelps 1981; Allendorf 1983, 1986; Chesser 1983; Selander 1983; Slatkin 1987). While it is rarely possible to determine the contribution of each process to the differentiation of populations (Kimura 1968; Kimura and Ohta 1971; Nevo 1976, 1978; Sarich 1977; Allendorf and Phelps 1981; Allendorf 1983; Nei 1983; Moritz and Hillis 1990; Nevo and Beiles 1992a, b), it is recognised that genetic drift becomes more important as the effective size of populations decreases because the fewer reproductive individuals there are, the less frequently each gene type occurs, and greater is the chance that gene types will not be reproduced (Soule 1976; Allendorf 1983; Futuyma 1986). Gene flow between populations effectively increases the number of individuals contributing to the population gene pool. Hence gene flow between populations acts to counteract their genetic divergence. The effectiveness of gene flow in homogenising the genetic identities of populations depends, however, on the force presented by the processes of selective advantage and genetic drift in relation to that of gene flow. Gene flow is expected to be less effective between populations that are separated by geographic distance or unsuitable environments (Thorpe 1982).

1.4.2 Measuring genetic variation

The genetic variation present within populations and the genetic differentiation among populations are examined using heritable markers provided by nucleic acids, chromosomes, and proteins. Different markers provide information at different levels, and the amount of time required to find and use each of them varies (see Moritz and Hillis 1990). For example, examination of chromosome number and structure is a relatively simple and quick procedure but it is not useful for assessing divergence between taxa which are either very similar or very different genetically because chromosome character is typically conservative among close relations, and different to the extent that comparison is uninformative between distantly related taxa. Comparison of nucleic acids, on the other hand, is a useful technique for detecting divergence between genetically similar taxa, but is very time-consuming and expensive. Examination of protein variants is quick and relatively cheap and has provided a lot of information on a variety of taxa concerning the genetic variability of conspecific and, at times, congeneric populations, and of taxa of more broad ecological classification (reviews in Selander and Johnson 1973; Bryant 1974; Avise 1975; Nevo 1978; Brown 1979; Hamrick et al. 1979; Avise and Aquadro 1982; Buth 1984; Ward et al. 1992). In particular, homologous protein analysis has proved particularly useful in the identification of genetic variability of conspecific lizard populations (Bezy et al. 1977; Gorman et al. 1977; Gorman and Renzi 1979; Miyamoto 1986; Milton 1990; Sarre 1989; Sarre et al. 1990), and further discussion here will be limited to analysis of genetic variation at the level of the protein.

Allozymes

Through chance gene duplication, the structure of functionally similar proteins may diverge. Gene duplication allows one copy of the gene to mutate without interfering in the genetic functioning of the organism (Ferguson 1980). Thus, multiple forms of a gene can persist within a group of interbreeding individuals. These gene variants are called 'alleles'. Individuals may contain either one or multiple allelic types at a locus. The frequencies with which each allele occurs in a population can be used to assess the genetic similarities of populations and the extent of gene flow occurring between them.

Individuals of different allelic constituent can be distinguished by using the technique allozyme electrophoresis. Proteins coded by allelic alternatives of a single gene locus (allozymes) differ in electrical charge, size or shape according to their particular amino acid characteristics (Lewontin 1974; Avise 1975; Nevo 1978; Thorpe 1982; Richardson et al. 1986). Allozyme electrophoresis is performed to segregate allozymes according to their mobility differences in charged, porous media.

The power of allozyme data to reflect genetic variation present within a population hinges on one major assumption (Richardson et al. 1986). It is assumed that variation within the structural portion of the genome reflects variation contained within the genome as a whole. Only a very small proportion of the genome encodes electrophoretically detectable proteins (Nevo 1978; Richardson et al. 1986), and so chances are high that estimates of genetic variation will be somewhat misrepresentative of genomic variability. As far as comparative analyses are concerned, however, allozymes provide a set of loci common to a variety of organisms among which relative levels of variation can be detected. Thus the use of allozyme electrophoresis for comparative studies of genetic variability can be informative.

1.4.3 Pattern

Low genetic diversity appears to be correlated with habitat specialisation in general (Selander and Kaufman 1973; Bryant 1974; Soule 1976; Nevo 1978; Brown 1979; Hamrick et al. 1979; Wyatt 1992) and with fossorial life-style in particular (Nevo and Shaw 1972; Nevo et al. 1974; Bezy et al. 1977; Gorman et al. 1977). Two processes could produce this pattern. One, individuals which are exposed to limited environmental heterogeneity may require only a limited genetic resource. Two, species which are wide-ranging organisms presumably encounter a greater number of potential mates than species composed of relatively sedentary individuals (Gorman et al. 1977; Chesson 1983; Selander 1983). Thus, the effective population sizes of species of high vagility may be greater. The smaller the population, the greater the potential for inbreeding and loss of genetic variation through genetic drift and the consequent fixation of particular alleles at loci (Soule 1976).

1.5 The pygopodid, *Delma impar*

The Pygopodidae are the Australian family of legless lizards. They have in common greatly reduced limbs and a very narrow, elongate body. Locomotion resembles that of snakes (Greer 1989).

1.5.1. Distribution and activity

Delma impar averages about 250mm in length and 3 - 4g in mass, and is of colouration similar to their brown-green grass environment. The species shows a strong association with lowland native grassland, having rarely been found in other habitat types (Kukolic 1993). Lowland native grassland constitutes tussocky, often dense vegetation (Coulson 1990; Kukolic 1993). These grasslands occur in valley plains where temperatures reach levels that are sufficiently cold that trees cannot establish themselves. They function in a seasonal climate i.e. they occur in areas which are subject to cold winters, moderate autumns, hot dry summers and wet springs. Lowland native grassland is found in the southeast of Australia, primarily in Victoria and in the ACT. The known distribution of *D. impar*, shown in Figure 1, corresponds to this pattern.

The denseness of the grassland habitat makes direct observation of *D. impar* activities difficult, but this difficulty may be enhanced by a semi-fossorial habit of the animal. The evolution of limblessness in lizards is apparently correlated with the development of fossorial habit (Greer 1980; see also Clark 1969; Withers 1981). *D. impar* is primarily diurnal (Martin 1972; Coulson 1990) but is rarely seen active in the field. Although information regarding its precise area of activity within grasslands is largely lacking, specimens have been found both in the soil, woven among the roots of grass tussocks, and on the surface of the soil within the tussocks (Coulson 1990; Kutt 1991). My own observations of captive animals have shown the species to be active seekers of burrows or cracks in the soil. During late spring through to autumn, they were observed to occupy underground refuges from mid-afternoon until the following morning, when the sun began to heat the soil. They then emerged to bask and forage.

Seasonal activity appears marked. Animals are surface active from late spring to early autumn, with peak activity occurring between late spring and early-mid summer (Kutt 1991; Kukolic 1993). They probably over-winter in the soil or under rocks and logs when available. Most of what is known about daily movement comes from some limited tracking of animals using fluorescent dyes (Kutt 1993). Results of this work suggest that individuals travel an average of 5m per day. Recapture of animals, using the pitfall trapping technique, is an infrequent occurrence and has provided little information on mobility in the animal, or on population size. The maximum distances yet reported have been about 50 m in two months and, more recently, some 60m in two days (Kukolic unpub.). Most pygopodids display efficient movement in grass, various abilities to climb amongst thick vegetation, but poor locomotion on smooth surfaces (Greer 1989; pers. obs.).

Coulson (1990), in a review of past and present occurrences of *D. impar*, reports apparent localised extinctions of populations of the species, and notes that the distribution of the species has contracted over recent decades. This is presumably due to extensive loss of its grassland habitat. The role that habitat fragmentation plays in this is not known, but could be of great influence if individuals are unable to migrate between patches. Survival outside of its preferred habitat is, presumably, not easily accomplished, considering the very restricted distribution of the species to sites of lowland native grassland.

i.4 Project aims

In order to predict the impact that habitat fragmentation has on populations of *D. impar* in the absence of direct evidence, something of its habitat specificity must be learned, i.e. are individuals able to survive outside of their grassland habitat? Furthermore, something of their potential for activity must be learnt, i.e. are individuals capable of enduring activity, such as would be required for migration between populations? Accordingly, the aims of study were to determine whether *D. impar* exhibits physiological and genetic qualities indicating adaptation to the ground-layer environment, and to an active life-style. Specifically, the study involved assessments of temperature preferences, rates of metabolism during inactivity at different temperatures, and allelic distributions across populations. These properties of *D. impar* were then compared with those of fossorial and surface-active species of lizard and with thermal features of the ground environment.

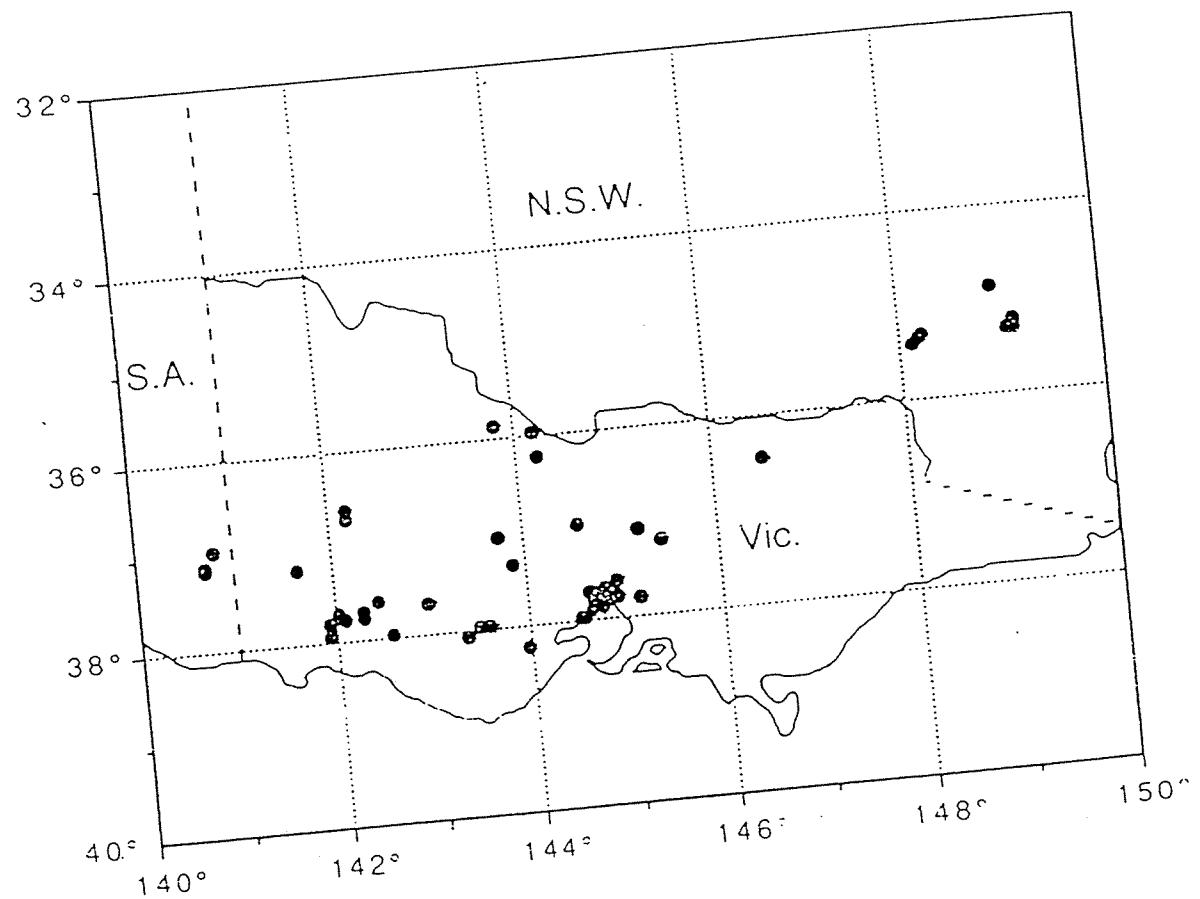


Figure 1. The known distribution of *Delma impar*.
From Coulson 1990

Chapter 2. Physiology

2.1 Introduction

Many physiological functions in ectotherms are dependent on temperature. Species which experience different thermal climates may be expected to possess physiological abilities and capacities of varying thermal dependence. An examination of the thermal relations of physiological processes in a species can be used to identify the range of temperatures within which the species is efficiently active and outside of which performance is likely to be constrained. In conjunction with field data on thermal climate, information thus obtained can be used to generate hypotheses concerning temporal and spatial patterns in activity.

Temperature preferences

When given a choice of temperatures in which to reside, most lizards will select temperatures to which they are operationally adapted, particularly those at which activity is optimal (Huey 1982). It may not be surprising, therefore, that the temperature preferences of species tend to reflect the temperatures to which they are accustomed. Species inhabiting hot environments prefer high temperatures relative to species that occupy cold environments (Spellerberg 1976; Arad et al. 1989; Greer 1989). In particular, fossorial species appear to prefer low temperatures relative to surface-dwelling species (Bury and Balgooyen 1976; Withers 1981; Bennett and John-Alder 1986).

Metabolism

The rate of metabolism of an organism is a measure of the rate at which energy is produced. Accordingly, a study of metabolic rate provides information on the energy requirements and energy expenditure of individuals. Among ectotherms, requirements for energy vary with temperature. Typically, energy requirements increase with increasing temperature. Energy requirements may thus be governed by daily and seasonal variations in temperature. Rates of metabolism and the thermal dependence of metabolic rate vary among ectothermic species. The different ecologies of species appears to account for some of this variation. One of the most striking correlations of metabolism and ecology is that concerning the extent of surface-activity shown by species. Relative to surface-dwelling lizards, fossorial lizards have slower standard rates

of metabolism (Bennett and Dawson 1976; Kamel and Gatten 1983; Andrews and Pough 1985).

Project objectives

The physiology of *Delma impar* was examined in order to identify the thermal qualities to which activity in the species is suited or by which it is constrained, so that microhabitats required by *D. impar* might be inferred. Specifically, physiological analyses were performed to quantify the temperature preferences, and resting rates and thermal dependence of metabolism of *D. impar*. These physiological characteristics were examined in relation to the temperatures available in the species' habitat, and to seasonal patterns in the activity of the species. The physiological properties of *D. impar* were also contrasted with those reported previously for other lizard species to determine whether or not *D. impar* exhibits fossorial-associated physiological characteristics, including low levels of activity.

2.2 Methods

Capture of animals

D. impar were captured at the Majura Valley grassland site of the ACT by pit-fall trapping, using six rows of ten 11 L tin cylinders at five metre spacings, joined by a mesh 'drift' fence of about 40cm in height. Trapping was conducted between 3 November 1993 - 5 March 1994. These dates represented almost the entire season of surface-activity shown by the species. Once trapped, individuals were transported to the lab, and weight, snout-vent length, and approximate age recorded. Individuals were nominated adults if they were of a snout-vent length greater than 78mm. Sex was unambiguously determined for gravid females only.

Pre-experiment treatment of individuals

Animals were housed individually in plastic containers at room temperature (20 - 25°C) and natural photoperiod (L:D 14:10). Moist, crumpled paper was provided for shelter and water, and was replaced with clean material as necessary. Animals were given crickets to eat, unless they were to be involved in metabolic analyses within five days. Only animals that maintained weight ($\pm 10\%$) were used in experiments.

Experiments were carried out usually within three weeks of capture. This was done to avoid bias of animal functioning through acclimation to laboratory conditions (see Brett 1970; Sievert and Hutchinson 1991). No influence of time in captivity on physiological attributes was detected.

Monitoring of field temperatures

Field temperatures were measured at 10 and 20cm depths of the soil, at the soil surface (shaded but on clear ground), at the base of grass tussocks, and 50cm in the air (shaded). Temperatures were recorded every 15 minutes between November and March using thermocouples attached to a data logger (Datataker^R) containing an internal cold reference.

