Patterns of Geographic Variation in *Acanthiza lineata* and *A. reguloides* (Aves: Acanthizidae)

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The research presented in this thesis is my original independent work except where otherwise acknowledged.

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Summary

This study describes the patterns of geographic variation in two species of thornbills, the Striated Thornbill (*Acanthiza lineata*) and the Buff-rumped Thornbill (*A. reguloides*), as revealed by analyses of plumage colouration, dimensional characters and protein electrophoresis. Possible explanations for the observed patterns of geographic variation are discussed. The patterns of geographic variation between the two species are compared.

Analyses of plumage colouration in both species have been conducted in terms of colour index of plumage, which is the sum of each score of a character state, due to the obvious correlation among the colour characters scored. The discontinuous patterns of plumage colouration, which are implied by the current subspecific division have not been confirmed by the present study. Instead, both species show general north-south clines in plumage colouration. Clines could result from selective adaptation or short-term plastic response to environmental gradients.

However, an abrupt change seems to be apparent for *A. lineata* in the area from Walcha south to the Putty Road, New South Wales, whereas no such stepped variation appears along the cline in plumage colouration for *A. reguloides*. This contrasts with the study of Ford and Simpson (1987), in which the pattern of plumage colouration for *A. reguloides* was revealed as a general cline but a stepped variation occurred in the McPherson Range. Great variability in plumage colouration could indicate a secondary intergradation.

Six dimensional characters (including the body weight) have been analysed. Sexual dimorphism has been found for nearly all the six dimensional characters for both species, but generally, the differences between the sexes are not affected by different geographic localities.

All six dimensional characters in *A. lineata* and 4 out of 6 in *A. reguloides* show similar north-south latitudinal clines of geographic variation. Analyses of correlograms generally confirm the clinal patterns revealed by regressions and indicate that along these clines, neighbouring populations are significantly similar to each other within 500-600 km. Selective adaptation to environmental gradients may be responsible for these clinal patterns.

Although in both species, correlations between nearly all pairs of dimensional characters are significant, two characters have not showed significant clines in *A. reguloides* and the extent of clines in different characters is different. In particular, the cline in the culmen length for *A. lineata* is more obvious, whereas the cline in the hindtoe length for *A. reguloides* is sharper, although these characters are supposed to be functionally important in both species. Whether these two characters are under stronger selection pressure could not be analysed.

Electrophoretically detectable genetic variability has been examined at 28 protein loci in 17 local populations across the natural range of *A. lineata*, and at 29 protein loci in 20 local populations across the natural range of *A. reguloides*. Variability is high compared to other passerine species, with the average heterozygosity (Nei, 1978) for the whole *A. lineata* population of

0.109 and that for the whole *A. reguloides* population of 0.097. Ten polymorphic loci, with a total of 48 alleles, have been found in *A. lineata* and 11 polymorphic loci, with the total of 43 alleles, have been found in *A. reguloides*.

Although the amount of genetic variability is relatively high, no significantly abrupt or clinal changes in allele frequencies have been detected in both species. The commonest allele at each locus is present at a high frequency throughout all localities. Many alternative alleles only occur at low frequencies at one or few localities. Judged on this variation pattern, each species seems to be geographically continuous and relatively panmictic. This phenomenon is consistent with those documented for other avian species in North America. Neutral selection, high levels of gene flow and large effective population size are usually considered as the causal factors to the panmixia variation in allozymes.

The geographic variation in average heterozygosity (Nei, 1978) across localities shows that the value is highest at one or two localities in each species. The high values of average heterozygosity, along with the commonly recognized hypothesis on past climatic and environmental changes in the Australia continent, may suggest that isolation of subpopulations probably happened during the period of the last glaciation (about 25000-15000 years BP) for each of the two species and each might have undergone a secondary intergradation later. Two additional lines of evidence support the supposition of the secondary contacts. First, these proposed intergradation zones for the two *Acanthiza* species are congruent with intergradation zones for some other Australian bird species. Second, the proposed contact area for *A.*

lineata is concordant with the abrupt change in colouration and the proposed contact zone for *A. reguloides* is consistent with that revealed from the pattern of plumage colouration in the study of Ford and Simpson (1987).

The differences between the geographic patterns of variation in plumage colouration, dimensional characters and allozymic characters seem to be consistent with the generally accepted view that different suites of characters often follow different evolutionary path ways. Plumage characters and dimensional characters are often influenced by environmental factors whereas molecular characters are often neutral and subject to temporal and demographic events and gene flow.

The two species basically show similar genetic structure, patterns of geographic variation in plumage colouration, body measurements and allozymes. As they are sympatric congeneric species, for most morphological characters they probably have reacted in a similar way to the same environmental agencies. However, as revealed by the concordance of variation in plumage colouration and average heterozygosity, both species must have been shaped by the same historical events.

The patterns of geographic variation revealed for the two *Acanthiza* species are similar to those documented for several other species. Namely, morphological characters follow Bergmann's and Gloger's rules. Historical events are confirmed to be important to geographic variation and even speciation.

Finally, based on the observed patterns of geographic variation , especially the great variability in plumage colouration and average heterozygosity at one or two given localities, this study suggests that two subspecies could be recognized within

each of the two species, with subspecific boundaries being located in the zones where great variability in plumage colouration and average heterozygosity has been observed.

Contents

CHAPTER 1 Introduction

1.1 Evolution and Geographic Variation

The origin and evolution of birds can be traced back at least 135 million years ago to *Archaeopteryx lithographica*, the earliest and most primitive bird species so far recorded. Since then, the evolution of birds has been shaped by the separation and drifting of land masses, historically climatic changes and the consequent expansions and contractions of species ranges. Presently, there are over 8600 extant species of birds, making them one of the largest vertebrate groups.

Birds evidently appeared in ancestral Australia about 110 million years ago (Talent et al., 1976; Rich and Baird, 1986). The traditional view of the origin of the modern Australian avifauna is that early colonies moved from Asia through New Guinea and into northern Australia, with a number of subsequent radiations within Australia. However, more recent research has suggested that at least some groups of Australian birds have a Gondwanan origin (Sibley and Ahlquist, 1985; Schodde, 1982, 1988). Today about 750 avian species are living in Australia.

For the purpose of either understanding evolution or executing conservation and management programs, biologists are interested in those processes that lead to the observed distribution patterns of species. Thus, the concept of species, either defined as a reproductively isolated population (the biological species concept), or as a phylogenetic taxon (Cracraft, 1983), is a basic unit in evolutionary analyses. Hence, speciation processes are vital to understanding evolution.

Speciation is generally viewed as an additive process through which the number of species increases in a clade, i.e., subsets of a species population differentiate to the point where the subsets become reproductively isolated. A variety of different modes of speciation has been postulated, including allopatric, parapatric, alloparapatric, stasipatric and sympatric speciation (Endler, 1977; Wiley, 1981; White, 1978). Despite differences between these processes, a prerequisite of each is that a panmictic ancestral species undergoes differentiation into a number of discrete populations. One process that can lead to such differentiation is geographic fragmentation of the ancestral species. This fragmentation will produce definable geographic variation.

Geographic variation is usually defined as variation in a set of characters, such as allozymes, morphology, physiology and ecology, among the subpopulations of a species.

Investigations of such variation have been given great importance in evolutionary biology. In fact, the foundation of most evolutionary theory rests on inferences drawn from geographic variation or on the verification of predictions made about it (Gould and Johnston, 1972). Mayr (1982) stated that "all characters which have been described as good species differences have been found to be subject to geographic variation, whenever they have been examined from such point of view, and that geographic variation not merely helps in producing differences, but that many of these differences, particularly those affecting physiological and ecological characters, are potential isolating mechanisms, which may reenforce an actual discontinuity between two isolated populations". Thus, studies of geographic variation

have an important role to play in attempting to elucidate evolutionary processes. Such studies usually involve clarification of the patterns of geographic variation and interpreting these patterns in light of the possible evolutionary processes that have led to them.

1.2 Geographic Variation in Birds

1.2.1 Phenomena of Geographic Variation

Geographic variation is a common phenomenon in birds. For example, Mayr and Short (1970) concluded that about 32% of the 607 North American bird species are polytypic to various degrees. A large proportion of Australian birds are also polytypic. Based on an analysis of geographic variation in 425 Australian bird species, Keast (1984) found that 44% of them are monotypic, 23% show patterns of clinal variation and 33% have morphologically differentiated isolates within the continental mainland.

In birds, geographic variation has been found in a wide variety of characters, such as body size, body shape, plumage pattern, epidermal and chitinous structures, internal anatomy, cytological organisation, sexual dimorphism, reproductive performance, moulting, temperature selection, habitat selection, foraging behaviour, life history, vocalization, migratory behaviour. However, except for features of external morphology, internal anatomy, vocalization and clutch size, other features have been studied insufficiently. It is possible that these features may be as important as those characters most frequently studied. The reason for so few studies on these characters resides primarily in the technical and experimental difficulties associated with their investigation. For example, chromosomal analysis is often

confounded by the difficulty in distinguishing a large number of microchromosomes in birds despite differential banding methods (Stock and Bunch, 1982; Shields, 1987).

By tradition, the plumage colouration and body dimensional measurements have been analysed in studies of avian geographic variation. Allozymic and DNA characters have been increasingly employed in studies of geographic variation. Features of these sets of characters will be discussed in the following two sections.

1.2.2 Geographic Variation in Morphological Characters

Morphological characters studied in avian geographic variation usually include plumage colouration, external measurements and skeletal measurements.

To postulate evolutionary processes, variation in characters which are used in studies of geographic variation must have a heritable component. Hence, it would be appropriate to verify that character variation under analysis, especially variation in morphological characters, has a definable heritability.

Despite the importance of verifying the genetic basis of character variation, the effects of environmental factors are simply assumed to be minimal in many studies. This is because it is traditionally assumed that birds, with their highly uniform internal environment (homoiothermy), exhibit a minimum of changeability through external causes (Mayr,1982). Another reason is the difficulty of identifying corresponding relationships of various characters and their genetic basis. This is due to the fact that a morphological character is usually determined by the interaction of more than two loci and a locus usually plays a

pleiotropic role in determining more than two characters. Most methods of estimating genetic heritability, such as hybridization experiments (e.g., Berthold and Querner, 1981), calculation of realized heritability (e.g., Boag and Grant, 1981), and transplant or explant experiments (e.g., James, 1983), are often practically limited due to the time and cost.

However, in the last decade, various experiments have been performed to examine the repeatability and heritability of external characters and reproductive features in wild avian populations (Boag and van Noordwijk, 1987). Most field studies have suggested that many morphological characters have significant heritabilities in the average range of 60-70% (e.g., Boag,1983; Grant, 1983; Smith and Zach, 1979; Smith and Dhondt, 1980; Dhondt, 1982; Alatalo and Lundberg, 1986), while most traits which are more closely related to reproductive performance have significant but lower heritabilities on average in excess of 30-40% (e.g., van Noordwijk, et al.,1980; Prince et al., 1970).

In contrast to the above studies, other studies have showed important environmental influences upon morphological characters (e.g., Boag, 1987; James, 1983; James and NeSmith, 1988; Wiggins, 1989) and plumage patterns (Slagsvold and Lifgeld, 1985). Hence, more studies are needed to obtain a more definitive picture of heritability in birds.

Three ecogeographic rules have been proposed to demonstrate the relationships of natural selection and phenotypic adaptation to environmental gradients. These are Bergmann's, Allen's and Gloger's rules.

Bergmann's rule states that the body size in homeotherms is positively correlated with decreasing temperature and humidity. It is supposed that larger animals have lower metabolic rates because the ratio of surface area to body size of larger animals is lower than that of smaller animals. Zink and Remsen (1986) reviewed the described geographic variation in body size of 92 North American bird species. They found that 42% varied according to Bergmann's rule, 12% were weakly supportive of it and 16% showed no association between latitude or altitude and body size. However, 29% of species were found to contradict expectations of Bergmann's rule. They concluded that in birds the empirical basis for Bergmann's rule is weak, but that bias may be produced when using the wing length or the body weight as an index of body size. It is suggested that the first principal component of a set of morphological characters is the most appropriate index of body size, because most morphological characters load highly and positively on that component (Johnston and Selander, 1971).

Allen's rule states that populations in cold areas show a reduction in relative appendage length. It is supposed that longer body parts cause more energy loss in the cold weather. As it is difficult to test this rule without an index of body size with which measures of extremity length are standardized, this rule remains untested in birds (Zink and Remsen, 1986).

Gloger's rule states that populations of a species in more humid areas are more heavily pigmented than those in drier areas. It has been postulated that these correlations may not be with humidity *per se*, but with other environmental factors associated with humidity, e.g., soil types (Miller and Miller, 1951).

Covariation between plumage colour and environmental backgrounds helps birds survive predation. This rule accounts for a considerable degree of phenotypic and environmental covariation in many bird species (Zink and Remsen, 1986).

Plumage colouration in birds has been postulated to be a vital component of the speciation process because it is one of the main recognition signals in mating systems and is also involved in selection against predators. For some species of birds, colours in different parts of the body may be controlled by independent loci and vary independently. In such a situation, the analysis of concordance of different colour characters will be particularly useful to determine if an intergradation zone is allopatrically originated. However, it seems that intraspecific variation in colour is continuous in most avian species (Barrowclough, 1982). Therefore, if the geographic variation in plumage colouration is genetically controlled and environmentally selected, as implyed by Gloger's rule, its genetic basis could be quantitative genes or affected by regulatory genes (Wilson, 1975). Few studies have been undertaken to verify the magnitude of heritability for variation in plumage colouration. Furthermore, Slagsvold and Lifjeld (1985) found that colour variation in the Great Tit (*Parus major*) is mainly affected by nongenetic factors. Thus, geographic variation in plumage colouration could be of phenotypic plasticity.

In summary, variation in most mensural characters in birds seems to have obvious heritability, but the universality of Bergmann's and Allen's rules cannot be completely approved due to the lack of a standard of body size measurements. Little evidence has been collected to verify the genetic basis of variation in

plumage colouration, despite the apparent agreement between empirical data and Gloger's rule.

1.2.3 Geographic Variation in Molecular Characters

In the last 20 years, many studies have used protein electrophoresis to determine patterns of geographic variation in birds (see Barrowclough, 1983 and Zink and Remsen, 1986 for reviews).

There are some considerable advantages to the use of protein electrophoresis for analyses of patterns of geographic variation. First, most proteins are coded by relatively simple structural genes. The direct translation products of those genes are analysed so that the genetic basis is relatively clear and problems of pleiotropy or complex genetic-environmental interactions during ontogeny are greatly reduced. Thus, the difficult breeding experiments or heritability studies needed to confirm the genetic basis in morphological or ecological characters are avoided. Second, a well-developed population genetic theory has been established for analyses of electrophoretic data. Third, most allozymes examined show no age variation or sex specificity, whereas sexual dimorphism and variation between juveniles and adults are common in morphological characters.

However, there are also some limitations to electrophoretic techniques. First, observed patterns of variation are sometimes caused by post-translational modifications of the proteins (Richardson et al., 1986). Second, the technique cannot detect those amino acid substitutions which do not alter the surface charge of proteins. Third, regulatory gene information is usually

not detected. Fourth, the analysis is mainly restricted to soluble proteins.

Barrowclough (1983), Corbin (1983), and Zink and Remsen (1986) have reviewed the electrophoretic analyses carried out on birds. From these analyses, two major features have emerged. First, Barrowclough (1983) found that although within-population genetic variation, measured as heterozygosity, is of a magnitude (5%) similar to that of other vertebrates, among-population variation is slight. The among-population difference, measured as F_{st} (Wright, 1978), is always less than 5%. This finding applies to species with and without phenotypic differentiation. Such an apparent low degree of genetic differentiation of enzyme loci was postulated to be due to natural selection, recency of common ancestry, high gene flow, high effective population size, high and stable body temperature and a relatively slow mutation rate (Avise, 1983).

Second, analyses of allelic and genotypic frequencies at allozyme loci have revealed that most avian populations are in Hardy-Weinberg equilibrium (Barrowclough et al., 1985; Zink, 1986). However, in several species, the equilibrium has not been achieved because of the excess of rare alleles, which could be caused by effect of bottlenecks (Nei et al., 1975). Barrowclough et al. (1985) also established that the origin and maintenance of enzyme polymorphisms within and among populations are consistent with a neutral model in which mutations and genetic drift determine the distribution of genetic polymorphisms. Other selection models such as overdominance, are not operating on genetic variation at loci coding for enzymes.

More recently, many researchers have employed DNA characters to study geographic variation in birds. Some methods, which are frequently used in systematic and evolutionary studies at higher taxonomic levels, such as DNA-DNA hybridization (e.g., Sibley and Ahlquist, 1983), are not appropriate for studying variation at or below the species level. Therefore, most studies have employed RFLP (restriction fragment length polymorphism) analysis of mitochondrial DNA to determine levels of geographic variation in birds. Theoretically, it is particularly useful for studying differentiation at or below the species level (Moritz et al., 1987; Avise et al., 1987; Shields and Helm-Bychowski, 1988; Quinn and White, 1987), because it is a relatively small molecule (14.3-19.5 kb), lacks many of the complicating features of nuclear DNA such as numerous linkage groups, introns, leader and trailer sequences, intergenic regions and repeated sequences, is maternally inherited, mostly selectively neutral and evolves rapidly. However, studies using mtDNA to determine patterns of geographic variation in birds have not detected as much variation as was originally anticipated. For example, Ball et al. (1988) employed 18 restriction endonucleases to detect mtDNA RFLP's in the Red-winged Blackbird (*Agelaius phoeniceus*). They found minor population differentiation although considerable geographic variation in morphology exists among the populations. Tegelstrom (1987) investigated mtDNA differences among three local populations of the Great Tit (*Parus major*) and found no obvious difference. Other examples of employing mtDNA to detect genetic variation include studies of Quinn and White (1987) on the Lesser Snow Goose (*Anser caerulescens caerulescens*), Shields and Wilson (1987) on five species of American geese.

In summary, compared with morphological characters, allozymic and DNA characters are basically exempt from environmental influence. Allozymic electrophoresis has been successfully employed in the studies of geographic variation. In birds, variation in allozymes seems to be low within species and an avian population is often in Hardy-Weinberg equilibrium and follows the evolutionary model of neutral selection. RFLP analysis of mtDNA is theoretically advantageous but few studies have been conducted for bird species.

1.3 Patterns of Geographic Variation and Evolutionary Processes

Several basic patterns of geographic variation have been described. First, the population may be panmictic in one or more character over its entire range. Second, continuous variation may occur either along a direction (simple cline) or from a central core toward the periphery of the range (depression). More complex forms of clines are also possible, e.g., "double depression" which is that two subpopulations with low values of character states are separated by subpopulations with high values, and "crazy quilt" in which high ranks are surrounded by low ranks and vice versa. Third, different subpopulations are allopatrically distributed and each allopatric population has undergone differentiation. In the case of birds their mobility commonly leads to range expansions and recontact of formerly isolated subpopulations to form hybrid zones. For example, avian hybrid zones are abundant among Australian bird species (Ford, 1974, 1987).

The patterns of geographic variation revealed from different suites of characters of the same species are not always concordant with each other. This is because different characters may be subject to different evolutionary processes. In birds, morphological characters are commonly involved in selection-adaptation processes such as those underlying the ecogeographic rules discussed in section 1.2.2. In contrast, allozyme characters are mostly assumed to be selectively neutral (see section 1.2.3).

Concerning the relationships between variation in genetic components and in morphological characters, Lerner (1954) predicted that within populations, increased heterozygosity will produce decreased morphological variance, owing to a buffering effect of heterosis during development. This prediction has been checked in several cases of birds. Zink et al. (1985) found no significant covariance between heterozygosity derived from protein electrophoresis and morphological variance for the Fox Sparrow (*Passerella iliaca*). Handford (1980), analysing the data from the Rufous-collared Sparrow (*Zonotrichia capensis*), also failed to support Lerner's prediction. However, the research of Fleischer et al. (1983) on the House Sparrow (*Passer domesticus*) supported the prediction.

There are many possible evolutionary processes to explain patterns of geographic variation. First, the theory of natural selection suggests that characters are selected by environmental factors. Hence, a patchy or gradual pattern of environmental variation will cause a patchy or gradual pattern of character variation. Second, the theory of neutral and genetic drift suggests that most allelic variation is non-selective, so that variation within a species may be due to random genetic drift or other

factors. Third, some biological features, such as gene exchange rate, may affect patterns of geographic variation. For example, in the isolation-by-distance model, individuals mate at random within a defined neighbourhood and are constrained from mating with other more distant members of the same species by limitations on their dispersal. This may result in a cline. Fourth, historic events also contribute to patterns of geographic variation. The so-called founder effect, the process that relative small populations of different types accidentally migrate and settle down, can lead to a patchy distribution. The diffusion of two or more formerly isolated populations may produce a character gradient between them. A systematic migration into an area by several populations that have differentiated elsewhere is also a possible historical cause of cline formation. Real situations in nature are likely to be mixtures of two or more of the above processes.

Intuitively, the relationships between observed patterns of geographic variation and their causal processes are of equifinality, i.e., more than one process can give rise to the same pattern of geographic variation. In addition, some stochastic processes, such as isolation-by-distance, exhibit multifinality, i.e., an identical single process yields different results during separate realizations (e.g., Sokal and Wartenberg, 1983; Sokal et al., 1989).

In summary, patterns of geographic variation in different characters are not always the same, because of the different evolutionary processes involved in different sets of characters. The lack of one-to-one relationships between patterns of

geographic variation and evolutionary processes make it more difficult to propose hypotheses on evolutionary tracks of species.

1.4 Methodology for Studying Geographic Variation

Many techniques have been employed to describe or summarize patterns of geographic variation. Usually used are the maps on which plots of numerical values, such as gene frequencies, means of morphometrics, are indicated at locality points (e.g., Jones et al., 1980), SYMAP contouring (e.g., Sokal and Riska, 1981), trend-surface analysis (e.g., Jorde, 1980) etc.

Cluster analyses, such as UPGMA (Sneath and Sokal, 1973) of numerical taxonomy, have been used when more than one variable has been measured so that locality samples can be treated as OTU's (operational taxonomic units). Sokal (1983), however, pointed out that in cases where the area is more or less continuous, a hierarchical cluster analysis is in fact a distortion of the potential relations and cannot be recommended except as a rapid means of inspection of the data for correspondence between geographic proximity and phenetic similarity of localities.

Various ordination methods are also used to analyse geographic variation. These are likely to be more preferable methods of displaying continuous geographic variation (Sokal, 1983). The most widely used are principal components analysis, principal coordinate analysis and non-metric multidimensional scaling.

An alternative approach is that of spatial autocorrelation analysis (Cliff and Ord, 1981; Goodchild, 1986; Sokal and Oden, 1978a, b; Bocquet-Appel and Sokal, 1989), a variation of the quadratic assignment method (Douglas and Endler, 1982; Hubert,

1983, 1987). Sokal (1983) stated that correlograms describe the underlying spatial relationships of patterns of geographic variation rather than their appearance and hence, they are probably more accurate guides to some of the processes that have generated the patterns of geographic variation than are the patterns themselves. Gabriel and Sokal (1969) and Sokal (1979) described several kinds of geographic distance matrices which are used to conduct spatial autocorrelation analyses. Sokal and Oden (1978b) showed that different types of artificially generated patterns of geographic variation yield characteristic spatial correlograms.

As discussed in section 1.3, inferences from observed patterns of geographic variation to processes bringing about the patterns, or *vice versa*, are often hampered by the lack of one-to-one correspondences between the patterns of geographic variation and the underlying evolutionary processes. Hence, in practice, other relationships in nature are often used to strengthen or reject an inference of an evolutionary process. For example, if variation in a character closely follows variation in an environmental variable, it is more possible that the pattern of geographic variation in the character (e.g., a cline) is caused by the variation pattern of an environmental factor (e.g., a gradient). Biological knowledge of a given species and its environment, its prey and predators, sometimes offers detailed inferences about how natural selection may vary geographically. For example, colour patterns in fishes are affected by predation (Endler, 1978). Knowledge of historical changes of climates, vegetation, geography etc. also helps to explain observed geographic patterns of character variation.

1.5 Geographic Variation of Two Species of Thornbills

The Striated Thornbill (*Acanthiza lineata*) and the Buff-rumped Thornbill (*A. reguloides*) are endemic Australian birds, confined primarily to woodlands of the east and south Australia, basically, east of the Great Divide (Figure 1.1).

Both species are sedentary and often occur sympatrically (Bell, 1985; Blakers et al., 1984). However, the two species show distinct ecological partitioning, evidenced by differences in foraging behaviour. *A. lineata* generally gleans in the forest canopy whereas *A. reguloides* usually gleans close to the ground, trunks and lower branches (Loyn, 1985; Recher and Holmes, 1985; Bell, 1985).

Geographic variation in plumage patterns of both *A. lineata* and *A. reguloides* has been documented (Mack, 1936; Mayr and Serventy, 1938; Ford and Simpson, 1987), although information on *A. lineata* has been derived from a limited number of specimens (Mack, 1936; Mayr and Serventy, 1938).

A. lineata is distributed over the coastal and sub-inland area of southeastern Australia (Blakers et al., 1984). Two, three, four and six subspecies have been separately proposed, but four subspecies are generally recognized (Mack, 1936; Mayr and Serventy, 1938; Mayr et al.,1986; MacDonald, 1973), viz., *A. l. alberti* in southeastern Queensland, *A. l. lineata* in eastern New South Wales, *A. l. chandleri* in Victoria and Kangaroo Island, and *A. l. clelandi* in the Fleurieu Peninsula and the Mount Lofty Ranges (Figure 1.1).

A. l. alberti differs from other subspecies by its apparently reduced black striation on the breast and the throat, more yellowish underparts, brown and greenish crown and pure green

back. *A. l. lineata* is green on the back, less brownish than *alberti* on the crown and has yellowish-olive flanks. *A. l. chandleri* has a brown crown and brownish-olive flanks. *A. l. clelandi* is, in some aspects, intermediate between typical *lineata* and *chandleri*. The subspecies characteristics are summarized in Table 1.1.

A. reguloides is distributed throughout eastern and southeastern Australia (Blakers et al., 1984). Several subspecies have been described but views on their status differ (Mack, 1936; Hall, 1974; MacDonald, 1973; Mayr and Serventy, 1938; Mayr et al., 1986). Mayr and Serventy (1938) suggested up to four subspecies, the distributions of which are shown in Figure 1.1. *A. r. squamata* of central eastern Queensland is brightest in colour with clear lemon-yellow underparts, lacking any ochraceous tinge. In this subspecies, scalloping on the forehead is pale grey and the tail tip is more whitish than other subspecies. *A. r. reguloides* which occurs in New South Wales, Victoria and eastern South Australia, has an ochraceous tinge to the underparts, ochraceous or bright buff scalloping on the forehead and dark tips to the tail feathers. *A. r. nesa*, which is distributed from southern Queensland to northern New South Wales, is intermediate in colouration between *A. r. squamata* and *A. r. reguloides*. *A. r. australis* is restricted to the Mount Lofty Ranges and differs from *A. r. reguloides* in its darker rump and brownish tail tips. The described plumage characteristics of these subspecies are summarized in Table 1.2.

Ford and Simpson (1987) studied pigment characters of *A. reguloides* and revealed a generally clinal distribution of plumage colour, with an abrupt change at the McPherson Range.

To summarize, variation patterns in plumage coloration for both *A. lineata* and *A. reguloides* have been documented. However, different authors have presented different views on the division of subspecies. Indeed, the question arises whether the described plumage variation is really subspecifically differentiated or merely clinal. Therefore, it remains to be shown whether the previously described patterns of variation are correct or whether alternative patterns exist.

1.6 Research Objectives

Since there is considerable confusion about the existence of patterns of geographic variation in plumage colouration for *A. lineata* and *A. reguloides*, and since the variation patterns in different suites of characters are not always concordant, two basic objectives are set out in the present study. First, the patterns of geographic variation in plumage coloration will be independently described based on the relatively complete samples across the species' ranges. The observed patterns will be compared with those previously described. Second, two more suites of characters, dimensional characters and allozymic characters will be employed to examine the patterns of geographic variation in these characters and compare the concordance of patterns of geographic variation in different suites of characters.

Basically, the following questions will be answered.

1). Can previously described patterns of geographic variation in plumage colouration and subspecies be corroborated by the patterns of geographic variation in plumage colouration revealed in the present study for *A. lineata* and *A. reguloides* ?

2). Do the different characters show concordant patterns of variation within each of the two species? What are possible hypotheses on the evolutionary processes underlying those patterns?

3). Is there concordance in patterns of geographic variation between the two species?

Table 1.1 Subspecific variation in plumage colouration in *A. lineata*. The description is based on those described by Mack (1936) and Mayr and Serventy (1938).

Character	*A. l. alberti*	*A. l. lineata*	*A. l. chandleri*	*A. l. clelandi*
crown colour tone	brown mixed with a large amount of green	brown with little green	brown	brown
back colour tone	brighter green	green	dark green with more brown	green with citrine tint
striation on throat and breast	much reduced	black	black	black
underparts colour tone	yellowish	yellow tinged with olive	brown tinged with olive	nearly yellowish olive

Table 1.2 Subspecific variation in plumage colouration in *A. reguloides*. The description is based on those described by Mack (1936) and Mayr and Serventy (1938).

Character	*A. r. squamata*	*A. r. nesa*	*A. r. reguloides*	*A. l. australis*
forehead scalloping	pale grey without ochraceous or bright buff colouring	whitish grey	buff	darker buff
back colour tone	olive	less olive	deep greyish olive	darker buff
rump colour tone	yellow	pale yellow	buff	darker buff
underparts colour tone	lemon yellow	cream yellow	buff	darker buff
tail tip colour tone	pale grey lacking ochraceous or bright buff colouring	pale buff	buff	darker buff

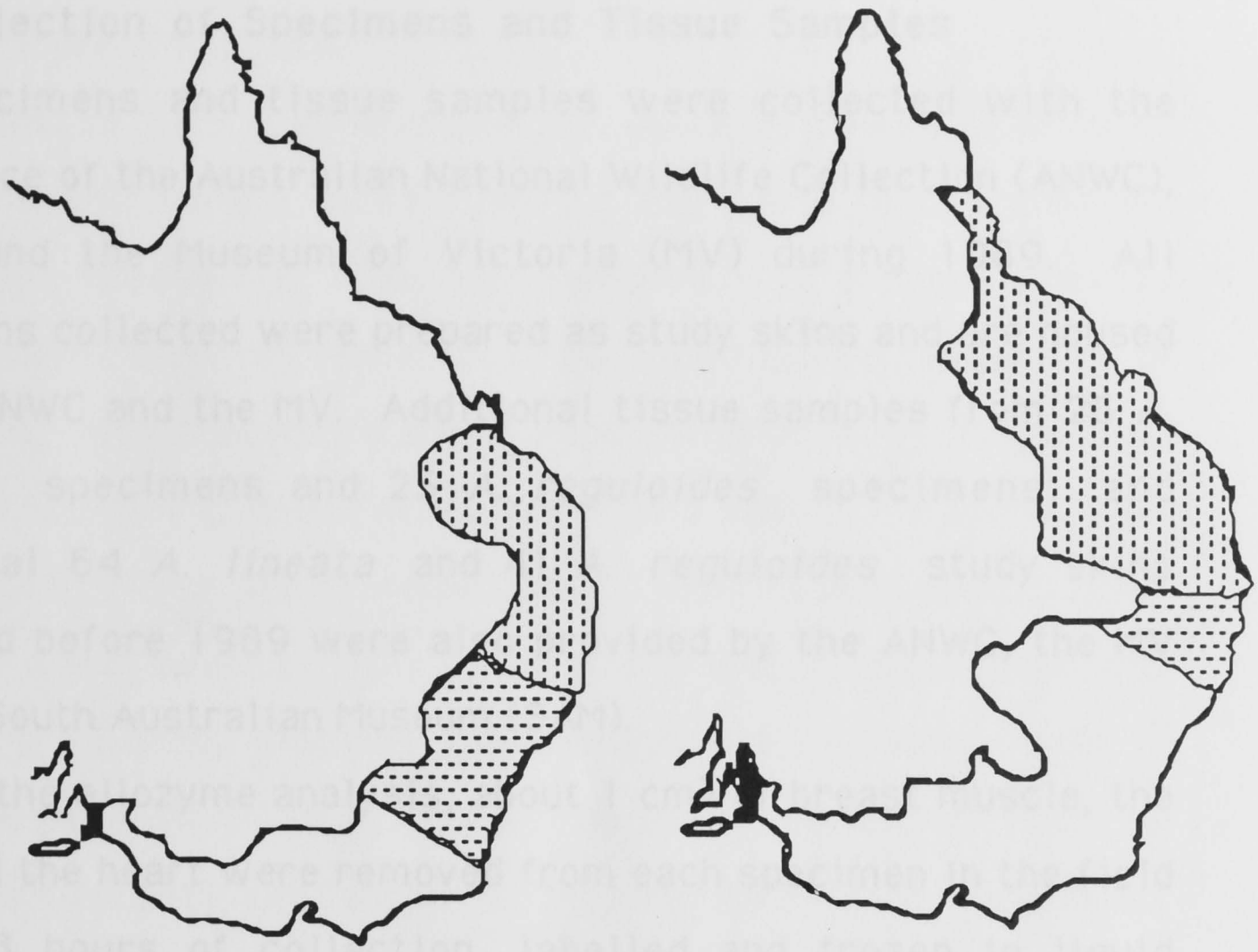


Figure 1.1 The approximate distribution ranges and subspecific division of *M. lineata* and *M. reguloides*.
a). *M. lineata*: *M. l. alberti*, *M. l. lineata*, *M. l. chandleri*, *M. l. clelandi*;
b). *M. reguloides*: *M. r. squamata*, *M. r. nesa*, *M. r. reguloides*, *M. r. australis*.

CHAPTER 2 Materials and Methods

2.1 Collection of Specimens and Tissue Samples

Specimens and tissue samples were collected with the assistance of the Australian National Wildlife Collection (ANWC), CSIRO and the Museum of Victoria (MV) during 1989. All specimens collected were prepared as study skins and are housed at the ANWC and the MV. Additional tissue samples from 26 *A. lineata* specimens and 25 *A. reguloides* specimens, and additional 64 *A. lineata* and 41 *A. reguloides* study skins collected before 1989 were also provided by the ANWC, the MV and the South Australian Museum (SAM).

For the allozyme analysis, about 1 cm^3 of breast muscle, the liver and the heart were removed from each specimen in the field within 3 hours of collection, labelled and frozen in liquid nitrogen. For long term storage, tissue samples were transferred from liquid nitrogen to an ultra-cold freezer (-70°C) upon return from the field.

A total of 188 specimens of *A. lineata* was assembled from 36 point sites covering its geographic range. From these specimens, 188 liver samples, 188 muscle samples and 186 heart samples were obtained. Since some point sites are very close to each other and within the same geographic zones and share the same habitats, both specimens and tissue samples from the 36 point sites were combined into 18 groups (sample sets or localities). Tissue samples were absent from one sample set (sample set 9). Therefore, 18 sample sets of specimens and 17 sample sets of tissues were used in analyses. The 18 localities representing these sample sets are shown in Figure 2.1a. The

localities, latitude, longitude and sample sizes are listed in Table 2.1. Each of the 18 sample sets were treated as a basic unit in the present study.

A total of 165 specimens of *A. reguloides* was assembled from 33 point sample sites covering the range of the species. From these specimens, 164 liver samples, 165 muscle samples and 165 heart samples were obtained. For similar reasons as in *A. lineata*, both specimens and tissue samples of *A. reguloides* from the 33 point sites were combined into 21 sample sets (localities). These are the basic units used in analyses. Tissue samples were absent from one sample set (sample set 16). Therefore, 21 sample sets of specimens and 20 sample sets of tissues were involved in analyses. The 21 localities representing these sample sets are shown in Figure 2.1b. The localities, latitude, longitude and sample sizes are listed in Table 2.2.

2.2 Scoring and Analysis of Plumage Colouration

In both species, there is no difference in plumage colouration between males and females. Juveniles were excluded from the analyses of plumage colouration because there is some difference in plumage colouration between adults and juveniles.

A total of 227 specimens of *A. lineata* and 183 specimens of *A. reguloides* were scored.

Colouration of each specimen was quantitatively assessed by dividing various characters into equally spaced character states, as scored in Table 2.3 for *A. lineata* and Table 2.4 for *A. reguloides*. The principle was to score the states from light to strong colour (from less brown tone to more brown tone). A

colour index for each specimens was produced by summarizing scores for all colour characters.

A linear regression analysis on the colour index against latitude and an F-test for significance were carried out.

2.3 Measurements of Dimensional Traits

Study skins measured include specimens preserved before 1989 and those prepared after the field work of this study. Those prepared before this study were examined from the ANWC and the SAM. Additional measurements of wings and tails from specimens in the Australian Museum, Sydney, the Queensland Museum, Brisbane and the MV, Melbourne were also provided by Dr R. Schodde.

Six dimensional traits measured for each species are listed in Table 2.5. Of these six traits, the culmen, tarsus and hindtoe lengths were measured to the nearest 0.1 mm with dial calipers. The wing and tail lengths were measured to the nearest 1.0 mm with a ruler. The body weight of the birds was taken immediately after a specimen was collected in the field. It was measured to the nearest 0.1 gram.

The sex and age (adult or juvenile) of birds were also recorded. Sex was used as a catalogue character and juveniles were excluded from the dimensional analyses.

2.4. Analysis of Dimensional Data

All statistic analyses were performed using the SAS package (SAS Institute, Inc., 1985). When the option existed, type III sums of squares (Freund et al, 1986) were chosen for the tests of significance, because the sizes of sample sets were not equal.

Unless indicated, the original measurements of the six traits were transformed to natural logarithms before any analysis was carried out. The reason for data transformation is to ensure that the distribution of the trait values is closer to the normal distribution, a necessary presumption for most statistical methods.

Pearson correlation coefficients among the six characters were calculated. Also calculated were significance levels of the correlation.

A two-way univariate variance analysis for each of the six traits was carried out to check whether the differences in each trait among the sample sets, between males and females and among the combination effects of sample sets and sexes were statistically significant.

A two-way multivariate variance analysis was carried out to check whether the overall trait difference among the sample sets and between males and females were statistically significant.

If trait differences between the sexes were significant, the measurements of males and females were separated for following analyses.

A cluster analysis (UPGMA) using the average trait values of males (or females) of sample sets was carried out.

A linear regression between measurements of each dimensional character and latitude was conducted and plotted. The significance of the linear regression was tested by an F-test.

For each character, a spatial autocorrelation analysis was carried out to display patterns of geographic variation. The spatial autocorrelation coefficient used was Moran's autocorrelation coefficient, I (Cliff and Ord, 1981; Sokal and

Oden, 1978a). The significance of Moran's I was tested by means of a Monte Carlo procedure (Hope, 1968). With the moderate number of sample sets involved (18 for *A. lineata* and 21 for *A. reguloides*), a complete enumeration of the 18! (21!) permutations was not computationally feasible. A random sampling of 2000 from the 18! (21!) permutations is sufficient for significance tests (Cliff and Ord, 1981). The connecting matrices used in the analyses were formed subject to the distance classes.

The geographic distance between any two localities (e.g. localities a and b) was calculated from the formula

$D_{ab}=\pi * 6367.4675 * \arccos(\cos AB)/180$

where $\pi=3.1416$; 6367.4675 is the average radius (km) of the earth; $\cos AB=\cos A\cos B+\sin A\sin B\cos C$; A is the difference (in degree) between the latitude of locality a and 90°, B is the difference between the latitude of locality b and 90°; C is the difference between the longitude of localities a and b.

For *A. lineata*, eight classes of distances (in km) were divided in autocorrelogram analysis. They are: class 1: (0, 300]; class 2: (300, 500]; class 3: (500, 700]; class 4: (700, 900]; class 5: (900, 1100]; class 6: (1100, 1300]; class 7: (1300, 1500]; and class 8: (1500, 1770]. Within each class, two localities were considered to be connected and had a value of 1 in connection matrix if the distance between the two localities falls into the range of that class. All other localities with distances that are equal to 0 or outside of the class range were not connected and were assigned to 0 in the connection matrix.

For *A. reguloides*, eight classes were derived. The range of each class is: (0, 300], (300, 600], (600, 900], (900, 1200], (1200, 1500], (1500, 1800], (1800, 2100] and (2100, 2400) in

order. The connecting method was the same with that used for *A. lineata*.

A FORTRAN program (Appendix) was written to carry out the calculation of locality distances, forming the connecting matrix, calculation of I-values and testing significance by permutation of 2000.

2.5 Electrophoresis Procedure

Sections of liver and muscle tissues were homogenized separately at 4°C in 300 µl of homogenizing buffer (Table 2.6) and centrifuged at 14000 rpm for four minutes in an Eppondort bench top centrifuge (Centrifugal force was approximately estimated to be greater than 10000G which is generally considered to be the minimum force needed). The supernatant was removed and stored in liquid nitrogen until the enzyme assays were performed.

Electrophoresis was performed on a cellulose acetate matrix (15x10 cm gel), as highlighted by Richardson et al. (1986). The recipes of running buffers are given in Table 2.6.

The staining procedures used were similar to those described by Richardson et al. (1986).

The scoring procedure used in this study is as follows: multiple alleles were designated alphabetically, beginning with the most anodal one. When two or more loci of an enzyme were active, they were numbered in sequence from the most anodally migrating to the least. Protein morphs with differing electrophoretic mobilities at a given locus were inferred to be the products of different alleles, represented in the form of individual genotypes.

Twenty-eight loci were resolved from samples of *A. lineata*, and 29 from *A. reguloides*. The loci examined, the tissue specificity and running conditions are summarised in Table 2.7.

Gene frequencies of the multiple-locus esterase complexes in both species were still included in analyses although an inaccuracy may occur when null alleles were present. When a null allele occurs, the band representing a heterozygote genotype of a normal allele and a null allele might be scored as a band representing a homozygote genotype of the normal allele because the two types of bands were only different in staining intensity, which could not be identified appropriately by eyes. Also, while the band representing the heterozygote genotype of a normal allele and a null allele was very fuzzy, the genotype might be scored as a homozygote genotype of the null allele. However, it is reasonable to suppose that the chance of scoring a heterozygote genotype of a normal allele and a null allele into a homozygote genotype of the normal allele was the same with that of scoring the heterozygote genotype into a homozygote genotype of the null allele. Hence, this kind of inaccuracy only affected the estimation of genotypic frequencies. Estimation of allele frequencies and mean heterozygosity (Nei, 1978) was not affected seriously.

For each enzyme system, 18-20 samples from different localities across the range of each of the two species, and 21 samples of *A. lineata* and 22 samples of *A. reguloides* from the Australian Capital Territory were examined first. If these samples were found to be monomorphic, that locus was considered to be monomorphic without examining further samples. If the zymogram showed variation in an allele frequency, all

samples were examined for that locus. This regimen of examining only part of samples for some loci may lead to a bias against rare alleles. But for the purpose of estimating genetic distance, it has no significant effect (Barrowclough et al., 1985).

2.6 Analysis of Electrophoretic Data

Analyses were performed using the BIOSYS-1 package of Swofford and Selander (1981). The following indices were calculated: allele frequencies for each sample set (locality), average heterozygosity (Nei, 1978) over all loci assayed for each sample set, Nei's (1978) and Rogers' (1972) genetic distances between each pair of sample sets.

Each polymorphic locus at each sample set was examined for a departure from Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test. When more than two alleles were present at a locus, the genotypes were pooled into three classes (homozygotes for the commonest allele, heterozygotes for the commonest allele and another allele, and all other genotypes) to circumvent problems in χ^2 tests caused by the fact that expected frequencies of some classes of genotypes were low. Levene's (1949) correction for small sample size was applied.

Cluster analyses were performed using the UPGMA (Sneath and Sokal, 1973) from a matrix of either Nei's (1978) or Rogers' (1972) genetic distance. A Distance Wagner analysis (Farris, 1972) was carried out from a matrix of Rogers' (1972) genetic distance.

Table 2.1 Localities and sample sizes of *Acanthiza lineata*. Tissue sample sizes at some localities are given as ranges because not all systems could be scored for all samples. Sample sizes of specimens at some localities are given as ranges because not all dimensional traits and plumage characters could be measured for all study skins.

Locality (sample set) code No.	Locality	Latitude (south)	longitude (east)	Sample size (tissue)	Sample size (male specimens)	Sample size (female specimens)
1	Blackdown Tableland, Qld.	23°44'	149°06'	7-8	4	3
2	Dawes Range, Qld.	24°37'	150°50'	8	5	3
3	Jimna Range (incl. Esk), Qld.	26°53'	152°20'	16	9-12	8-10
4	Warwick, Qld.	28°10'	152°18'	4	3-9	0
5	New England Plateau, NSW	29°24'	151°23'	10	10-13	1-2
6	Grafton-Dorrigo, NSW	30°01'	152°50'	6	2	4
7	Walcha-Wilson River, NSW	30°59'	151°01'	8-12	7-8	3-4
8	Taree-Putty Road, NSW	32°35'	151°35'	17	11-16	5-8
9	Sydney Area, NSW	33°53'	151°13'	0	1-16	0-15
10	Sutton-ACT, NSW	35°17'	149°13'	25	17-32	12-23
11	NSW-Vic. border area	36°40'	148°30'	3	3-7	3-6
12	Merton, Vic	36°59'	145°43'	7	2-3	4-5
13	Central Victoria area	37°49'	144°58'	23	9-20	12-15
14	Mt. Avoca, Vic	37°05'	143°02'	17	7-8	9
15	Vic-SA border area	38°16'	141°26'	5	1-4	3-4
16	Southeastern area of SA	36°58'	140°45'	9	3-10	5-7
17	Kangaroo Island. SA	35°50'	137°15'	8	5-9	3-4
18	Mt. Lofty Ranges, SA	34°59'	138°43'	10	9-15	5-11

Table 2.2 Localities and sample sizes of *Acanthiza reguloides*. Tissue sample sizes at some localities are given as ranges because not all systems could be scored for all samples. Sample sizes of specimens at some localities are given as ranges because not all dimensional traits and plumage characters could be measured for all study skins.

Locality (sample set) code No.	Locality	Latitude (south)	Longitude (east)	Sample size (tissue)	Sample size (male specimens)	Sample size (female specimens)
1	Atherton Tableland, Qld	17°31'	145°25'	3	2-3	1
2	Burra Range, Qld	20°36'	145°20'	6	3	3
3	Clarke Range, Qld	20°47'	148°10'	11-12	6-7	4-7
4	St. Lawrence-Rockhampton, Qld	22°51'	149°58'	5-6	3-4	2
5	Blackdown Tableland, Qld	23°44'	149°06'	8	4-5	2-3
6	Dawes Range, Qld	24°37'	150°50'	10-11	6-7	7
7	Carnarvon, Qld	25°16'	148°39'	6-7	2-3	4
8	Jimna Range, Qld	26°53'	152°20'	8-9	3-4	4-5
9	Warwick, Qld	28°10'	152°18'	4	3-11	0-2
10	New England Plateau, NSW	29°24'	151°23'	10	5-8	5
11	Grafton-Dorrigo, NSW	30°01'	152°50'	3-4	2	2
12	Attunga-Walcha, NSW	30°59'	151°01'	10	8	2
13	Taree-Putty Road, NSW	32°35'	151°35'	12	8-19	5-9
14	Sydney area, NSW	33°53'	151°13'	2	1-10	1-5
15	Sutton-ACT, NSW	35°17'	149°13'	25	19-22	7-12
16	NSW-Victoria border area	36°40'	148°30'	0	2-7	3-6
17	Merton, Vic	36°59'	145°43'	10	6-8	3-4
18	Central Victoria	37°49'	144°58'	13	9-12	3-6
19	Mt. Avoca, Vic	37°05'	143°02'	2	1-2	1-2
20	Victoria-SA border area	38°16'	141°26'	6	7-11	1-4
21	Mt. Lofty Ranges, SA	34°59'	138°43'	5	3-12	5-14

Table 2.3 Scoring criteria of colouration states in *A. lineata*. Three states are scored for the colour tones of the crown, the back and the striation on the breast and the throat. Five states are scored for the colour tone of the underparts.

Character	Character state				
	1	2	3	4	5
crown colour tone	grey brown	brown	darker brown	-	-
back colour tone	olive green	green washed with brown	darker green	-	-
striation on breast and throat	fine, grey brown	moderate, greyish black	thick, strong greyish black	-	-
underparts colour tone	bright yellow	pale yellow	grey yellow	darker greyish yellow	yellowish grey

Table 2.4 Scoring criteria of colouration states in *A. reguloides*. Three states are scored for the colour tones of the forehead scalloping, the back and the tail tip. Four states are scored for the rump colour tone. Five states are scored for the colour tone of the underparts.

Character	Character state				
	1	2	3	4	5
colour of forehead scalloping	grey with brown	brown	heavier brown	-	-
back colour tone	bright green	smoky green	green with strong smoke	-	-
rump colour tone	deep yellow	yellow with little brown	brown	deep brown	-
underparts colour tone	deep yellow	yellow	yellow washed with grey	pale brown	brown washed with grey
colour tone of tail tips	grey	grey washed with brown	brown	-	-

Table 2.5 Six dimensional characters measured and the total sample sizes.

Character	Definition	Total sample size of *A. lineata*	Total sample size of *A. reguloides*
culmen length	length from tip of bill to base of skull	219	177
tarsus length	length from notch of intertarsal joint to lower edge of last complete scale before toes diverge	226	181
hindtoe length	length from tip of hindtoe claw to joint point between hindtoe and other toes	225	181
wing length	length of flattened chord	321	283
tail length	length from tip of central rectrices to the base	272	227
weight	body weight in grams	198	173

Table 2.6 Recipes for homogenizing and running buffers. (Abbreviations: Tris= Tris (hydroxymethyl) aminothanol, EDTA=Ethylenediaminetetra-acetic acid disodium salt, NADP=Nicotinamide Adenine Dinucleodite Phosphate, NAD= Nicotinamide Adenine Dinucleodite).

Buffer	Composition
homogenizing buffer	20 ml stock solution (0.1 M Tris, 0.001M EDTA, PH to 7.0 with HCl) 10 µg NADP 10 µl b-mecaptoethanol
5 litre TEM 50 (50 mM Tris EDTA maleate). NAD or NADP was added when running some systems.	Tris 30.25 g (50 mM) $Na_2EDTA.2H_2O$ 9.30 g (5 mM) $MgCl_2.6H_2O$ 1.0 g (1 mM) PH to 7.8 with Maleic acid
5 litre TEB15 (15 mM Tris EDTA Borate)	Tris 9.00 g (15 mM) $Na_2EDTA.2H_2O$ 3.72 g (2 mM) $MgCl_2.6H_2O$ 0.4 g (0.4 mM) PH to 8.2 with Boric acid
4 litres TC (0.1 M Tris-citrate)	Tris 48.4 g (0.1M) PH to 8.2 with Citric acid
5 litres CP (0.01 M Citrate-phosphate)	Na_2HPO_4 7.10 g (10 mM) PH to 6.4 with Citric acid
4 litre TEB 25 (25 mM Tris EDTA borate)	Tris 12.10 g (25mM) $Na_2EDTA.2H_2O$ 7.44 g (5 mM) $MgCl_2.6H_2O$ 0.80 g (1mM) PH to 8.20 with Boric acid

Table 2.7 Loci examined, tissue specificity and running conditions used in electrophoresis. All loci were resolvable for both *Acanthiza lineata* and *A. reguloides* except for PK (2.7.1.40) which was scorable only for *A. reguloides*. L=liver, M=muscle. Enzyme abbreviations follow those listed by Richardson et al. (1986). Substrate used in PEP-2 staining is the tri-peptide leucine-glycine-glycine. For abbreviations of running buffers, see Table 2.6.

Enzyme (locus) Abbreviation	E.C. Number	Tissue Used	Running Buffer	Voltage (Volts) D.C.	Duration of Run
ACON	4.2.1.3	L	TC	200	2
AK	2.7.4.3	M	TEM50	200	2
ALD-1	4.1.2.13	M	TEM50	200	2
ALD-2	4.1.2.13	L	TEM50	200	2
CK	2.7.3.2	M	TEM50	200	2
EST-1	3.1.1.1	L	TEM50	150	2
EST-2	3.1.1.1	L	TEM50	150	2
EST-3	3.1.1.1	L	TEM50	150	2
FUM	4.2.1.2	L	TC	200	2.5
GAPD	1.2.1.12	L	TEM50	200	2.5
GDA	3.5.4.3	L	TEM50+NADP	150	5/6
GDH	1.4.1.3	M	TEM50	200	2
GOT	2.6.1.1	L	TC	200	2.5

Table 2.7 (continued).

αGPD	1.1.18	L	TEM50	200	2
GPI	5.3.1.9	L	TEM50	200	2
IDH	1.1.1.42	L	TC	200	2
LDH-1	1.1.1.27	M	TEM50	200	3
MDH-1	1.1.1.37	M	TEM50	200	2
MDH-2	1.1.1.37	M	TEM50	200	2
ME-2	1.1.1.40	L	TEM50	200	2
MPI	5.3.1.8	L	TEM50+NADP	200	2
NP	2.4.2.1	L	CP	200	1.5
PEP-2	3.4.11 or 13*	L	TEB15	150	1.5
6PGD	1.1.1.44	L	TEM50+NADP	200	2
PGK	2.7.2.3	L	TEM50	200	2
PGM	2.7.5.1	M	TEM50	200	2.5
PK	2.7.1.40	M	TEB25	100	4
SOD	1.15.1.1	L	TEM50	200	2.5
TPI	5.3.1.1	L	TC	200	2.5

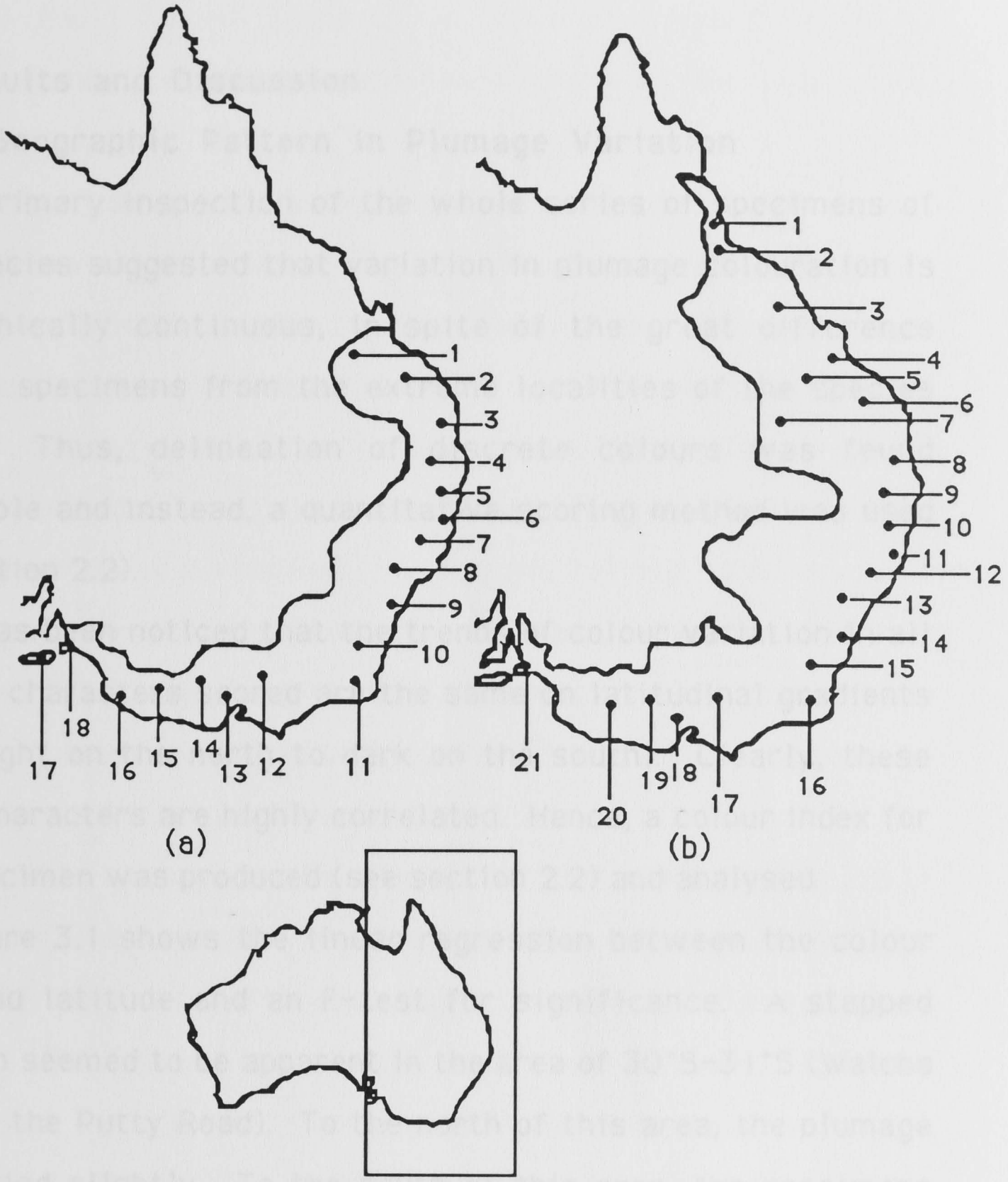


Figure 2.1 The distributions and sample sites for the two thornbill species: *A. lineata* (a) and *A. reguloides* (b). For site locations, see Table 2.1 and Table 2.2.

CHAPTER 3 Geographic Variation in the Striated Thornbill

3.1 Results and Discussion

3.1.1 Geographic Pattern in Plumage Variation

A primary inspection of the whole series of specimens of both species suggested that variation in plumage colouration is geographically continuous, in spite of the great difference between specimens from the extreme localities of the species ranges. Thus, delineation of discrete colours was found impossible and instead, a quantitative scoring method was used (see section 2.2).

It has been noticed that the trends of colour variation in all plumage characters scored are the same on latitudinal gradients (from light on the north to dark on the south). Clearly, these colour characters are highly correlated. Hence, a colour index for each specimen was produced (see section 2.2) and analysed.

Figure 3.1 shows the linear regression between the colour index and latitude and an F-test for significance. A stepped variation seemed to be apparent in the area of 30°S-31°S (Walcha south to the Putty Road). To the north of this area, the plumage tone varied slightly. To the south of this area, the specimens were increasingly brown-hued with the increasing latitude. The significance of the regression may be caused only by the population south of the area where the abrupt variation was identified.

The stepped variation in plumage colouration may indicate a contact zone. If this area is a zone of intergradation, the origin of the zone needs explaining. However, a distinction between

primary and secondary contacts usually cannot be made (Endler, 1982, 1983; Barton and Hewitt, 1985), except in few cases (e.g., Thorpe, 1984). Nevertheless, as will be argued below, a secondary contact seems to be more likely in the case of *A. lineata*.

Firstly, the population genetic structure of birds is not conducive to parapatric divergence and parapatric speciation (Templeton, 1981; Barrowclough, 1983). The only suggested intergradation zones with parapatric origins for Australian birds are from Hughes (1982) for the Australian Magpie (*Gymnorhina tibicen*) and Ford (1981) for the intergrades between the Black-eared Miner (*Manorina melanotis*) and the Yellow-throated Miner (*M. flavigula*). However, these primary intergradations have also been questioned by Ford (1987), Schodde (1981) and Joseph (1986). Therefore, secondary contact is usually proposed to explain stepped intergradation in birds.

Secondly, it is generally postulated that a series of arid to pluvial oscillations and glaciations with increasing aridity occurred from 2.5 million years ago. The last glacio-aridity of 25000 to 15000 years ago was the most severe in term of aridity and caused the expansion of the arid core in the Australian continent (Bowler, 1982). Such climatic changes were associated with vegetation changes of great magnitude, and therefore, have been proposed to have caused range contraction of many avian species when the climate became dry and cold and range expansion when the climate became mild. Keast (1961) and Ford (1987) postulated a number of major refuges (as the results of range contractions) around the continental periphery and major geographical barriers between those refuges. These barriers

were usually stretching zones of aridity from the continental interior, with low rainfall and open vegetation, which were inhospitable to some avian species. Many recognized contact zones are consistent with these barriers (Ford, 1987). Therefore, it is possible that the supposed stepped intergradation in *A. lineata* is also related to the last glaciation-aridity.

Of the barriers suggested by Keast (1961) and Ford (1987), the Hunter River valley is the closest to the assumed contact zone between the northern and southern populations of *A. lineata*. Short et al. (1983) supposed that this area, as a dry east-west strip during the last glaciation, was a former barrier that separated the "White-headed" Varied Sittella (*Daphoenositta chrysoptera leucocephala*) and the "Orange-winged" Varied Sittella (*D. c. chrysoptera*), and cited several taxa which now do not cross this barrier despite the climatic improvement since the last glaciation to support their supposition.

However, a past barrier to the north of the Hunter River (e.g., the McPherson Range) cannot be dismissed. As summarized by Barton and Hewitt (1985), many factors (e.g., differential rates of dispersal) could lead to the movement of contact zones (or tension zones). For example, if the dispersal of *A. lineata* from the north was faster than from the south during any past diffusion process, the movement of the contact zone to the south is possible. Furthermore, some contact zones of other avian species have been identified at the McPherson Range (Ford, 1987). For example, Ford (1980) supposed that the rather cold upland region of the McPherson Range was the barrier that separated *Daphoenositta chrysoptera leucocephala* and *D. c. chrysoptera*.

Irrespective of which geographic barrier might have been significant, in the sense of vicariance it is more likely that *A. lineata*, with some other avian species, was subdivided because of the emergence of a physical barrier during the period of 25000-15000 years ago. Then, the more or less isolated subpopulations have secondarily come into contact when this barrier subsequently broke down.

In summary, the analysis of plumage colouration has not corroborated the pattern of geographic variation suggested by the various divisions of subspecies, the discontinuous distribution of plumage colouration states. Variation in plumage colouration has been found to generally follow Gloger's law, i.e., a north-south cline. However, a stepped variation has been identified and this may indicate a past secondary intergradation.

3.1.2 Geographic Patterns in Dimensional Variation

3.1.2.1 Variation between Sexes

Figures 3.2-3.7 show the means (in original form) and standard errors of the six dimensional characters for males and females at each locality. Usually, the means for males were larger than those for females. However, the variation trends of characters in both males and females were basically similar.

Table 3.1 shows the results of an analysis of variance (F-tests) for the differences in each of the six characters among localities, between sexes and among the interaction of localities and sexes. The differences in the culmen, hindtoe, wing and tail lengths and the body weight were significant between males and females, but the difference in the tarsus length between males and females was not. The differences in each of the six

characters among the interaction of localities and sexes were not significant (The significance level in the hindtoe length was less than 0.05 but is greater than 0.01).

The Pearson correlation coefficients between each pair of characters, the significance levels of the correlation between the pair of characters, and the number of observations are shown in Table 3.2. All six characters were highly correlated with each other.

The six characters have been checked simultaneously because of the high correlations among them (Table 3.2). The results of multivariate analyses of variance are shown in Table 3.3. All four types of statistics showed significant differences in overall characters both among localities and between the two sexes.

Strong sexual dimorphism has been shown in five of the six characters (Table 3.1). The statement by Mayr and Serventy (1938) that there was no difference between males and females seems to be inaccurate, probably because their conclusion was based on only a few specimens. However, the tarsus length was similar in both sexes (Table 3.1), though this character was correlated to the other five characters (Table 3.2). Nevertheless, the difference in characters between sexes, whether significant or not, was not related to localities (Table 3.1). This is also shown in Figures 3.2-3.7, on which geographic variation trends of both sexes were proximately similar.

Differences in characters between males and females could be caused by sex-linked loci or by different selective regimes of the two sexes during ontogeny or both.

Unlike the other five characters, the tarsus length did not show significant sexual dimorphism although all six characters

were correlated (Table 3.2). This may indicate that not only the body size, but also the body shape was different between the sexes. However, scaling (allometry) analysis was not carried out to test this hypothesis.

In summary, although five of the dimensional characters (and the overall dimensional character) show significant sexual differences, the variation trends of both sexes are approximately similar. In other words, no evidence indicates that differences in dimensional characters between sexes are related to geographic locations.

3.1.2.2 Variation among Localities

The difference in each of the six characters among localities was significant (Table 3.1). The overall difference in all characters among localities was also significant (Table 3.3). On the other hand, a very tiny proportion of multiple comparisons (either Tukey's pairwise comparisons based on the studentized ranges or Duncan's multiple-range tests) of characters of males (or females) between each pair of localities, was significant (Results are not presented due to the huge number of comparisons made). Failure to reject the hypothesis that two means are equal implies only that the difference between the two locality means, if any, is not large enough to be detected with the given sample size. It should not lead to the conclusion that the locality means are in fact equal (SAS Institute, Inc., 1985). However, it is more likely that the variation in characters among localities was not abrupt, despite several reversals of means in Figures 3.2-3.7.

Cluster analyses (UPGMA) from matrices of character means of either males or females at the 18 localities showed no

discernible patterns. Therefore, these results are not shown. Reasons for the distortion will be discussed in section 3.1.3.3.

Figures 3.8a-f show the simple regressions between transformed character values of male individual specimens and latitude, and the results of F-tests which were used to check the significance of the established linear regressions. All six characters had larger values in the south than in the north. All the regressions were statistically significant, despite the gentle appearance of the regression lines. Similar results were found for female *A. lineata* and therefore, are not displayed.

Figure 3.9 shows the results of spatial autocorrelation analyses for the six morphological characters for male *A. lineata*. Results obtained for females were very similar and are therefore not presented. For each Moran's I computed at a distance class for a character, a Monte Carlo permutation of 2000 was carried out to decide whether the value of Moran's I is significant at the level of 0.05. Usually, a positive significant I-value indicates that the neighbouring populations within the range indicated by a distance class are similar and a negative significant I-value indicates that the neighbouring populations within the range are dissimilar.

The I-values for the culmen length at the first two distance classes were positive and significantly different from the expectation. The I-values at other distance classes were negative but not significant.

The correlogram for the wing length was very similar to that for the culmen length. The autocorrelation coefficients at the first two classes were significantly positive. Others were nonsignificantly negative.

For the tarsus length, the autocorrelation coefficients at the first two classes were significantly positive. Those at the third and fourth classes were nonsignificantly positive. Those at other classes were negative but not significant.

For the tail length, the first three autocorrelation coefficients were positive. Of these three coefficients, the first two were also significant. Autocorrelation coefficients at other distance classes were nonsignificantly negative.

The I-values for the hindtoe length and the body weight were either positive or negative but none of them was significant. Most of these values oscillated around zero. From these two correlograms, it is hard to deduce any pattern of variation.

In general, the correlograms for the culmen, wing, tarsus and tail lengths are similar. That is, as the distance increases, the autocorrelation coefficients decrease from positive values to negative values (Figure 3.9). This is similar to the correlogram pattern of a gradual cline simulated by Sokal and Oden (1978b), although in the present case, the negative coefficients are not significant. Patterns revealed from correlograms are parallel to the patterns uncovered in Figures 3.8a-b, 3.8d-e, the gentle but statistically significant clines. In addition, significantly positive I-values at the first two distance classes in these characters may indicate that neighbouring populations within 500 km (the upper limit of distance class 2) exchange genes more than those which are 500 km apart.

The clines in the morphological characters could be formed through several processes. Firstly, a process of isolation-by-distance may apply. The dispersal ability of *A. lineata* may be limited so that the genetic exchange could only be conducted

within a limited distance (500 km is suggested by the spatial autocorrelation analyses). The second possible process is selection in response to some type of environmental gradients. As Bergmann's rule suggests, body size (defined loosely as the body weight or the other five dimensional characters in the present study) may increase as the temperature decreases. The third process is that environmental gradients affect ontogeny and cause character clines. The fourth possible process is the diffusion of two or more genetically differing subpopulations one into the other. In other words, the species might be separated into two subpopulations for historical reasons. The more or less isolated subpopulations then came into contact later. This hypothesis is consistent with that posed to explain the pattern of geographic variation in colour index of plumage (see section 3.1.1). In the absence of information on dispersal rates, the mechanisms of species-environment interaction, and powerful evidence of historical separation, it is impossible to determine which process is most likely. However, environmental selection is usually supposed to be the most possible process (see discussion on heritability of morphological characters and Bergmann's rule in section 1.2.2).

In summary, both linear regression and correlogram analyses show that most of the six dimensional characters display clinal variation although the clines are not steep. The correlogram analyses also show that neighbouring populations within 500 km are significantly similar.

3.1.3 Geographic Patterns in Allozymic Variation

3.1.3.1 Variation in Allele Frequencies

Of the 28 loci which could be appropriately scored for samples of *A. lineata*, only ten showed variation in allele frequencies at at least one locality. Table 3.4 summarizes the frequencies of these ten loci at the 17 localities (Tissue samples are absent from locality 9).

Contingency χ^2 analysis is usually used to test significance of difference in allele frequencies, but it was not performed to test the difference in allele frequencies among localities. This is because in the current study, many alternative alleles only occurred at few localities at low frequencies, the number of alleles at a polymorphic locus was usually high and the sample size was limited. Under such conditions, expected values on many cells of contingency analysis tables were less than five and even less than one and the χ^2 tests would be of little meaning. Hence, contingency χ^2 analysis was not performed to test the significance of heterogeneity in allele frequencies among local populations. Alternatively, allele frequencies were analysed qualitatively as follows.

At the ALD-2 locus, two alternative alleles to the common allele (C) were detected. Allele B occurred at a northern locality (locality 2) and two central localities (localities 8 and 10); allele A occurred at a southern locality (locality 14) and a central locality (locality 7).

At the GPI locus, the common allele is C. The most obvious variation occurred in the area of Mt. Lofty (including the Fleurieu Peninsula), where the frequency of allele D (0.6) was higher than the common allele (C) at other localities. In addition to the Mt.

Lofty Ranges, allele D was found only at one other locality at a low frequency (0.022). Allele A was found at three adjacent localities, i.e., the area from the Jimna Range south to the New England Plateau. Allele B occurred at localities 7 and 13. The frequency of allele B at locality 7 was relatively high (0.333) but was low at locality 13. Allele E was only detected at the northernmost locality (the Blackdown Tableland).

The alternative allele A to the common allele B at the ME-2 locus was found only at two localities at frequencies of about 0.06. These two localities are not adjacent.

At the PEP-2 locus, three alleles were identified. Allele B occurred at all localities. Allele A occurred in populations from southern New South Wales and the border area between Victoria and South Australia. Allele C was restricted to New South Wales localities.