Temperature preferences

Temperature preferences were assessed for 22 animals. Seven of these were gravid females, two were juveniles. Animals ranged in mass from 1.4 - 6.5g (mean 3.0g) for non-gravid adults, 5.5 - 8.3g (mean 7.1g) for gravid females, and 1.5 and 2.2g for juveniles.

Temperature preference is assessed, to the exclusion of confounding variables present in the field, using artificial temperature gradients. The animal is placed in an area in which all conditions but temperature are kept as homogeneous as possible. Those temperatures in which the animal consistently chooses to reside are nominated as its preferred temperature range (Pough and Gans 1982). While the relevance of laboratory studies of thermal preferenda to behaviour in the field may be questioned, it does appear that species generally select temperatures in artificial gradients corresponding to those they would in the field (Licht et al. 1966; Bennett and John-Alder 1986; Huey 1991; Rosen 1991), including species that have naturally restricted access to heat (Patterson 1990), in contrast to the wide range of temperatures provided during assessment of temperature preference.

The experimental set-up consisted of a wooden box 150x40x20cm. The inside bottom was layered with sand coarse enough to permit free movement of animals. The sand was replaced after individual trials so that animals would not avoid certain areas of the gradient because of 'territorial' markings. While workers have often heated temperature gradients using heat lamps suspended above the sand, some species may be photophobic and thus may behave unnaturally in the presence of intense light i.e. select temperatures below their thermal preference (Licht et al. 1966; Spellerberg 1974; Sievert and Hutchinson 1991). Accordingly, heat was supplied to the sand surface from below the

the substrate. The box was kept in a room held at a constant 15°C, and a temperature gradient of 15°C to 45°C was maintained across the sand surface. This range encompasses the temperature preferences previously demonstrated by lizards (Spellerberg 1976; Greer 1989). A clear perspex lid placed on top of the box allowed for observation of lizards while preventing air currents from disturbing the thermal gradient and keeping potential disturbance of animals through noise to a minimum. A line of shelving and watering sites across the length of the gradient provided lizards with shelter and water at any given temperature. Biases associated with particular shelters or drinking points were not apparent. Although the provision of water may be associated with an increase in temperature preference (Bury and Balgooyen 1976), water deprivation of animals can have a greater effect on their behaviour, as some species rapidly lose condition with dehydration (Bury and Balgooyen 1976; Andrews 1994). *D. impar* seems susceptible to water loss because animals drink frequently and lose weight relatively quickly when removed from water for more than a few days (pers. obs.). Light was uniform across the gradient and was regulated according to the natural summer photoperiod, L:D 14:10.

While the most accurate measures of body temperature are made using cloacally-inserted thermocouples, this is an unnecessary cause of discomfort and possible injury where the temperature preferences of small animals are of interest. Small ectotherms readily take on the temperature of their surroundings because of their large surface area relative to volume. Accordingly, body temperature was inferred from the temperature at the surface of the sand upon which the animal lay, using fine thermocouples. While animals may maintain body temperatures below ambient temperature through evaporative cooling, this indirect method has been reported to provide accurate measures of body temperature in lizards of variable weight (0.9 to 39g) which occupy sheltered environments (Daut and Andrews 1993; Andrews 1994), and so while these species may have been expected to cool evaporatively in warm and exposed environments, body temperature measured directly was consistent with indirect measures of body temperature. Body temperature was estimated as the mean of measurements taken at three points along the body to account for any differentiation of body temperature along the length of the animal (see Johnson 1977). Care was taken to note whether the animal was lowering its body temperature below that of the substrate by raising its body off the sand or through respiratory cooling by panting. The latter was not observed to occur, but animals frequently held their heads in the air. The positioning of thermocouples was adjusted accordingly on such occasions by being suspended alongside the animal's head.

Estimates of body temperature were made between the hours 8:00 and 18:00. Animals were introduced into the gradient the evening before measurements were taken so that they could adjust to the conditions. Body temperature estimates were recorded over two -

four day periods per individual. Shorter durations may not be long enough for animals to demonstrate the range of their normal thermoregulatory behaviours, while animals may modify their behaviour in response to the gradient environment, in contrast to their natural habitat, if trials are of longer duration (Bennett and Dawson 1976; Evans 1990). Recordings were made at regular intervals throughout the day, usually every 30 to 40 minutes. This was sufficient to determine any temporal patterns in temperature selection, as noted by Spellerberg (1974), Rosen (1991), Daut and Andrews (1993), and Andrews (1994), while giving the animal substantial periods of disturbance-free time so that movement was not unnecessarily inhibited.

Metabolism

Because oxygen is used in metabolic processes, metabolic rate can be (and typically is) estimated by determining the rate at which an individual consumes oxygen (Schmidt-Nielsen 1983). Oxygen consumption of seven resting adult *D. impar* weighing between 3.1 and 3.7g (mean 3.3g) was measured using a flow-through respirometry system (Figure 3). Experimentation was performed in the evening, during the primary period of inactivity in the species. Because the digestive state and health of individuals can influence metabolic rate (Bradshaw et al. 1980; Taylor and Davies 1981), subjects included in analyses were non-gravid, were fasted for at least five days, and had not recently shed or suffered injury. Oxygen consumption was measured at 15, 20 and 30°C. The latter two temperatures are likely to incorporate the temperature preferences of the species, according to reviews of temperature preferences in various ecological classes of lizard (Licht et al. 1966; Spellerberg 1976; Bennett and John-Alder 1986), while an examination of metabolic rate at a lower temperature (i.e. 15°C) is of interest because the species may display adaptation to low temperatures given the cold thermal climate it experiences for most of the year.

During experimentation, animals were contained individually within 30cm lengths of plastic tubing immersed in a water bath maintained at the required temperature $\pm 0.5^{\circ}\text{C}$. An identical empty chamber was used as a reference cell. Room air was drawn through the system by a flowmeter at 50ml/min. Air was circulated around the water bath to adjust it to the correct temperature before being passed first through the experimental chambers, then through containers of Drierite and silica gel for the removal of water and carbon dioxide respectively, which otherwise influence oxygen concentrations (Withers 1977), before being passed for analysis into the oxygen sensor of an Applied Electrochemistry Oxygen Analyser (model S-3A). The difference in oxygen content of air which had passed through a chamber containing a lizard and that leaving the reference chamber was considered due to consumption by the animal. Materials and connections

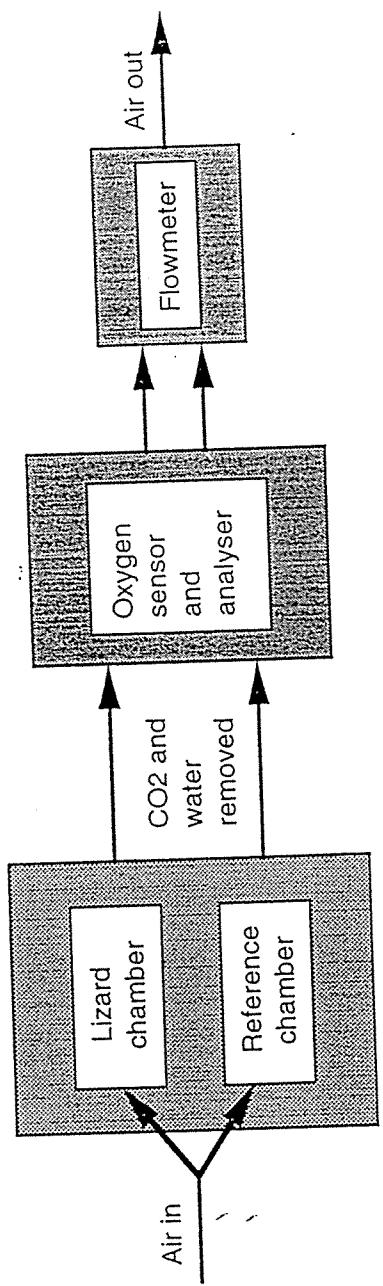


Figure 3. Flow-through respiratory system

were used so as to facilitate the passage of air and minimise air leaks i.e. equipment pieces were connected by relatively impermeable tubing of narrow diameter and minimal length. Animals were given 40 minutes to equilibrate to experimental temperature, and 20 minutes to adjust to the controlled air flow before oxygen consumption was recorded.

The minimum oxygen content difference maintained for at least 30 minutes between the reference and experimental chamber, and during which trial the animal was not observed to be active, was judged as representing the standard rate of oxygen consumption. Animals were not held in chambers for longer than five hours. Rates of oxygen consumption were calculated according to the difference in oxygen content of the two chambers and rate of air flow using the formula provided by Withers (1977). Estimates were corrected for variation in temperature and pressure.

2.3. Results

Trapping

Figure. 4 shows the temporal pattern of captures and temperature over the season. The majority of animals were caught during November and December. Captures declined substantially in January and very few animals were trapped beyond mid-summer. Gravid animals were trapped between late November and early January.

Field temperatures

Mean air temperatures increased gradually from November to February at which point they began to drop again. Maximum air temperatures were highest in January and February, when they reached an average of about 32°C , while minimum air temperatures reached their peak in mid-December, dropped suddenly in late December and remained low throughout January. Table 1 shows the distribution of temperatures throughout the ground strata. The temperature regime within the soil is less extreme and less influenced by air temperature than that affecting the soil surface and grass. On average, temperatures were higher within the grass tussocks and on the soil surface (21.6°C), than within the soil (20.2°C).

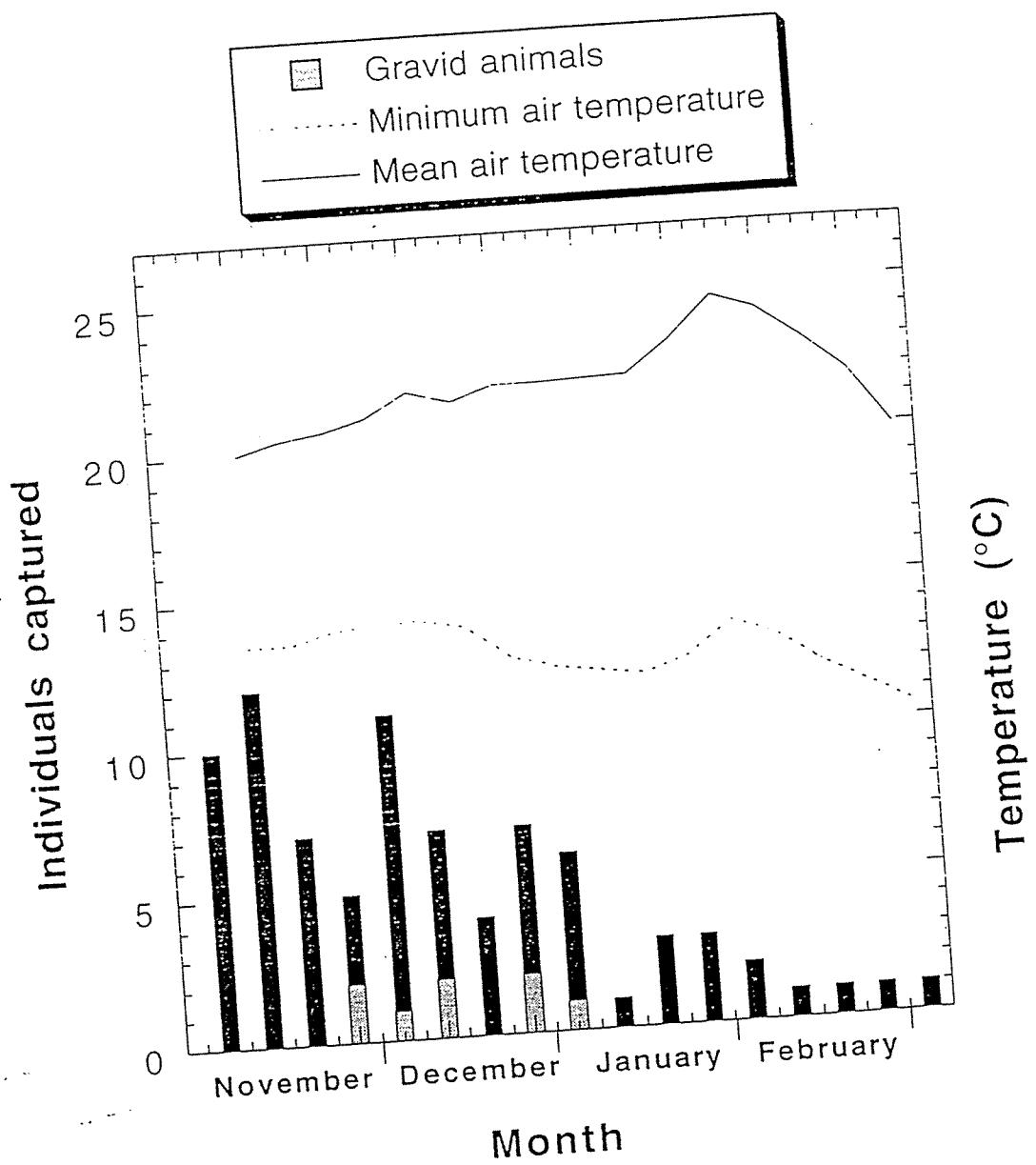


Figure 4. Temporal pattern of captures and temperature

Table 1

Spatial and temporal distribution of temperatures in ACT lowland native grassland 1993-4

	Level	Air	Soil surface	Tussock	10 cm soil depth	20 cm soil depth
	Temperature (°C)					
November	Mean	20.3	21.1	20.3	19.2	19.8
	Minimum	13.6	14.9	14.3	16.1	16.9
	Maximum	27.5	29.5	27.1	20.9	20.0
December	Mean	21.5	22.0	21.3	18.5	18.0
	Min.	13.7	15.2	14.9	16.0	16.3
	Max.	27.1	29.5	25.1	21.2	20.4
January	Mean	22.3	23.2	22.5	20.8	21.2
	Min.	12.2	13.7	12.4	18.1	18.9
	Max.	31.3	32.6	31.8	22.8	22.1
February	Mean	23.3	23.4	22.5	22.9	23.4
	Min.	12.8	15.8	13.3	19.6	20.0
	Max.	31.8	31.0	31.3	24.7	24.2
March	Mean	19.9	20.5	19.1	19.1	18.8
	Min.	10.5	12.6	11.7	17.3	18.0
	Max.	28.1	28.7	28.0	23.5	22.6

Temperature preferences

The temperature preferences of *D. impar* are presented in Table 2. Non-gravid animals demonstrated a mean temperature preference of 26.0°C. The majority (70%) of recordings of temperature selection fell between 24.5 and 27.5°C, although animals were observed to be active in a wide range of temperatures, including that available in the gradient. Temperature preferences were consistent throughout the day. Gravid animals preferred higher temperatures than non-gravid animals (Mann-Whitney $U' = 92.0$; $p < 0.01$). The temperature preferences of the two juveniles examined did not deviate from those of non-gravid adults. A difference between the preferences of non-gravid animals caught in December and those caught in February was apparent and may indicate a seasonal change in temperature preferences, although insufficient sampling of animals in these months does not permit conclusions to be drawn.

Metabolism

Standard metabolic rate increased about two-fold over a 10° increase in temperature (referred to as 'Q10' of metabolism) between 15 and 30°C in inactive *D. impar* (Table 3). Rates of increase were faster between 20 and 30°C than between 15 and 20°C.

2.4 Discussion

2.4.1 Temperature preference and field behaviour

D. impar demonstrated a preference for temperatures between 24.5 and 27.5°C. Considering this temperature range is likely to represent the temperatures at which physiological performance is optimal (see Huey 1982), it is interesting to determine whether it is possible for individuals to access and maintain these temperatures in the field. Mean temperatures at all levels examined at the ground layer remained below the preferred range throughout the summer (Table 1). However, temperatures within the preferred range and higher were available on the surface during the day throughout the season. At night, temperatures were highest within the soil. Thus, individuals could most closely attain their preferred temperatures by being surface active during the day and spending nights within the soil layer. It is not known whether such a pattern of activity occurs in the field. Field observations of individuals are lacking, primarily because of the denseness of the vegetation and the cryptic colouration of the animals. The few reports of field activity suggest that the lizard is primarily diurnal (Coulson 1990; Kutt 1991).