Three alleles were identified at the LDH-1 locus. The uncommon alleles (B and C) were located mainly in New South Wales localities.

At the PGM locus, up to seven alleles were identified. Allele B was found at all localities. Allele C was absent from most Queensland locations. Allele D was present at two New South Wales localities (Localities 7 and 8) and a Victorian locality (Locality 14). Allele A was found only at locality 2. Allele E only occurred at locality 5. Alleles F and G were present at locality 7.

Up to ten alleles were identified at the NP locus. Alleles F and G, and to some extent, alleles D and H, appeared most frequently. Allele A only occurred at locality 8. Allele B was absent from Queensland and South Australia. Allele C was absent

from all localities of Queensland and northern New South Wales. Other alleles were present at several localities.

At the EST-1 locus, the common allele was C. Allele E was a null allele and appeared at most localities. Allele A was absent from most localities of Victoria and from South Australia. Allele B was absent from localities of Queensland. Allele D appeared at most localities.

At the EST-2 locus, alleles B and A occurred at nearly all localities. Allele D was found in a single sample and its overall frequency was calculated as zero (Table 3.4). Allele E only occurred at locality 7. Allele C appeared at several discrete localities.

At the EST-3 locus, allele B was most commonly detected. Allele E was a null allele and appeared at most localities. The other three alleles were mainly distributed at localities in New South Wales and Victoria.

Summarizing Table 3.4, three major points can be noticed. First, common alleles at ALD-2, GPI, ME-2, PEP-2, LDH-1 and PGM loci were shared with high frequency (usually over 0.8) throughout all of the localities. Alleles F and G of the NP locus, C of the EST-1 locus, B and A of the EST-2 locus and B of the EST-3 locus also appeared throughout the species range at relatively high frequencies. Subsequently, it seems appropriate to conclude that there are no obvious fixed differences in alleles between local populations. The species can be considered to be panmictic with respect to the electrophoretic characters currently studied.

Second, although a considerable number of alternative alleles was detected, they usually appeared at low frequencies. Some alternative alleles were only found at one locality. Others

appeared at either some continuous or separate localities. Consequently, it is not possible to clearly relate these uncommon alleles with a geographic pattern. However, it seems that many such alleles occurred more frequently at New South Wales localities than localities in Queensland, Victoria and South Australia. In particular, many are absent from Queensland populations.

Third, some alternative alleles, with higher frequencies were found. This may indicate that some unique variation occurred at the locality where the allele was found. Allele D at the GPI locus, which was found in the Mt. Lofty Ranges, is an example.

Several explanations on the panmictic variation in *A. lineata* are conceivable. First, dispersal with or without interbreeding can cause the homogeneity of a population. However, several banding studies have showed that *A. lineata* is sedentary in northeastern New South Wales (Bell, 1982) and other areas (Blakers et al., 1984). *A. lineata* usually occurs in clans of 10-20 permanent member birds and maintains a clan territory of about 5-9 ha throughout the non-breeding period. In breeding season (July-November) the clan territory is divided among smaller breeding groups, which hold the same breeding territory each year (Bell, 1985; Recher et al., 1987). It has also been found that *A. lineata* never appears in pine plantations and open land (Recher et al., 1987). Therefore, large patches of pine plantations or open land may inhibit its dispersal. So, it seems to be unlikely that individuals of *A. lineata* disperse across long distances. The effect of gene flow on the similarity of *A. lineata* population may not be as prominent as those on other avian species with higher mobility.

The second explanation is that *A. lineata* may have a large effective population size and low mutation rate. Effective population size in birds is usually considered to be large, typically in the order of 10^2 or larger (Barrowclough and Shields, 1984). *A. lineata* is an abundant and widely distributed species (Recher et al., 1987). In addition, the mutation rate of bird lineages is among the slowest (Britten, 1986).

The third explanation concerns the evolutionary history of the species. A short history of evolution may account for the lack of stepped variation. According to an analysis of DNA-DNA hybridization (Sibley and Ahlquist, 1985), the *Acanthiza* lineage emerged about 10-15 million years ago. The formation of *A. lineata* and *A. reguloides* must therefore be more recent. However, during the span in this order, it is possible that more or less isolation might happen in history, but the premating or postmating reproductive isolation was not complete, so that the latter diffusion of the subpopulations could be relatively thorough.

The discontinuity of the observed distribution of uncommon alleles among the localities could be due to several possibilities. First, since frequencies of these alleles were low, their gene flow among local populations of this species would not be high. Consequently, the sample size at each locality may not have been large enough to ensure that these rare alleles were drawn from all localities. Second, the rises or maintenance of these alleles are highly affected by local environmental factors. This is less likely because most allozymes are only mildly subject to environmental factors.

The avifauna of the Mt. Lofty Ranges has been considered isolated from other areas of Victoria and South Australia, and the *A. lineata* population in this area has been described as a unique subspecies (*A. l. clelandi*). The high frequency of allele D at the GPI locus in the area seems to be consistent with this interpretation. However, Blakers et al. (1984) showed no break in the distribution of mainland populations. But there is indeed a restriction in connection between the populations of southeastern South Australia and the Mt. Lofty Ranges across the Lower Murray Basin (mostly covered by mallee scrub). The gene flow between these two populations may therefore be low. Moreover, the population density at a peripheral locality and the gene flow between this locality and other localities would be low even without geographic restriction, and the mutation rate therefore could be high. Hence, it is more likely that the possible low density and restriction of gene flow at the periphery of the species range, rather than complete isolation, cause the higher frequency of allele D at the GPI locus in the Mt. Lofty Ranges.

In summary, allozymic variation is relatively panmictic. A considerable amount of alternative alleles are found but they are only detected at only a few localities and at low frequencies. The frequency of allele D at the GPI locus in the Mt. Lofty Ranges is high, but this variation alone cannot confirm that the population in the area is isolated.

3.1.3.2 The Assumption of a Panmictic Sample Set

Although *A lineata* usually forms foraging flocks in the non-breeding season (Bell, 1985; Recher et al., 1987) when most of specimens in this study were collected, probably, few sibling

individuals would have been collected because only one or two individuals were taken from each feeding flock. The foraging flocks are also made up of more than one nesting group (Bell, 1985; Recher et al., 1987). Lastly, some of the 17 sample sets were actually formed by combining individuals taken from very close but different point sample sites. Therefore, ecologically and practically, the chance that closely related individuals were included in a sample set is not large.

Genotypic frequencies were tested for conformity to Hardy-Weinberg equilibrium within each population at a locality. Pooled genotypes and Levene's (1949) correction for small sample sizes were applied (for details, see section 2.6). The significance levels, at which a locus is in Hardy-Weinberg equilibrium, are summarized in Table 3.5. If a sample set is drawn from an inbreeding population or from different subpopulations with different gene frequencies, divergence from Hardy-Weinberg expectation, either being of more homozygote or being of more heterozygote, will be detected.

Judging from Table 3.5, the ALD-2, ME-2 and LDH-1 loci were unlikely to be in Hardy-Weinberg equilibrium. In fact, these three loci were polymorphic at only a few localities. Furthermore, the frequencies of alternative alleles were very low. It is possible that this effect was caused by relatively small sample sizes rather than inbreeding or the Wahlund effect. The three esterase loci were polymorphic at almost all localities and χ^2 tests showed significant divergence from Hardy-Weinberg expectation at most localities. However, because the presence of null alleles at two of these loci (allele E at the EST-1 locus and allele E at the EST-3 locus) may lead to the overestimate of homozygote

genotypes of a normal allele or homozygote genotypes of a null allele (see section 2.5), the detected significance levels may not necessarily indicate the actual deviation from Hardy-Weinberg equilibrium. The remaining four loci, NP, PEP-2, PGM and GPI, showed no divergence from Hardy-Weinberg equilibrium at most localities. As these loci, especially the NP and PEP-2 loci, were polymorphic at most localities and the estimation of their genotype frequencies was not hampered by null alleles, it is reasonable to suppose that those loci are more reliable in calculation of testing Hardy-Weinberg equilibrium. Therefore, it would appear that the specimens in a sample set in this study were drawn independently from a genetically homogeneous population.

In population genetics, it is important that each sample set is drawn independently from a single panmictic population. If a sample set consists of more closely related individuals, a comparison between sample sets may overestimate real genetic differences between them because the actual number of independent genes is smaller than the number of genes taken from the population. On the other hand, if a sample set includes individuals from more than one genetically different subpopulation, a comparison between the sample set and other sample sets will generate a spurious result because the allele frequency of the sample set is biologically heterogeneous.

The assumption that genes of a sample set are independently taken from a genetically homogeneous population could also be tested by comparing the difference in allele frequencies between replicate sample sets. Owing to the limitation of time and cost,

most of samples in this study were collected within a year during the non-breeding season. Such a comparison cannot be conducted.

The currently obtained conclusion that *A. lineata* populations are likely to be in Hardy-Weinberg equilibrium is consistent with that stated by Barrowclough et al. (1985) and Zink (1986), viz., genotype frequencies in most avian populations are in Hardy-Weinberg equilibrium.

In summary, each population at a locality in this study can be considered to be in Hardy-Weinberg equilibrium, so that allozymic analyses are not biased.

3.1.3.3 Variation in Genetic Distances

Table 3.6 shows Rogers' (1972) genetic distance and Nei's (1978) unbiased genetic distance between each pair of localities.

Two cluster analyses (UPGMA) using either Nei's (1978) or Rogers' (1972) genetic distances and a Distance Wagner analysis from Rogers' (1972) genetic distance did not provide dendrograms that could be related to the geographic sequence of the localities (Figures 3.10-3.12).

The relatively small sample sizes might be a problem in these analyses. However, it is generally accepted that only a few individuals are needed if about 30 or more loci are screened (Nei, 1978; Nei et al., 1983), although Archie et al. (1989) stated that in the context of stability and accuracy of dendrograms, increasing the number of loci does not necessarily compensate for having inadequate samples of individuals. In the present study, more than 20 individuals were sampled at a single locality (the Australian Capital Territory) and allele frequencies and average heterozygosity at this locality seem not to be significantly

different from those at most other localities. Hence, evidence for the effect of small sample size is not significant. Sokal (1983) pointed out that the distortion of population relationships outlined by dendrograms is caused by the continuity of allozymic characters over the geographic range of the species. This is more likely in the current study. As shown in section 3.1.3.1, *A. lineata* is relatively panmictic without apparent diagnostic alleles between sample sets. Meanwhile, a considerable number of rare alleles exists and this may contribute to a distorted dendrogram.

To overcome the above problem, local populations could also be pooled together by using a G-test to check the deviation of F_{st} values from zero (Corbin, 1987). However, many of alternative alleles occurred at low frequencies at only a few scattered localities, therefore the G-test cannot be conducted in the present study.

In summary, dendrograms constructed from genetic distances are not informative mainly due to the relative panmixia of allele frequencies.

3.1.3.4 Variation in Average Heterozygosity and Polymorphism

Figure 3.13a illustrates the geographic variation in average heterozygosity (Nei, 1978) and its standard error at each of the 17 localities. The percentage of polymorphic loci (A locus is considered to be polymorphic if the frequency of the most common allele does not exceed 0.95) at each locality is shown in Figure 3.13b.

The values of average heterozygosity were the highest at localities 7 and 8 (the area from Walcha south to the Putty Road).

Similarly, the values of per cent polymorphism were the highest at these localities. The average heterozygosity at other localities varied slightly except for those at localities 2 and 15, where the values of heterozygosity were low.

From the variation in average heterozygosity, it could be hypothesized that the area from Walcha to the Putty Road (localities 7 and 8, latitude of 31°S to 32°S), might represent an area of past intergradation between distinct forms.

Corbin (1981, 1983, 1987) pointed out that average heterozygosity is maximal when allele frequencies at loci are approximately equal or when the average number of polymorphic loci is maximized. In a contact zone, allele frequencies at a given locus will converge towards intermediate values as the result of increasing gene exchange and heterozygous individuals. Furthermore, the increased presence of rare alleles in contact zones is common and this may be due to higher mutation rate, intragenic recombination and even relaxed selection, provided that the rare alleles are neutral or only slightly deleterious (Barton and Hewitt, 1985). Given these conditions, heterozygosity usually rises in a zone of intergradation, whether primary or secondary, but is usually greater within the latter.

Corbin (1981) presented an example of using genic heterozygosity as an index to the possible intergradation between subspecies of the White-crowned Sparrow (*Zonotrichia leucophrys*). The heterozygosity he calculated was based on the proportion of polymorphic loci. Heterozygosity calculated from allele frequencies (Nei, 1978) was used in the current study, because local populations were shown not to deviate significantly from Hardy-Weinberg equilibrium (section 3.1.3.2), because not

all loci could be processed for all samples in this study, and because this form of heterozygosity should be more sensitive to variation of allele frequencies at a locus.

The higher mean heterozygosity in the area from Walcha to the Putty Road appears to be due to the fact that some alternative alleles mainly occurring at either more northerly or southerly localities were also found in this area (e.g., alleles A and B of the ALD-2 locus, allele B of the GPI locus, allele A of the ME-2 locus, allele A of the PEP-2 locus, allele D of the PGM locus, allele C of the NP locus and alleles A and B of the EST-1 locus) and that more alternative alleles were evident in this area (e.g., allele C of the PEP-2 locus, alleles B and C of the LDH-1 locus, alleles F and G of the PGM locus, allele A of the NP locus, allele E of the EST-2 locus etc.).

However, there was no obvious difference in allele frequencies between the localities to the north and south of the proposed intergradation zone. Therefore, the assumed contact zone, if it exists, must have decayed a great deal since its origin, leaving only remnant alternative alleles that have not secondarily introgressed into the parental populations.

The proposed contact zone is very close to that revealed by the variation in colour index of plumage (section 3.1.1). Primary intergradation of independent characters could be produced by linked or co-adapted loci or the shift of independent clines into a region of low population density. No evidence indicates the existence of these situations for *A. lineata*. It is most likely that genes controlling the plumage colouration and genes coded for allozymes are not correlated. Hence, as two genetically independent suites of features exhibit the same pattern of abrupt

variation, the former (and probably still current) secondary contact zone is more likely.

The historical aspect of secondary intergradations of the Australian birds, the concordance between the proposed intergradation zone and those of other avian species were discussed in section 3.1.1. Either the Hunter Valley or the McPherson Range could be the past physical barrier.

In summary, the high values of mean heterozygosity in the area from Walcha to the Putty Road suggest a past secondary intergradation zone in *A. lineata*. The concordance of patterns revealed from both plumage colouration and mean heterozygosity, the Australian biogeographic history and the presence of intergradations of other species support the hypothesis. Whichever the past barrier is, the intergradation zone has long broken down and only remnants are left.

3.2 General Discussion and Conclusions

The analyses of the three suites of characters reveal different patterns of geographic variation. Variation in the plumage colouration, in spite of generally following Gloger's rule, shows a stepped pattern, probably indicating a secondary contact zone. Mensural morphological characters display gradual clines. Variation in allozymic loci is relatively panmictic, without apparently abrupt changes or clines in allele frequencies. However, the variation in mean heterozygosity (Nei, 1978) shows a possible former contact zone, which is concordant with that seen for colour index of plumage.

Obviously, the discordance of the patterns of geographic variation is due to the fact that different suites of traits usually

vary independently. This is because both differential selection at a locus and differing selective regimes among loci cause gene flow to vary among loci. Furthermore, allozyme loci are usually considered to be neutral (Barrowclough et al., 1985; Zink 1986), and dimensional and plumage characters to be environmentally selective as suggested by Bergmann's and Gloger's rules.

The possibility that the variation in dimensional and plumage characters is mainly shaped by environmental factors cannot be excluded completely, because no experiment on heritability or breeding experiment has been conducted for *A. lineata*. The genetic basis of the infraspecific variation in the dimensional and plumage characters currently studied is simply assumed.

Turning to the questions posed in the introduction (section 1.6), the following conclusions can be drawn.

1). Although plumage colouration in *A. lineata* becomes darker from north to south, a high level of variation has been found in the area of Walcha south to the Putty Road, possibly indicating a contact zone. To the north of this zone, the colour index changes only marginally, whereas to the south of this zone, a gradual cline is displayed. The pattern of plumage colouration revealed by this study has not corroborated the proposed existence of subspecies (Mack, 1936; Mayr and Serventy, 1938).

2) As deduced from the dimensional measurements, the population of *A. lineata* shows a gradual cline. All dimensional characters studied have increased values with increasing latitude, possibly indicating the mechanism of adaptive selection to some environmental variation which are related to a latitudinal gradient. Judging from the allozymic data, all samples of *A. lineata* are derived from the same panmictic population.

3). The analyses of the geographic patterns of the colour index of plumage and average heterozygosity and some alternative (rare) alleles, together with the generally accepted knowledge of avian history in Australia, suggest a possible historical secondary intergradation between subpopulations originally located in the north and the south of the area from Walcha to the Putty Road. The two subpopulations diffused so completely that no fixed allelic differences have been detected and no abrupt mensural morphometric variation is evident.

4). The panmixia of the allozymic characters, the clines of morphometric characters and the stepped cline in the plumage colouration probably indicate that these suites of characters underwent different evolutionary processes. The first is likely to be neutral and affected mainly by gene flow, effective population size and historical events, whereas the second and third probably reflect some environmental selection or non-selective environmental effects.

Table 3.1 Results of analyses of variance. Shown in the table are F-tests of the differences in dimensional characters among localities, between sexes and among the interaction of localities and sexes for *A lineata*. "*" indicates a probability being less than 0.05. "**" indicates a probability being less than 0.01. The calculations were based on the natural logarithm values of the original measurements.

Character		Locality	Sex	locality x Sex
Culmen	df	17	1	15
	F-values	4.37	9.00	0.86
	P	0.0001**	0.0031**	0.6116
Tarsus	df	17	1	15
	F-values	6.34	1.65	0.26
	P	0.0001**	0.2011	0.9979
Hindtoe	df	17	1	15
	F-values	6.72	21.71	1.87
	P	0.0001**	0.0001**	0.0287*
Wing	df	17	1	16
	F-values	6.67	23.89	0.99
	P	0.0001**	0.0001**	0.4664
Tail	df	17	1	16
	F-values	5.32	9.09	1.12
	P	0.0001**	0.0028**	0.3370
Weight	df	17	1	15
	F-values	7.88	5.29	1.15
	P	0.0001**	0.0226*	0.3200

Table 3.2 Pearson correlation coefficients, significance levels of correlation and the number of observations (in order) of the dimensional characters. The calculations were based on the natural logarithm values of the original measurements.

	Culmen	Tarsus	Hindtoe	Wing	Tail	Weight
Culmen	1.0000 0.0000 219					
Tarsus	0.3266 0.0001 219	1.0000 0.0000 226				
Hindtoe	0.3372 0.0001 218	0.2796 0.0001 225	1.0000 0.0000 225			
wing	0.2702 0.0001 219	0.3066 0.0001 226	0.4193 0.0001 225	1.0000 0.0000 321		
Tail	0.3005 0.0001 218	0.3128 0.0001 225	0.4187 0.0001 224	0.6258 0.0001 272	1.0000 0.0000 272	
Weight	0.4312 0.0001 191	0.3907 0.0001 198	0.5494 0.0001 198	0.4063 0.0001 198	0.4159 0.0001 197	1.0000 0.0000 198

Table 3.3 Multivariate analyses of variance of six dimensional characters for *A. lineata*. "**" indicates a probability being less than 0.01. The calculations were based on the natural logarithm values of the original measurements.

Hypothesis of no overall locality effect				
Statistics	value	F	Df	PR>F
Wilks' λ	0.1263	4.089	102	0.0001**
Pillai's trace	1.5736	3.576	102	0.0001**
Hotelling-Lawley trace	2.9180	4.701	102	0.0001**
Roy's greatest root	1.6300	16.396	17	0.0001**
Hypothesis of no overall sex effect				
Statistics	value	F	Df	PR>F
Wilks' λ	0.8112	6.437	6	0.0001**
Pillai's trace	0.1888	6.437	6	0.0001**
Hotelling-Lawley trace	0.2327	6.437	6	0.0001**
Roy's greatest root	0.2327	6.437	6	0.0001**

Table 3.4 Allele frequencies of 10 polymorphic loci at 17 localities (locality 9 is absent) and sample sizes (N). For locality details see Table 2.1. For locus abbreviations, see Table 2.7.

	LOCALITY																
LOCUS	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18
ALD-2																	
(N)	8	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.000	0.000
B	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.059	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	0.875	1.000	1.000	1.000	1.000	0.917	0.941	0.960	1.000	1.000	1.000	0.941	1.000	1.000	1.000	1.000
GPI																	
(N)	8	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	0.000	0.000	0.063	0.125	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
C	0.875	1.000	0.938	0.875	0.950	1.000	0.667	1.000	1.000	1.000	1.000	0.957	1.000	1.000	1.000	1.000	0.400
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.600
E	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ME-2																	
(N)	7	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	0.938	1.000	1.000	1.000	1.000	0.941	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEP-2																	
(N)	8	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.029	0.020	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000
B	1.000	1.000	1.000	0.875	0.800	0.917	0.667	0.912	0.960	1.000	1.000	0.935	1.000	1.000	0.944	1.000	1.000
C	0.000	0.000	0.000	0.125	0.200	0.083	0.000	0.059	0.020	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000

Table 3.4 (Continued)

LDH-1																	
(N)	8	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	1.000	1.000	1.000	1.000	0.800	0.667	0.583	0.706	0.960	1.000	0.857	1.000	1.000	1.000	1.000	0.750	1.000
B	0.000	0.000	0.000	0.000	0.200	0.333	0.375	0.235	0.040	0.000	0.143	0.000	0.000	0.000	0.000	0.250	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

PGM																	
(N)	8	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	0.875	1.000	0.875	0.900	1.000	0.667	0.882	0.960	0.833	1.000	0.957	0.794	1.000	0.944	0.688	0.850
C	0.000	0.000	0.000	0.125	0.050	0.000	0.083	0.088	0.040	0.167	0.000	0.043	0.176	0.000	0.056	0.312	0.150
D	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.029	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

NP																	
(N)	8	8	16	4	10	6	12	17	25	3	7	23	17	5	9	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.050	0.083	0.083	0.059	0.000	0.000	0.071	0.000	0.088	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.059	0.080	0.000	0.000	0.043	0.088	0.000	0.111	0.000	0.000
D	0.250	0.000	0.063	0.125	0.150	0.167	0.292	0.147	0.180	0.333	0.071	0.217	0.118	0.300	0.000	0.125	0.300
E	0.000	0.063	0.000	0.000	0.000	0.000	0.167	0.059	0.020	0.000	0.000	0.022	0.088	0.000	0.278	0.250	0.000
F	0.563	0.688	0.250	0.125	0.200	0.417	0.083	0.265	0.240	0.167	0.357	0.239	0.324	0.300	0.389	0.125	0.100
G	0.188	0.188	0.281	0.500	0.300	0.250	0.125	0.118	0.140	0.167	0.214	0.130	0.118	0.400	0.222	0.000	0.050
H	0.000	0.063	0.281	0.250	0.200	0.083	0.000	0.206	0.180	0.167	0.143	0.283	0.118	0.000	0.000	0.438	0.150
I	0.000	0.000	0.094	0.000	0.100	0.000	0.083	0.059	0.120	0.167	0.143	0.043	0.029	0.000	0.000	0.062	0.400
J	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.022	0.029	0.000	0.000	0.000	0.000

Table 3.4 (Continued)

EST-1																	
(N)	8	8	16	4	10	6	12	17	25	3	7	22	17	5	9	8	10
A	0.063	0.000	0.094	0.000	0.000	0.000	0.167	0.000	0.020	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.333	0.167	0.059	0.080	0.000	0.000	0.068	0.000	0.000	0.000	0.125	0.000
C	0.625	1.000	0.469	1.000	1.000	0.667	0.500	0.559	0.480	0.167	0.500	0.659	0.294	0.500	0.722	0.875	0.400
D	0.063	0.000	0.063	0.000	0.000	0.000	0.000	0.088	0.100	0.167	0.071	0.000	0.059	0.500	0.056	0.000	0.300
E	0.250	0.000	0.375	0.000	0.000	0.000	0.167	0.294	0.320	0.667	0.286	0.273	0.647	0.000	0.222	0.000	0.300
EST-2																	
(N)	8	8	16	4	10	6	12	17	25	3	7	22	17	5	9	8	10
A	0.500	0.125	0.594	0.250	0.250	0.667	0.292	0.471	0.420	0.667	0.500	0.545	0.706	0.000	0.556	0.312	0.100
B	0.375	0.875	0.406	0.750	0.750	0.333	0.625	0.529	0.480	0.333	0.500	0.432	0.294	1.000	0.444	0.688	0.900
C	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST-3																	
(N)	8	8	16	4	10	6	8	17	25	3	7	22	17	5	9	8	10
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.060	0.333	0.000	0.068	0.000	0.000	0.000	0.000	0.000
B	0.750	0.500	0.750	0.750	0.850	1.000	0.688	0.706	0.640	0.500	0.643	0.614	0.824	1.000	0.889	0.875	0.800
C	0.000	0.000	0.063	0.000	0.050	0.000	0.063	0.088	0.020	0.000	0.071	0.091	0.088	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.029	0.000	0.000	0.000	0.000
E	0.250	0.500	0.188	0.250	0.100	0.000	0.250	0.176	0.280	0.000	0.286	0.227	0.059	0.000	0.111	0.125	0.200

Table 3.5 P values of χ^2 significance tests for deviation from Hardy-Weinberg equilibrium for *A. lineata* using pooled genotypes with Levene's (1949) correction for small sample sizes. "-" indicates that no test was carried out because the locus is monomorphic at the locality. Localities are as given in Table 2.1. For locus abbreviations, see Table 2.7. The last row shows the test results for the whole population.

Locality	ALD-2	GPI	ME-2	PEP-2	LDH-1	PGM	NP	EST-1	EST-2	EST-3
1	-	<0.001	-	-	-	-	0.564	0.002	0.108	0.001
2	<0.001	-	-	-	-	0.782	0.573	-	<0.001	0.002
3	-	0.853	<0.001	-	-	-	0.021	<0.001	0.123	<0.001
4	-	1.000	-	1.000	-	1.000	0.021	-	0.655	0.007
5	-	1.000	-	0.502	<0.001	0.808	0.745	-	0.405	0.017
6	-	-	-	1.000	0.006	-	0.877	0.006	0.301	-
7	<0.001	<0.001	-	<0.001	<0.001	0.303	0.121	<0.001	0.075	0.024
8	<0.001	-	<0.001	0.748	<0.001	0.639	0.908	<0.001	<0.001	0.002
10	<0.001	-	-	0.884	<0.001	0.884	0.699	0.001	0.0.058	<0.001
11	-	-	-	-	-	1.000	0.564	0.021	0.021	0.346
12	-	-	-	-	<0.001	-	0.699	0.036	0.560	0.039
13	-	<0.001	-	0.787	-	0.879	0.782	<0.001	<0.001	0.001
14	<0.001	-	-	-	-	0.328	0.456	<0.001	0.002	0.339
15	-	-	-	-	-	-	0.206	0.110	-	-
16	-	-	-	1.000	-	1.000	0.279	0.015	0.001	<0.001
17	-	-	-	-	0.001	0.259	0.021	<0.001	0.024	<0.001
18	-	0.043	-	-	-	0.656	0.530	0.485	0.808	<0.001
Σ	<0.001	<0.001	<0.001	<0.001	<0.001	0.919	0.465	<0.001	<0.001	<0.001

Table 3.6 Genetic distances (below diagonal: Rogers' (1972) genetic distance; above diagonal: Nei's (1978) unbiased genetic distance) among *A. lineata* populations at 17 localities. For locality code number, see Table 2.1.

Locality	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18
1	*****	0.013	0.002	0.009	0.012	0.006	0.020	0.004	0.001	0.007	0.000	0.001	0.008	0.018	0.001	0.017	0.027
2	0.057	*****	0.023	0.007	0.012	0.028	0.031	0.017	0.017	0.040	0.012	0.017	0.039	0.022	0.015	0.019	0.037
3	0.030	0.070	*****	0.009	0.014	0.009	0.022	0.002	0.001	0.000	0.000	0.001	0.004	0.021	0.003	0.016	0.026
4	0.052	0.049	0.052	*****	0.000	0.013	0.017	0.008	0.008	0.024	0.006	0.005	0.026	0.010	0.006	0.004	0.024
5	0.061	0.056	0.056	0.026	*****	0.009	0.015	0.006	0.011	0.028	0.008	0.009	0.028	0.013	0.009	0.004	0.027
6	0.050	0.082	0.052	0.069	0.052	*****	0.017	0.004	0.010	0.018	0.005	0.009	0.016	0.024	0.005	0.011	0.042
7	0.081	0.098	0.083	0.079	0.073	0.080	*****	0.009	0.015	0.023	0.013	0.018	0.026	0.028	0.019	0.015	0.027
8	0.046	0.069	0.037	0.057	0.047	0.045	0.061	*****	0.000	0.004	0.000	0.001	0.007	0.018	0.003	0.005	0.024
10	0.032	0.058	0.028	0.052	0.053	0.054	0.069	0.027	*****	0.000	0.000	0.000	0.006	0.017	0.004	0.012	0.021
11	0.057	0.089	0.045	0.077	0.081	0.069	0.097	0.058	0.045	*****	0.001	0.003	0.000	0.028	0.011	0.025	0.025

Table 3.6 (Continued)

12	0.030	0.059	0.025	0.056	0.050	0.045	0.071	0.029	0.022	0.052	*****	0.000	0.005	0.016	0.001	0.011	0.022
13	0.030	0.061	0.026	0.046	0.050	0.049	0.075	0.031	0.021	0.045	0.028	*****	0.008	0.023	0.003	0.010	0.026
14	0.047	0.079	0.037	0.072	0.072	0.057	0.087	0.047	0.041	0.030	0.045	0.043	*****	0.033	0.007	0.024	0.035
15	0.056	0.063	0.059	0.058	0.058	0.061	0.099	0.069	0.057	0.075	0.057	0.062	0.070	*****	0.018	0.022	0.021
16	0.031	0.059	0.033	0.050	0.050	0.044	0.082	0.041	0.034	0.056	0.035	0.028	0.040	0.053	*****	0.011	0.031
17	0.066	0.066	0.064	0.051	0.039	0.057	0.072	0.048	0.057	0.077	0.055	0.056	0.066	0.069	0.052	****	0.028
18	0.069	0.081	0.067	0.067	0.076	0.099	0.088	0.074	0.063	0.074	0.070	0.069	0.075	0.062	0.074	0.076	****

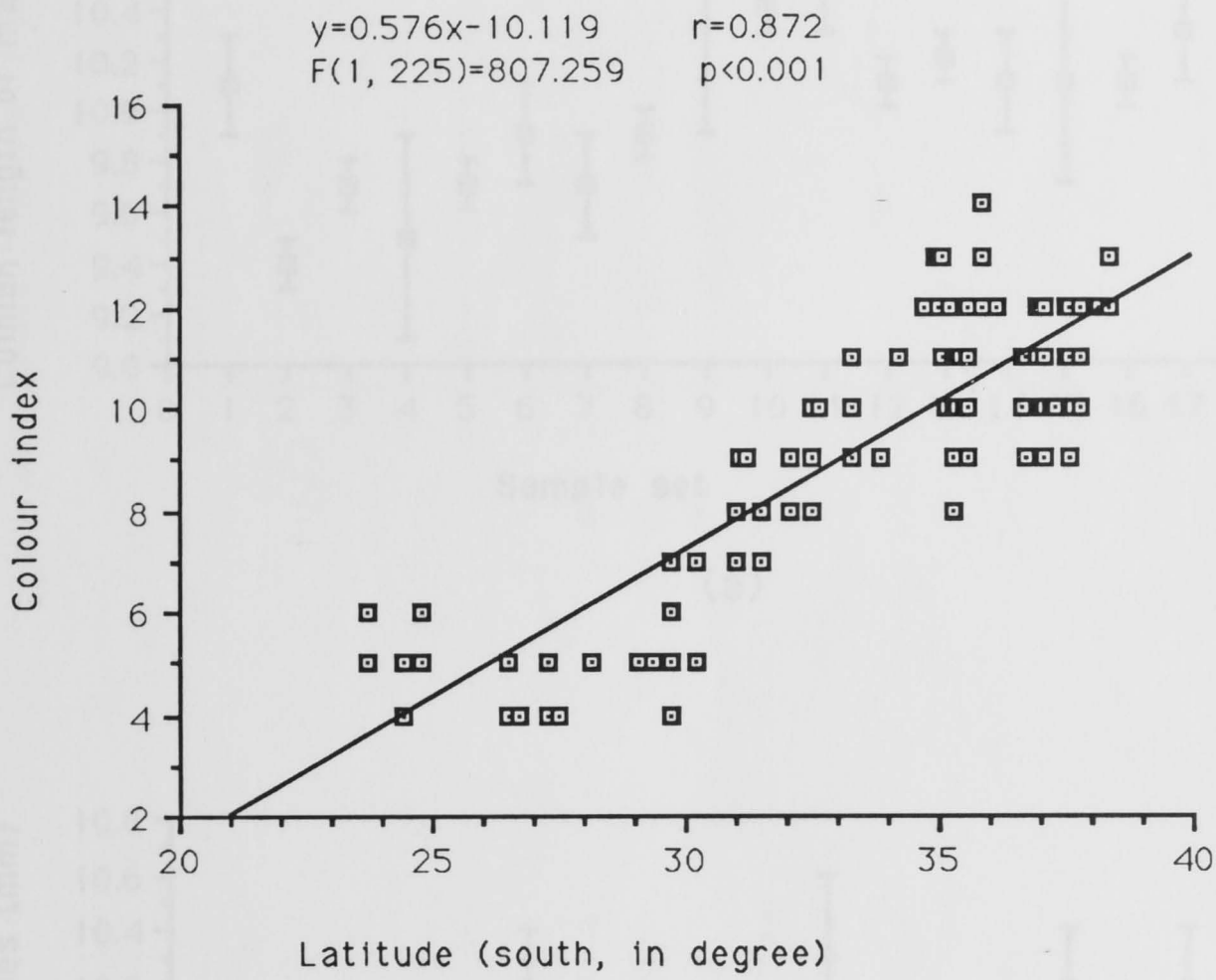
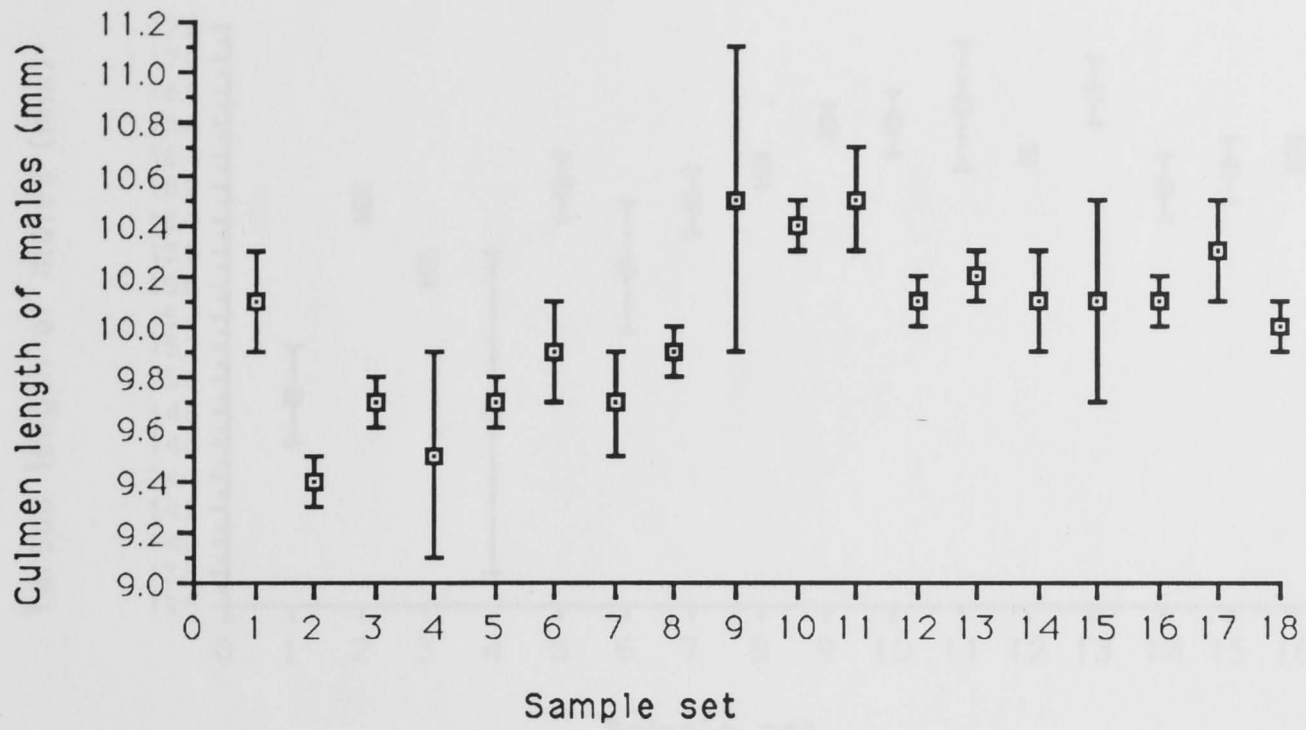
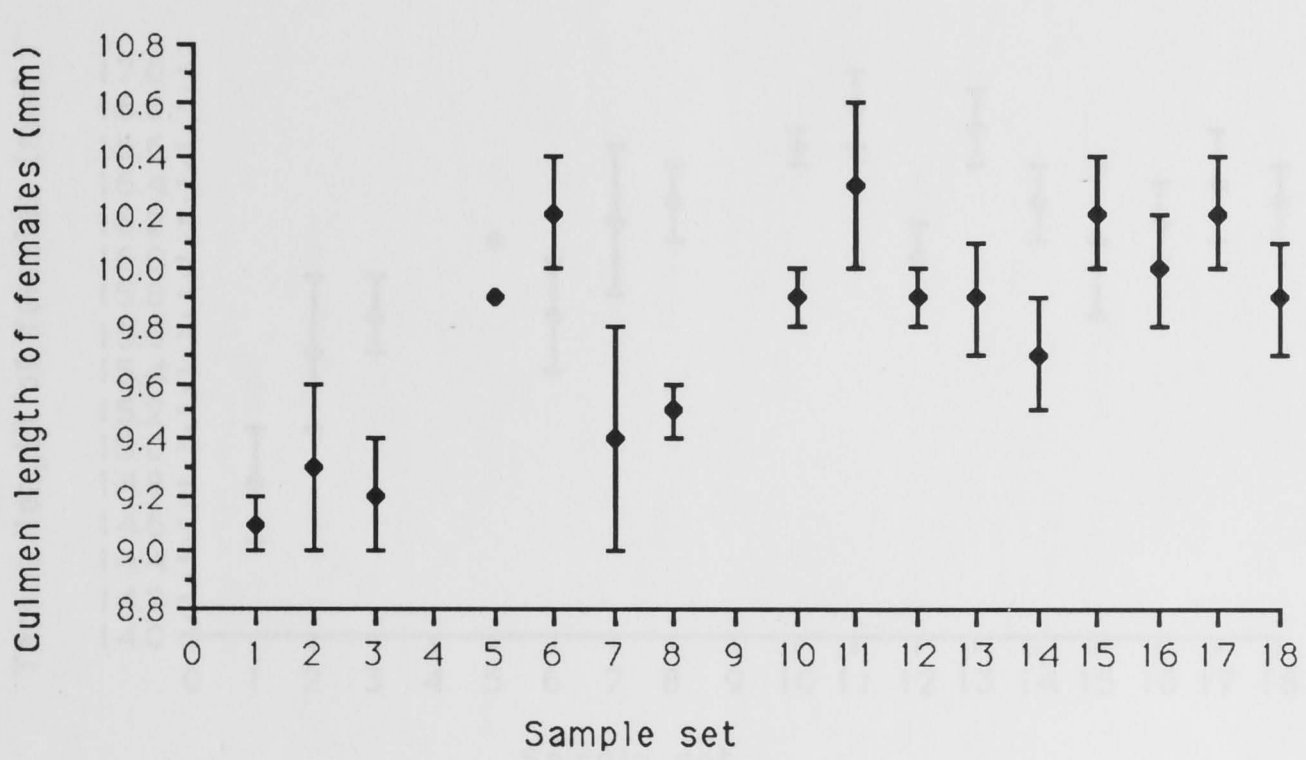


Figure 3.1 Linear regression between the colour index of *A. lineata* and latitude. Many individuals have the same values of the colour index, so that they are hidden on the plot.

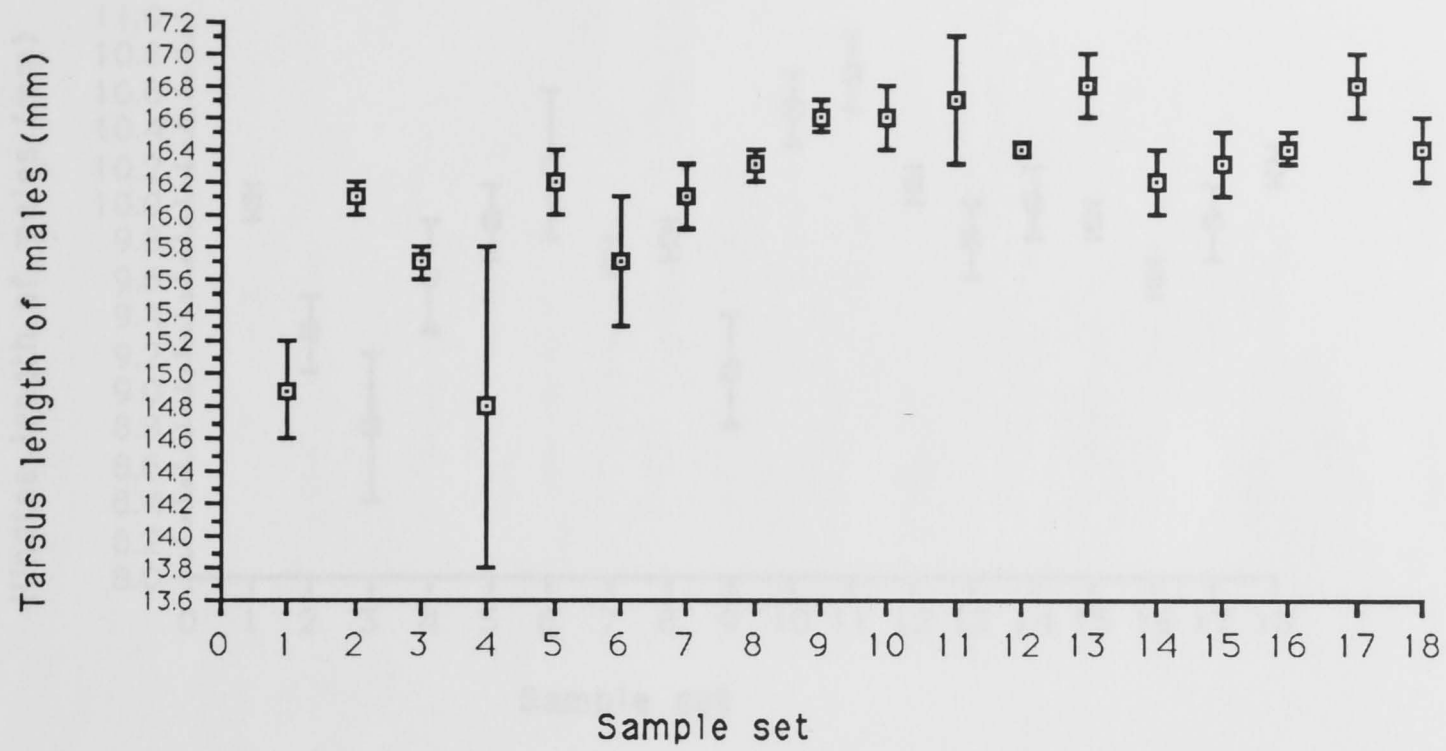


(a)

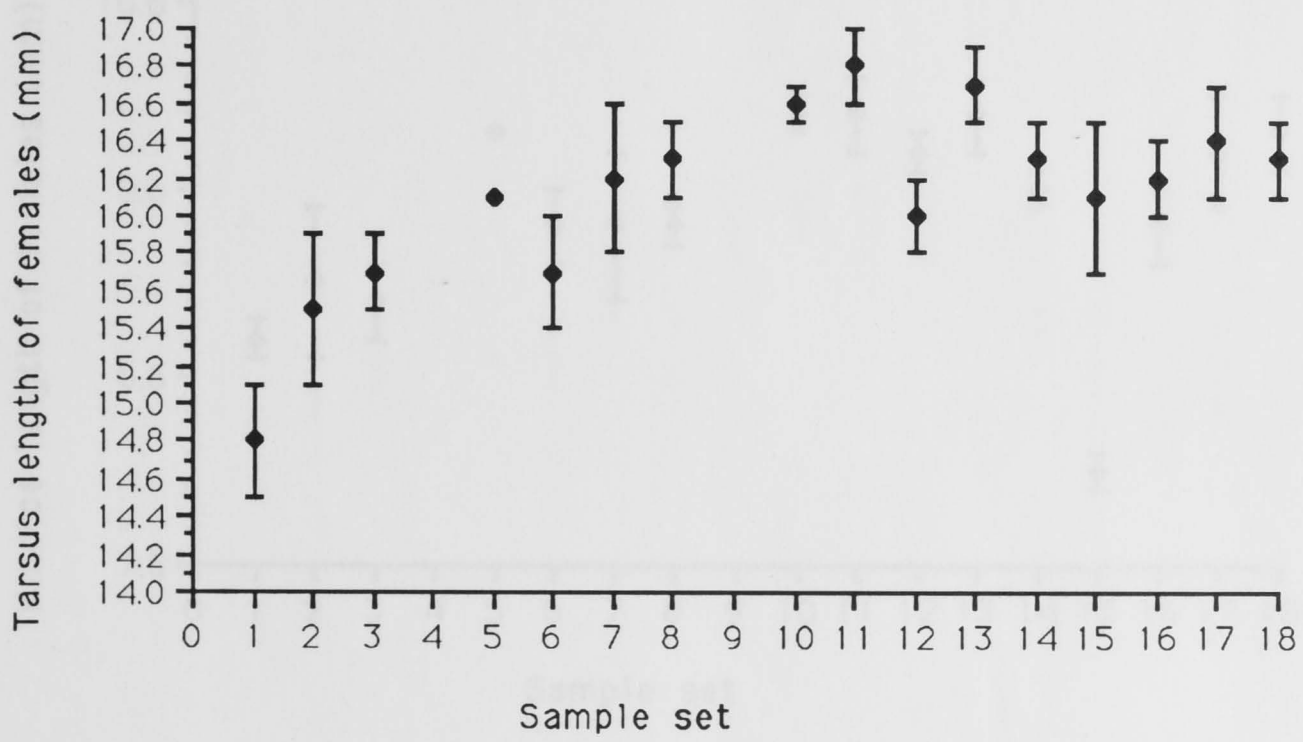


(b)

Figure 3.2 (a). Geographic variation in the culmen length in male *A. lineata*. (b). Geographic variation in the culmen length in female *A. lineata*. Localities are as given in Table 2.1.

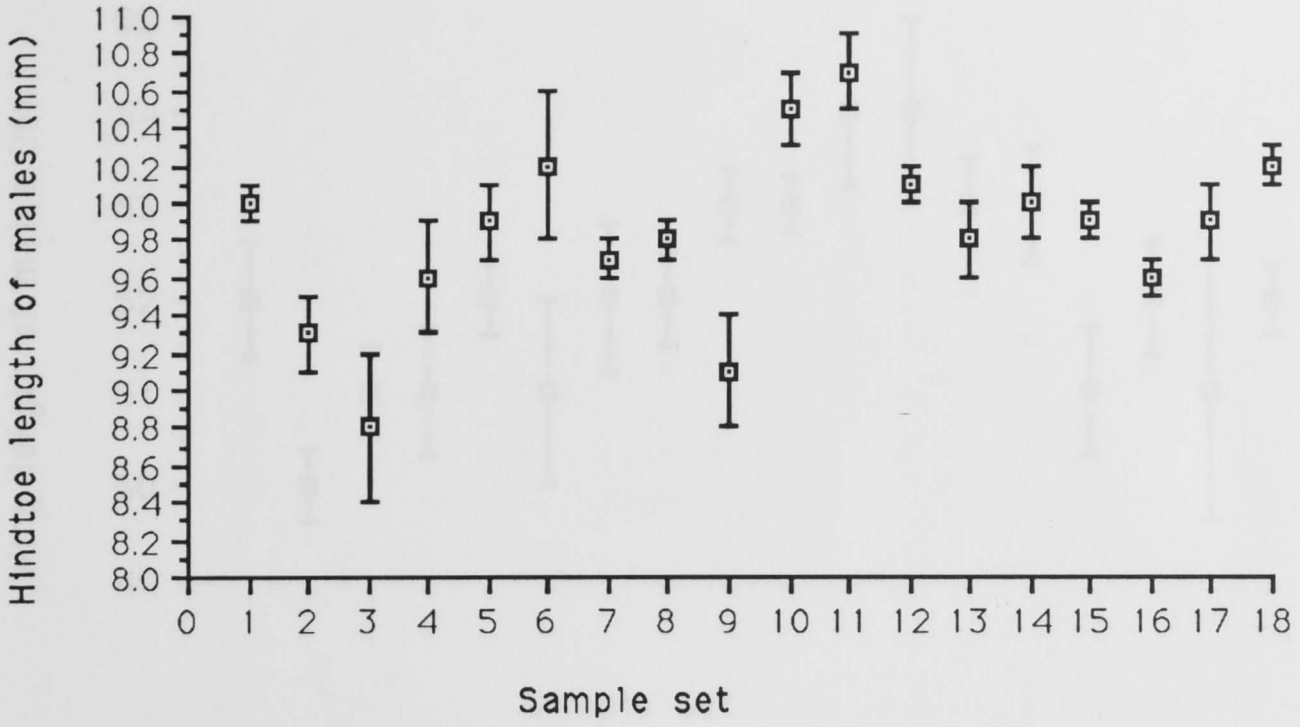


(a)

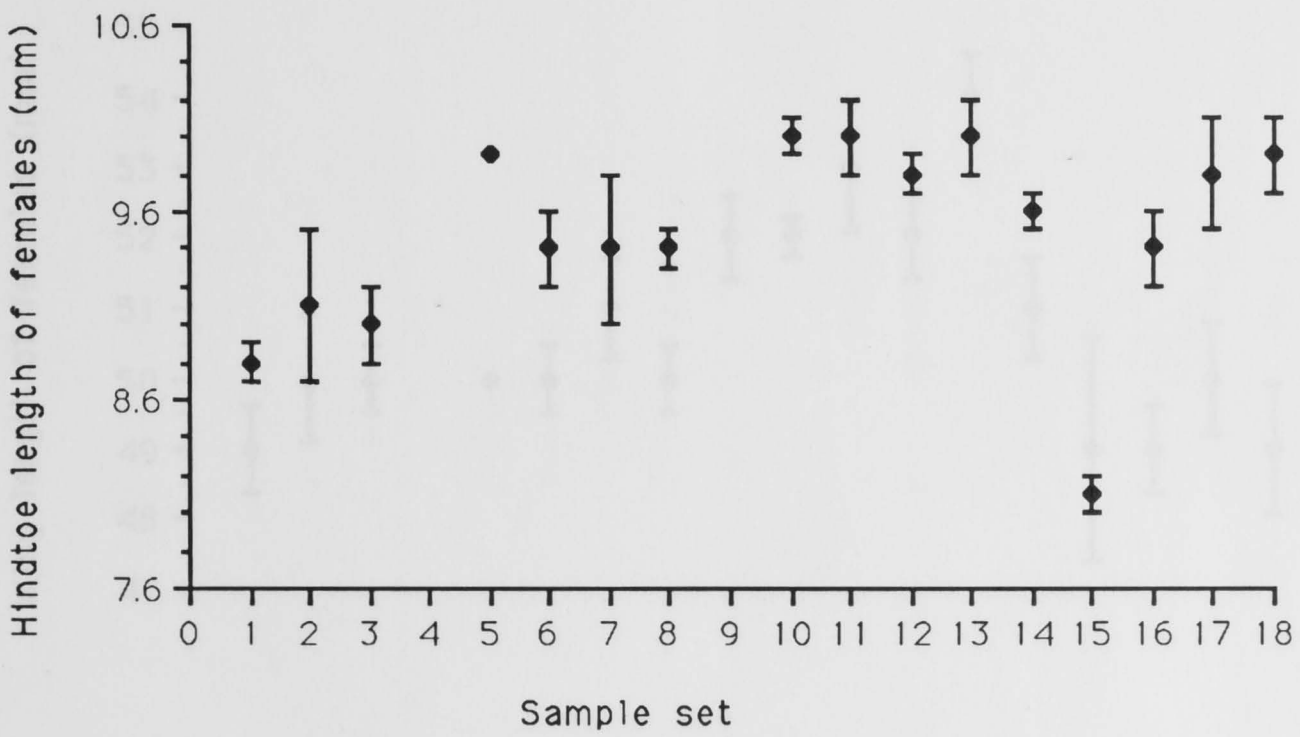


(b)

Figure 3.3 (a). Geographic variation in the tarsus length in male *A. lineata*. (b). Geographic variation in the tarsus length in female *A. lineata*. Localities are as given in Table 2.1.

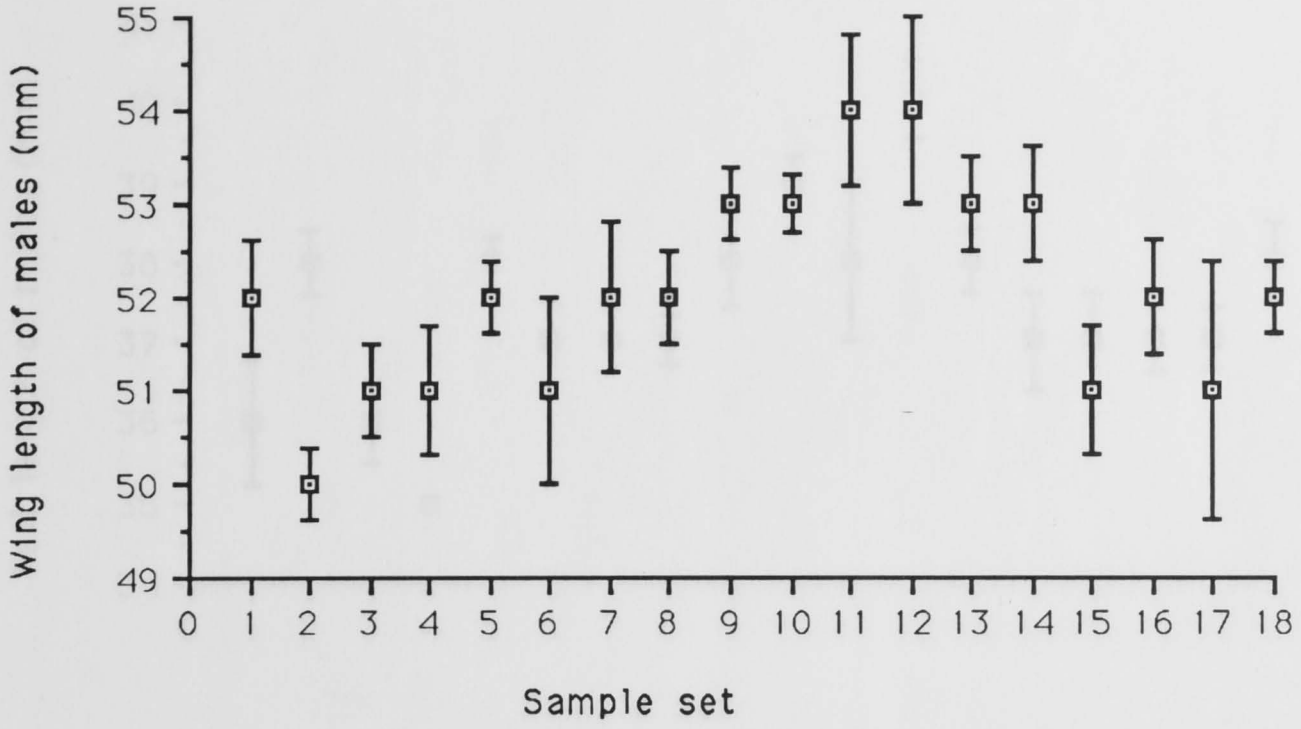


(a)

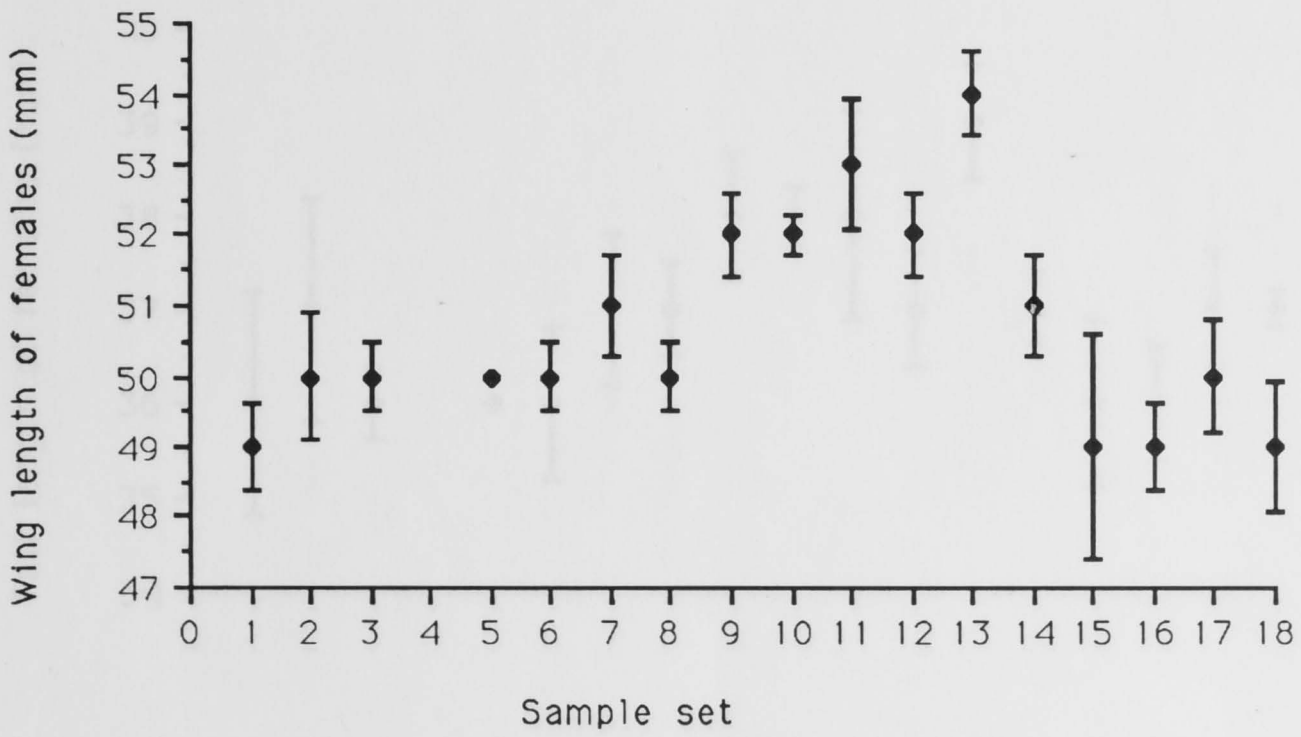


(b)

Figure 3.4 (a). Geographic variation in the hindtoe length in male *A. lineata*. (b). Geographic variation in the hindtoe length in female *A.lineata*. Localities are as given in Table 2.1.

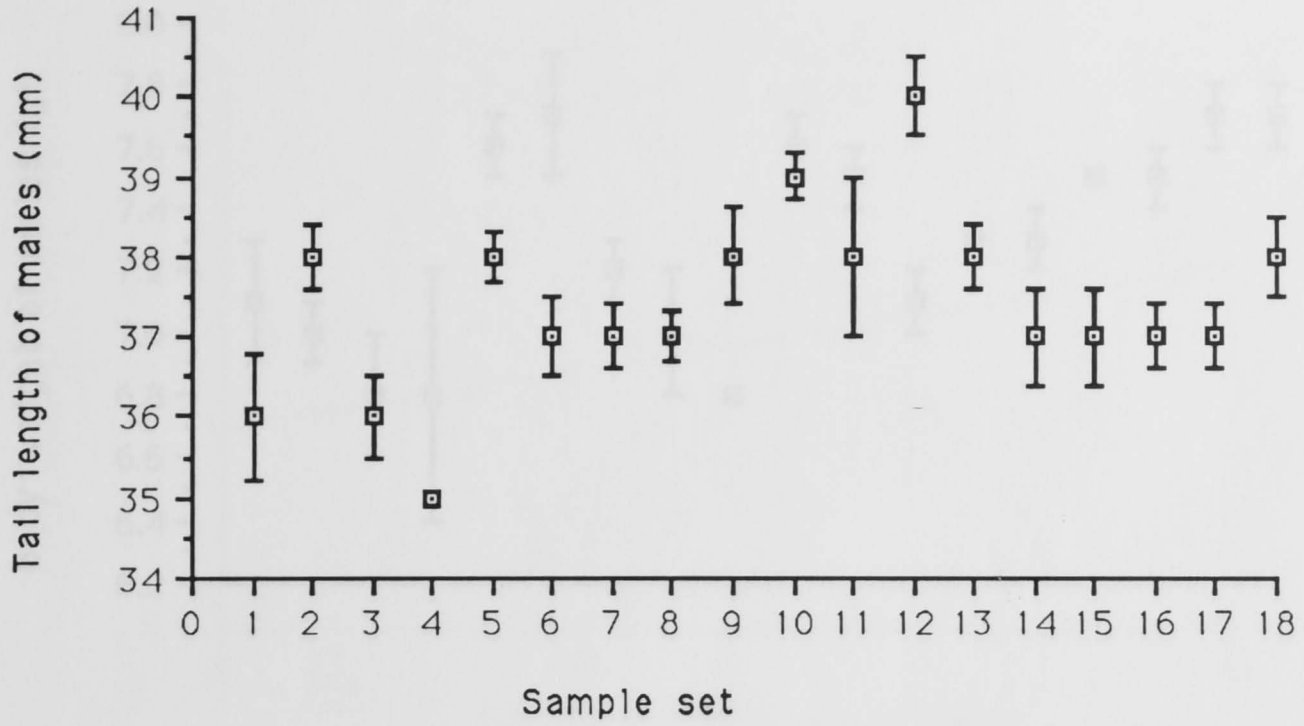


(a)

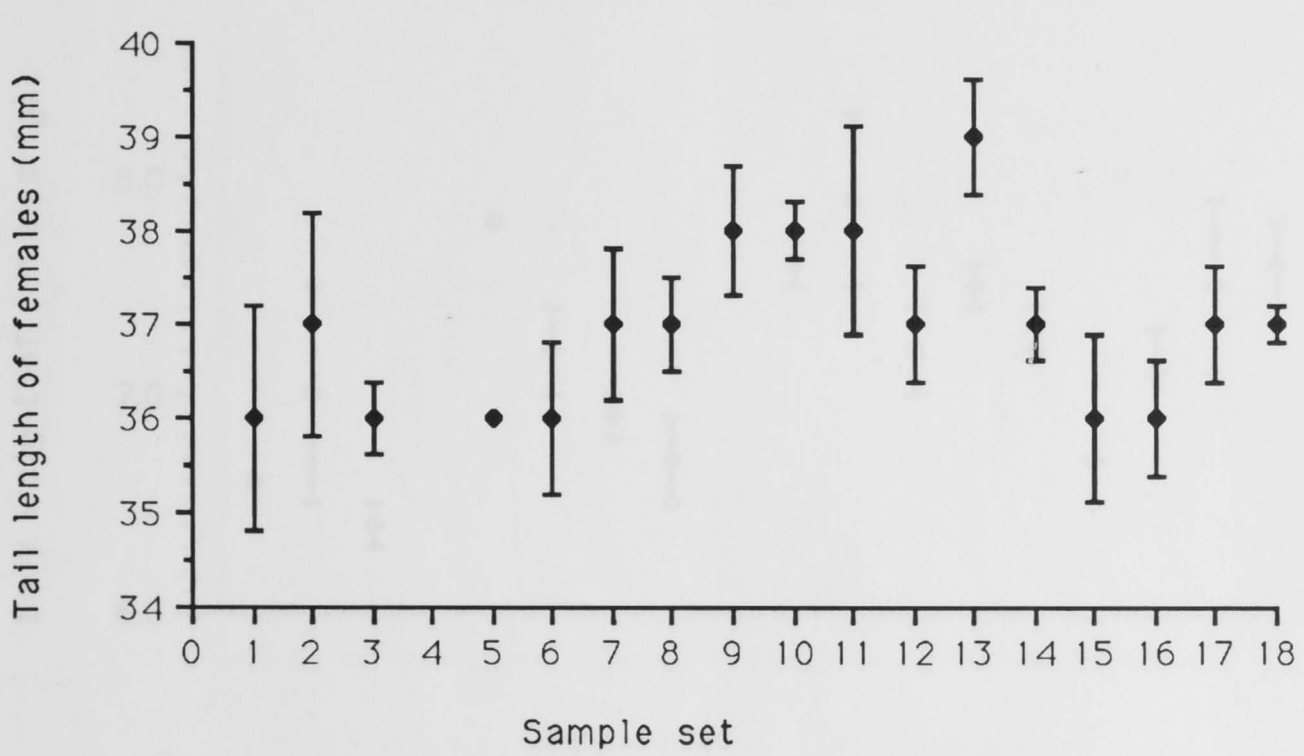


(b)

Figure 3.5 (a). Geographic variation in the wing length in male *A. lineata*. (b). Geographic variation in the wing length in female *A. lineata*. Localities are as given in Table 2.1.

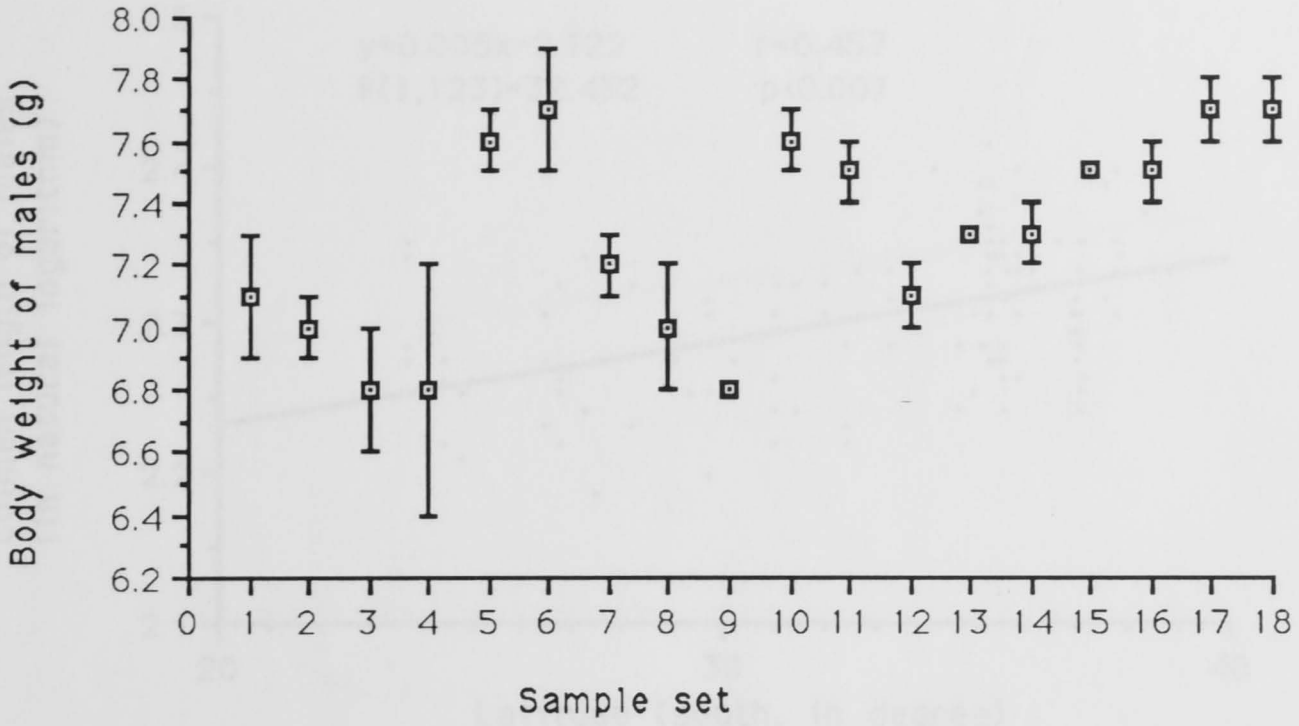


(a)

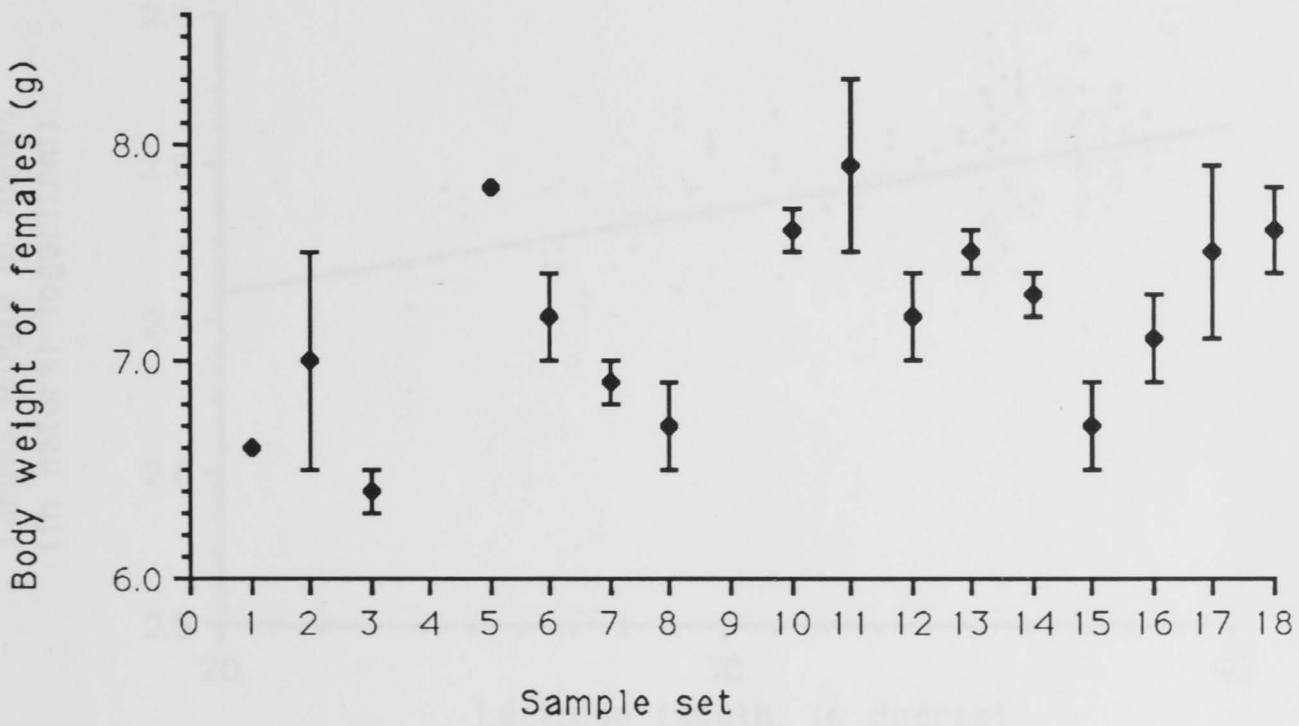


(b)

Figure 3.6 (a). Geographic variation in the tail length in male *A. lineata*. (b). Geographic variation in the tail length in female *A. lineata*. Localities are as given in Table 2.1.