Table 2
Mean preferred temperature (MPT) of *Delma impar*

	Variable	MPT (s.d.) (°C)	N
State	non-gravid	26.0 (1.5)	15
	gravid	29.3 (2.2)	7
Month of capture (non-gravid only)	December	27.3 (0.35)	2
	January	25.8 (1.6)	11
	February	25.5 (0.71)	2

Table 3
Temperature dependence of standard metabolic rate (SMR) in *Delma impar*

Temperature (°C)	SMR (s.d.) (ml O ₂ /hr)	Body mass (g)	Q ₁₀
15	0.23 (0.05)	3.1	
20	0.29 (0.07)	3.1	1.74
30	0.67 (0.23)	3.7	2.31

Observations of captive animals have included individuals basking in sunlight and actively foraging during the day (Martin 1972; pers. obs.). While the species has been reported to over-winter in cracks and root crevices within the soil and under rocks and logs (Coulson 1990; Kutt 1991), my own observations of captive animals demonstrate that they also submerge in diel rhythm, in the late afternoon, and re-emerge the following morning as temperatures increase. Temperature has been reported as influential on diel submergence behaviour in some skinks, by which maintenance of relatively high body temperatures may be achieved (Spellerberg 1972). While diel cycles in activity correspond to rhythms in temperature selection in some species (Rosen 1991; Daut and Andrews 1993; Andrews 1994), temperature preference was consistent during the day in *D. impar*, presumably because the species maintains a relatively homogeneous body temperature during the day and night, in contrast to species which are typically heliothermic and maintain high (>30°C) diurnal body temperatures.

Individuals were captured most frequently during November and December. This is consistent with trapping results of previous years (Kutt 1991; Kukolic 1993). November and December were outstanding during the study period because of relatively high minimum temperatures. Assuming that the decline in captures was not indicative of inadequacies in trapping methodology, such that animals learnt to avoid the traps (the pit sites used have been in place for a few years, however), activity may have declined in response to a decrease in minimum temperatures. Seasonal patterns in minimum temperatures appear to be influential on activity in ectotherms inhabiting cold environments (Spellerberg 1976; Macartney et al. 1989). Individuals which do not respond early to decreasing temperatures can be killed by sudden advancement of cold weather (Spellerberg 1976).

— or reduced
population
size, due to
sampling.

On the other hand, seasonal activity patterns may correspond to the reproductive period, which is in itself probably regulated by seasonal variations in thermal climate. Gravid animals appeared during December and early January. This is consistent with previous findings (Kutt 1991; Kukolic 1993) and seems to be typical of pygopodids (Patchell and Shine 1986). It is assumed that reproductive activity would have been completed during January, at which time animals ceased to be captured at any great frequency. Gravid females consistently preferred higher temperatures than non-gravid animals in the thermal gradient and this indicates that optimal temperatures for embryo development are different to those optimal for activity in non-gravid adults, and this has been noted in other species of lizard, particularly viviparous taxa (Daut and Andrews 1993). Activity associated with reproduction in *D. impar*, therefore, may be expected to occur during the hottest period of the year. Indeed, eggs were laid just before mean daily temperatures reached their

maximum for the season which, although below the optimal temperatures indicated by temperature preferences of gravid females, provided eggs with the most optimal conditions available for rapid development.

The temperature preferences demonstrated by *D. impar* in this study may only apply to the summer thermal regime. Temperature preferences have been reported as seasonally variable in some reptilian species (Patterson and Davies 1978; van Damme et al. 1986; Rosen 1991; Daut and Andrews 1993). This is probably a factor of the seasonal variance of temperatures available in the field and of activities of the animals, such as reproduction. The higher temperature preferences of gravid females may be, in part, an artefact of a general increase in temperature preference in all animals during December, considering that the two non-gravid animals caught in December displayed preference for relatively high temperatures. Some authors choose to avoid seasonal bias by acclimatising their specimens to standard temperature and light conditions over a period of time from weeks (Kitchell 1969) to months (Arad et al. 1989). This approach avoids temporal bias but limits the relevance of results to natural situations and so was not performed here.

Temperature preference and fossoriality

A compilation of published data on the thermal preferences of lizards (Table 4) confirm that lizards which display fossorial habit tend to prefer lower temperatures than surface-dwelling lizards (Mann-Whitney $U' = 16.0$; $p < 0.05$). This test was carried out by averaging the temperature preferences of species grouped by family. Temperature preferences of the Scincidae were segregated according to whether or not they represented fossorial or surface-active species, and averaged independently. Furthermore, amphisbaenians, burrowing fossorial reptiles which are very rarely surface active, also have low temperature preferences (Al-Sadoon and Spellerberg 1985). The association of low temperature preference with fossoriality appears largely independent of phylogeny because when the analysis is confined to species within the family Scincidae, mean temperature preference of congeneric species are lower in fossorial genera ($n=6$) than in surface-active genera ($n=10$) (Mann-Whitney $U' = 59.5$; $p < 0.01$). [In performing these analyses, data have been selected according to comparable and appropriate methodologies. In particular, preference was given to studies which examined fossorial and nocturnal species with heat supplied from below the substrate rather than above, in a way similar to that which they would naturally experience in their sunshine-deficient environments. Licht et al. (1966) found that some species selected higher temperatures when heat was supplied from beneath the substrate rather than from a suspended heat lamp. For species for which multiple estimates of temperature preference

Table 4
Mean preferred temperature (MPT) of lizards

Family	Species	Habit*	MPT (°C)	N**	Reference
Agamidæ	<i>Amphibolurus barbatus</i> subspp	S	36		Licht et al. 1966
	<i>A. caudicinctus</i>	S	37.7		Licht et al. 1966
	<i>A. inermis</i>	S	36.4		Licht et al. 1966
	<i>A. maculatus</i>	S	37		Licht et al. 1966
	<i>A. muricatus</i>	S	36		Licht et al. 1966
	<i>A. reticulatus</i>	S	36.9		Licht et al. 1966
	<i>A. scutulatus</i>	S	38.2		Licht et al. 1966
	<i>Moloch horridus</i>	S	36.7		Licht et al. 1966
	<i>Physignathus longirostris</i>	S	37.1		Licht et al. 1966
Anguidæ	<i>Anguis fragilis</i>	F	23	4	Spellerberg 1976
Anniellidae	<i>Anniella pulchra</i>	F	24.0		Bury and Balgooyen 1976
Gekkonidae	<i>Gehyra punctata</i>	S	34.6		Licht et al. 1966
	<i>G. variegata</i>	S	35.3		Licht et al. 1966
	<i>Phyllurus miliaris</i>	S	21.6		Licht et al. 1966
	<i>Phyllodactylus marmoratus</i>	S	27.1		3
	<i>Rhynchoedura ornata</i>	S	34.0		Licht et al. 1966
	<i>Strophurus spinigerus</i>	S	34.6		Licht et al. 1966
Lacertidae	<i>Lacerta agilis agilis</i>	S	31		Spellerberg 1976
	<i>L. sicula sicula</i>	S	34		Spellerberg 1976
	<i>L. viridis</i>	S	33		Spellerberg 1976
	<i>L. vivipara</i>	S	32		Spellerberg 1976
Pygopodidae	<i>Delma impar</i>	U	26.0		present study
	<i>Lialis burtonis</i>	S	35.1		Bradshaw et al. 1980
Scincidae	<i>Acontias meleagris</i>	F	28.1		Withers 1981
	<i>Chalcides ocellatus</i>	F	32.5		Daut and Andrews 1993
	<i>Ctenotus regius</i>	S	35.6		Bennett and John-Alder 1986
	<i>C. robustus</i>	S	34.4		Bennett and John-Alder 1986
	<i>C. taeniolatus</i>	S	35.3		Bennett and John-Alder 1986
	<i>C. uber</i>	S	35.3		Bennett and John-Alder 1986
	<i>Eremiascincus fasciolatus</i>	F	21.2		Bennett and John-Alder 1986
	<i>Egernia carinata</i>	S	34.7		Licht et al. 1966
	<i>E. cunninghami</i>	S	33.3		Licht et al. 1966
	<i>E. depressa</i>	S	34.0		Licht et al. 1966
	<i>E. stokesi</i>	S	32.6		Licht et al. 1966
	<i>E. striolata</i>	S	32.7		Bennett and John-Alder 1986
	<i>E. whitii</i>	S	34.1		Bennett and John-Alder 1986
	<i>Hemiergis decresiensis</i>	F	17.6		Bennett and John-Alder 1986
	<i>H. quadrilineatum</i>	F	26.5	3	Licht et al. 1966
	<i>H. peroni</i>	F	20.3		Bennett and John-Alder 1986
	<i>Lampropholis delicata</i>	S	26		Spellerberg 1976
	<i>Lerista bougainvillii</i>	S	31		Spellerberg 1976
	<i>Leiolopisma trilineata</i>	S	32		Spellerberg 1976

Table 4 continued

Family	Species	Habit*	MPT (°C)	N**	Reference
Scincidae	<i>Leiolopisma entrecasteauxii</i>	S	31		Spellerberg 1976
	<i>L. metallica</i>	S	29		Spellerberg 1976
	<i>L. ocellatum</i>	S	31		Spellerberg 1976
	<i>L. pretiosa</i>	S	29		Spellerberg 1976
	<i>Mabuya</i> spp	S	34.5		Spellerberg 1976
	<i>Nannoscincus maccoyi</i>	F	21.1		Withers 1981
	<i>Neoseps reynoldsi</i>	F	30		From Greer 1989
	<i>Scelotes gronovii</i>	F	30.4		Andrews 1994
	<i>Sphenomorphus kosciuskoi</i>	S	29.8		Withers 1981
	<i>S. quoyi</i>	S	28.8		Bennett and John-Alder 1986
	<i>S. tympanum</i>	S	29.6		Bennett and John-Alder 1986
	<i>S. labillardieri</i>	S	32.2		Bennett and John-Alder 1986
	<i>S. lesueuri</i>	S	32.6		Licht et al. 1966
	<i>Tiliqua occipitalis</i>	S	32.9	4	Licht et al. 1966
	<i>T. casuarinae</i>	S	32.6		Licht et al. 1966
	<i>T. nigrolutea</i>	S	35		Bennett and John-Alder 1986
	<i>Trachydosaurus rugosus</i>	S	31.9		Spellerberg 1976
					Bennett and John-Alder 1986
Varanidae	<i>Varanus gouldii</i>	S	37		From Greer 1989
	<i>V. gilleni</i>	S	34.7		From Greer 1989

* S = surface-dwelling

F = displays fossorial habit

U = unknown habit

** for sample sizes less than five

have been published, estimates for which sample sizes were greatest, and for which methods were most comparable were included in analyses.]

It is apparent that fossorial species of lizard vary according to the amount of time they spend actually in the soil. Some are almost completely subterranean, emerging periodically at dusk to forage under leaf litter and scrub (Huey 1974; see also Bennett and Dawson 1986). Others obtain heat from beneath cover during the day (Bury and Balgooyen 1976; Patterson 1990; Andrews 1994). And there are species which utilise the underground environment at night or during extreme temperatures, while spending substantial periods of time on the surface, typically in dense ground vegetation (Al-Sadoon and Spellerberg 1985; see also Kamel and Gatten 1983). So, the temperatures accessed by fossorial lizards will vary between species. Accordingly, thermal adaptation of species may be expected to vary. The species that do not access high temperatures but remain well down in the substrate and appear to be largely nocturnal (e.g. *Hemiergis*, *Eremiascincus* spp) tend to have lower temperature preferences than species that access heat from beneath cover during the day (e.g. *Anguis fragilis*, *Anniella pulchra*) which in turn have lower preferences than species which may emerge from cover during the day to bask (e.g. *Chalcides ocellatus*).

The temperature preferences demonstrated by *D. impar* are much more similar to those of fossorial lizards than surface-dwelling species (Figure 5). *D. impar* has a mean temperature preference similar to fossorial species of median thermophily, or surface-activity. This supports the theory outlined above, generated by a consideration of the temperature preferences of and field temperatures available to *D. impar*, which proposes that the species emerges from underground during the day to obtain heat. The mean temperature preference of *D. impar* is much lower than that of its fellow-pygopodid *Lialis burtonis*, a species which is active among leaf litter in a variety of habitats ranging from coastal forests to the desert interior of Australia (Cogger 1992). It is also apparent that the mean temperature preference of *D. impar* is equal to that of the skink *Lampropholis delicata* with which it is sympatric in some areas, and the two are likely to experience similar environmental pressures. This provides further support to the trend that ecology often appears more important than phylogeny in determining the physiological adaptations of ectotherms.

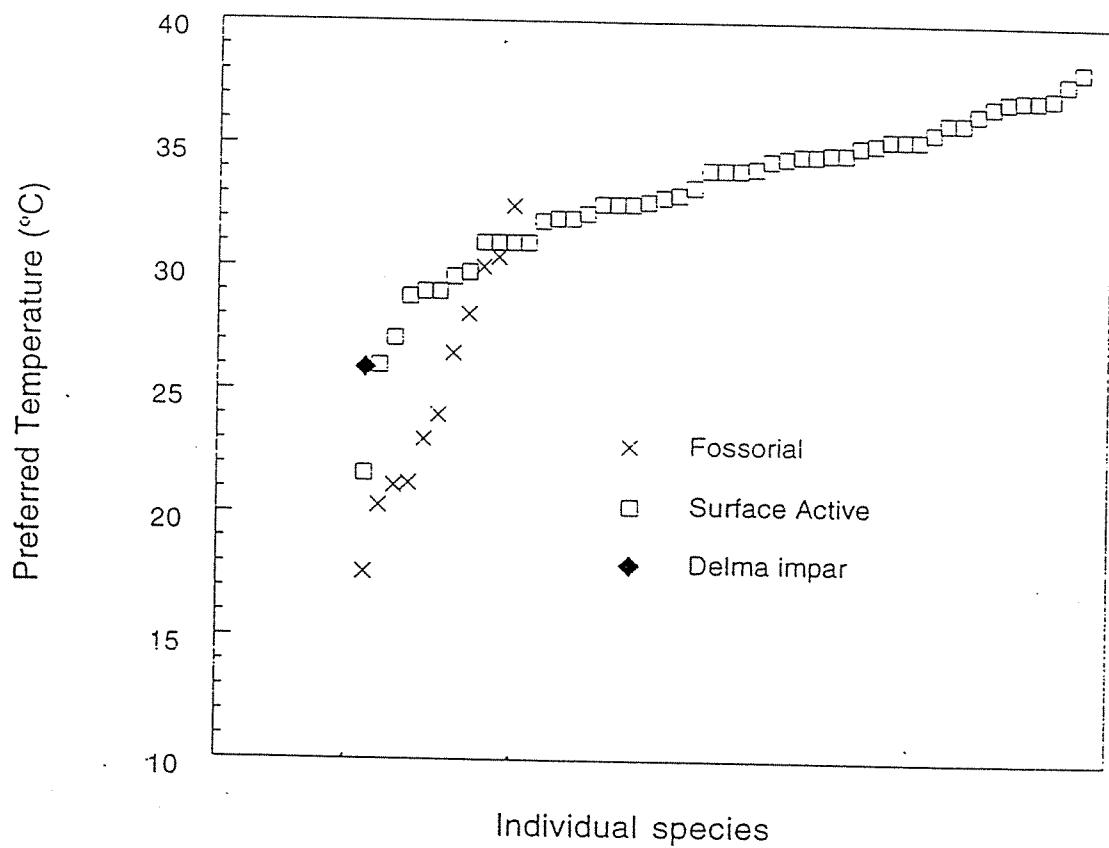


Figure 5. Temperature preferences of *Delma impar* and fossorial and surface active lizards

2.4.2. Metabolism

Standard metabolic rates observed in *D. impar* are similar to values expected considering body mass, temperature and phase of the activity cycle (Andrews and Pough 1985). Nevertheless, between 15 and 20°C the thermal dependence of metabolic rate is low in *D. impar* compared with the mean Q₁₀ displayed by reptiles of between two and three (Bennett and Dawson 1976). Considering the low temperature preference demonstrated by *D. impar* individuals, the low thermal dependence of metabolism at low temperatures may be an adaptation to a cold climate. This relative thermal independence of metabolism at cold temperatures has been noted to occur in several lizard species which inhabit cool climates. It is suggested that this enables activity to be prolonged at colder temperatures without sacrificing efficiency of physiological performance when temperatures are sufficiently warm (e.g. Aleksuk 1971). Accordingly, *D. impar* can maintain relative functional efficiency at cool temperatures, and thus important physiological operations, such as digestion, can proceed throughout the lower temperatures experienced during its active season. (15 to 20°C corresponds well with minimum temperatures recorded in the soil.)