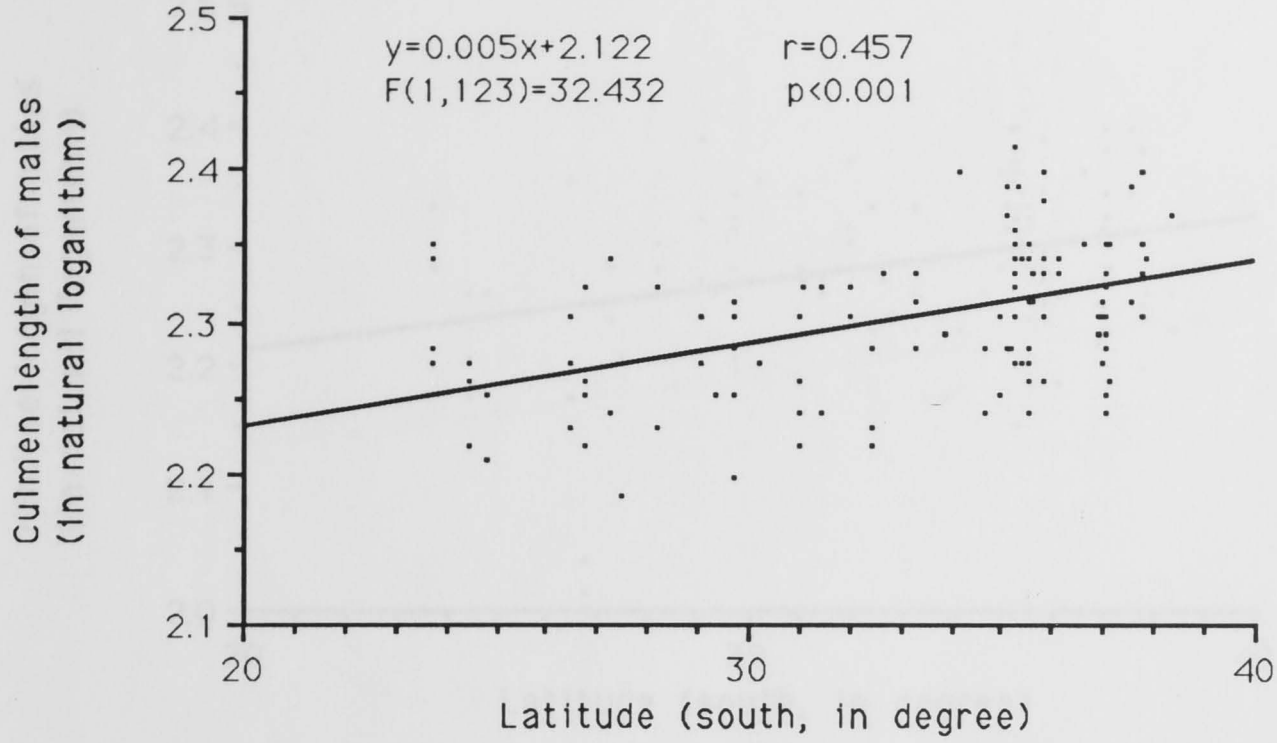


(a)

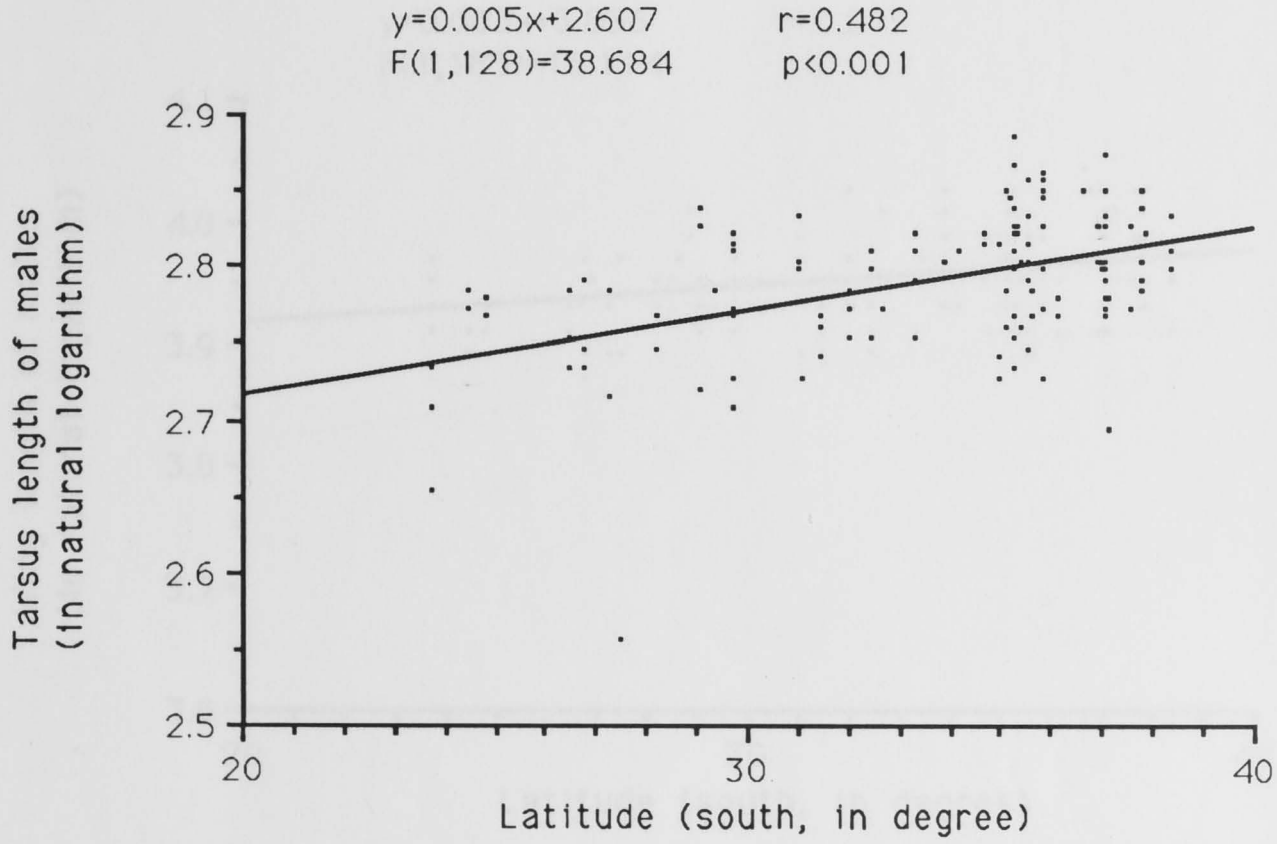


(b)

Figure 3.7 (a). Geographic variation in the body weight in male *A. lineata*. (b). Geographic variation in the body weight. in female *A. lineata*. Localities are as given in Table 2.1.

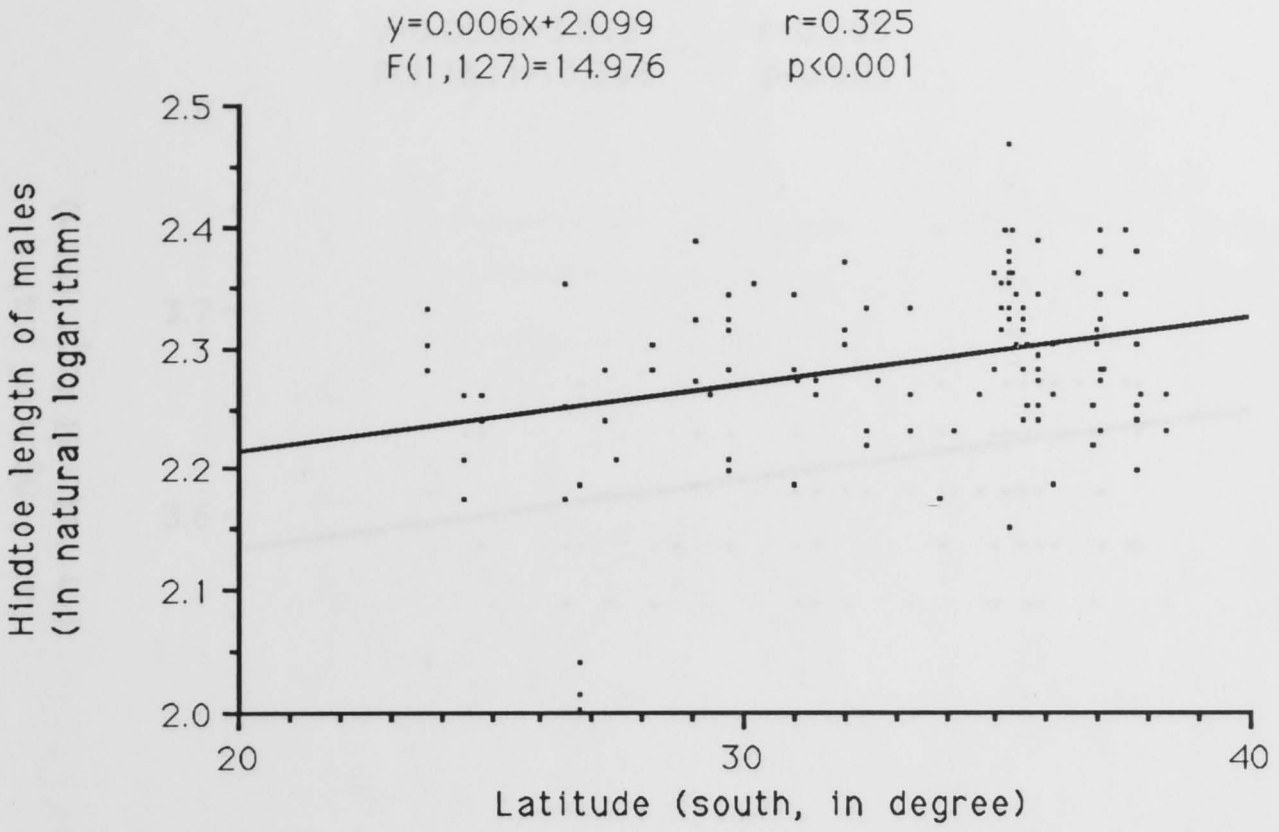


(a)

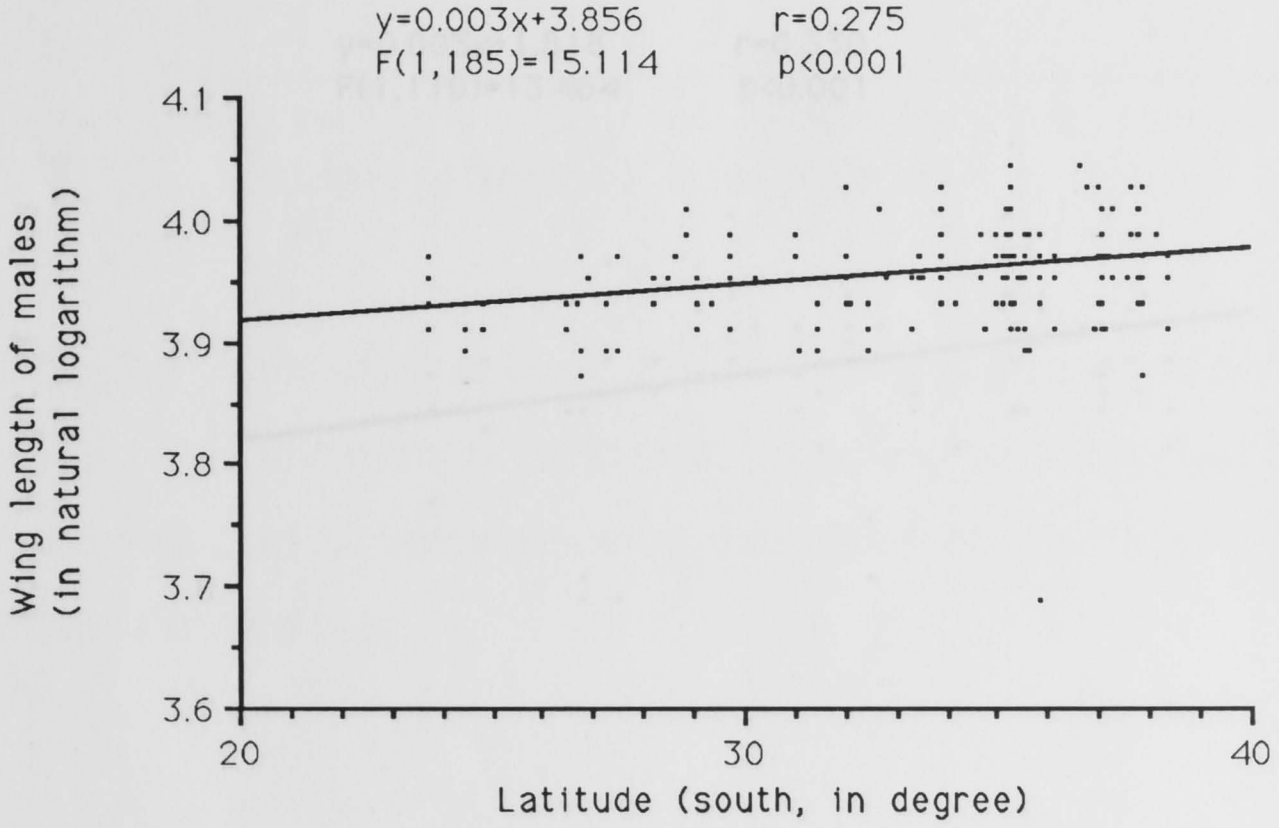


(b)

Figure 3.8 (a). Linear regression between the culmen length of male *A. lineata* and latitude. (b). Linear regression between the tarsus length of male *A. lineata* and latitude.

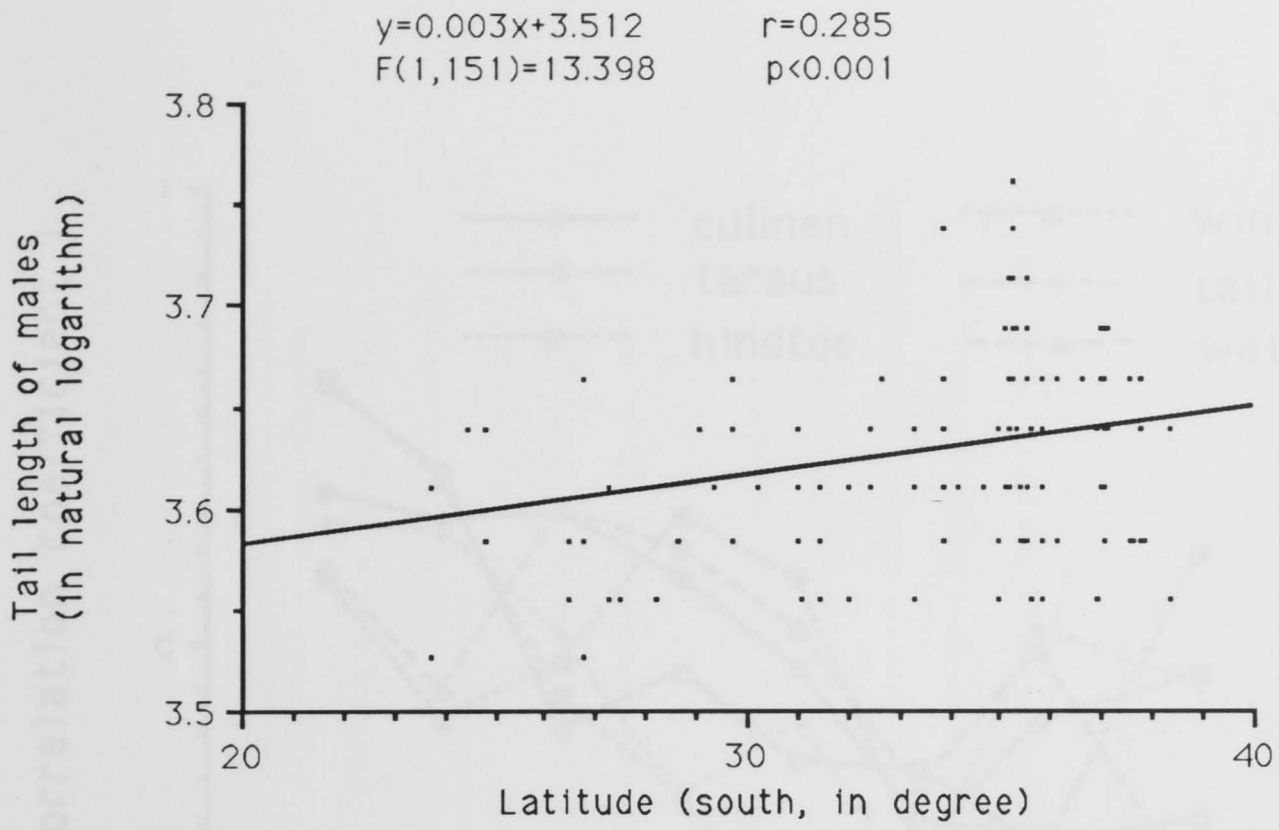


(c)

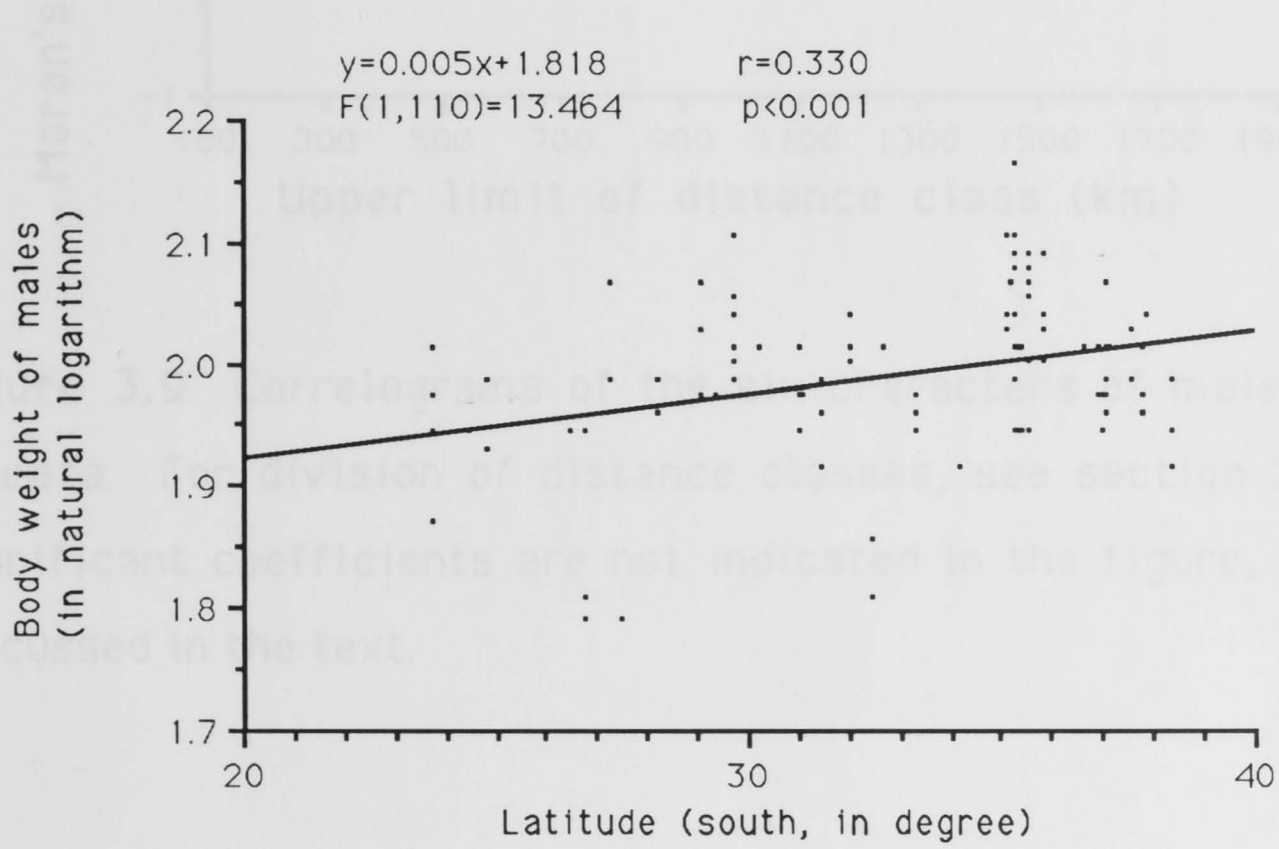


(d)

Figure 3.8 (continued) (c). Linear regression between the hindtoe length of male *A. lineata* and latitude. (d). Linear regression between the wing length of male *A. lineata* and latitude.



(e)



(f)

Figure 3.8 (continued) (e). Linear regression between the tail length of male *A. lineata* and latitude. (f). Linear regression between the body weight of male *A. lineata* and latitude.

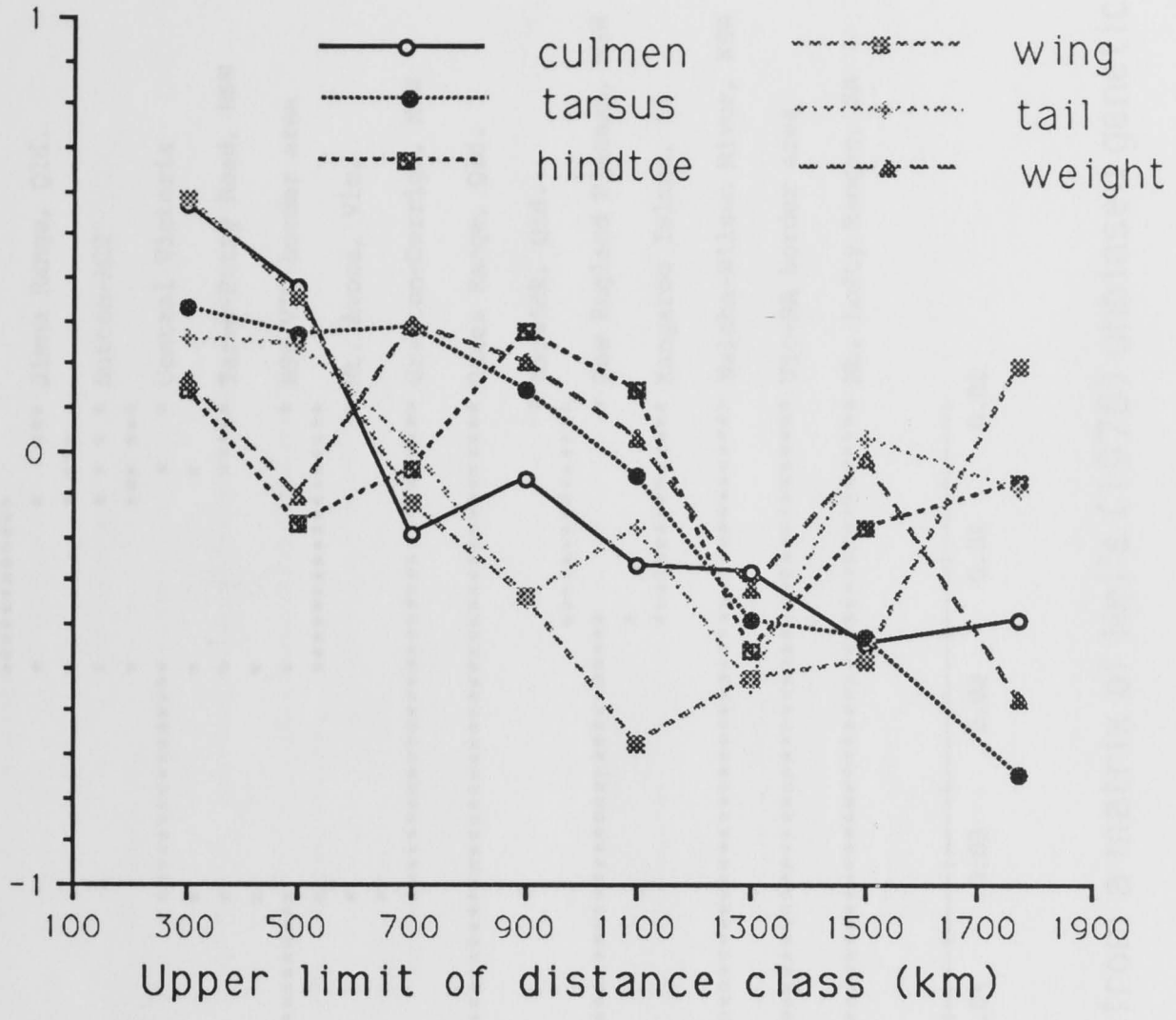


Figure 3.9 Correlograms of the six characters of male *A. lineata*. For division of distance classes, see section 2.5. Significant coefficients are not indicated in the figure, but discussed in the text.

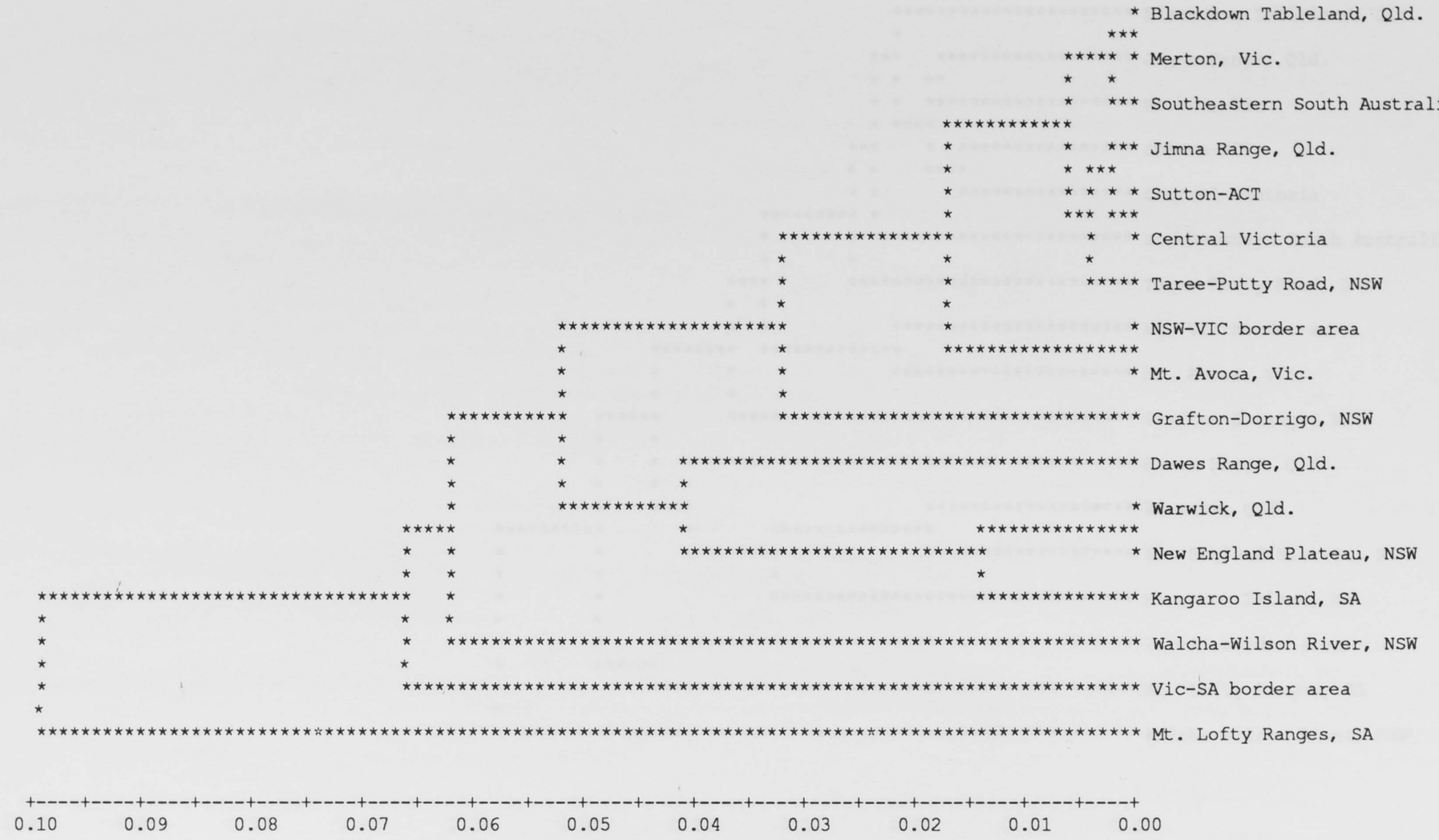


Figure 3.10 Dendrogram reduced by UPGMA from a matrix of Nei's (1978) unbiased genetic distance for *A. lineata*.

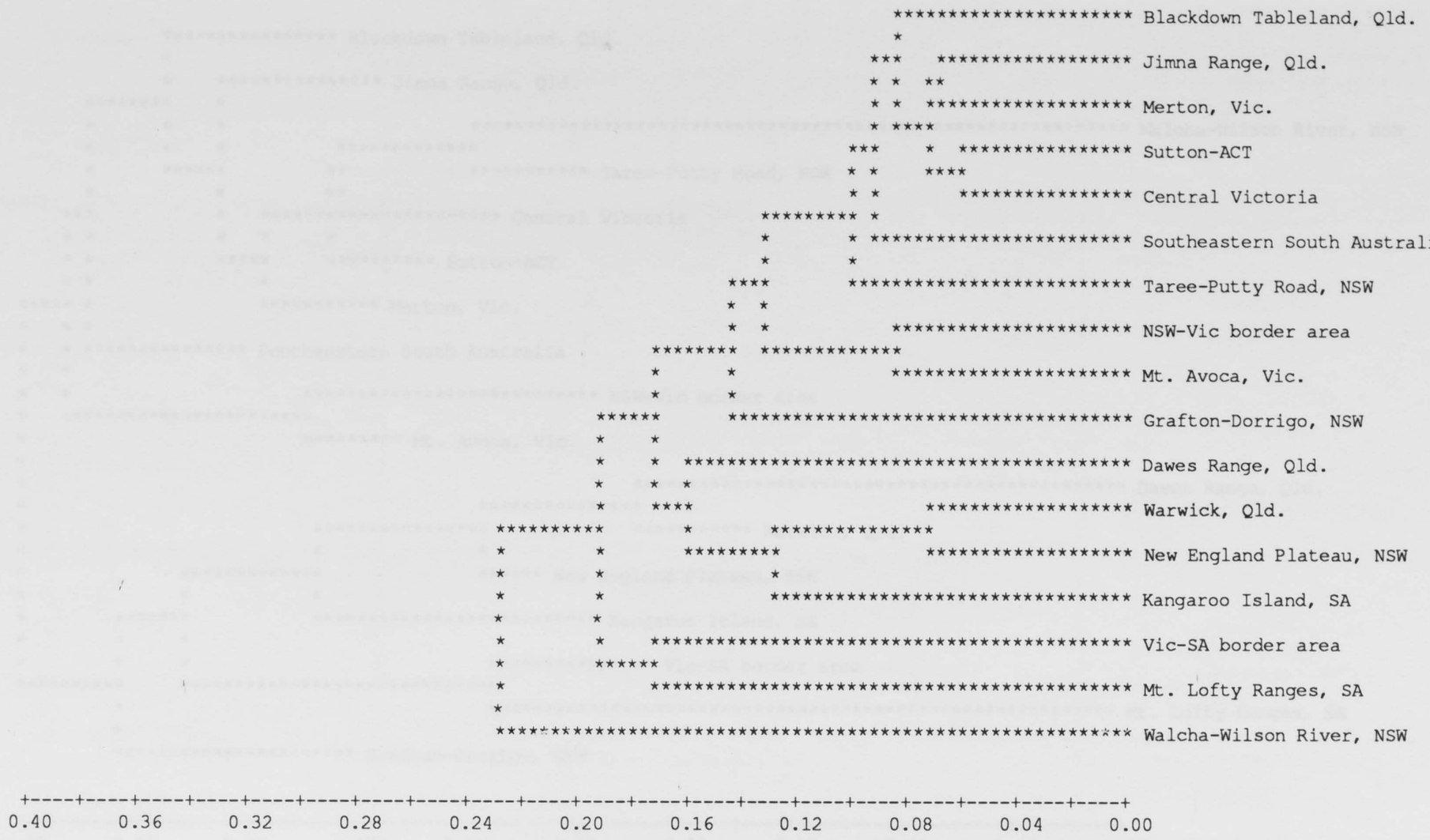


Figure 3.11 Dendrogram reduced by UPGMA from a matrix of Rogers' (1972) genetic distance for *A. lineata*.

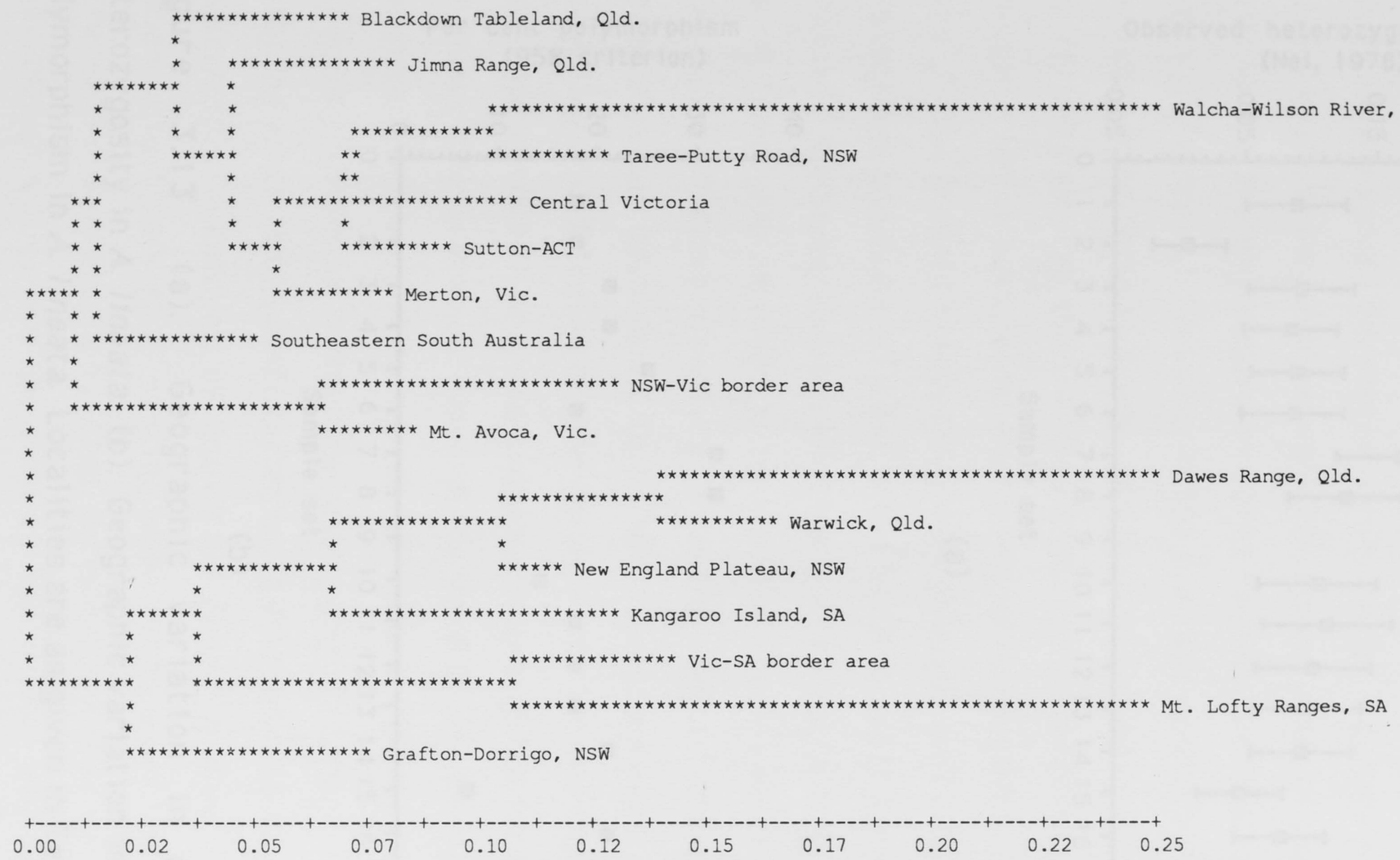
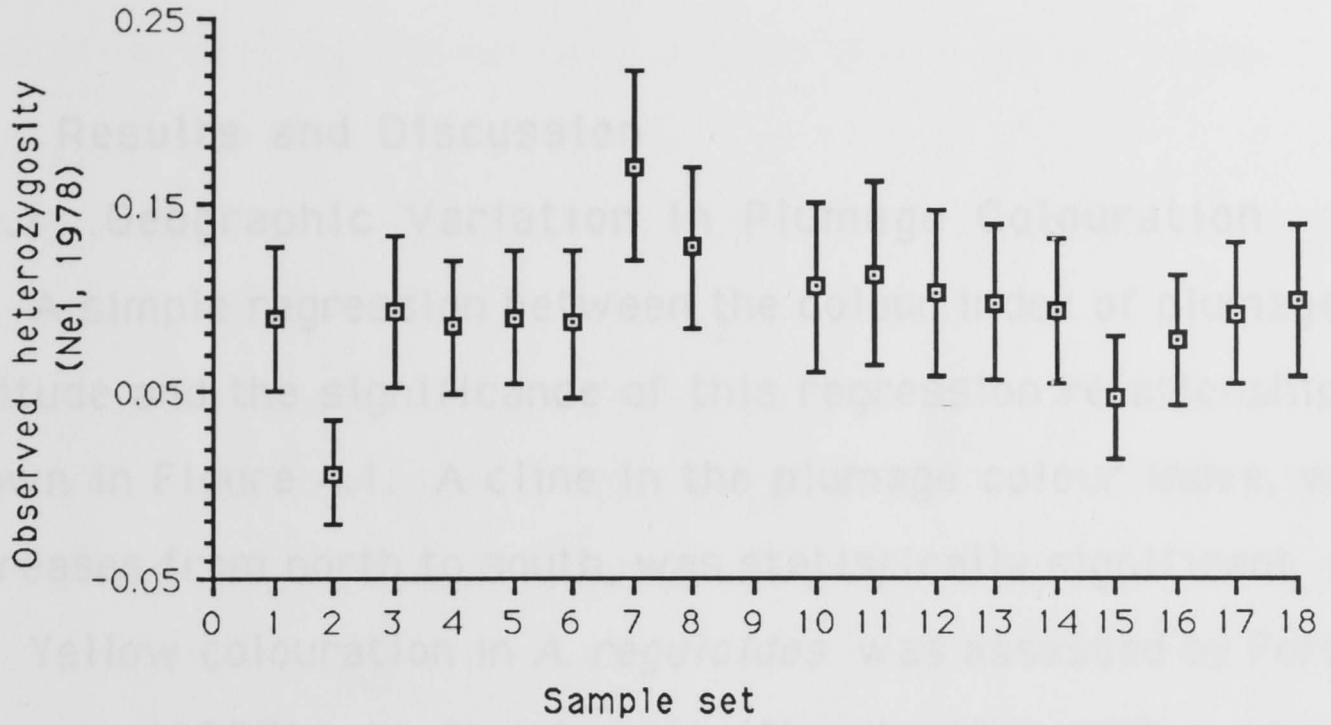
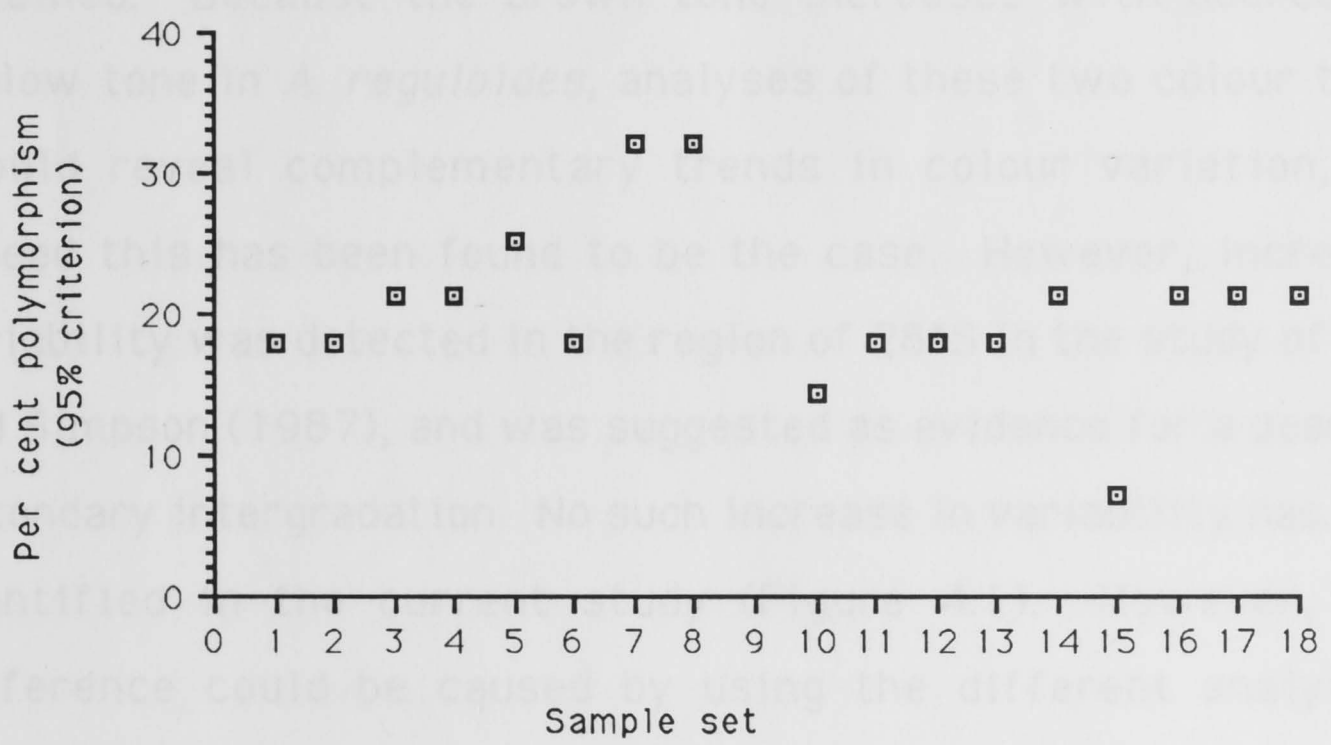


Figure 3.12 Dendrogram reduced by Distance Wagner analysis from a matrix of Rogers' (1972) genetic distance for *A. lineata*. The tree is rooted at the midpoint of the longest path.



(a)



(b)

Figure 3.13 (a). Geographic variation in observed heterozygosity in *A. lineata*. (b). Geographic variation in per cent polymorphism in *A. lineata*. Localities are as given in Table 2.1.

CHAPTER 4 Geographic Variation in the Buff-rumped Thornbill

4.1 Results and Discussion

4.1.1 Geographic Variation in Plumage Colouration

A simple regression between the colour index of plumage and latitude and the significance of this regression relationship are shown in Figure 4.1. A cline in the plumage colour index, which increases from north to south, was statistically significant.

Yellow colouration in *A. reguloides* was assessed by Ford and Simpson (1987) who found a significant cline with a negative slope. In the present study, the brown colouration in *A. reguloides* was scored and a significant cline with a positive slope was obtained. Because the brown tone increases with decreasing yellow tone in *A. reguloides*, analyses of these two colour tones should reveal complementary trends in colour variation, and indeed this has been found to be the case. However, increased variability was detected in the region of 28°S in the study of Ford and Simpson (1987), and was suggested as evidence for a possible secondary intergradation. No such increase in variability has been identified in the current study (Figure 4.1). However, this difference could be caused by using the different analytical methods rather than by measuring the different colour tones. The possible disadvantages of analysing colour scores, as in the present study, are that scoring may vary subjectively among different observers, and that although scores are ranked measurements they are analysed as continuous data. In such a situation, it seems more objective to employ quantitative measurements, such as those obtained by using a reflectance

spectrophotometry (e.g., Selander et al., 1964) and pigmental measurements (e.g., Ford and Simpson, 1987). However, the limitation of time did not allow such an analysis in the present study.

The cline in the plumage colouration revealed in the present study may be the results of several processes. For instance, isolation-by-distance process, selective adaptation to an environmental gradient, as suggested by Gloger's rule, environmental influence during ontogeny or historical events could contribute to a cline.

In summary, colour variation in *A. reguloides* is found to be clinal. This pattern has not corroborated that implied by the division of subspecies (e.g., Mack, 1936; Mayr and Serventy, 1938). It also contradicts the abrupt variability found by Ford and Simpson (1987).

4.1.2 Geographic Variation in Dimensional Characters

4.1.2.1 Difference between Sexes

Figures 4.2-4.7 show the means for each of the six characters (in original form of measurements) in males and females at the 21 localities. Analyses of variance (Table 4.1) showed significant sexual dimorphism in all six characters. The overall difference between sexes shown by multivariate analyses of variance was also significant (Table 4.3). In general, males were larger than females at a given locality, but the trends of geographic variation in both sexes were similar (Figures 4.2-4.7). This is also shown in Table 4.1, in which, the joint effects of locality and sex on difference of means in all six characters were estimated as being nonsignificant (All possibilities were larger

than 0.05, except for the possibility for the hindtoe length, which was less than 0.05 but larger than 0.01).

Whether the body shape is different between the sexes was not analysed in this study.

Summarily, the sexual dimorphism in dimensional measurements for *A. reguloides* is not affected by geographic localities.

4.1.2.2 Difference among Localities

Table 4.1 also shows that the means among the 21 localities were significantly different in all characters except for the tail length. Multivariate analyses of variance (Table 4.3) also showed significant overall heterogeneity among localities.

Table 4.2 lists the correlation coefficients among the six characters. Correlations between all pairs of characters were significant, except for the correlation between the culmen length and the tail length.

Although significant differences in most characters among localities have been shown by both univariate and multivariate analyses of variance, multiple comparisons using either Tukey's pairwise comparisons (based on the studentized ranges) or Duncan's multiple-range tests for each character in males or females showed that only four or five pairs of localities had significantly different character means (The results are not presented because of the huge number of comparisons made). However, failure to reject the hypothesis that two means are equal implies only that the difference between the two locality means, if any, is not large enough to be detected with the given sample size.

Dendrograms of cluster analyses (UPGMA) using character means of either males or females at the 21 localities were not discernible and are not presented.

From the plots of character means against localities (Figures 4.2-4.7), the existence of north-south clines cannot be investigated accurately because some localities in Victoria and South Australia are located north of some localities in New South Wales. Therefore, plots of each log-transformed trait value of an individual male specimen against the latitude of its collection locality are given in Figures 4.8a-f. F-tests for the significance of the linear regressions were carried out and the results are also presented in these figures. Analyses of female specimens produced similar results and therefore are not presented.

Significant north-south clines were found for the tarsus, hindtoe and wing lengths and the body weight. In particular, the hindtoe length varies more markedly.

No evidence for clines was found for the culmen and tail lengths. In particular, the slope of the regression for the tail length was negative. This trait was not correlated with the culmen length and was less correlated with the other traits (Table 4.2).

Figure 4.9 shows the correlograms for the six characters of male *A. reguloides*. Correlograms for females were similar to those of males and are not presented.

The correlogram for the hindtoe length showed a typical clinal pattern, with positive and larger coefficients at low distance classes and negative coefficients at high distance classes. Among the positive coefficients, only those at the first two classes were significant.

The correlogram for the wing length was very similar to that for the hindtoe length. The main difference is that the coefficient in the hindtoe length changed from positive to negative values at distance class 4, while this change occurred at distance class 5 in the correlogram for the wing length.

The correlograms for the culmen length and the body weight were similar. The coefficients at the first distance class were not significant but those at the second distance class were. However, coefficients at both classes were close and positive. The coefficient changed to a negative value at class 4 for the culmen length and at class 5 for the body weight.

Changes of coefficients in the correlograms for the tarsus and tail lengths were not regular. No significant coefficient was found in the correlograms for these two characters.

The analyses of correlograms of the hindtoe and wing lengths, and to some extent, the culmen length and the body weight, suggested that character means at localities were similar within 600 km (the upper limit of distance class 2).

Similar patterns were observed from the regressions and correlograms for the hindtoe, wing and tail lengths and the body weight. For the hindtoe and wing lengths and the body weight, clines were found and for the tail length, no clinal pattern was revealed.

Different patterns were observed from the regressions and correlograms for the culmen and tarsus lengths. The regression line for the culmen length was not significant (Figure 4.8a), but its correlogram showed a clinal pattern, despite this pattern was not as typical as those in the hindtoe and wing lengths (Figure 4.9). In contrast, the regression for the tarsus length was

significant (Figure 4.8b) but no clinal pattern was revealed by its correlogram (Figure 4.9). The discordance between patterns revealed by regressions and correlograms for the culmen and tarsus lengths was probably due to the fact that the clines of traits in this study are generally smooth, and that the regressions mainly express the variation trends with latitude whereas the correlograms mainly show the variation trends as a function of distances between localities.

As discussed in section 3.1.2.2, the causative processes of clines could be the restriction of gene flow, selective or ontogenetic response to environmental gradients or a diffusion of differentiated local populations. Usually, selectively environmental factors are proposed to be responsible.

In summary, the above analyses suggest that the variations in dimensional characters are significant but not abrupt. Instead, the patterns of the north-south clines have been identified for the four characters (the hindtoe, tarsus and wing lengths and the body weight). Along these clines, neighbouring populations within 600 km are proposed to exchange genes more frequently than those which are more than 600 km apart. This distance limit may also reflect the environmental gradient scale.

4.1.3 Geographic Variation in Allozymic Characters

4.1.3.1 Difference in Allele Frequencies

Of the 29 loci assayed, 11 loci were found to be polymorphic at at least one locality. Allele frequencies of these 11 polymorphic loci for all localities are shown in Table 4.4 (Locality 16 was excluded because no allozymic data were collected).

At the NP locus, up to six alleles were found. Of these six alleles, B, C and D were present at almost all localities, but there were no obvious frequency clines across localities. Allele E occurred at most localities, but was absent from most northern localities. Allele A only occurred in the Clarke Range (locality 3). Allele F only occurred at three localities, two in Queensland and one in northern New South Wales.

Four alleles were detected at the αGPD locus. Allele A was found only in the ACT area (locality 15) at a frequency of 0.020. The sample size at this locality (25) is the largest for all localities, and its low frequency indicates that it was very rare. Allele B only occurred at two localities in the New England and Sydney areas. Allele D occurred at only two northern Queensland localities, also at low frequencies.

At the GPI locus, four alleles were found. Allele C was the most common allele and allele D also occurred at most localities. Allele B was found at localities in Queensland and New South Wales, while allele A only occurred in the Mount Lofty Ranges.

Four alleles were found at the PEP-2 locus. The most common allele was C. Allele B was present at many localities but appeared to be more frequent at northern localities than at southern localities. Allele D was absent from northern Queensland (localities north of the Jimna Range) but present at almost all other localities. Allele A was present at only one locality (the Jimna Range).

Five alleles were present at the ME-2 locus. Except for the common allele (C), each of the other four alleles only occurred at low frequencies at one or few localities.

At the PGM locus, the common allele was A. Each of the other three alleles was found only at a different locality at a low frequency.

At the MPI locus, allele A was the common one. Allele B was scattered at low frequencies at several localities.

At the LDH-1 locus, only two alleles were detected. The alternative allele (A) occurred at only two disjunct Queensland localities (localities 4 and 8).

Three alleles were found at the EST-1 locus. Allele C was a null allele. All three alleles appeared at most localities.

At the EST-2 locus, allele D was a null allele. Allele B was found to be common. The other two alleles were found scattered throughout localities.

At the EST-3 locus, the null allele (E) did not appear at localities south of the Warwick area. Allele A only occurred in the Taree-Putty Road area. The common allele (B) appeared at higher frequencies at all localities. The other two alleles (C and D) appeared at only a few localities.

From the above analysis, it appears that at all polymorphic loci, a major allele occurred at a high frequency throughout all localities. Hence, the population of *A. reguloides* is relatively panmictic. Meanwhile, many alternative alleles occurred, but most of them were at low frequencies, and an obvious geographic pattern could not be inferred from the frequencies of these alleles. Finally, the distribution of some alleles (e.g., that of alleles A and D at the PEP-2 locus and allele A at the GPI locus) may provide some information on the unique variation of alleles at given localities. For instance, the presence of the unique alternative allele A, albeit at low frequency, at the GPI locus in

the population of the Mt. Lofty Ranges may indicate that some unique genetic variability exists in this area (see section 3.1.3.1). However, in the absence of concordance of variation at several loci, it could not be concluded that significant variation occurred in this area.

Possible explanations for the lack of stepped or clinal variation in this species are analogous to those for *A. lineata* (see section 3.1.3.1). Although *A. reguloides* is sedentary, no detailed banding study has been carried out to reveal its dispersal ability. Unlike *A. lineata*, *A. reguloides* can inhabit relatively open areas. The lack of eucalypt forest may not inhibit its dispersal. Thus, dispersal may be an important factor leading to relatively panmixia. Explanations for the occurrence of scattered low frequency alternative alleles and the presence of some unique alleles at some localities are similar as those for *A. lineata* (see section 3.1.3.1)

In summary, the population of *A. reguloides* is relatively panmictic. Alternative alleles are at low frequencies and show no discernible geographic pattern. Allele variation at some localities seems to be unique but because of low frequencies of these alleles and lack of concordant variation, conclusion of abrupt variation could not be obtained.

4.1.3.2 The Assumption of a Panmictic Sample Set

Analogous to the discussion for *A. lineata* (see section 3.1.3.2), a possibility that sibling individuals were included in a sample set of *A. reguloides* is not high.

Table 4.5 shows significance levels of χ^2 tests, with which the hypothesis that a given locus at a locality is in Hardy-

Weinberg equilibrium was tested. The tests were carried out after pooling of genotypes into three categories and using Levene's (1949) correction for small sample sizes (see section 2.6).

The results of χ^2 tests for the NP, αGPD, PEP-2 and MPI loci at the localities, where these loci were polymorphic, were not significantly different from the expected. Hence, these four loci were in Hardy-Weinberg equilibrium.

The χ^2 significance levels for the GPI, ME-2, PGM, LDH-1 loci and the three esterase loci were not consistent between localities. The LDH-1 locus was only found to be polymorphic at two localities. Although the frequencies of the alternative allele at the two localities were greater than 0.05, these alleles were rare across the entire range of the species. The PGM locus was only polymorphic at three localities with unique alleles at each. As such, they could be considered as rare alleles. The estimation of the genotype frequencies of the three esterase loci might be distorted because of the inaccuracy of detecting genotypes involving null alleles (see section 2.5). Therefore, the χ^2-tests for these three loci might be more inaccurate.

Hence, it seems that the results from the NP, PEP-2, αGPD and MPI loci are more reliable than those from other loci. Therefore, it is acceptable that each sample set was drawn from a genetically homogeneous population.

The bottom row in Table 4.5 shows the significance levels of χ^2-tests for the whole *A. reguloides* population. The values for the NP, PEP-2, αGPD and MPI loci indicated that the whole population was in Hardy-Weinberg equilibrium. This is an indirect evidence for the panmixia of the whole population.

In summary, each sample set can be considered to be drawn from a genetically homogeneous population of *A. reguloides*. Comparisons in allozymic characters between localities would not be seriously biased.

4.1.3.3 Difference in Genetic Distances

Table 4.6 shows the Nei's (1978) and Rogers' (1972) genetic distances between each pair of the 20 localities.

As was found in *A. lineata*, both kinds of genetic distances between each pair of *A. reguloides* sample sets showed no apparent pattern. Cluster analyses (UPGMA) using either Nei's (1978) or Rogers' (1972) genetic distance, and a Distance Wagner analysis using Rogers' (1972) genetic distance were carried out. The results are shown in Figures 4.10-4.12. None of the dendrograms produced demonstrates any pattern that can be interpreted in a geographic meaningful fashion.

The reasons that dendrograms may distort population relationships were discussed in section 3.1.3.3. The lack of stepped genetic differentiation, the presence of some alternative alleles which were only found at few localities, and less possibly, the relatively small sample sizes of the sample sets, may contribute to the distortion of the dendrograms.

In summary, due to the panmixia of allele variation and irregularly scattered rare alleles, dendrograms have no discernible meaning.

4.1.3.4 Difference in Average Heterozygosity and Polymorphism

Figure 4.13a shows the distribution of average heterozygosity (Nei, 1978) by locality. Figure 4.13b shows the percentage of polymorphic loci at each of the 20 localities (A locus is considered to be polymorphic if the frequency of the most common allele does not exceed 0.95).

The values of heterozygosity and the percentages of polymorphism were the highest at one locality, the Jimna Range (latitude of 27°S).

As discussed for *A. lineata* (section 3.1.3.4), a secondary intergradation is also possible for *A. reguloides*. Apart from the generally accepted hypothetical history of the changes of climate, environment, flora and fauna in Australia (see section 3.1.1), two additional discoveries support the hypothesis of the secondary intergradation.

Firstly, by analysing integumentary pigments in feathers of *A. reguloides*, Ford and Simpson (1987) found a latitudinal cline, apparently with the increased variability in the region of 28°S (the McPherson Range). They concluded that this may indicate the possibility of a secondary intergradation. The possible secondary intergradation zone revealed in the present study is to the north of that speculated by Ford and Simpson. But the two speculated zones are very close. Also, it is possible that clines in independent suites of characters are discordant across zones of primary and secondary intergradation, because gene flow varies at different loci.

Secondly, at least four hybrid zones in other pairs of avian taxa have been identified near the McPherson Range (Ford, 1980,

1985), namely, those between Varied Sittellas (*Daphoenositta chrysoptera leucocephala* and *D. c. chrysoptera*), Striated pardalotes (*Pardalotus striatus melanocephalus* and *P. s. ornatus*), the Pale-headed Rosella (*Platycercus adscitus*) and the Eastern Rosella (*P. eximius*), and White-browed Scrub-wrens (*Sericornis frontalis frontalis* and *S. f. laevigaster*). In particular, both *Sericornis frontalis* and *A. reguloides* are foraging in understorey of forests. With the similar ecological behaviour, the concordance of their possible intergradation zones is reasonable evidence for the existence of the zones *per se*. Since it is more likely that both species were contracted to the same refuges, and their rejoining areas should be closer due to their closer dispersal rates and the following of the expanding of suitable environments.

On the other hand, the high heterozygosity in the zone near the McPherson Range coincides with the possible high biological diversity in the area. Burbidge (1960) defined the area as the McPherson-Macleay Overlap, where the tropical and temperate elements of vegetation overlap. However, allozymes are generally considered to be independent of environmental selection. Hence, it seems to be unlikely that the high heterozygosity reflects a primary intergradation rising from selective adaptation to the diverse environmental factors in the overlap area.

To sum up, the high values of average heterozygosity and percentage polymorphism near the McPherson Range could indicate a secondary intergradation for *A. reguloides*. This hypothesis is consistent with the great variability in plumage

colouration revealed by Ford and Simpson (1987) and intergradation zones of some Australian birds.

4.2 General Discussion and Conclusions

A steep cline in plumage colouration and gradual clines in 4 of 6 dimensional characters have been identified. On the other hand, the population of *A. reguloides* has been found to be relatively panmictic in allozymic characters. The discordance of the patterns of these three sets of characters implies that they are subject to different evolutionary processes or under different degrees of selective pressure. Accumulated data in avian allozymic studies demonstrate that genes coding for allozymic loci are usually neutral (Barrowclough et al., 1985). Morphometric characters are usually considered to be affected by selection, as expressed in the three ecogeographic rules (see section 1.2.2) or environmental influence during ontogeny.

Given the fact that the pattern of geographic variation in the pigmental character revealed by Ford and Simpson (1987) is concordant with the pattern of geographic variation in the average heterozygosity revealed in the present study, the hypothesis of past (and perhaps still present) secondary intergradation is favoured. Accordingly, it is possible that the diffusion of the subpopulations after the secondary contact also contributes to the clines in morphological characters. The actual situation is probably that both historical and ecological factors determined the present patterns of geographic variation in *A. reguloides*. In the absence of abrupt variation in both allele frequencies and morphological characters, the ecological factors

seem to contribute more to shape the present patterns of geographic variation.

In summary, the conclusions made from the analyses on patterns of geographic variation in *A. reguloides* and the hypothesis of evolutionary mechanisms are as follows.

1). Basically, the plumage colouration, as scored in the present study, shows a steep cline. This is in contrast to the result found by Ford and Simpson (1987), i.e., a generally clinal trend with a high level of variation in the McPherson Range. However, the discrete pattern of plumage colouration suggested by division of subspecies has not been confirmed by this study.

2). Of the six morphological characters studied, four (the hindtoe, wing and tarsus lengths and the body weight) also show clinal patterns, but these clines are much smoother than that found for plumage colouration. The distribution of allele frequencies reveals a geographical panmixia across the whole population.

3). Geographic variation in the average heterozygosity, consistent with the geographic variation in the pigmental character revealed by Ford and Simpson (1987), suggests a secondary intergradation, probably in the McPherson Range. However, the species' dispersal and large effective population size probably have overcome the zone of secondary intergradation and led to the lack of abrupt changes in the dimensional and allozymic characters.

4). The differences in geographic patterns between plumage, dimensional and allozymic characters may confirm the view that morphological (plumage and dimensional) characters are more

selectively or environmentally dependant and allozymic characters are usually neutral to selection.

Table 4.1 Results of analyses of variance. Shown in the table are F-tests of the differences in dimensional characters among localities, between sexes and among the interaction of localities and sexes for *A. reguloides*. "*" indicates a probability being less than 0.05. "**" indicates a probability being less than 0.01.

Character		Locality	Sex	locality x Sex
Culmen	df	20	1	19
	F-value	2.96	16.52	1.26
	P	0.0001**	0.0001**	0.2228
Tarsus	df	20	1	19
	F-value	3.45	9.61	1.04
	P	0.0001**	0.0023**	0.4210
Hindtoe	df	20	1	19
	F-value	4.98	44.31	1.73
	P	0.0001**	0.0001**	0.0382*
Wing	df	20	1	20
	F-value	2.43	103.66	0.93
	P	0.0008**	0.0001**	0.5495
Tail	df	20	1	20
	F-value	1.45	62.28	0.96
	P	0.1031	0.0001**	0.5178
Weight	df	20	1	19
	F-value	5.04	30.36	0.74
	P	0.0001**	0.0001**	0.7679

Table 4.2 Pearson correlation coefficients, significance levels of correlation and the number of observations (in order) of the dimensional characters for *A reguloides*. The calculations were based on the natural logarithm values of the original measurements.

	Culmen	Tarsus	Hindtoe	Wing	Tail	Weight
Culmen	1.0000 0.0000 177					
Tarsus	0.3452 0.0001 176	1.0000 0.0000 181				
Hindtoe	0.3506 0.0001 177	0.3910 0.0001 180	1.0000 0.0000 181			
wing	0.3022 0.0001 177	0.4607 0.0001 181	0.4255 0.0001 181	1.0000 0.0000 283		
Tail	0.1302 0.0907 170	0.2898 0.0001 174	0.2451 0.0011 174	0.6339 0.0001 227	1.0000 0.0000 227	
Weight	0.3182 0.0001 168	0.4079 0.0001 172	0.5172 0.0001 172	0.4727 0.0001 173	0.2392 0.0019 167	1.0000 0.0000 173

Table 4.3 Multivariate analyses of variance of six dimensional characters for *A. reguloides*. "**" indicates a probability being less than 0.01. The calculations were based on the logarithm values of the original measurements.

Hypothesis of no overall locality effect				
Statistics	value	F	Df	PR>F
Wilks' l	0.1729	2.312	120	0.0001
Pillai's trace	1.3974	2.110	120	0.0001
Hotelling-Lawley trace	2.3254	2.564	120	0.0001
Roy's greatest root	1.2930	8.986	20	0.0001
Hypothesis of no overall sex effect				
Statistics	value	F	Df	PR>F
Wilks' l	0.5272	20.029	6	0.0001
Pillai's trace	0.4728	20.029	6	0.0001
Hotelling-Lawley trace	0.8968	20.029	6	0.0001
Roy's greatest root	0.8968	20.029	6	0.0001

Table 4.4 Allele frequencies of 11 polymorphic loci at 20 localities (locality 16 is absent) and sample sizes (N) for *A. reguloides*. For locality details see Table 2.2. For locus abbreviations, see Table 2.7.

	LOCALITY																			
LOCUS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21
NP (N)	3	6	11	5	8	11	6	8	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.167	0.500	0.273	0.300	0.438	0.182	0.417	0.188	0.000	0.100	0.000	0.250	0.542	0.750	0.280	0.400	0.385	0.250	0.333	0.700
C	0.500	0.083	0.227	0.300	0.438	0.409	0.083	0.375	0.500	0.350	0.250	0.400	0.083	0.000	0.260	0.300	0.346	0.750	0.250	0.100
D	0.333	0.333	0.364	0.300	0.125	0.273	0.500	0.125	0.375	0.350	0.375	0.250	0.250	0.000	0.360	0.200	0.192	0.000	0.333	0.000
E	0.000	0.000	0.045	0.000	0.000	0.136	0.000	0.313	0.125	0.150	0.375	0.100	0.125	0.250	0.100	0.100	0.077	0.000	0.083	0.200
F	0.000	0.083	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
αGPD																				
(N)	3	6	12	5	8	11	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	0.917	0.958	1.000	1.000	1.000	1.000	1.000	1.000	0.850	1.000	1.000	1.000	0.750	0.980	1.000	1.000	1.000	1.000	1.000
D	0.000	0.083	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI																				
(N)	3	6	12	5	8	11	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
B	0.000	0.167	0.000	0.000	0.063	0.045	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	0.833	0.917	1.000	0.688	0.773	1.000	0.722	1.000	0.400	0.750	0.800	0.833	0.750	0.960	1.000	0.923	1.000	1.000	0.900
D	0.000	0.000	0.083	0.000	0.250	0.182	0.000	0.222	0.000	0.600	0.250	0.200	0.167	0.000	0.040	0.000	0.077	0.000	0.000	0.000

Table 4.4 (continued)

PEP-2

(N)	3	6	12	5	8	11	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.167	0.083	0.292	0.500	0.250	0.091	0.071	0.000	0.125	0.000	0.000	0.100	0.000	0.000	0.040	0.050	0.077	0.000	0.000	0.100
C	0.833	0.917	0.708	0.500	0.750	0.909	0.929	0.833	0.750	0.700	0.875	0.850	0.875	0.750	0.900	0.950	0.769	1.000	0.917	0.800
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.125	0.300	0.125	0.050	0.125	0.250	0.060	0.000	0.154	0.000	0.083	0.100

ME-2

(N)	3	6	12	5	8	11	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000	0.250	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000
C	1.000	0.833	0.917	1.000	1.000	0.909	1.000	0.889	1.000	1.000	0.750	1.000	1.000	1.000	0.920	1.000	0.923	1.000	0.833	1.000
D	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.077	0.000	0.000	0.000

PGM

(N)	3	6	12	5	8	11	7	8	4	10	4	10	12	2	25	10	13	2	6	5
A	1.000	1.000	1.000	1.000	1.000	0.955	1.000	0.938	1.000	1.000	1.000	1.000	1.000	1.000	0.960	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MPI

(N)	3	6	12	6	8	11	7	9	4	10	3	10	12	2	25	10	13	2	6	5
A	1.000	1.000	1.000	0.917	0.938	0.955	1.000	0.944	1.000	1.000	1.000	0.900	0.958	1.000	0.940	0.900	0.962	1.000	0.833	0.900
B	0.000	0.000	0.000	0.083	0.063	0.045	0.000	0.056	0.000	0.000	0.000	0.100	0.042	0.000	0.060	0.100	0.038	0.000	0.167	0.100

LDH-1

(N)	3	6	12	6	8	11	7	9	4	10	3	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	0.917	1.000	1.000	1.000	0.889	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 4.4 (continued)

EST-1																				
(N)	3	6	12	5	8	10	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.208	0.800	0.563	0.500	0.786	0.333	0.750	0.550	0.750	0.600	0.333	0.500	0.560	0.700	0.538	0.500	0.250	0.300
B	0.333	0.333	0.208	0.000	0.188	0.300	0.071	0.667	0.250	0.450	0.000	0.200	0.417	0.500	0.360	0.100	0.385	0.000	0.583	0.500
C	0.667	0.667	0.583	0.200	0.250	0.200	0.143	0.000	0.000	0.000	0.250	0.200	0.250	0.000	0.080	0.200	0.077	0.500	0.167	0.200

EST-2																				
(N)	3	6	12	5	8	11	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.250	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.040	0.000	0.000	0.000	0.167	0.300
B	1.000	0.750	0.917	0.800	1.000	0.818	0.786	0.722	0.375	0.800	1.000	0.900	0.750	1.000	0.720	1.000	0.846	1.000	0.583	0.700
C	0.000	0.000	0.042	0.200	0.000	0.000	0.214	0.167	0.125	0.200	0.000	0.000	0.000	0.000	0.160	0.000	0.077	0.000	0.083	0.000
D	0.000	0.000	0.000	0.000	0.000	0.182	0.000	0.111	0.500	0.000	0.000	0.100	0.000	0.000	0.080	0.000	0.077	0.000	0.167	0.000

EST-3																				
(N)	3	6	12	5	8	10	7	9	4	10	4	10	12	2	25	10	13	2	6	5
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	0.792	0.600	1.000	0.750	0.500	0.722	0.750	0.800	0.500	0.950	0.917	1.000	0.920	0.800	0.885	1.000	0.833	0.700
C	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.200	0.125	0.000	0.042	0.000	0.060	0.150	0.115	0.000	0.000	0.300
D	0.000	0.000	0.125	0.400	0.000	0.050	0.357	0.167	0.000	0.000	0.375	0.050	0.000	0.000	0.020	0.050	0.000	0.000	0.167	0.000
E	0.000	0.000	0.083	0.000	0.000	0.100	0.143	0.111	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.5 P values of χ^2 significance tests for deviation from Hardy-Weinberg equilibrium for *A. reguloides* using pooled genotypes with Levene's (1949) correction for small sample sizes. "-" indicates that no test was carried out because the locus is monomorphic at the locality. For localities see Table 2.2. For locus abbreviations, see Table 2.7. The last row shows the test results for the whole population.