Ecological correlates of standard metabolic rate

The specific ecological correlates of standard metabolic rate in ectotherms are largely uncertain (Andrews and Pough 1985). Standard rates of metabolism have been associated with general activity levels of species, primarily as indicated by foraging mode. Sit-and-wait predatory species appear to have slower resting rates of metabolism than widely-foraging predators (Anderson and Karasov 1981; Bennett 1983; Andrews and Pough 1985; Hailey and Davies 1986). Andrews and Pough (1985) suggest that lower metabolic rates may be associated with restricted levels of activity. While a slow metabolic rate during inactivity is expected to substantially reduce daily energy requirements, given that reptiles are active for large portions of the day (Huey 1982), it is uncertain whether metabolic costs suffered during inactivity constitute the majority of total energy requirements (Nagy and Degen 1988; Nagy and Knight 1989). A correlation between standard metabolic rate and metabolic rates achieved during activity has been reported in some species (Taigen 1983; Hailey and Davies 1986), although the physiological properties that permit fast rates of metabolism during activity are not necessarily associated with an obligatory increase in standard metabolic rates (Kamel and Gatten 1983). Very little is known about activity in *D. impar*. Individuals have been observed to be highly agile in the field (Coulson 1990), and will rapidly travel through the grass upon release, particularly when of a reasonably high body temperature (i.e. 20°C+). My own observations of captive animals suggest that they perform both sit-and-wait and

active predatory methodologies. They also display a degree of territorial behaviour involving aggressive striking and biting activity and occasional chasings, although vocalisations appear to replace physical attacks on occasions. (Of course, unnaturally high densities in captivity may have encouraged territorial behaviour which may be absent in natural populations). These notes indicate that the species is of at least intermediate activity levels; however, further work on distances travelled daily is required before any conclusions can be drawn.

Reduced energy requirements may be relatively advantageous in the soil environment if resources, such as oxygen (Withers 1981; Kamel and Gatten 1983) are limited. The fact that rates of reproduction and growth appear slow in fossorial species (Huey 1974; Greer 1989) suggests that they may be limited by some resource, probably heat for activity, and it has been experimentally demonstrated that individuals sacrifice growth as a method of conserving energy in the cold (Cabanac 1985).

A reduced thermal dependence of metabolic rate (i.e. small Q₁₀) would also conserve energy while maintaining physiological performance if Q₁₀ was minimal around preferred temperatures i.e. if metabolic costs were relatively low at temperatures adopted by species for much of the time. The relatively thermal-independent metabolism occurring at temperatures just below mean preferred temperatures in some species is suggested to be a method of energy conservation (Al-Sadoon and Spellerberg 1985; Evans 1990). This being the case, we would expect different species to show relative thermal independence of metabolic rate at different temperatures, corresponding to preference for different temperatures. Table 5 shows the variations in thermal dependence of metabolism in different temperature ranges among fossorial and surface active species of a variety of reptilian taxa. I have not tested for statistically significant differences because there are very few observations for fossorial species and for surface-active species at lower temperatures. The lack of estimates of metabolism at multiple temperature intervals for species makes the recognition of occurring trends difficult, but it is apparent that a particular area of low thermal dependence occurs in two species of gecko and in the iguanas. In the two geckoes, these periods of thermally independent metabolism occur at temperatures below the preferred temperatures of at least one of the species (Arad et al. 1989), and occur probably more or less at the preferred temperature range of the iguanas (see Grant 1990). A more striking pattern, however is the occurrence of relatively large Q₁₀s in relation to temperature preferences. In fossorial species thermal dependence reaches a peak below about 20°C, while in surface active species, this occurs typically above 20°C, although this is confounded by the fact that few studies of surface-active metabolism have considered temperatures less than 20°C. This pattern is in accordance with the differentiation of temperature preferences shown by

Table 5

Thermal-dependent variation in Q10 for standard metabolic rate in lizards

Family	Species	Ecological category ¹	Q10	Temperature range (°C)	Reference
Agamidae	<i>Physignathus lesueuri</i>	S	2.42 2.22	20-30 30-37	From Andrews and Pough 1985
Anguidac	<i>Anguis fragilis</i>	F	1.83 4.9 1.4 1.53 1.65 1.74	5-10 10-15 15-20 20-25 25-30 30-35	Al-Sadoon and Spellerberg 1985
Gekkonidae	<i>Bunopus tuberculatus</i>	S	1.4 0.76 1.39 1.83 1.87	10-15 15-20 20-25 25-30 30-35	Al-Sadoon and Abdo 1989
	<i>Hemidactylus frenatus</i>	S	5.09 2.05	20-26 26-30	From Andrews and Pough 1985
	<i>Ptyodactylus hasselquistii</i>	S	1.48 1.69 0.99 5.37	10-15 15-20 20-25 25-30	Al-Sadoon and Abdo 1989
	<i>Tarentola mauritanica</i>	S	2.02 1.61 2.03 1.84 1.92 2.09	5-10 10-15 15-20 20-25 25-30 30-35	Al-Sadoon and Spellerberg 1985
Iguanidae	<i>Dipsosaurus dorsalis</i>	S	2.13 4.59 1.44	25-30 30-35 35-40	From Andrews and Pough 1985
	<i>Sceloporus undulatus</i>	S	5.94 7.53 1.76	20-25 25-30 30-35	From Andrews and Pough 1985
Lacertidae	<i>Acanthodactylus bosianus</i>	S	1.09 1.08 1.33	25-30 30-35 35-40	From Andrews and Pough 1985
Lacertidae	<i>Lacerta vivipara</i>	S	2.01 1.98 2.46 1.86 2.53 3.65	5-10 10-15 15-20 20-25 25-30 30-35	Al-Sadoon and Spellerberg 1985
Pygopodidae	<i>Delma impar</i>		1.74 2.31	15-20 20-30	present study
Scincidae	<i>Acontias meleagris</i>	F	1.42 1.66	15-23 23-33	Withers 1981
	<i>Chalcides ocellatus</i>	F	2.59 5.03 1.91 1.43 2.66	10-15 15-20 20-25 25-30 30-35	Al-Sadoon and Spellerberg 1985
	<i>Eumeces obsoletus</i>	S	3.4 2.69	20-30 30-37	From Andrews and Pough 1985
	<i>Mabuya spp</i>	S	4.98 2.57	15-23 23-33	Withers 1981
	<i>Scelotes gronovii</i>	F	4.61 1.39	15-23 23-33	Withers 1981
	<i>Trachydosaurus rugosus</i>	S	3.07 1.82	20-30 30-37	From Andrews and Pough 1985
Teiidae	<i>Cnemidophorus murinus</i>	S	2.25 2.56	20-30 30-37	From Andrews and Pough 1985

Table 5 continued

Family	Species	Ecological category	Q10	Temperature range (°C)	Reference
Trogonophidae ²	<i>Blanus cinereus</i>	F	1.93 1.04 1.38 1.1 1.95 3.54	5-10 10-15 15-20 20-25 25-30 30-35	Al-Sadoon and Spellerberg 1985
Varanidae	<i>Varanus gouldi</i>	S	3.21 1.69	20-30 30-37	From Andrews and Pough 1985
Xantusiidae	<i>Klauberina riversiana</i>	S	4.06 2.24	20-25 25-30	From Andrews and Pough 1985
	<i>Lepidophyma gaigeae</i>	S	1.36 1.98	20-25 25-30	From Andrews and Pough 1985
	<i>Xantusia henshawi</i>	S	2.01 1.51	20-25 25-30	From Andrews and Pough 1985

¹ S = surface-dwelling

F = displays fossorial habit

U = unknown habit

² an amphisbaenian

the two groups; surface active species have relatively high temperature preferences. Thus, the period of maximum thermal dependence corresponds with temperatures that are within normal access, and there is a tendency for species to prefer temperatures at which physiological performance is most efficient, rather than maximal. A high thermal dependence of metabolism at low temperatures allows for flexible physiological performance that may be altered quickly depending on thermal conditions. It also enables considerable conservation of energy for reptiles in cool surroundings during long-term inactivity, such as hibernation (Bennett and Dawson 1976), i.e. animals can readily minimise metabolic costs by occupying a relatively cool environment.

Table 6 presents data from Andrews and Pough (1985) on the standard metabolic rates for lizards. These were used to determine the range of variation in standard metabolic rates of fossorial and surface-dwelling lizards so that resemblance of metabolism in *D. impar* to the two groups could be determined. The standardised residuals represent the magnitude of deviation in the observed metabolism of lizard species from rates expected according to the multiple regression equation produced by Andrews and Pough (1985). The equation has been used to standardise observed rates of metabolism according to adult body mass, temperature (between 20 and 40°C), and activity state (standard or resting). Metabolic rate during inactivity is greater in surface-dwelling skinks ($n=6$) than in fossorial skinks ($n=4$), and in surface-dwelling species in general ($n=8$; $n=3$ fossorial families) (Mann-Whitney $U' = 23.0$; $p < 0.05$). Average standardised residuals of both fossorial species and surface-active species within each family only were considered in analyses to minimise phylogenetic influence. Mean standardised residuals for fossorial species were consistently less than predicted, and were variable among surface dwelling species. Standardised rates of metabolism in *D. impar* are at a level corresponding to average rates for surface-dwelling lizards and are faster than those observed for fossorial species (Figure 6).

Although phylogenetic influence on metabolism appears to be relatively small, its consideration is warranted where the relative rate of a single species is of interest. In the absence of data on metabolism in other pygopodids, the extent of phylogenetic influence on rates of metabolism in *D. impar* may be assessed by considering the rates of metabolism recorded for the closest relative of the Pygopodidae, the geckoes. Standardised residuals averaged -0.461 in geckoes. Accordingly, the relatively high rate of metabolism in *D. impar* does not appear to be an artefact of phylogeny, but suggests that the species is similar to surface-active lizards in its metabolic processes.

Table 6

Ecological correlates in standard metabolic rate. Standardised residuals indicate deviations in observed rates from predicted.

Family	Species	Ecological category*	Standardised residuals	mass (g)
Agamidae	<i>Amphibolurus barbatus</i>	S	.678	373.0
	<i>Physignathus lesueuri</i>	S	.151	504.0
Anguidae	<i>Anguis fragilis</i>	F	-1.933	11.4
Anguidae	<i>Ophisaurus ventralis</i>	F	-1.115	32.2
Anniellidae	<i>Anniella pulchra</i>	F	-.412	4.5
Gekkonidae	<i>Anarbylus switaki</i>	S	-.082	9.5
	<i>Cosymbotus platyurus</i>	S	-.250	3.5
	<i>Coleonyx variegatus</i>	S	1.185	3.6
	<i>Gekko gekko</i>	S	-.401	61.5
	<i>Gonatodes antillensis</i>	S	-1.062	1.8
	<i>Hemidactylus frenatus</i>	S	-.265	4.4
	<i>Sphaerodactylus beattyi</i>	S	.683	.4
	<i>Sphaerodactylus cinereus</i>	S	-1.249	.5
	<i>Sphaerodactylus macrolepis</i>	S	-.431	.5
	<i>Sphaerodactylus notatus</i>	S	-1.483	.3
Iguanidae	<i>Anolis acutus</i>	S	1.098	4.3
	<i>Anolis bonairensis</i>	S	-.845	12.0
	<i>Anolis carolinensis</i>	S	.434	4.5
	<i>Anolis limifrons</i>	S	.180	1.5
	<i>Crotaphytus collaris</i>	S	.755	30.0
	<i>Dipsosaurus dorsalis</i>	S	-.588	35.0
	<i>Iguana iguana</i>	S	.628	795.0
	<i>Phrynosoma cornutum</i>	S	.029	51.0
	<i>Phrynosoma douglassi</i>	S	.165	28.0
	<i>Phrynosoma m'calli</i>	S	.247	16.0
	<i>Sauromalus hispidus</i>	S	-.247	574.0
	<i>S. obesus</i>	S	-.740	150.0
	<i>Sceloporus graciosus</i>	S	-.134	5.0
	<i>Sceloporus occidentalis</i>	S	.930	10.0
	<i>Sceloporus olivaceus</i>	S	1.258	20.0
	<i>Sceloporus undulatus</i>	S	.958	4.0
	<i>Uta stansburiana</i>	S	-.413	14.0
Lacertidae	<i>Acanthodactylus bosianus</i>	S	.543	7.8
	<i>Acanthodactylus erythrurus</i>	S	2.073	9.0
	<i>Acanthodactylus pardalis</i>	S	2.167	9.7
	<i>Acanthodactylus schreiberi</i>	S	.197	10.9
	<i>Acanthodactylus scutellatus</i>	S	.508	6.6
	<i>Lacerta sicula</i>	S	.145	8.0
	<i>Lacerta trilineata</i>	S	.072	60.0

Table 6 continued

Family	Species	Ecological category*	Standardised residuals	mass (g)
	<i>Lacerta viridis</i>	S	-.379	25.0
	<i>Lacerta vivipara</i>	S	.163	4.0
Pygopodidae	<i>Delma impar</i>	U	.200	3.3
Scincidae	<i>Acontias meleagris</i>	F	-1.809	7.3
	<i>Chalcides ocellatus</i>	F	-1.284	25.0
	<i>Ctenotus labillardieri</i>	S	-.560	2.8
	<i>Egernia cunninghami</i>	S	.002	261.0
	<i>Eumeces fasciatus</i>	S	.837	7.0
	<i>Eumeces inexpectatus</i>	S	-.169	9.6
	<i>Eumeces obsoletus</i>	S	.527	30.0
	<i>Scelotes gronovii</i>	F	-.254	1.1
	<i>Scincella lateralis</i>	S	.407	1.0
	<i>Sphenops sepsoides</i>	F	-1.412	7.4
	<i>Tiliqua scincoides</i>	S	.152	493.0
	<i>Trachydosaurus rugosus</i>	S	.400	461.0
Teiidae	<i>Cnemidophorus murinus</i>	S	-.448	85.0
	<i>Cnemidophorus tigris</i>	S	.019	18.0
Varanidae	<i>Varanus albigularis</i>	S	1.128	963.0
	<i>Varanus bengalensis</i>	S	1.206	3440.0
	<i>Varanus exanthematicus</i>	S	-.167	7500.0
	<i>Varanus gouldi</i>	S	.636	674.0
	<i>Varanus varius</i>	S	.111	4410.0
Xantusiidae	<i>Klauberina riversiana</i>	S	-.717	19.0
	<i>Lepidophyma gaigeae</i>	S	-.189	5.0
	<i>Lepidophyma smithi</i>	S	-1.698	25.0
	<i>Xantusia henshawi</i>	S	.037	3.5
	<i>Xantusia vigilis</i>	S	-.175	1.5

* F = fossorial

U = undetermined

S = surface-active

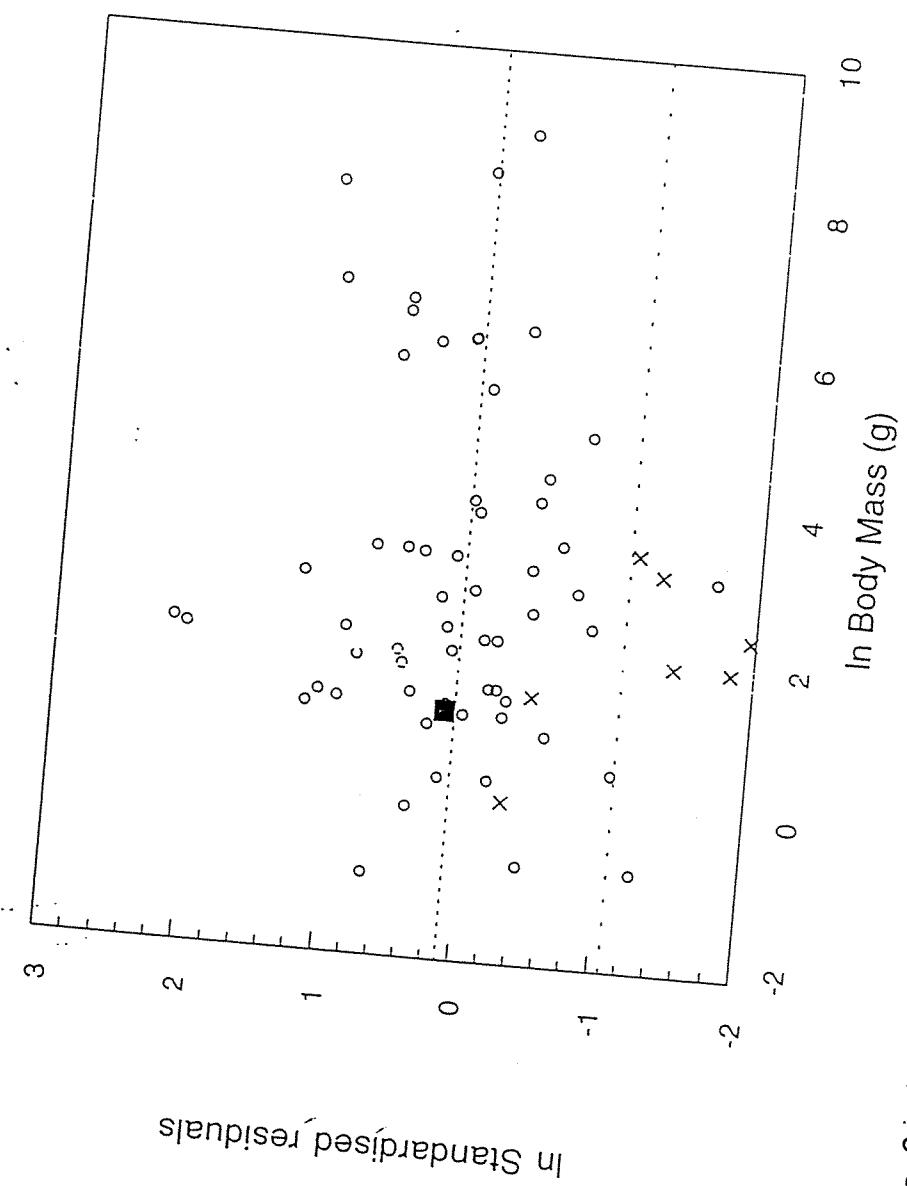


Figure 6. Deviations in standard rates of metabolism from expected rates for *D. impar* (■), and fossorial (x) and surface-dwelling (o) lizards.

Conclusions

D. impar appears to be physiologically adapted to a cool and relatively homogeneous thermal climate, and probably requires well-sheltered environments for efficient physiological operation. Standard rates of metabolism suggest that *D. impar* is largely surface-dwelling and displays moderate levels of activity.

Chapter 3. Genetics

3.1 Introduction

Exchange of genetic material among isolated populations by interbreeding serves to counteract genetic differentiation of populations. In contrast, where interbreeding is only slight or absent, genetic differences, either in the frequencies with which alleles occur or in the allelic composition of allozymes, can be observed. Comparing genetic differences among populations using allozymes permits the determination of the interbreeding of isolated populations, and can be used as an indication of the mobility of species.

Delma impar occurs primarily in the ACT and Victoria. The distribution of the species is confined to areas of lowland native grassland. The continuity and size of areas of these grasslands have greatly decreased over the last century through urban and agricultural development. It may be that former populations have been divided into reproductively isolated subpopulations in consequence. The recent history of lowland native grassland in Australia is reasonably well known and it is of interest to compare the genetic identities of populations in the ACT and Victoria, which appear to have been separated for varying periods of time. Consideration of the genetic similarities of *D. impar* populations and a comparison of genetic parameters determined for the species with previously reported values of lizards permits hypotheses concerning the mobility of *D. impar* to be generated.

3.2 Methods

3.2.1 Tissue sampling

Genetic analyses were based on tissue samples from 120 *D. impar* individuals representing the three populations occurring in the ACT and groups occurring in several Victorian localities. Livers were taken from 40 individuals in all populations except for the Museum population of the ACT. Muscle tissue constituted the majority of tissue sampled and was taken from all 120 individuals. Muscle was obtained from tails, which subsequently could be regenerated. Tail tips were removed by applying firm pressure with fingers to a section near the end of the tail at which point the animal usually attempted to escape, autotomizing the tail in the process. Otherwise, tail fracture was

induced by gently twisting the tail end. Most of the tails sampled were primary growth, but several constituted tail regrowth. Secondary growth does not contain bone. The tissues examined were largely sampled during the time of study and were stored at -70°C immediately upon removal. The livers and some tails of Victorian animals had been sampled and stored previously at the Melbourne Zoo.

3.2.2 Allozyme electrophoresis

Electrophoresis was performed using cellulose acetate gels following the methods described in Richardson et al. (1986). While still frozen from storage, the skin of tails was removed to facilitate muscle homogenisation. Tissues were thawed slightly on ice quickly before being mechanically homogenised in equal volumes of lysis solution, and then centrifuged for ten minutes at 10 000 rpm. The supernatant was collected in 20-25ml capillary tubes and loaded directly onto gels by carefully wiping the tip of the capillary tube across a loading site of the gel so that a thin film of solution was released. Some 50 samples per gel were loaded. Prior to loading, gels had been immersed in the appropriate running buffer for at least 20 minutes and blotted. The buffers and running times used for each protein system are given in Appendix 1, with all buffer recipes and stains following the recommendations of Richardson et al. (1986). Gels were run at 5°C using 200V. Stain ingredients were mixed and the solution spread thinly over a flat sheet of plastic just prior to staining. The active side of the gel only was immersed in the staining solution until the gel surface was evenly stained. Gels were then blotted and the bands of activity, as they became visible, scored using visible or UV light as appropriate. Unless bands appeared immediately, gels were incubated at 37°C to accelerate enzyme activity. Gels were photocopied throughout the scoring process and either fixed by freezing or by soaking in formalin. Alleles were labelled alphabetically according to anodal migration distance. Comparison gels were run to check the consistency of enzyme activity and allele segregation. The clarity of bands was improved, where necessary, by modifying the buffer, running time, or substituting cathodal loading sites with anodal ones.

3.2.3 Analysis

Intrapopulation variation

Three measures of genetic diversity were made: 1) the proportion of loci exhibiting polymorphism. This measure distinguishes between loci which have variant and invariant allelic constitutions and determines whether or not the genetic variation present

is widely distributed across loci within the population; 2) the mean number of alleles per locus. This is a very crude measure of genetic variation and so varies relatively little between populations (Nevo 1978). Nevertheless, its generality and simplicity means that it is a measure through which large differences between taxa can be easily detected. Put simply, populations with many alleles per locus have great scope for variation through different allelic combinations, while populations with a mean number of alleles per locus of essentially one show very low genetic diversity and; 3) genetic variability may be measured by calculating the proportion of individuals heterozygous at each locus, averaged over all loci. In contrast to the other measures, estimates of mean heterozygosity are relatively insensitive to the number of individuals which have been sampled per population. For instance, where sample size is small, mean heterozygosity estimates will be affected only slightly if a rare allele is missed through sampling error, whereas values of mean allele number and proportion of polymorphic loci could be doubled or halved. All three measures, however, provide different information and it is useful to assess each of them.

Average heterozygosity estimates were obtained for each population by direct count, and compared with expected values under Hardy-Weinberg equilibrium, applying Nei's (1978) unbiased estimate and Levene's (1949) correction for small sample size. Deviations from Hardy-Weinberg equilibrium were determined with the chi-square test. Because the chi-square test cannot be used for data points of less than five, the frequencies of alleles for which expected frequencies were low were pooled. Where less than seven individuals had been sampled, calculations of exact significance probabilities replaced the chi-square test because the latter shows high variance at small sample sizes (Elston and Forthofer 1977).

Interpopulation variability

Genetic relations among populations were quantified using Nei's (1978) unbiased genetic identity and Rogers' (1972) coefficient of genetic similarity. Both of these measures have been widely used and thus their calculation is useful for comparison of taxa. The measures are derived from gene frequency data and they assess the distributional patterns of alleles at loci across populations. Populations sharing the same alleles at a given locus and exhibiting those alleles at similar frequencies are genetically similar and are considered to be closely related. Nei's (1978) coefficient was employed in a cluster analysis using UPGMA.

The genetic relations of populations have also been assessed using Wright's (1969, 1978) 'F' statistics. These are a measure of the genetic differentiation of populations at

individual loci. The genetic variability within populations (F_{IS}) is contrasted with the genetic variability among populations (F_{IT}). F_{ST} summarises the genetic differentiation over all populations. Substantial divergence of populations, perhaps due to lack of gene flow, is indicated where interpopulation variability is greater than intrapopulation variability.

The genetic relations among Victorian groups could not be assessed in this study because of sample size limitations. However, individuals sampled at the different localities were segregated to avoid mixing the genetic identities of populations. The results thus obtained may serve to guide future work involving these populations.

In order to discount the possibility that genetic divergence of populations was due to isolation of populations by distance rather than geographical barriers, the two geographical extremes (two or three hundred metres separate) of the ACT's most widespread population, Gungahlin, were considered, in separate analyses of genetic differentiation of ACT populations, both as separate groups and as belonging to one population. Any differentiation of the two subpopulations would indicate that geographical barriers are not the only obstruction to gene flow, such that populations may be distinct genetically in the absence of habitat fragmentation.

The statistical package Biosys-1 (Swofford and Selander 1981) was used in the analysis of electrophoretic data.

3.3 Results

General

The allele frequencies at loci, and the sample sizes per locus per population are given in Table 1. Twenty nine presumptive loci were scorable in liver, and all of these excluding Pgm-1 were scorable in muscle. Of the 29 loci, 11 exhibited polymorphism in at least one population. Nine of these were scorable in muscle tissue, and eight polymorphic loci were examined in all individuals. Individuals for which both muscle and liver were examined were of the same genotype in each tissue. In no instance were fixed allelic differences between populations observed. Estimates of genetic variability were derived from information for all 29 loci except for those values representing the Museum population of the ACT, for which Pgm-1 was not scored.

Table 1
Population allele frequencies for polymorphic loci in *Delma impar*
Sample sizes included

LOCUS	POPULATION*								
	VS	VD	VL	VDP	VLtn	VU	G	Ma	Mu
Est-1									
(N)	5	8	1	1	1	2	45	50	6
A	1.000	1.000	1.000	1.000	1.000	1.000	0.944	0.880	0.917
B	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.120	0.083
Gpi									
(N)	5	8	1	2	1	2	45	50	6
A	1.000	0.875	1.000	1.000	1.000	1.000	0.935	1.000	1.000
B	0.000	0.125	0.000	0.000	0.000	0.000	0.065	0.000	0.000
Idh-1									
(N)	5	8	1	2	1	2	44	50	4
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.210	0.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.790	1.000
Idh-2									
(N)	5	8	1	2	1	2	44	50	5
A	0.000	0.063	0.000	0.000	0.000	0.000	0.801	0.830	0.300
B	1.000	0.875	1.000	1.000	1.000	1.000	0.144	0.170	0.700
C	0.000	0.063	0.000	0.000	0.000	0.000	0.055	0.000	0.000
Lap									
(N)	3	8	1	2	1	2	39	47	4
A	0.500	0.688	0.500	0.500	0.500	0.250	1.000	0.968	1.000
B	0.500	0.313	0.500	0.500	0.500	0.750	0.000	0.032	0.000
Me									
(N)	5	8	1	2	1	2	44	50	4
A	0.500	0.500	0.500	0.250	0.000	0.750	0.947	1.000	1.000
B	0.500	0.500	0.500	0.750	1.000	0.250	0.053	0.000	0.000
PepA									
(N)	5	8	1	2	1	2	44	50	4
A	0.300	0.188	0.500	0.250	0.000	0.000	0.060	0.030	0.000
B	0.700	0.813	0.500	0.750	1.000	1.000	0.940	0.970	1.000
Pgdh									
(N)	5	8	1	2	1	2	43	48	3
A	0.800	0.875	0.500	0.750	1.000	0.750	0.966	0.979	0.667
B	0.200	0.125	0.500	0.250	0.000	0.250	0.034	0.121	0.333
Pgk									
(N)	4	8	1	2	1	2	11	10	6
A	0.875	0.938	1.000	0.500	1.000	1.000	0.955	0.950	1.000
B	0.125	0.063	0.000	0.500	0.000	0.000	0.045	0.050	0.000
Pgm-1									
(N)	5	8	1	2	1	2	11	10	
A	1.000	1.000	1.000	1.000	1.000	1.000	0.591	0.400	
B	0.000	0.000	0.000	0.000	0.000	0.000	0.227	0.200	
C	0.000	0.000	0.000	0.000	0.000	0.000	0.182	0.400	
Pgm-2									
(N)	5	8	1	2	1	2	11	10	5
A	1.000	1.000	0.500	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000

* Codes prefixed with 'V' indicate Victorian populations

VS: Sunshine
VD: Derrimut
VDP: Deer Park
G: Gungahlin

VLtn: Laverton
VL: Lara
VU: unknown
Ma: Majura
Mu: Museum

There was substantial differentiation of loci between populations, much of which was concordant with the Victorian/ACT division of animals.

3.3.1 ACT - Victorian comparison

Measures of genetic diversity are provided in Table 2. Overall, ACT groups exhibited polymorphism in a proportion of loci similar to that in Victorian groups, but polymorphism was not confined to the same loci in each group. *Idh-2* was the only locus at which differential fixation of alleles between the two groups was suggested, the 'a' allele being predominant in the ACT populations, but rare in the Victorian populations. For populations of sample size greater than four, the percentages of polymorphic loci were 17.2 and 24.1% in Victorian populations, and ranged between 10.7 and 27.6% in the ACT populations. Victorian animals display greater allelic variation at each locus than ACT animals. Mean heterozygosity per locus (direct count) was 6.1 and 7.3% in Victorian populations, and between 3.3 and 4.8% in ACT groups.

The populations were organised through UPGMA into two distinct groups according to Victorian and ACT origins (Figure 1). Victorian and ACT populations are estimated to be about 90 and 95% similar, for Rogers' (1972) and Nei's (1978) measures respectively (Table 3).

The F statistics indicate substantial divergence at the *Idh-2*, *Lap*, and *Me* loci (Table 4). Locus *Idh-2* showed differentiation twice as great as the average of all loci among populations. F_{IS} values are, on average, less than zero and show that heterozygotes are slightly more common than expected. F_{IT} values tend to be considerably greater than zero as do F_{ST} estimates and indicate that gene flow has been more substantial within than between Victorian and ACT groups.