Locality	NP	αGPD	GPI	PEP-2	ME-2	PGM	MPI	LDH-1	EST-1	EST-2	EST-3
1	0.346	-	-	1.000	-	-	-	-	0.021	-	-
2	0.297	1.000	0.739	1.000	1.000	-	-	-	0.006	0.085	-
3	0.380	1.000	<0.001	0.121	<0.001	-	-	-	<0.001	0.827	0.004
4	0.131	-	-	0.841	-	-	1.000	1.000	0.002	0.705	0.011
5	0.564	-	0.024	0.425	-	-	1.000	-	0.021	-	-
6	0.371	-	0.006	0.819	<0.001	1.000	1.000	-	0.039	<0.001	0.010
7	0.297	-	-	1.000	-	-	-	-	0.057	0.567	0.036
8	1.000	-	0.015	0.633	<0.001	1.000	1.000	<0.001	0.001	0.015	0.015
9	0.083	-	-	0.655	-	-	-	-	0.007	0.021	0.007
10	0.120	0.656	0.001	0.223	-	-	-	-	0.007	<0.001	<0.001
11	0.343	-	0.007	1.000	0.007	-	-	-	0.007	-	0.773
12	0.530	-	<0.001	0.656	-	-	0.808	-	0.043	<0.001	1.000
13	0.061	-	<0.001	0.692	-	-	1.000	-	0.015	<0.001	0.827
14	1.000	1.000	1.000	1.000	-	-	-	-	0.046	-	-
15	0.915	1.000	<0.001	0.622	<0.001	<0.001	0.796	-	<0.001	0.002	0.709
17	0.530	-	-	1.000	-	-	0.808	-	0.062	-	0.502

Table 4.5 (continued)

18	0.346	-	<0.001	0.516	<0.001	-	1.000	-	0.129	<0.001	0.709
19	1.000	-	-	-	-	-	-	-	0.046	-	-
20	0.388	-	-	1.000	0.001	-	0.739	-	0.064	0.064	0.001
21	0.131	-	1.000	0.705	-	-	1.000	-	0.110	0.450	0.131
Σ	0.282	0.796	<0.001	0.761	<0.001	<0.001	0.525	<0.001	<0.001	<0.001	<0.001

Table 4.6 Genetic distances (below diagonal: Rogers' (1972) genetic distance; above diagonal: Nei's (1978) unbiased genetic distance) among *A. reguloides* populations at 20 localities. For locality code number, see Table 2.2.

Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21
1	*****	0.001	0.000	0.021	0.007	0.006	0.024	0.015	0.024	0.027	0.021	0.007	0.009	0.017	0.011	0.012	0.009	0.001	0.007	0.015
2	0.039	*****	0.003	0.029	0.013	0.012	0.024	0.019	0.032	0.032	0.026	0.014	0.003	0.009	0.013	0.017	0.013	0.014	0.006	0.007
3	0.034	0.045	*****	0.009	0.006	0.005	0.012	0.014	0.020	0.023	0.012	0.006	0.007	0.012	0.009	0.009	0.008	0.007	0.007	0.010
4	0.068	0.087	0.056	*****	0.008	0.010	0.002	0.020	0.011	0.026	0.007	0.007	0.019	0.018	0.011	0.007	0.009	0.015	0.017	0.018
5	0.041	0.060	0.045	0.057	*****	0.001	0.014	0.010	0.017	0.011	0.012	0.000	0.005	0.002	0.006	0.003	0.001	0.002	0.011	0.009
6	0.050	0.060	0.046	0.063	0.038	*****	0.008	0.001	0.003	0.008	0.003	0.000	0.004	0.006	0.001	0.002	0.000	0.004	0.001	0.008
7	0.062	0.067	0.054	0.036	0.060	0.052	*****	0.017	0.012	0.025	0.005	0.009	0.013	0.014	0.007	0.005	0.009	0.018	0.011	0.016
8	0.072	0.074	0.064	0.072	0.059	0.041	0.069	*****	0.009	0.007	0.011	0.005	0.006	0.004	0.004	0.013	0.003	0.017	0.001	0.007
9	0.062	0.086	0.065	0.063	0.065	0.050	0.053	0.066	*****	0.019	0.015	0.007	0.018	0.022	0.005	0.013	0.007	0.016	0.008	0.022
10	0.075	0.086	0.070	0.079	0.062	0.054	0.071	0.060	0.067	*****	0.012	0.009	0.015	0.011	0.013	0.020	0.010	0.027	0.018	0.022
11	0.072	0.080	0.063	0.063	0.058	0.051	0.051	0.061	0.071	0.071	*****	0.005	0.017	0.018	0.010	0.006	0.010	0.013	0.017	0.022

Table 4.6 (continued)

12	0.041	0.059	0.044	0.053	0.022	0.024	0.049	0.050	0.051	0.055	0.051	*****	0.004	0.003	0.001	0.000	0.000	0.001	0.005	0.009
13	0.051	0.040	0.047	0.070	0.042	0.040	0.054	0.051	0.065	0.061	0.065	0.035	*****	0.000	0.003	0.007	0.002	0.013	0.000	0.000
14	0.067	0.065	0.071	0.090	0.052	0.068	0.077	0.071	0.083	0.059	0.082	0.057	0.049	*****	0.003	0.004	0.000	0.014	0.005	0.000
15	0.049	0.054	0.045	0.055	0.045	0.032	0.042	0.045	0.048	0.053	0.062	0.030	0.035	0.059	*****	0.003	0.000	0.009	0.000	0.007
17	0.043	0.062	0.047	0.045	0.033	0.038	0.035	0.063	0.054	0.066	0.051	0.027	0.043	0.060	0.034	*****	0.001	0.002	0.008	0.007
18	0.047	0.058	0.042	0.055	0.035	0.031	0.050	0.046	0.049	0.049	0.060	0.028	0.033	0.049	0.021	0.030	*****	0.006	0.001	0.003
19	0.031	0.060	0.051	0.067	0.038	0.052	0.057	0.075	0.064	0.080	0.065	0.040	0.054	0.066	0.050	0.033	0.048	*****	0.013	0.017
20	0.057	0.057	0.053	0.067	0.064	0.046	0.057	0.050	0.060	0.075	0.075	0.048	0.040	0.075	0.034	0.047	0.043	0.064	*****	0.001
21	0.064	0.058	0.060	0.072	0.057	0.054	0.064	0.061	0.070	0.072	0.081	0.051	0.030	0.055	0.050	0.047	0.043	0.068	0.046	*****

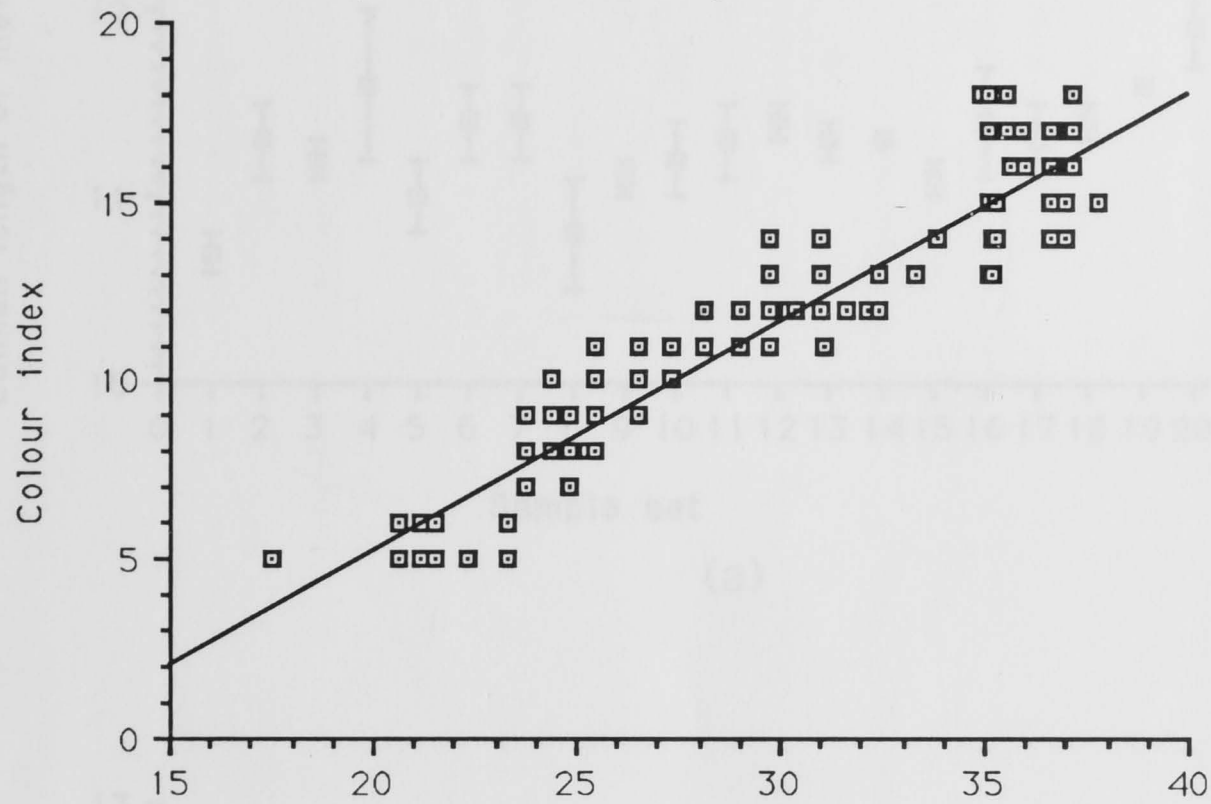
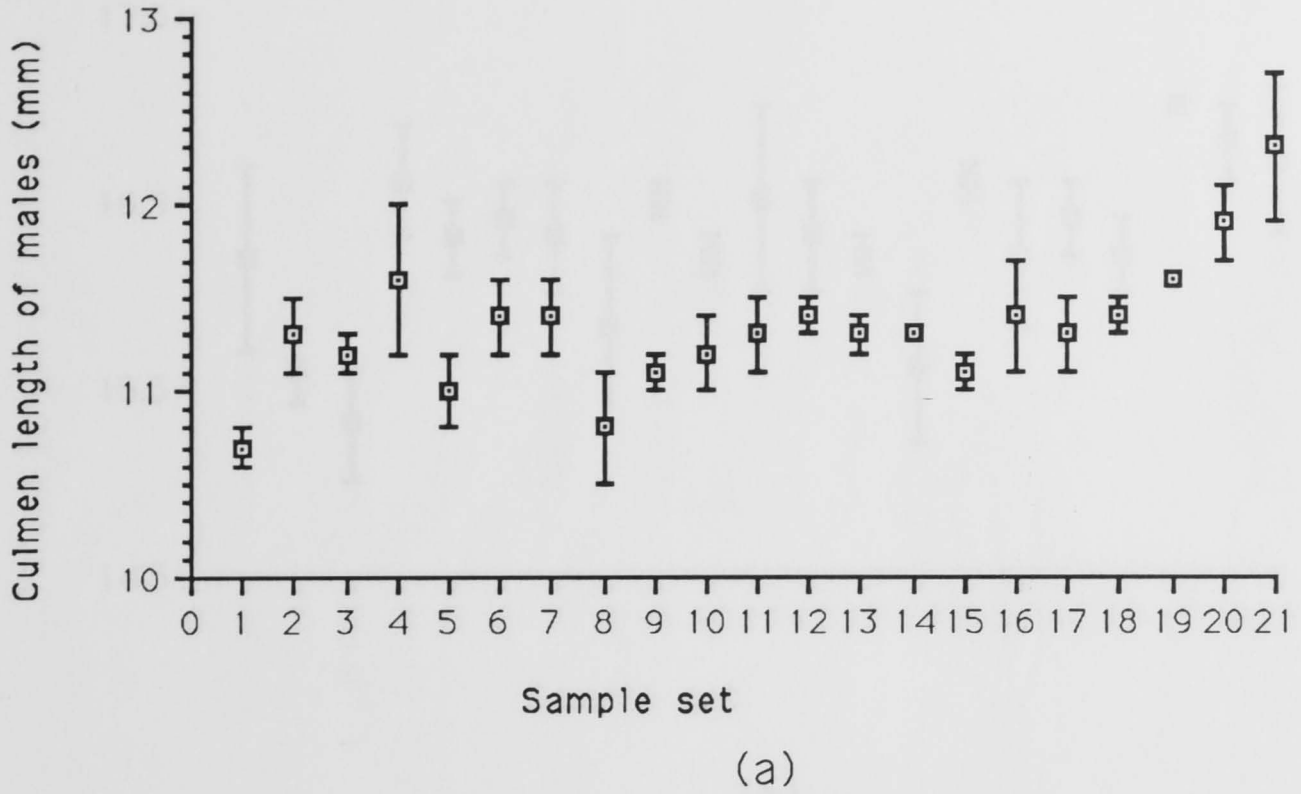
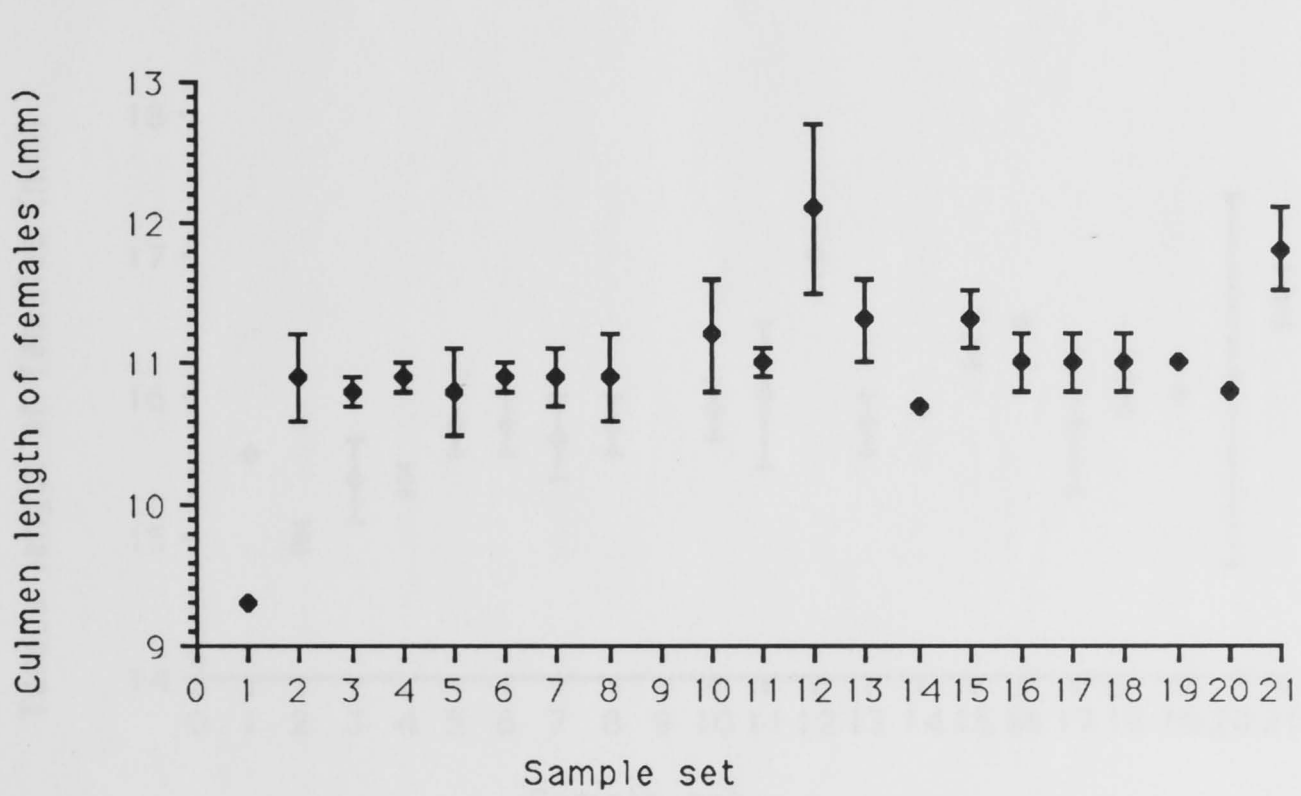


Figure 4.1 Linear regression between the colour index of *A. reguloides* and latitude. Many individuals have the same values of the colour index, so that they are hidden on the plot.

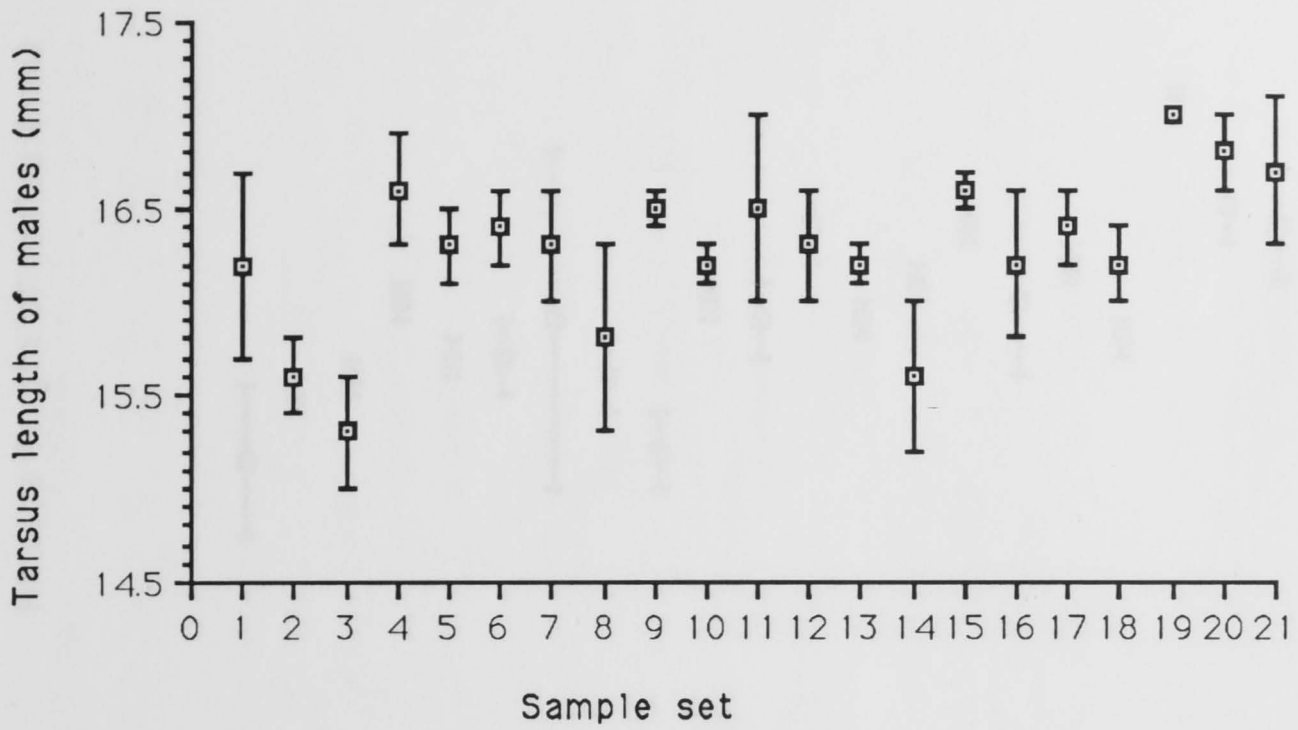


(a)

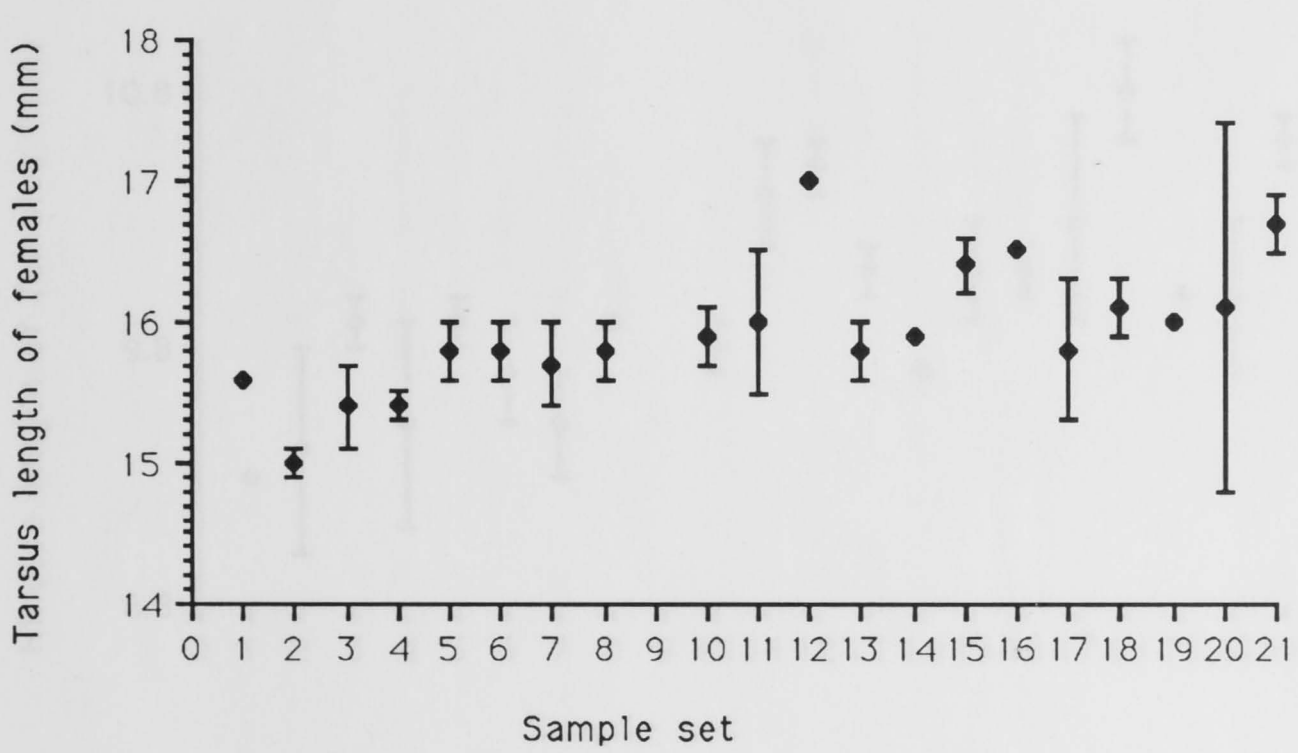


(b)

Figure 4.2 Geographic variation in the culmen length in *A. reguloides* ((a): male; (b): female) Localities are as given in Table 2.2.

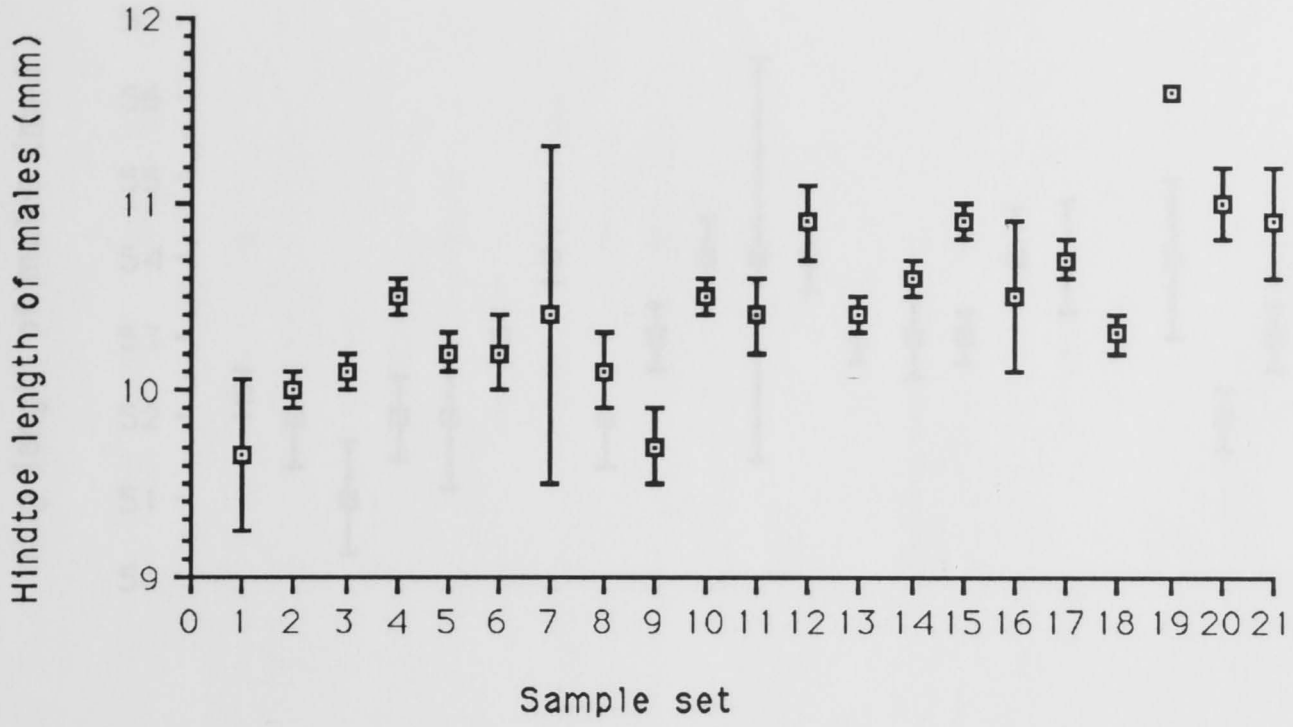


(a)

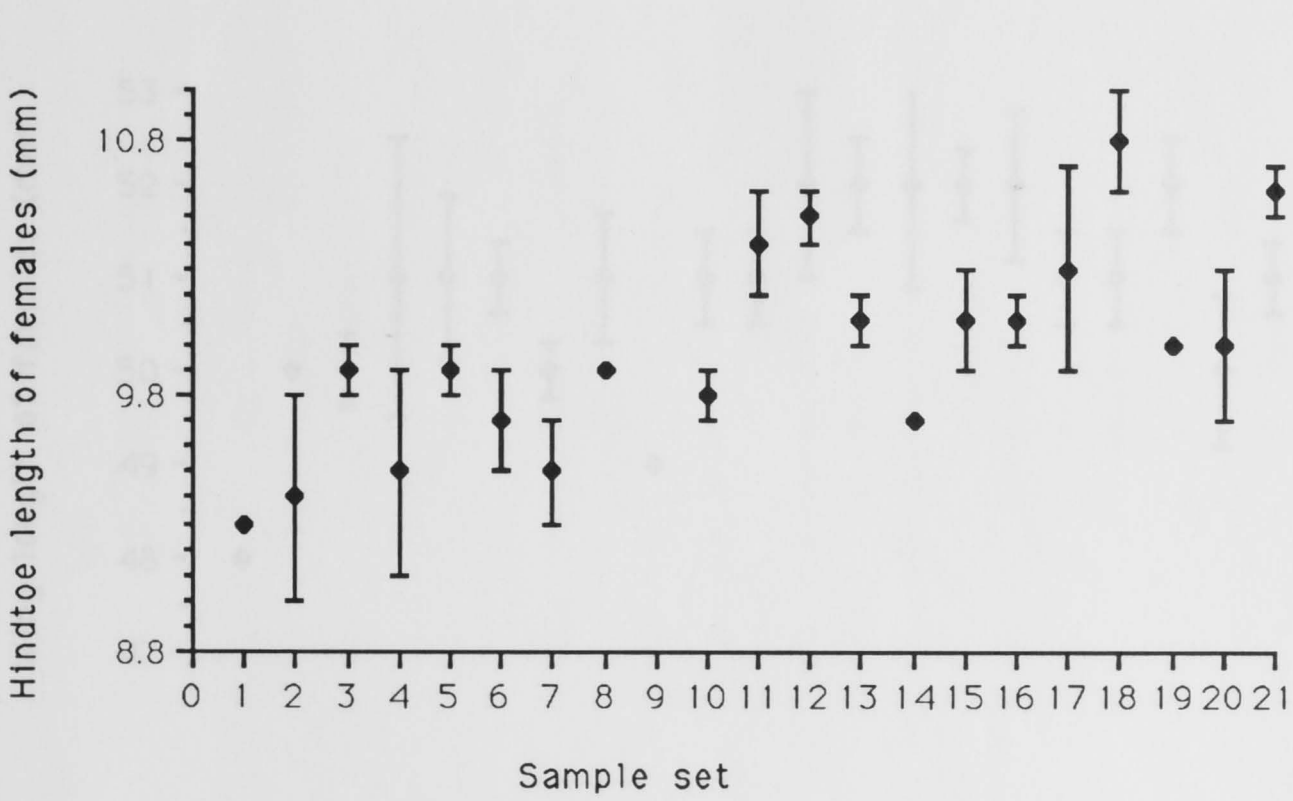


(b)

Figure 4.3 Geographic variation in the tarsus length in *A. reguloides* ((a): males; (b): females). Localities are as given in Table 2.2.



(a)



(b)

Figure 4.4 Geographic variation in the hindtoe length in *A. reguloides* ((a): males; (b): females). Localities are as given in Table 2.2.