3.3.2 ACT group comparison

The Museum population was differentiated from the Majura and Gungahlin populations in displaying polymorphism in relatively few loci, and in the relative frequency with which alleles occurred at loci *Idh-2* and *Pgdh*. However, the relatively few individuals sampled at this population may have resulted in an exaggeration of its genetic distinction. This will be discussed further in section 3.4. Both Majura and Gungahlin populations contained within them unique alleles. Majura was distinct at the *Idh-1* and *Lap* loci, while Gungahlin was distinct at loci *Gpi*, *Idh-2*, and *Me*.

Table 2

Genetic variability estimates for populations of *Delma impar* at 29 loci
 s.e. in parentheses

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY DIRECT-COUNT	MEAN HETEROZYGOSITY HDYWBG EXPECTED**
1. Sunshine	4.9 (0.1)	1.2 (0.1)	17.2		
2. Derrimut	8.0 (0.0)	1.3 (0.1)	24.1	0.061 (0.028)	0.077 (0.033)
3. Lara	1.0 (0.0)	1.2 (0.1)	17.2	0.073 (0.027)	0.074 (0.028)
4. Deer Park	2.0 (0.0)	1.2 (0.1)	17.2	0.172 (0.071)	0.172 (0.071)
5. Laverton	1.0 (0.0)	1.0 (0.0)	17.2	0.086 (0.043)	0.098 (0.041)
6. Unknown	2.0 (0.0)	1.1 (0.1)	3.4	0.034 (0.034)	0.034 (0.034)
7. Gungahlin	10.9 (0.1)	1.2 (0.1)	10.3	0.052 (0.029)	0.052 (0.029)
8. Majura	10.0 (0.0)	1.3 (0.1)	20.7	0.033 (0.014)	0.052 (0.025)
9. Museum***	5.5 (0.2)	1.1 (0.1)	27.6	0.048 (0.017)	0.065 (0.027)
			10.7	0.037 (0.025)	0.042 (0.025)

A locus was considered polymorphic if more than one allele was observed
 Unbiased estimate (see Nei 1978)
 * Estimates for the Museum population calculated from 28 loci only

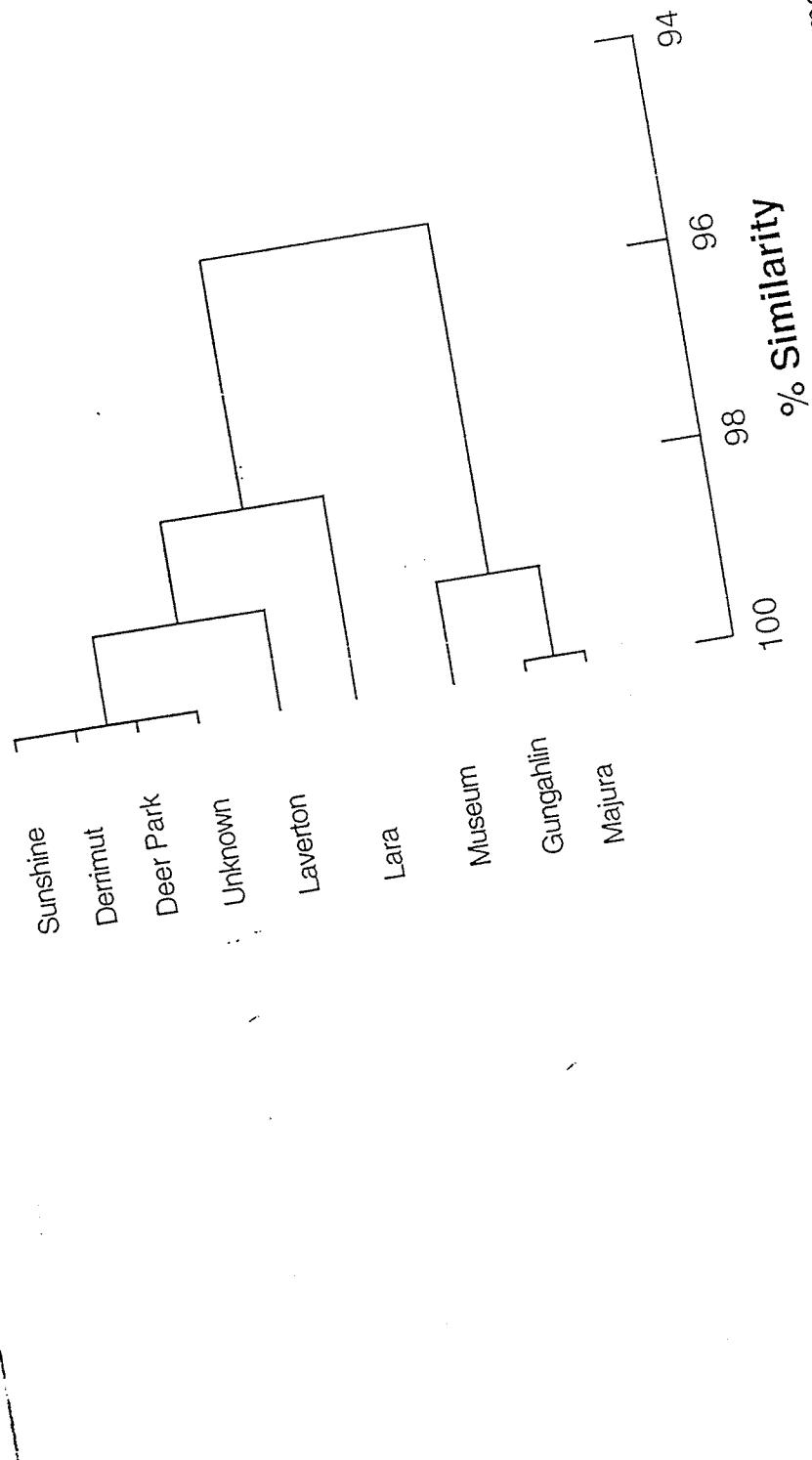


Figure 1 UPGMA cluster of Nei (1978) genetic similarity measures among ACT and Victorian populations of *Delma impar*

Table 3
Matrix of genetic similarity coefficients for *Delma impar*: all populations

Below diagonal: Rogers (1972) genetic similarity
Above diagonal: Nei (1978) unbiased genetic identity

POPULATION	1	2	3	4	5	6	7	8	9
1 Sunshine	*****	1.000	0.990	1.000	0.991	1.000	0.961	0.958	0.981
2 Derrimut	0.976	*****	0.981	1.000	0.989	0.997	0.972	0.967	0.987
3 Lara	0.960	0.940	*****	0.987	0.963	0.981	0.933	0.932	0.960
4 Deer Park	0.974	0.954	0.938	*****	0.998	0.997	0.950	0.947	0.970
5 Laverton	0.960	0.954	0.929	0.955	*****	0.982	0.933	0.929	0.949
6 Unknown	0.965	0.954	0.938	0.946	0.955	*****	0.961	0.958	0.983
7 Gungahlin	0.912	0.935	0.878	0.890	0.910	0.922	*****	1.000	0.992
8 Majura	0.912	0.926	0.877	0.889	0.902	0.918	0.979	*****	0.989
9 Museum	0.931	0.940	0.909	0.912	0.921	0.948	0.966	0.961	*****

Matrix of genetic similarity coefficients for *Delma impar*: ACT populations
Derived from muscle tissue (8 loci only)

POPULATION	1	2	3
1 Gungahlin	*****	0.993	0.956
2 Majura	0.929	*****	0.950
3 Museum	0.871	0.856	*****

Table 4
Summary of F statistics at individual loci for ACT and Victorian populations of *Delma impar*

LOCUS	ACT and Victorian populations			ACT populations only			ACT populations excluding Museum		
	F _{IS}	F _{TR}	F _{ST}	F _{IS}	F _{TR}	F _{ST}	F _{IS}	F _{TR}	F _{ST}
Est-1	-0.104	-0.069	0.032	-0.096	-0.085	0.010	-0.098	-0.083	0.013
Gpi	-0.064	-0.031	0.032	-0.071	-0.033	0.035	-0.071	-0.045	0.024
Idh-1	-0.266	-0.055	0.166	-0.266	-0.055	0.166	-0.266	-0.075	0.151
Idh-2	0.214	0.562	0.443	0.150	0.365	0.253	-0.021	0.016	0.036
Lap	0.143	0.430	0.335	-0.033	-0.008	0.024	-0.033	-0.011	0.022
Me	0.023	0.417	0.403	0.514	0.539	0.051	0.514	0.534	0.042
PepA	0.017	0.111	0.096	-0.065	-0.039	0.024	-0.065	-0.053	0.011
Pgdh	-0.344	-0.167	0.131	-0.370	-0.118	0.184	-0.033	-0.030	0.002

MEAN	-0.020	0.271	0.285	-0.029	0.135	0.160	-0.034	0.010	0.040
------	--------	-------	-------	--------	-------	-------	--------	-------	-------

Gungahlin and Majura were estimated to be 98 and 100% similar according to Rogers' (1972) genetic similarity coefficient and Nei's (1978) unbiased genetic identity respectively, and the Museum population was estimated to be 96 and 99% similar to these two populations, for the same coefficients (Table 3). The relative genetic similarities of ACT populations were consistent whether derived from data for 28 loci or for the eight loci scored in tail muscle displaying polymorphism alone. Therefore muscle, which can be sampled without the sacrifice of animals, can be used for future genetic work on *D. impar* with very little loss of information relative to that derived from liver. It has also been noted that enzyme activity in tail regrowth was comparable to that occurring in primary growth.

Average heterozygosity values appeared significantly different from those expected under Hardy-Weinberg equilibrium in one instance. Heterozygotes are less common than expected in the Gungahlin population at Pgm-1. Three alleles occurred at this locus; all three were present in homozygous form, while only one 'ab' heterozygote occurred. These results indicate that genetic divergence resulting from isolation by distance may be slightly effective. Individuals from Gungahlin South appear distinct from Gungahlin North individuals at the Idh-2 locus (Table 5). It could not be determined whether individuals from the two areas were differentiated at Pgm-1 because no liver samples were available for Gungahlin South individuals.

F statistics for the ACT groups indicate relatively little genetic differentiation of individual populations (Table 4). Values of F_{ST} and F_{IT} for the Gungahlin and Majura populations are both small, suggesting that gene flow between them is effective. Relatively large F_{IT} values may indicate that gene flow between the Museum population and the Majura and Gungahlin populations has not been sufficient to keep them genetically similar.

3.4 Discussion

3.4.1 Sampling restrictions

Before the relevance of results can be discussed, the ambiguity of results associated with sampling deficiencies should first be considered. Estimates of genetic variability in populations can only be as accurate as that permitted by the data from which they are derived. If the genetic characteristics of the individuals sampled in a population at the particular loci examined do not represent the genetic variability present, estimates of

Table 5

Allele frequencies for polymorphic loci in Gungahlin North (GN) and Gungahlin South (GS) populations of *Delma impar*

LOCUS	POPULATION			
	GN	GS	Ma	Mu
Est-1				
(N)	27	18	50	6
A	0.944	0.944	0.880	0.917
B	0.056	0.056	0.120	0.083
Gpi				
(N)	27	18	50	6
A	0.926	0.944	1.000	1.000
B	0.074	0.056	0.000	0.000
Idh-1				
(N)	26	18	50	4
A	0.000	0.000	0.210	0.000
B	1.000	1.000	0.790	1.000
Idh-2				
(N)	26	18	50	5
A	0.712	0.889	0.830	0.300
B	0.231	0.056	0.170	0.700
C	0.058	0.056	0.000	0.000
Lap				
(N)	22	17	47	4
A	1.000	1.000	0.968	1.000
B	0.000	0.000	0.032	0.000
Me				
(N)	27	17	50	4
A	0.981	0.912	1.000	1.000
B	0.019	0.088	0.000	0.000
PepA				
(N)	26	18	50	4
A	0.038	0.083	0.030	0.000
B	0.962	0.917	0.970	1.000
Pgdh				
(N)	25	18	48	3
A	0.960	0.972	0.979	0.667
B	0.040	0.028	0.021	0.333

genetic variability will be in error. The most effective way to avoid sampling bias is to sample a large number of loci (Nei and Roychoudhury 1974; Nei 1978; Gorman and Renzi 1979). Biases arise because different proteins have different rates of evolution with the result that different loci have different probabilities of being polymorphic (Sarich 1977; Avise and Aquadro 1982). Reviewers of allozyme data for estimates of gene heterozygosity tend to consider only studies which involve more than 14 loci (e.g. Nevo 1978; Gorman and Renzi 1979; Ward et al. 1992), and an examination of many more than 14 loci is encouraged (Nei and Roychoudhury 1974; Nei 1978). Sampling bias is also associated with insufficient sampling of individuals, such that a substantial proportion of electrophoretically detectable allelic diversity at loci present within a population remains unsampled (Nei and Roychoudhury 1974; Nei 1978; Nevo 1978; Gorman and Renzi 1979). While the present study considered a relatively large number of loci, there were several instances where the number of individuals sampled at a population were few. Some indication of the errors associated with variance in the number of individuals sampled for estimates of average heterozygosity is provided by Gorman and Renzi (1979). These authors examined data obtained from some 30 species of lizard representing seven genera. They considered studies for which 17 to 30 loci had been examined so that variation observed in estimates was largely attributable to differences in the number of individuals sampled rather than differences in loci selection. Because the error associated with sample size variation is dependent on the level of heterozygosity within the group concerned (Nei 1978), I have considered only information for species exhibiting heterozygosity levels similar to *D. impar* (i.e. 3 - 8%). Sample sizes of between eight and 12 individuals yielded estimates of average heterozygosity similar to those derived from larger sample sizes of between 20 and 41 individuals, with an average variance of about 20%. Sample sizes of four and less were associated with a much larger error, yielding heterozygosity estimates averaging in deviation from estimates obtained using larger samples more than 30%.

In accordance with these findings, results regarding Victorian populations represented by two individuals or less will be removed from further discussion, while results for the Museum population of the ACT at loci for which three and four individuals were examined will be treated as ambiguous. Of particular concern are results for the Pgdh locus. The relative frequency of alleles at this locus in the Museum population was very different from those in the Majura and Gungahlin populations, and had high influence on FST values. Sampling bias may be responsible for this apparent differentiation. In contrast, the difference between the Museum population and the other two populations observed at the Idh-2 locus for which five Museum individuals were sampled is more likely to reflect a real difference in genetic relations of the populations. Sampling bias may also explain the relatively low proportion of polymorphic loci exhibited by the

Museum population. Rare alleles present in the populations may not have been detected because they were not present in the small number of Museum individuals sampled. One final observation is that the lack of polymorphic loci within the Museum population effectively increased the weight contributed by each polymorphic locus to estimates of genetic variability. Consequently, the absence of data for the Pgm-1 locus is likely to have lowered measures of genetic variability relative to the Gungahlin and Majura populations, given that this locus exhibited a high degree of variation in these two populations. This last point illustrates well the importance of examining a large number of loci in analyses of population genetic structure.

3.4.2 Genetic similarity and geographic history of ACT populations

Although the three ACT populations of *D. impar* are genetically very similar, genetic distinction of the Museum population is apparent. The observed distinction of the Museum population, whether or not exaggerated by sampling bias, can be considered consistent with the history of native lowland grassland in the ACT, as proposed by the Wildlife Research Unit, ACT Parks and Conservation Service (Wildlife Research Unit 1992). Grassland is thought to have been continuous between the Gungahlin and Majura areas prior to development of the Canberra city, some 50 years ago. In contrast, grassland at the Museum site is thought to have been separated from the Majura-Gungahlin grassland for a large portion of its history by woodland associated with higher altitudes. This separation may have acted to obstruct gene flow between the populations, resulting in their genetic divergence.

The Museum population could also have diverged for reasons associated with small population size. Trapping results (Kukolic unpub.) suggest that the Museum population is comparatively small. This may be due to the establishment of Lake Burley Griffin adjacent to the population site in 1963. Genetic drift or bottleneck circumstances (Soule 1976) within the Museum population may explain why some of the rare alleles present in the Majura and Gungahlin populations were not observed in this population. The genetic differentiation of the Museum population and the other two populations does not necessarily indicate the absence of gene flow between them. Rather, gene flow may be a chance event such that the rate at which it occurs proves to be relatively ineffective in homogenising the genetic identities of the Museum population relative to the Majura and Gungahlin populations because of genetic loss associated with drift.

3.4.3 Does gene flow occur between populations?

The genetic similarities of ACT and Victorian populations overall are relatively high in the range observed for conspecific populations of reptile, which spans some 69-98% and averages 85% (Rogers' 1972 similarity coefficient) (reviews in Selander and Johnson 1973; Avise 1975) and for conspecific populations in general (more recently reviewed by Thorpe 1982), relative to the 90% similarity of *D. impar* populations. The lack of extensive differentiation of ACT populations relative to Victorian groups at 29 loci suggests that gene flow between the groups has been effective. Nevertheless, it may be expected that the same genetic adaptations have evolved in both Victorian and ACT individuals, and that some of their genetic similarity is maintained through similar environmental selective pressures. Lowland native grasslands are a distinct habitat. For instance, the dense grasses maintain a temporally stable thermal climate at the ground level (chapter 2), and the biotic community is reported to be of unusually low diversity in lowland native grasslands (Kukolic unpub.). Accordingly, the particular genetic resources that are advantageous in grasslands of the ACT are also likely to be beneficial in Victorian grasslands. While the operation of selective differences or similarities at particular loci in different populations can only be determined through detailed analysis of the functional properties of polymorphic proteins in relation to the distribution of relevant environmental variables (see Selander and Johnson 1973), inferences regarding levels of gene flow occurring between populations of *D. impar* can be made by considering the degree of vagility shown by the species - i.e. how likely is it that individuals travel the distance between populations?

In the absence of direct evidence of migrational movement, this question can be first addressed by considering whether geographically separate individuals are genetically differentiated in the absence of physical barriers. The presence of reproductive barriers between individuals may be indicated when allele frequencies within the group do not conform to Hardy-Weinberg equilibrium (Futuyma 1986). Within the Gungahlin population, heterozygotes are less common than expected at Pgm-1. It may be that individuals which are homozygous at this locus are in some way advantaged over heterozygous individuals (Futuyma 1986), given that the 'a' allele is fixed in Victorian animals. However, all genotypes are represented as expected in the Majura population, and so homozygous advantage is probably not responsible for the deviation apparent in the Gungahlin population. An alternative explanation is that the Gungahlin group may represent more than one reproductively discrete population. For instance, if individuals from two populations are homozygous for different alleles, and the two populations are mistaken as one group, homozygotes will be detected but there will not be any heterozygotes of the two alleles present because the two populations do not interbreed to

any great extent. If individuals had a tendency to mate with others in close proximity only, i.e. isolation by distance (see Brown 1979; Chesson 1983), individuals occupying a continuous stretch of habitat may diverge genetically. Results of the present study indicate that genetic divergence resulting from isolation by distance may be slightly effective. Individuals from Gungahlin South appear distinct from Gungahlin North individuals at the *Idh-2* locus (Table 5).

The mobility capabilities of a species could also be assessed where levels of activity are indicated in genetic attributes. The level of vagility of species seems to be reflected in patterns of genetic variability within populations such that heterozygosity levels increase with increasing vagility (Nevo and Shaw 1972; Nevo et al. 1974; Gorman et al. 1977). Moreover, populations of individuals exhibiting low vagility are expected to exhibit a relatively high level of interpopulation variation because of lack of gene flow among populations (Brown 1979; Gorman and Kim 1975).

Genetic correlations of vagility in lizards provides a means of inferring vagility levels in *D. impar*. Fossorial lizards are habitat specialists and appear to be of extremely limited inmobility (Miller 1944; Huey et al. 1974). Low allelic heterozygosity seems to characterise fossorial species of a variety of taxa (Nevo and Shaw 1972; Nevo et al. 1974; Bezy et al. 1977; Kim et al. 1976- cited in Gorman et al. 1977) and while the genetic variability of only two fossorial reptiles has been examined, levels are much lower than those of more typical (vagile, surface-active) reptiles (Table 6). *D. impar*, in comparison, has mean heterozygosity values comparable to the sit-and-wait predatory reptiles (relatively inactive surface-dwellers) included in the study of Gorman et al. (1977). Furthermore, homozygotes are less common than expected according to Hardy-Weinberg equilibrium, as shown by negative *FIT* values (Table 4), and thus inbreeding does not seem to operate to any great extent. Clearly, however, more work needs to be done if we are ever able to infer with accuracy the activities and ecologies of species through genetic data.

Conclusions

Because *D. impar* populations are highly similar genetically, and intrapopulation genetic variability is substantial and corresponds more closely to levels contained within vagile, surface-active lizards than to inactive and inbred species, there seems to be no evidence suggesting that populations have been reproductively isolated.

Table 6

Ecological correlates of genetic diversity in reptiles

Ecological category	Mean heterozygosity (%)	Range of heterozygosity (%)	N (spp)	Reference
General	7.8	s.e. = 0.7	85	Ward et al. 1992
Fossorial	1.1	0 - 3.2	3	Cited in Gorman et al. 1977
Sit-and-wait	4.3	0 - 10	6	Cited in Gorman et al. 1977
Active forager	11.9	5.9 - 12.9	2	Cited in Gorman et al. 1977
<i>Delma impar</i>	5.0	3.3 - 7.3	-	present study

Chapter 4. General Discussion

4.1 Thermal constraints on activity in *Delma impar*

It is apparent that *D. impar* is not operationally confined to the soil environment, and that individuals have been able to migrate between populations. The occurrence of genetic differences between populations, however, suggests that gene flow is restricted to some extent. How might activity of *D. impar* be constrained?

Physiological examination of *D. impar* indicates that the species prefers low temperatures, between 24 and 28°C, and suffers reduced performance as temperatures decrease below this level. Many lizard species which are active at low temperatures appear to have relatively poor tolerance of high temperatures (Bury and Balgooyen 1976; Greer 1980). *D. impar* spent comparatively little time at temperatures above 27.5°C. *Anniella pulchra*, a species which prefers temperatures similar to *D. impar*, avoids temperatures over 30°C (Bury and Balgooyen 1976). Furthermore, moisture appears essential to the existence of a number of lizards occupying the sheltered ground layer environment (Miller 1944; Bury and Balgooyen 1976; Al-Sadoon and Spellerberg 1985). While individuals living either within the soil or in dense cover at the surface can function relatively independently of the more climatically variable surface environment, migration of individuals between disjunct populations necessitates movement across the surface layer. Thus, hot and desiccating conditions are likely to be effective constraints on the migrational activity of species which are typically active in sheltered microhabitats. In addition, the microclimate experienced by *D. impar* when retreating underground at night permits individuals to maintain higher body temperatures. Surface exposure at night may mean that body temperature decreases as heat is lost to the environment, and would result in reduced performance of important processes such as digestion.

The lack of suitable shelter outside of *D. impar*'s preferred habitat may limit the successful migration of individuals between populations. Obstructions to migration restrict the amount of gene flow that occurs between populations, and so influence the genetic identities of populations. By considering how the genetic similarities of populations vary with regard to the type of environment that separates them, we may be able to infer the specific habitat requirements of the species.

4.2 Habitat requirements

Gene flow between the Museum population, and the Gungahlin and Majura populations of the ACT appears to be restricted. The Museum population is geographically separated from the other two populations by dry sclerophyll woodland. In contrast to the dense vegetation, and basaltic or sedimentary soil types characteristic of native lowland grassland (Coulson 1990; W. Osborne pers. comm.), the ground layer of dry sclerophyll woodland constitutes relatively sparse ground vegetation, and dry stony soil (pers. obs.). *D. impar* individuals attempting to cross these woodlands between populations may have difficulties finding shelter sites that maintain temperature and humidity to an extent comparable to the grass and soil of native grasslands. Similar restrictions are probably experienced in agriculturally-modified areas of land. Agricultural development has played a major role in the loss and fragmentation of lowland native grassland (Coulson 1990). Domestic livestock compact the soil, and the establishment of pastoral crops also invariably involves modification of soil structure and composition through ploughing and nutrification (Bock et al. 1990). The reduced abundance of suitable soil shelters resulting from urban and rural development has been linked to the decline in abundance of the Adelaide pygmy bluetongue, *Tiliqua adelaidensis* (Armstrong 1992). Similarly, the reduction in ground cover attributable to grazing and pasture plantations is thought to have deprived the North American iguana *Sceloporus scalaris* of its grass tussock shelter sites, without which survival appears reduced (Bock et al. 1990). Clearly, suitable forms of shelter are essential for the survival of lizards.

4.3 Assessments of gene flow and life-history traits

The more fragmented habitats become, the greater the distance and probable variety of habitats between remnant populations. Migration is likely to become more difficult as land modification becomes more extensive. Because lowland native grassland has been modified at a very fast rate, the patterns in genetic variation among populations may reflect levels of gene flow that occurred sometime in the past, at times when migration between populations may have been comparatively easy and frequent. The rate at which the genetic composition of populations change is dependent on generation times. *D. impar* has very low reproductive potential. Females lay only one clutch of two eggs in a season, and probably do not lay in consecutive years (see Greer 1989; Kukolic pers. comm.). Although longevity is uncertain, a currently captive individual has persisted for more than

four years (Kukolic pers. comm.), and thus may be relatively long-lived for a reptile of this size (see Greer 1989). Hatchlings probably take at least three years to reach reproductive maturity (Kukolic pers. comm.). Perhaps less than twenty generations of *D. impar* have probably passed since the development of Canberra sub-divided native grasslands in the ACT, some thirty to forty years ago. Depending on population size, of course, that is little time for genetic divergence of populations (see Sarich 1977; Thorpe 1982). Hence, changes in patterns of gene flow are not likely to be reflected in the genetic constitutions of populations for some time after the changes occur.

4.4 Studies of ectothermal ecology

A common approach to describing and explaining patterns in nature involves identifying similarities between taxa. Species which occupy the same habitat type and function in similar ways are likely to be influenced by the same environmental pressures and to have traits of common adaptive value. This approach is particularly useful in an interpretation of the functional qualities of ectotherms because all ectotherms have in common a dependence of activity on temperature. By examining the thermal requirements for activity of an ectotherm we are assessing an aspect central to its survival. Its ability to forage, to avoid predation, and to acquire mates are all related to how it performs in the thermal climate in which it lives.

The physiology of organisms can be examined at different levels, each level providing different information. Some, such as metabolism, indicate abilities to perform. Others indicate functional capacities. In order to distinguish the specific factors which influence an ectotherm's physiology, a comparative approach is useful i.e. information from a variety of sources should be combined.

The results of the present study illustrate the ambiguity involved in indirect assessments of ecology. *Delma impar* exhibits temperature preferences similar to those of the relatively sedentary, fossorial lizards. Genetic similarities of populations, on the other hand, correspond to those of active, surface-dwelling lizards. If only one or the other of these analyses had been conducted, conclusions reached concerning the activity of the species could have been conflicting. By expanding analyses to include a variety of activity-indicative traits, and the functional adaptations of other ecological taxa, patterns regarding ecology begin to emerge because results begin to complement one another. For instance, the high rate of metabolism but low thermal dependence of metabolism,

particularly at lower temperatures, demonstrated by *D. impar* are probably indicative of an active animal adapted to cool temperatures. This information can be used to aid interpretation of temperature preference results, i.e. that preference for low temperatures of *D. impar* are probably indicative of adaptation to a cool thermal regime rather than to the soil environment *per se*.

Studies which consider both physiological and genetic qualities of species are absent from the literature. This study, however, shows that when they are used in combination they are a valuable means of identifying the adaptive significance of operational characteristics, and thus the factors which constrain activity and distribution of organisms.

Acknowledgements

Special thanks to Paul Cooper (ANU) for introducing me to reptiles, and for his extraordinary enthusiasm, practical sense and support; to Kruno Kukolic and Will Osborne (ACT Parks and Conservation Service) for their eagerness to share their knowledge; to Peter Robertson (Arthur Rylah Institute for Environmental Research, Melbourne) and Chris Banks (Melbourne Zoo) for their support; to Steven Donnellan (Museum of South Australia) for his help with the genetic work; to Stephen Sarre (ANU) for his advice and critical review of my work; to Terry Murphy for his time and talent for innovation; to the ANU 'workshop crew' for construction and maintenance of equipment; to Tim Marples for his space; to Keith Herbert for processing film; to the ACT Herpetological Society and friends for their assistance in establishing and checking pitfall traps; and to my family for their interest.

Many thanks also to ACT Parks and Conservation Service for their financial support.

Appendix 1

Protein systems scored and electrophoretic conditions used in genetic analyses of *Delma impar*

Protein system	E.C. number	Buffer*; running time (hr)
Aconitate hydratase (Acon)	4.2.1.3	A; 2.5
Adenylate kinase (Ak)	2.7.4.3	B; 2.5
Aspartate aminotransferase (Aat)	2.6.1.1	B; 2
Carbonic anhydrase (Ca)	4.2.1.1	C; 2
Dipeptidase (PepA)	3.4.13.11	A; 2
Esterase (Est)	3.1.1.1	B; 1.5
Fructose diphosphatase (Fdp)	3.1.3.11	B; 2.5
Glycerol-3-phosphate dehydrogenase (G3pdh)	1.1.1.8	C; 2
Glucose-phosphate dehydrogenase (Gpdh)	1.1.1.49	C; 2
Glucose-phosphate isomerase (Gpi)	5.3.1.9	C; 2
Isocitrate dehydrogenase (Idh)	1.1.1.42	A; 2.5
Lactate dehydrogenase (Ldh)	1.1.1.27	C; 2
Leucine aminopeptidase (Lap)	3.4.11.1	A; 1.5
Malate dehydrogenase (Mdh)	1.1.1.37	A; 2.5
Malic enzyme (Me)	1.1.1.40	B; 2.5
Mannose-phosphate isomerase (Mpi)	5.3.1.8	C; 2
Phosphoglucomutase (Pgm)	2.7.5.1	B/D; 2.5
Phosphogluconate dehydrogenase (Pgdh)	1.1.1.44	C; 2.5
Phosphoglycerate mutase (Pgamt)	2.7.5.3	A; 1.5
Phosphoglycerate kinase (Pgk)	2.7.2.3	A; 2
Sorbitol dehydrogenase (Sordh)	1.1.1.14	B; 2.5
Superoxide dismutase (Sod)	1.15.1.1	C; 2
Triose-phosphate isomerase (Tpi)	5.3.1.1	C; 2.5

*A 0.01M Citrate-phosphate pH 6.4

B 0.05M Tris-Maleate pH 7.8

C 0.02M Phosphate pH 7.0

D 0.02M Tris-EDTA-Citrate pH 7.5

REFERENCES

- Armstrong, G. and Reid, J. (1992). The rediscovery of the Adelaide pygmy bluetongue *Tiliqua adelaidensis*, (Peters, 1863). *Herpetofauna* 22: 3-7
- Al-Sadoon, M. K. and Spellerberg, I. F. (1985). Effect of temperature on the oxygen consumption of lizards from different climatic regions. *Amphibia-Reptilia* 6: 241-258.
- Al-Sadoon, M. K. and Abdo, N. M. (1989). Temperature effects on oxygen consumption of two nocturnal geckos, *Ptyodactylus hasselquistii* (Donndorff) and *Bunopus tuberculatus* (Blanford) (Reptilia: Gekkonidae). *Journal of Comparative Physiology* 159B: 1-4.
- Aleksuk, M. (1971). Temperature-dependent shifts in the metabolism of a cool temperate reptile, *Thamnophis sirtalis parietalis*. *Comparative Biochemistry and Physiology* 39A: 495-503.
- Allendorf, F. W. (1983). Isolation, gene flow, and genetic differentiation among populations. In: *Genetics and conservation. A reference for managing wild animal and plant populations*. (Ed. C. M. Schonewald-Cox, S. M. Chambers and B. Macbryde). Benjamin / Cummings Publishing Co.: London. Pp. 51 - 65.
- Allendorf, F. W. and Phelps, S. R. (1981). Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Science* 38: 1507 - 1514.
- Allendorf, F. W. and Leary, R. F. (1986). Heterozygosity and fitness in natural populations of animals. In: *Conservation biology: the science of scarcity and diversity*. (Ed. M. E. Soulé). Sinauer Assoc. Inc.: Massachusetts. Pp. 57 - 76.
- Anderson, R. A. and Karasov, W. H. (1981). Contrasts in energy intake and expenditure in sit-and-wait and widely foraging lizards. *Oecologia* 49: 67 - 72.
- Andrews, R. M. (1994). Activity and thermal biology of the sand-swimming skink *Neoseps reynoldsi*: diel and seasonal patterns. *Copeia* 1994: 91-99.
- Andrews, R. M. and Pough, H. F. (1985). Metabolism of squamate reptiles: allometric and ecological relationships. *Physiological Zoology* 58: 214-231.
- Arad, Z., Raber, P. and Werner, Y. L. (1989). Selected body temperature in diurnal and nocturnal forms of *Ptyodactylus* (Reptilia: Gekkonidae) in a photothermal gradient. *Journal of Herpetology* 23: 103 - 108.
- Avise, J. C. (1975). Systematic value of electrophoretic data. *Systematic Zoology* 23: 465 - 481.
- Avise, J. C. and Aquadro, C. F. (1982). A comparative summary of genetic distances in the vertebrates. *Evolutionary Biology* 15: 151 - 185.
- Begon, M., Harper, J. L. and Townsend, C. R. (1990). *Ecology: individuals, populations and communities*. Blackwell Scientific Publications, London.
- Bennett, A. F. (1980). Thermal dependence of lizard behaviour. *Animal Behaviour* 28: 752-762.
- Bennett, A. F. (1983). Ecological consequences of activity metabolism. In: *Lizard ecology: studies of a model organism*. (Ed. R. B. Huey, E. R. Pianka and T. W. Schoener). Harvard University Press: Cambridge. Pp. 11 - 23.

- Bennett, A. F. and Dawson, W. R. (1976). Metabolism. In: *Biology of the Reptilia* (Ed. C. Gans and W. R. Dawson). Academic Press: London. Pp. 127-235.
- Bennett, A. F. and John-Alder, H. (1986). Thermal relations of some Australian skinks (Sauria: Scincidae). *Copeia* 1986: 57 - 64.
- Bezy, R. L., Gorman, G. C., Kim, Y. J. and Wright, J. W. (1977). Chromosomal and genetic divergence in the fossorial lizard family Anniellidae. *Systematic Zoology* 26: 57 - 71.
- Bock, C. E., Smith, H. M. and Bock, J. H. (1990). The effect of livestock grazing upon abundance of the lizard, *Sceloporus scalaris*, in southeastern Arizona. *Journal of Herpetology* 24: 445-446.
- Bradshaw, S. D., Gans, C. and Saint Girons, H. (1980). Behavioural thermoregulation in a pygopodid lizard, *Lialis burtonis*. *Copeia* 1980: 738 - 743.
- Brett, J. R. (1970). Temperature: animals: fishes. In: *Marine Ecology* (Ed. O. Kinne). Wiley: New York. Pp. 515-560.
- Brown, A. H. D. (1979). Enzyme polymorphism in plant populations. *Theoretical Population Biology* 15: 1 - 42.
- Brown, J. S. (1990). Habitat selection as an evolutionary game. *Evolution* 44: 732-746.
- Bryant, E. H. (1974). Of the adaptive significance of enzyme polymorphisms in relation to environmental variability. *American Naturalist* 108: 1 - 28.
- *Bury, R. B. and Balgooyen, T. G. (1976). Temperature selectivity in the legless lizard, *Anniella pulchra*. *Copeia* 1976: 152 - 155.
- Buth, D. G. (1984). The application of electrophoretic data in systematic studies. *Annual Review of Ecology and Systematics* 15: 501 - 522.
- Cabanac, M. (1985). Strategies adopted by juvenile lizards foraging in a cold environment. *Physiological Zoology* 58: 262-271.
- Chesser, R. K. (1983). Isolation by distance: relationship to the management of genetic resources. In: *Genetics and conservation. A reference for managing wild animal and plant populations*. (Ed. C. M. Schonewald-Cox, S. M. Chambers and B. Macbryde). Benjamin / Cummings Publishing Co.: London. Pp. 66 - 77.
- Clark, D. R. (1969). Experiments into selection of soil type, soil moisture level, and temperature by five species of small snakes. *Transactions of the Kansas Academy of Science* 70: 490 - 496.
- Cogger, H. G. (1992). *Reptiles and amphibians of Australia*. Reed Books, Chatswood, NSW.
- Coulson, G. (1990). Conservation of the striped legless lizard (*Delma impar*). An initial investigation. *Arthur Rylah Institute for Environmental Research. Technical Report Series No. 106*.
- Daut, E. F. and Andrews, R. M. (1993). The effect of pregnancy on thermoregulatory behaviour of the viviparous lizard *Chalcides ocellatus*. *Journal of Herpetology* 27: 6-13.
- Elston, R. C. and Forthofer, R. (1977). Testing for Hardy-Weinberg equilibrium in small samples. *Biometrics* 33: 536-542.

- Evans, D. O. (1990). Metabolic thermal compensation by rainbow trout: effects on standard metabolic rate and potential usable power. *Transactions of the American Fisheries Society* 119: 585-600.
- Ferguson, A. (1980). *Biochemical systematics and evolution*. Wiley and Sons, London.
- Futuyma, D. J. (1986). *Evolutionary biology*. Sinauer Associates Incorporated, Massachusetts.
- Gorman, G. C. and Kim, Y. J. (1975). Genetic variation and genetic distance among populations of *Anolis* lizards on two Lesser Antillean island banks. *Systematic Zoology* 24: 369 - 373.
- Gorman, G. C., Kim, Y. J. and Taylor, C. E. (1977). Genetic variation in irradiated and control populations of *Cnemidophorus tigris* (Sauria, Tiliidae) from Mercury, Nevada with a discussion of genetic variability in lizards. *Theoretical and Applied Genetics* 49: 9 - 14.
- Gorman, G. C. and Renzi Jr., J. (1979). Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. *Copeia* 1979: 242 - 249.
- Grant, B. W. (1990). Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. *Ecology* 71: 2323-2333.
- Greer, A. E. (1980). Critical thermal maximum temperatures in Australian scincid lizards: their ecological and evolutionary significance. *Australian Journal of Zoology* 28: 91 - 102.
- Greer, A. E. (1989). *The biology and evolution of Australian lizards*. Surrey Beatty and Sons, Pty Ltd, Chipping Norton, NSW.
- Hailey, A. and Davies, P. M. C. (1986). Lifestyle, latitude and activity metabolism of natricine snakes. *Journal of Zoology, London* 209A: 461-476.
- Hamrick, J. L., Linhart, Y. B. and Mitton, J. B. (1979). Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics* 10: 173 - 200.
- Huey, R. B. (1974). Behavioural thermoregulation in lizards: importance of associated costs. *Science* 184: 1001 - 1003.
- Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In: *Biology of the Reptilia* (Ed. C. Gans and F. H. Pough). Academic Press: New York. Pp. 25-91.
- Huey, R. B. (1991). Physiological consequences of habitat selection. *American Naturalist* 137: S91-S115.
- Huey, R. B., Pianka, E. R., Egen, E. and Coons, L. W. (1974). Ecological shifts in sympatry: Kalahari fossorial lizards (*Typhlosaurus*). *Ecology* 55: 304 - 316.
- Johnson, C. R. (1977). Thermoregulation in four Australian lizards of the genus *Egernia* (Sauria: Scincidae). *Zoological Journal of the Linnean Society* 60: 381 - 390.
- Kamel, S. and Gatten, R. E. (1983). Aerobic and anaerobic activity metabolism of limbless and fossorial reptiles. *Physiological Zoology* 56: 419-429.
- Kimura, M. (1968). Evolutionary rate at a molecular level. *Nature* 217: 624 - 626.

- Kimura, M. and Ohta, T. (1971). *Theoretical aspects of population genetics*. Princeton University, Princeton.
- Kitchell, J. F. (1969). Thermophilic and thermophobic responses of snakes in a thermal gradient. *Copeia* 1969: 189 - 191.
- Kukolic, K. (1993). Survey of the striped legless lizard, *Delma impar*, in the Gungahlin Town Centre and North Watson proposed development areas. *Wildlife Research Unit, ACT Parks and Conservation Service. Internal Report 93/1*.
- Kutt, A. J. (1991). Report of the survey results for the 1990/91 *Delma impar* trapping programme in the Derrimut Grasslands Reserve. *Unpublished report to the Melbourne Region Department of Conservation and Environment*.
- Kutt, A. (1993). A preliminary evaluation of the use of fluorescent pigments to track the movements of the striped legless lizard *Delma impar* (Reptilia: Pygopodidae). In: *Herpetology in Australia: a diverse discipline*. (Ed. D. Lunney and D. Ayers). Royal Zoological Society of New South Wales: Mosman, NSW. Pp. 179-183.
- Levene, H. (1949). On a matching problem arising in genetics. *Annals of Mathematical Statistics* 20: 91-94.
- Lewontin, R. C. (1974). *The genetic basis of evolutionary change*. Columbia University Press, New York.
- Licht, P., Dawson, W. R., Shoemaker, V. H. and Main, A. R. (1966). Observations on the thermal relations of Western Australian lizards. *Copeia* 1966: 97 - 110.
- Macartney, J. M., Larsen, K. W. and Gregory, P. T. (1989). Body temperatures and movements of hibernating snakes (*Crotalus* and *Thamnophis*) and thermal gradients of natural hibernacula. *Canadian Journal of Zoology* 67: 108-114.
- Martin, K. (1972). Captivity observations of some Australian legless lizards. *Herpetofauna* 5: 5-6.
- *Miller, C. M. (1944). Ecological relationships and adaptations of the limbless lizards of the genus *Anniella*. *Ecological Monographs* 14: 271 - 289.
- Milton, D. (1990). Genetic evidence for sympatric differentiation between two colour morphs of the skink *Egernia whitii*. *Australian Journal of Zoology*. 38: 117 - 130.
- Miyamoto, M. M., Hayes, M. P. and Tennant, M. R. (1986). Biochemical and morphological variation in Floridian populations of the Bark Anole (*Anolis distichus*). *Copeia* 1986: 76 - 86.
- Moritz, C. and Hillis, D. M. (1990). Molecular systematics: context and controversies. In: *Molecular systematics* (Ed. D. M. Hillis and C. Moritz). Sinauer Associates, Inc.: Massachusetts. Pp. 1-10.
- Nagy, K. A. and Degen, A. A. (1988). Do desert geckos conserve energy and water by being nocturnal? *Physiological Zoology* 61: 495-499.
- Nagy, K. A. and Knight, M. H. (1989). Comparative field energetics of a Kalahari skink (*Mabuya striata*) and gecko (*Pachydactylus bimaculatus*). *Copeia* 1989: 13-17.
- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583 - 590.

- Nei, M. (1983). Genetic polymorphism and the role of mutation in evolution. In: *Evolution of genes and proteins*. (Ed. M. Nei and R. K. Koehn). Sinauer Associates, Incorporated.: Massachusetts. Pp.
- Nei, M. and Roychoudhury, A. K. (1974). Sampling variances of heterozygosity and genetic distance. *Genetics* 76: 379 - 390.
- Nevo, E. (1976). Adaptive strategies of genetic systems in constant and varying environments. In: *Population genetics and ecology*. (Ed. S. Karlin and E. Nevo). Academic Press: New York. Pp. 159 - 184.
- Nevo, E. (1978). Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology* 13: 121 - 177.
- Nevo, E. and Shaw, C. R. (1972). Genetic variation in a subterranean mammal *Spalax ehrenbergi*. *Biochem. Genetics* 7: 235 - 241.
- Nevo, E., Kim, Y. J., Shaw, C. R. and Thaeler Jr., C. S. (1974). Genetic variation, selection and speciation in *Thomomys talpoides* pocket gophers. *Evolution* 28: 1 - 23.
- Nevo, E. and Beiles, A. (1992a). Amino-acid resources in the wild progenitor of wheats, *Triticum dicoccoides*, in Israel- polymorphisms and predictability by ecology and isozymes. *Plant Breeding* 108: 190 - 201.
- Nevo, E. and Beiles, A. (1992b). Selection for class II Mhc heterozygosity by parasites in subterranean mole rats. *Experientia* 48: 512 - 515.
- Patchell, F. C. and Shine, R. (1986). Food habits and reproductive biology of the Australian legless lizards (Pygopodidae). *Copeia* 1986: 30-39.
- Patterson, J. W. (1990). Field body temperatures of the lizard *Anguis fragilis*. *Amphibia-Reptilia* 11: 295-306.
- Patterson, J. W. and Davies, P. M. C. (1978). Preferred body temperature: seasonal and sexual differences in the lizard *Lacerta vivipara*. *Journal of Thermal Biology* 3: 39 - 41.
- Pough, F. H. and Gans, C. (1982). The vocabulary of reptilian thermoregulation. In: *Biology of the Reptilia* (Ed. C. Gans and F. H. Pough). Academic Press: London. Pp. 17-23.
- Richardson, B. J., Baverstock, P. R. and Adams, M. (1986). *Allozyme electrophoresis. A handbook for animal systematics and population studies*. Academic Press, Sydney.
- Robertson, I. C. and Weatherhead, P. J. (1992). The role of temperature in microhabitat selection by northern water snakes (*Nerodia sipedon*). *Canadian Journal of Zoology* 70: 417 - 422.
- Rogers, J. S. (1972). Measures of genetic similarity and genetic distance. *Studies in genetics, University of Texas Publication* 7213: 145 - 153.
- Rosen, P. C. (1991). Comparative field study of thermal preferenda in garter snakes (*Thamnophis*). *Journal of Herpetology* 25: 301-312.
- Sarich, V. M. (1977). Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature* 265: 24-28.
- Sarre, S. (1989). *Conservation genetics of the sleepy lizard Trachydosaurus rugosus*. Masters Thesis, Canberra College of Advanced Education.

van Damme, R., Bauwens, D. and Verheyen, R. F. (1986). Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. *Journal of Thermal Biology* 11: 219 - 222.

Ward, R. D., Skibinski, D. O. F. and Woodwork, M. (1992). Protein heterozygosity, protein structure, and taxonomic differentiation. *Evolutionary Biology* 26: 73-141.

Wildlife Research Unit. (1992). Recovery Plan. Lowland native grassland ecosystems in the Australian Capital Territory. *Progress report to the Australian National Parks and Wildlife Service, Endangered Species Unit*.

Withers, P. C. (1977). Measurement of VO₂, VCO₂, and evaporative water loss with a flow through mask. *Journal of Applied Physiology* 42: 120-123.

* Withers, P. C. (1981). Physiological correlates of limblessness and fossoriality in Scincid lizards. *Copeia* 1981: 197 - 204.

Wright, S. (1969). *The theory of gene frequencies*. University of Chicago Press, Chicago.

Wright, S. (1978). *Variability within and among natural populations*. University of Chicago Press, Chicago.

Wyatt, R. (1992). Conservation of rare and endangered bryophytes: input from population genetics. *Biological Conservation* 59: 99-107.