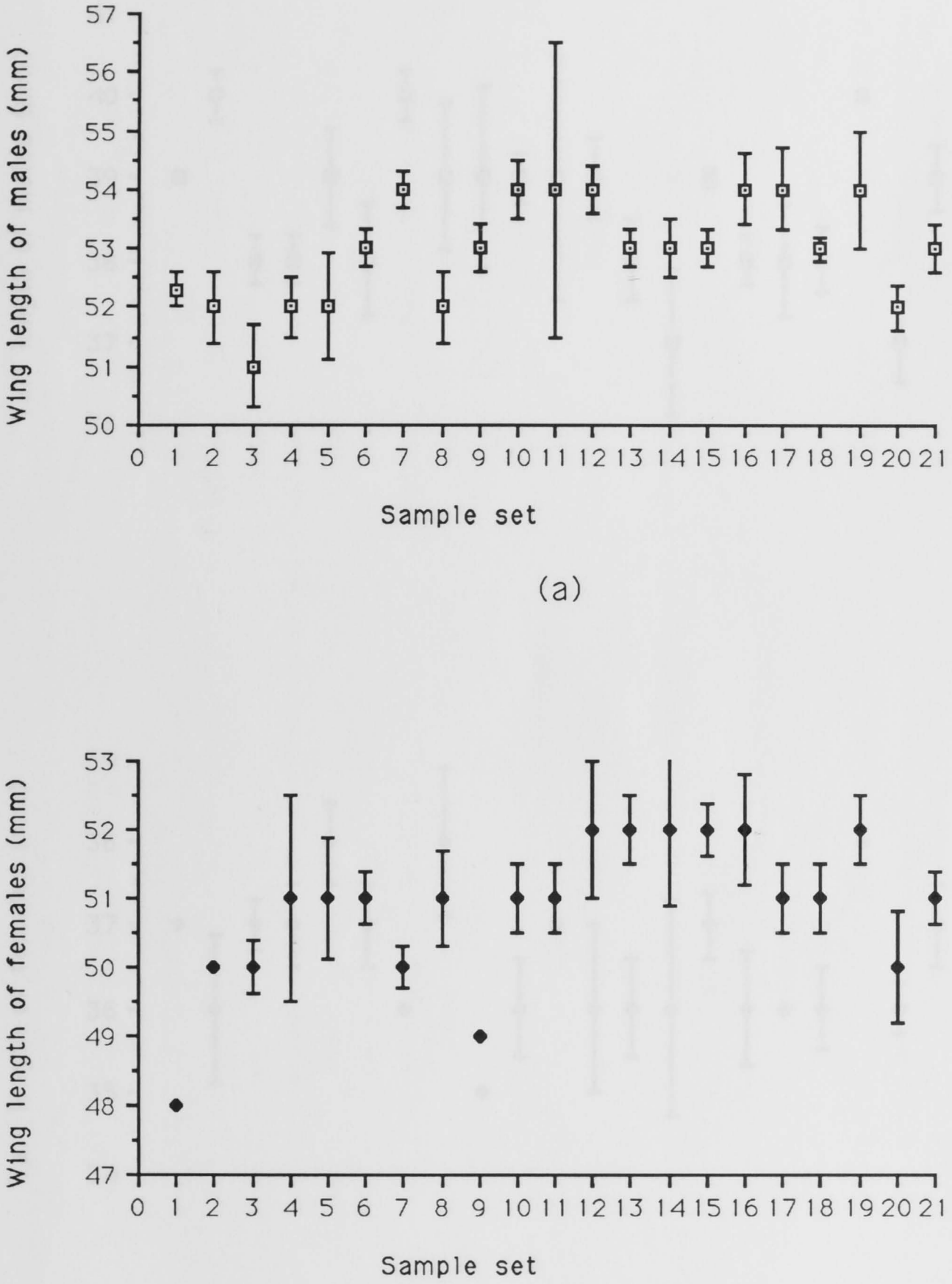
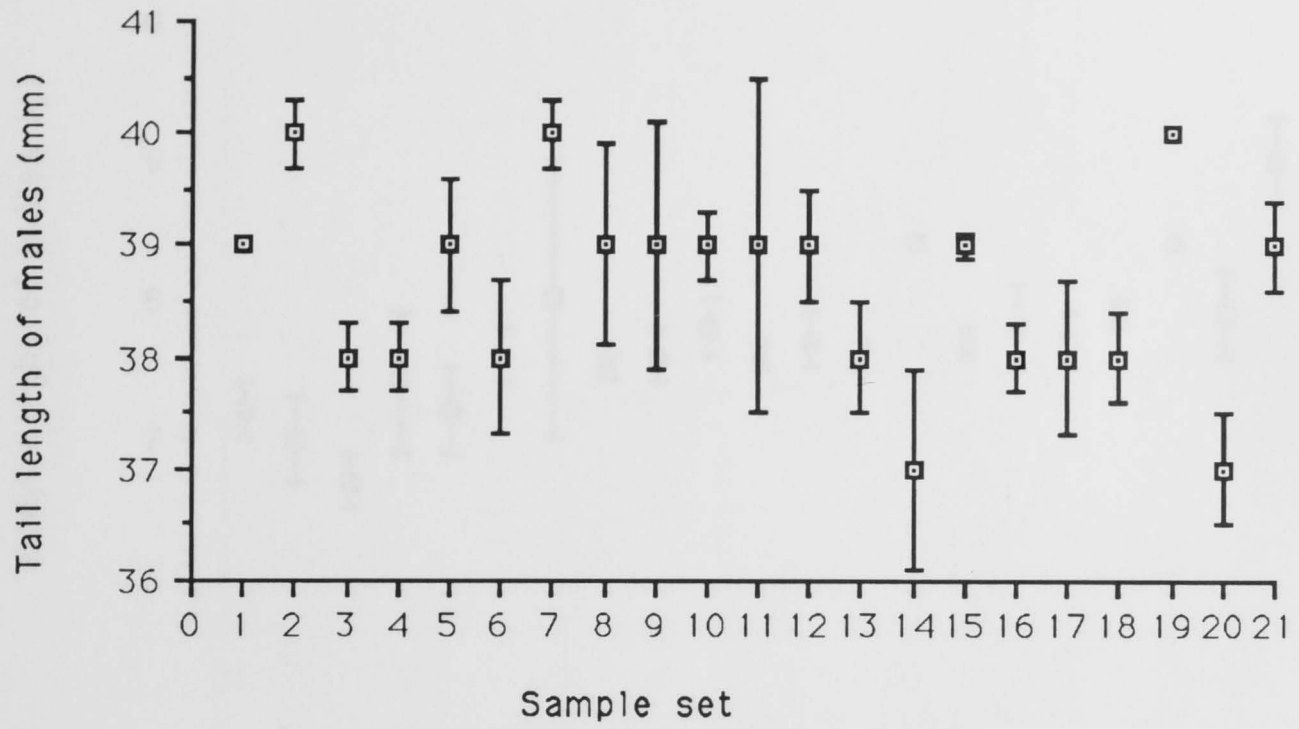
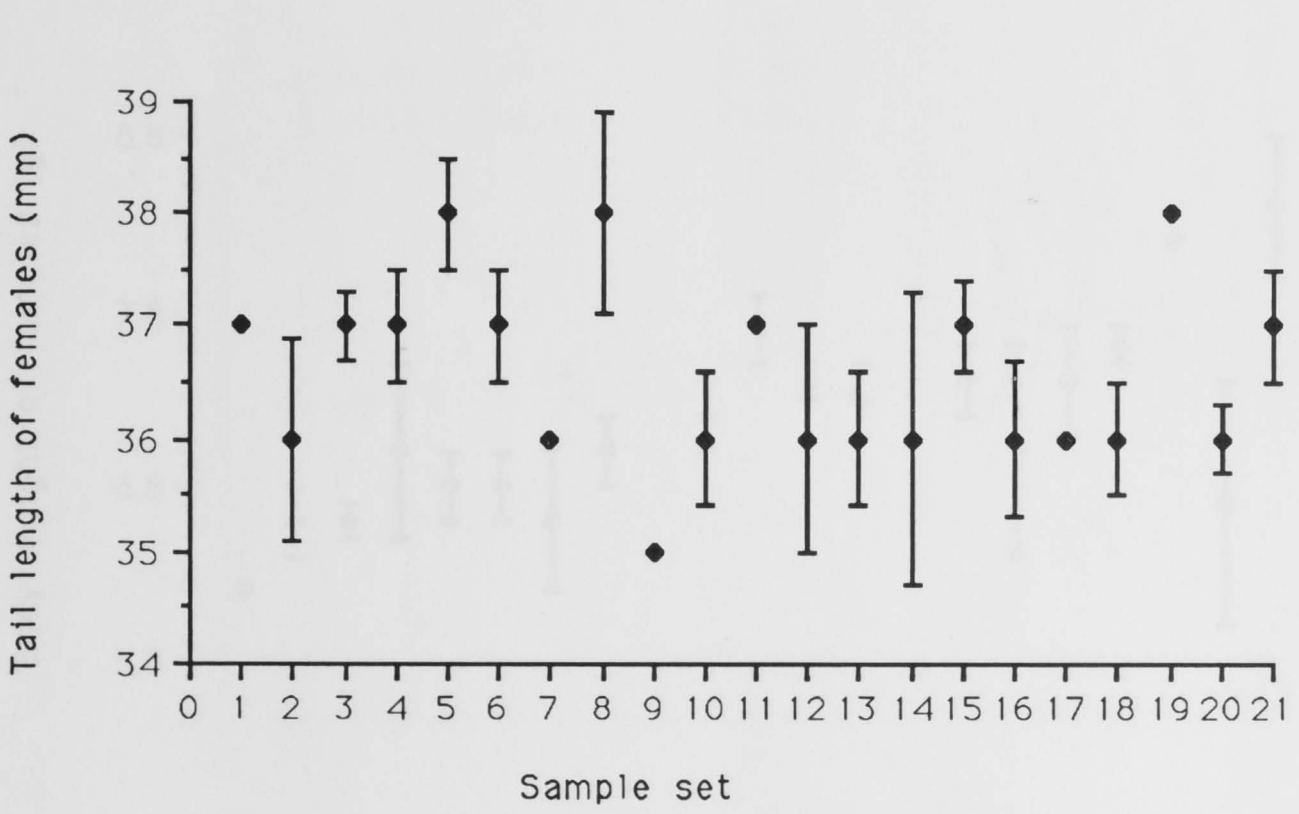


Figure 4.5 Geographic variation in the wing length in *A. reguloides* ((a): males; (b): females). Localities are as given in Table 2.2.

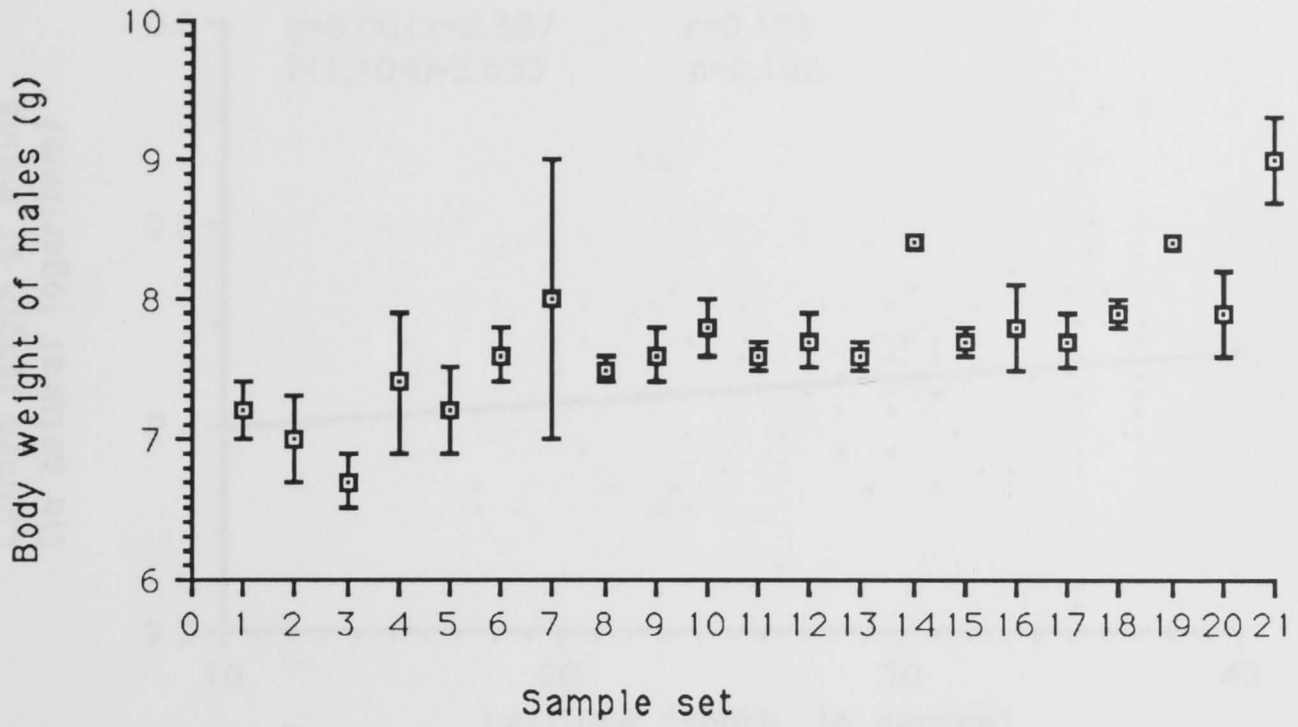


(a)

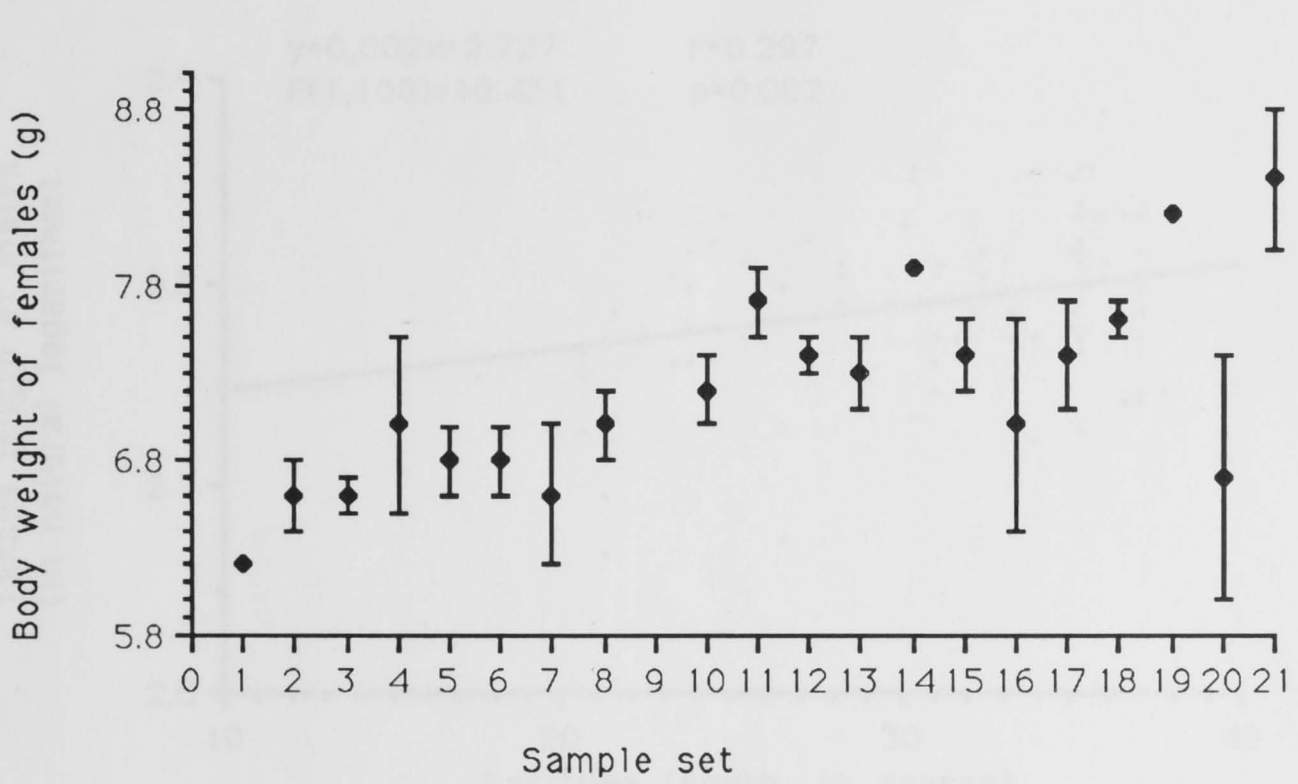


(b)

Figure 4.6 Geographic variation in the tail length in *A. reguloides* ((a): males; (b): females). Localities are as given in Table 2.2.

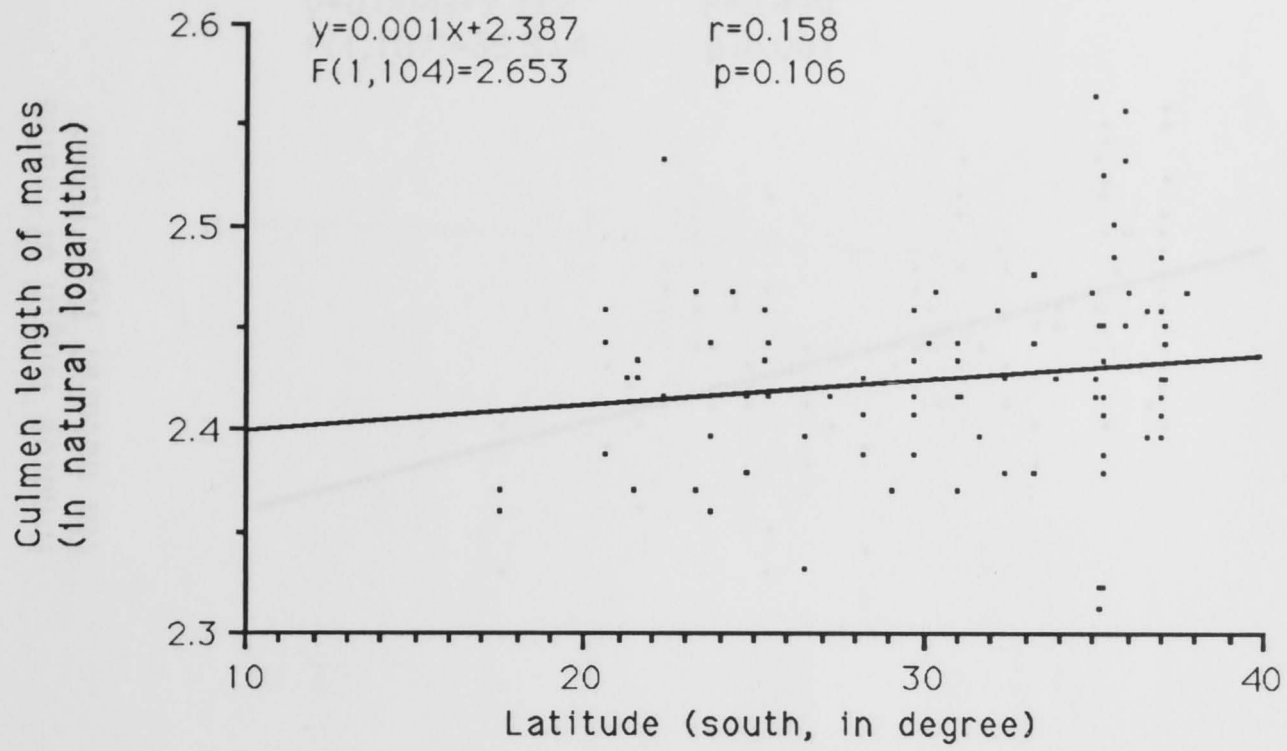


(a)

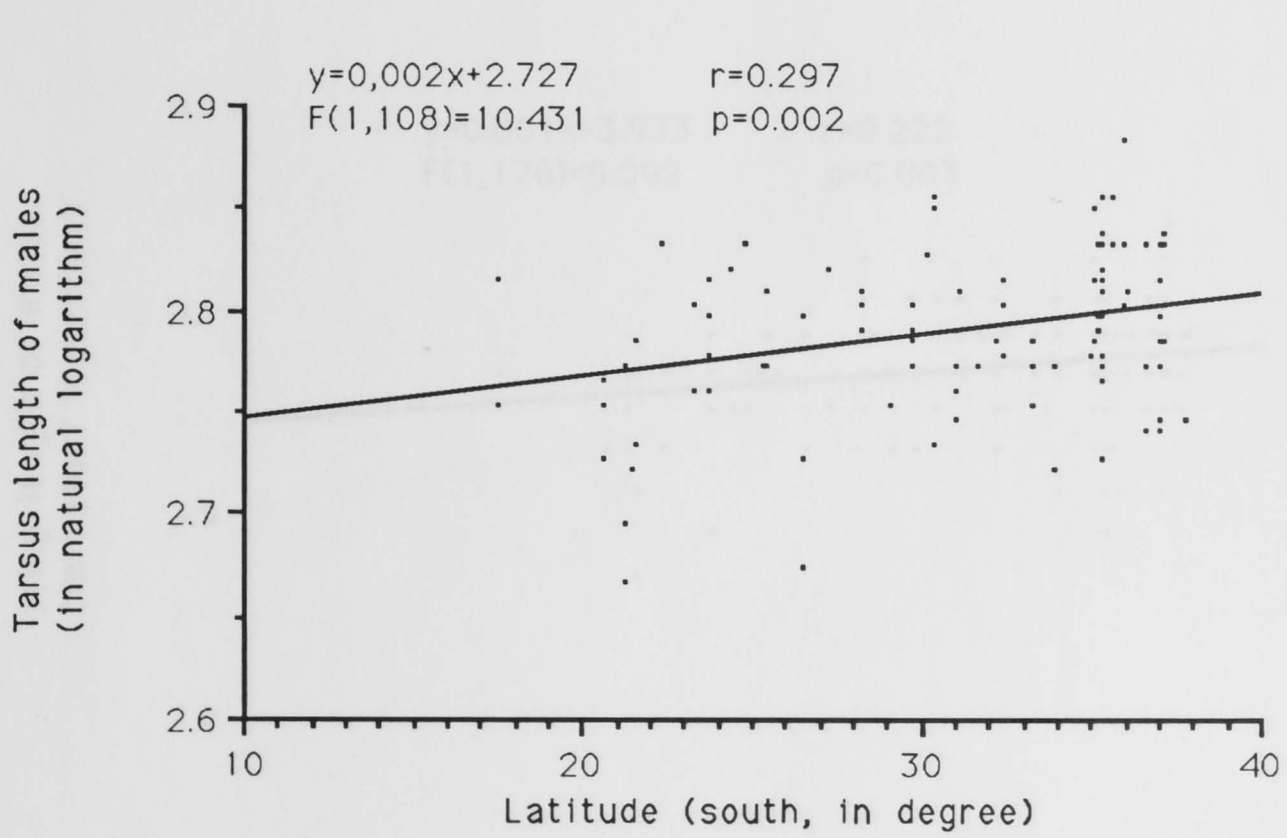


(b)

Figure 4.7 Geographic variation in the body weight in *A. reguloides* ((a): males; (b): females). Localities are as given in Table 2.2.

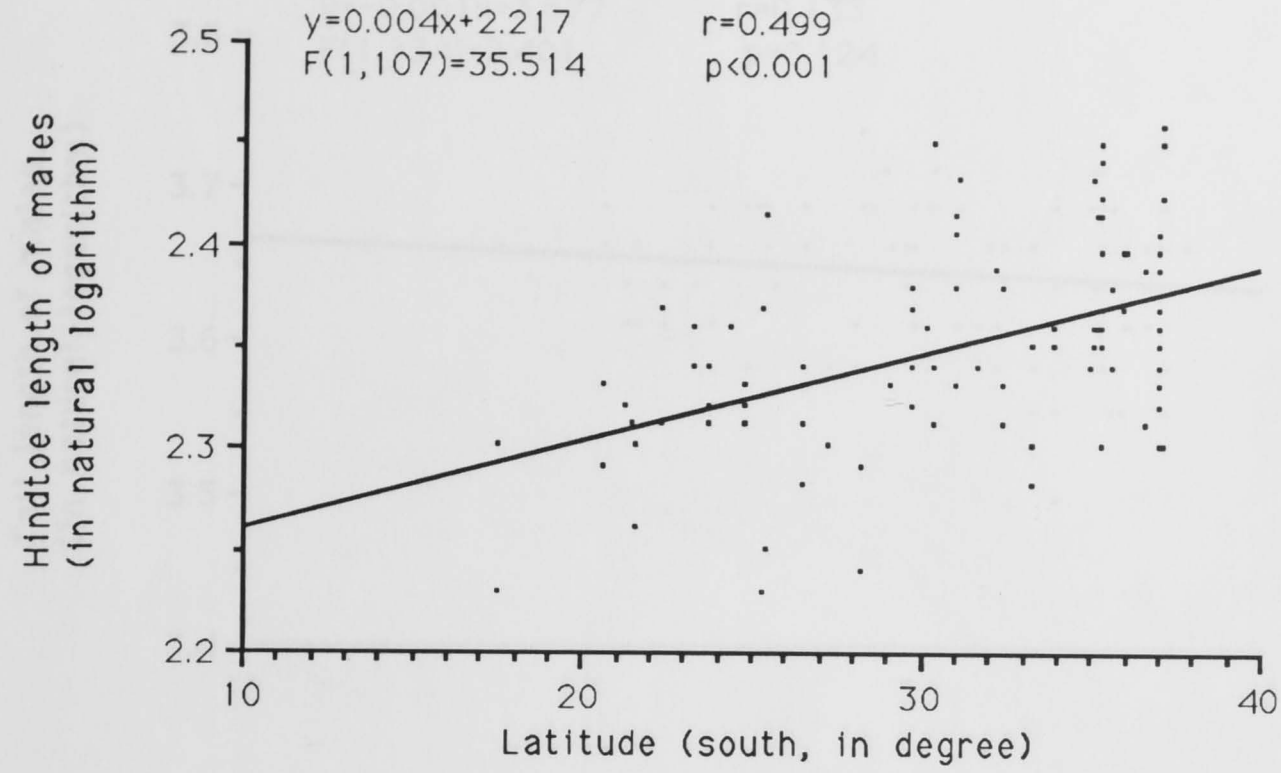


(a)

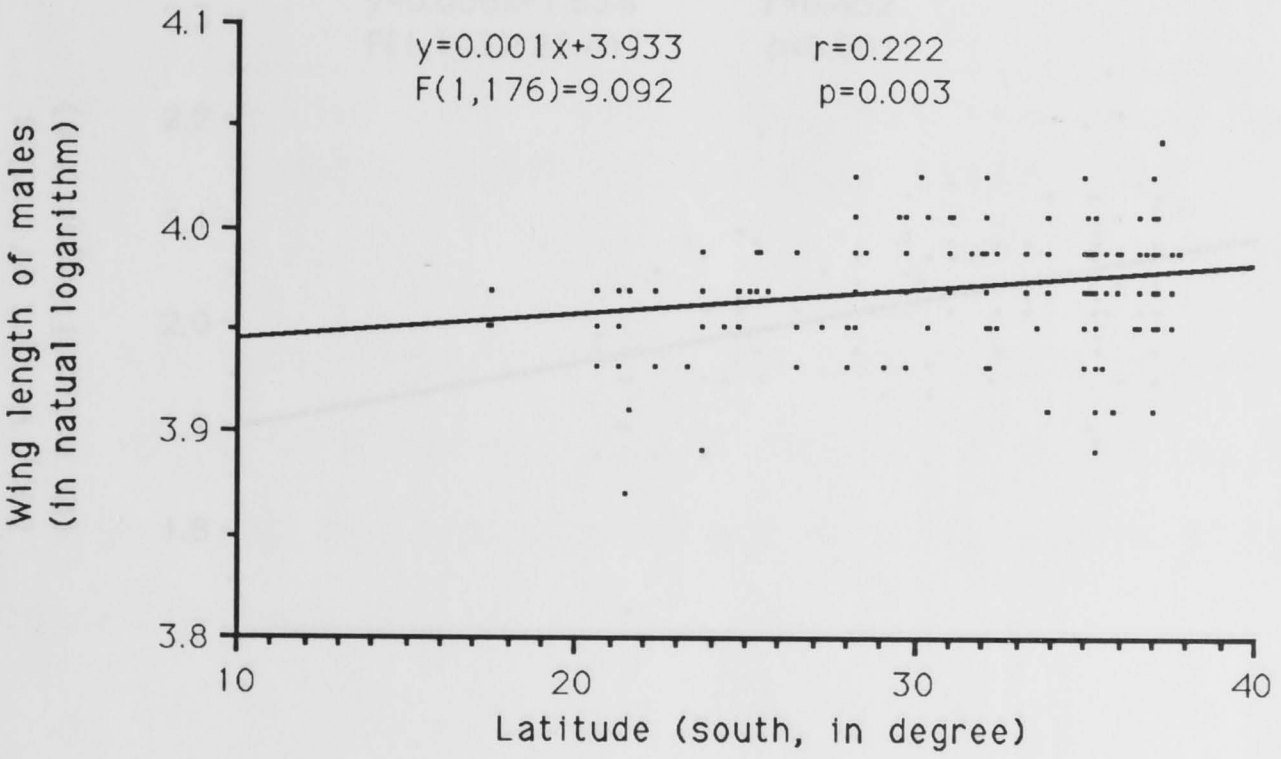


(b)

Figure 4.8a-b (a). Linear regression between the culmen length of male *A. reguloides* and latitude. (b). Linear regression between the tarsus length of male *A. reguloides* and latitude.

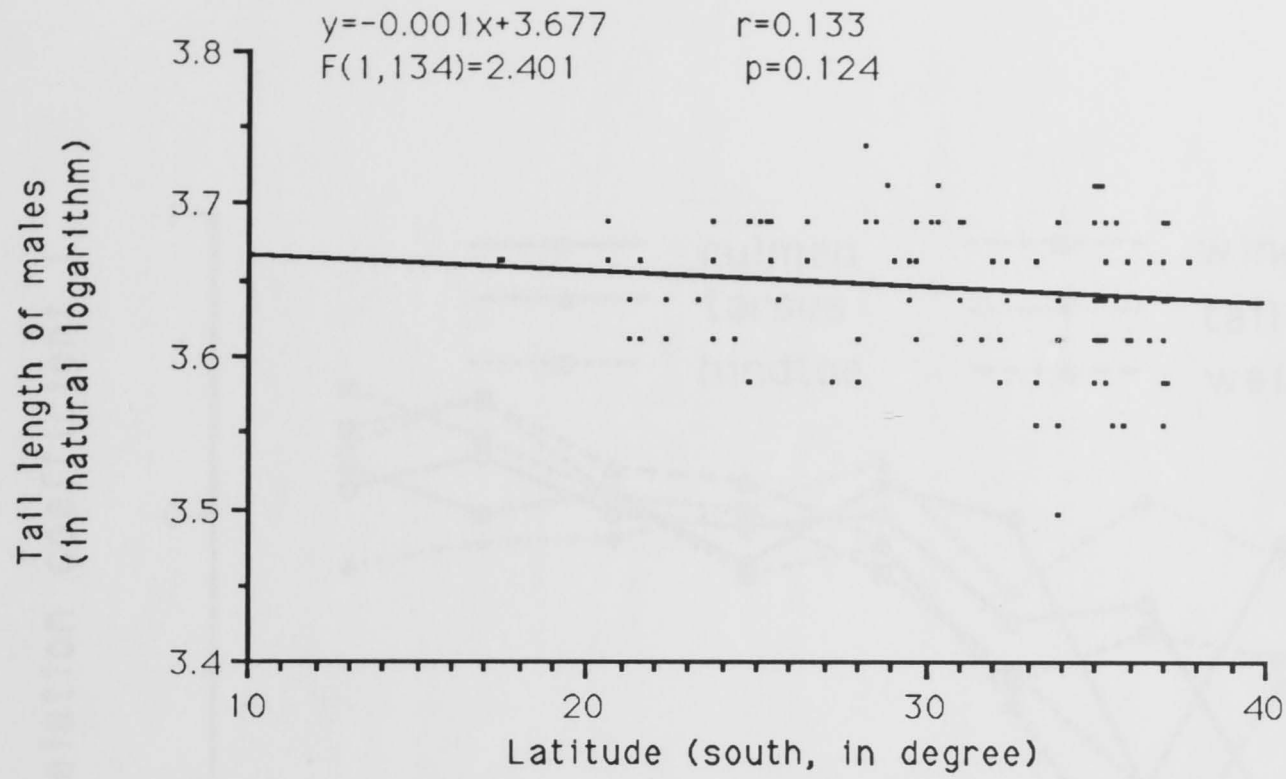


(c)

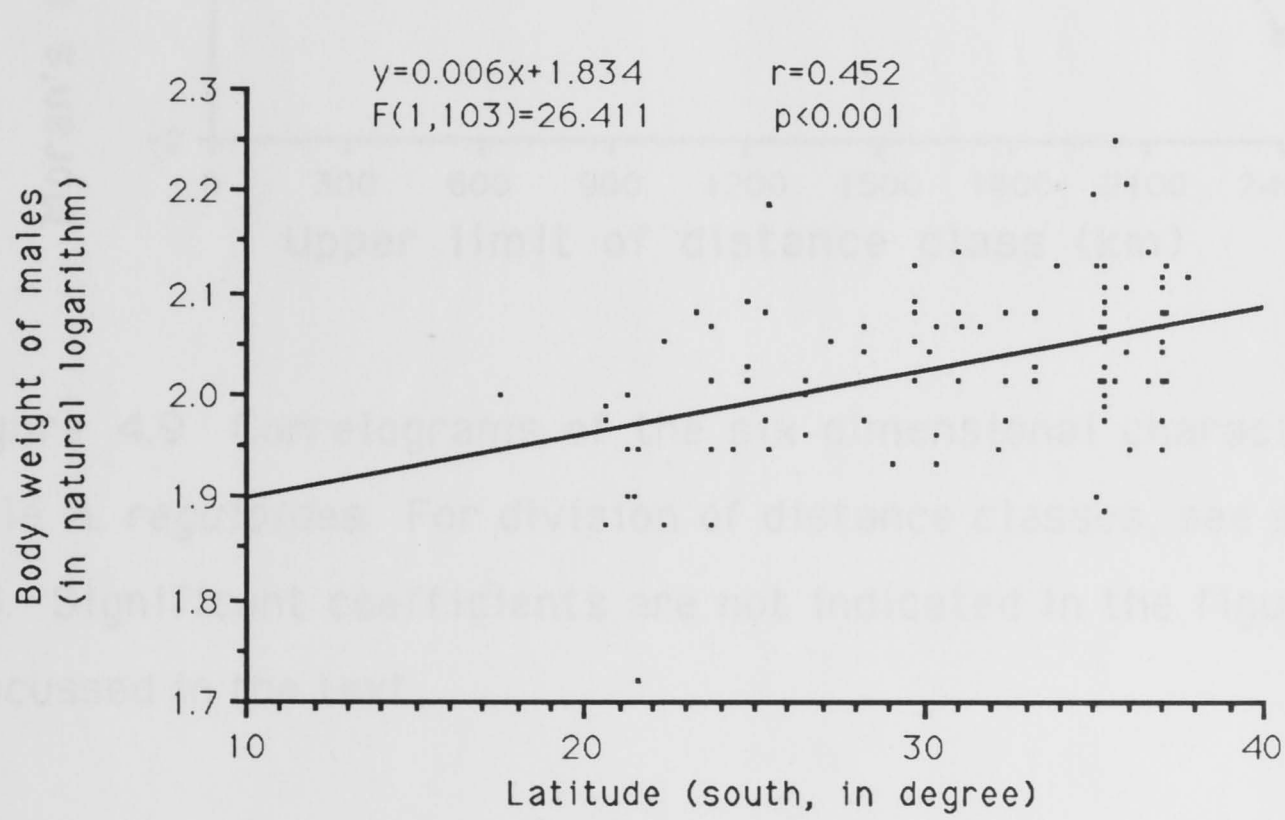


(d)

Figure 4.8c-d (a). Linear regression between the hindtoe length of male *A. reguloides* and latitude. (b). Linear regression between the wing length of male *A. reguloides* and latitude.



(e)



(f)

Figure 4.8e-f (a). Linear regression between the tail length of male *A. reguloides* and latitude. (b). Linear regression between the body weight of male *A. reguloides* and latitude.

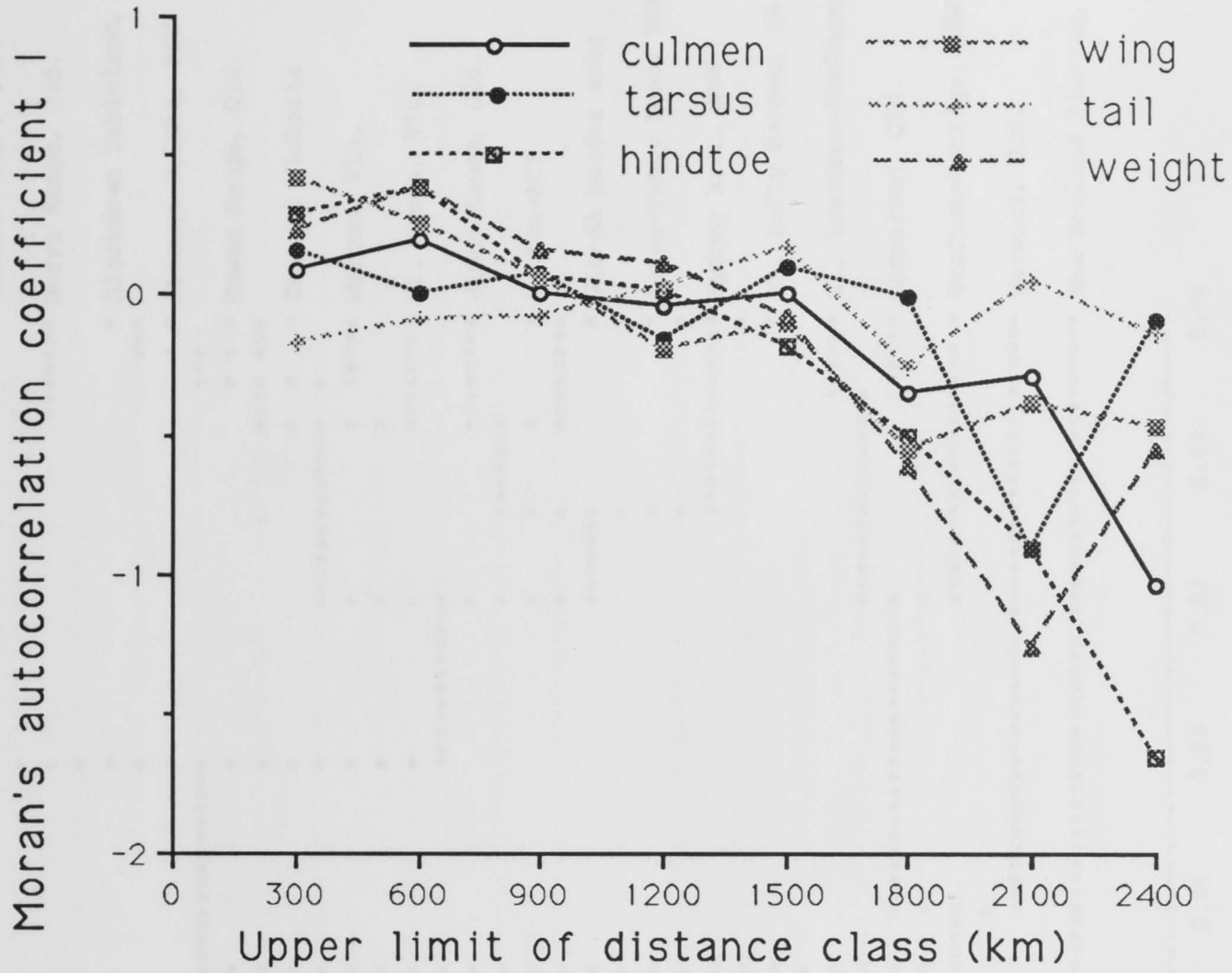


Figure 4.9 Correlograms of the six dimensional characters of male *A. reguloides*. For division of distance classes, see section 2.4. Significant coefficients are not indicated in the figure, but discussed in the text.

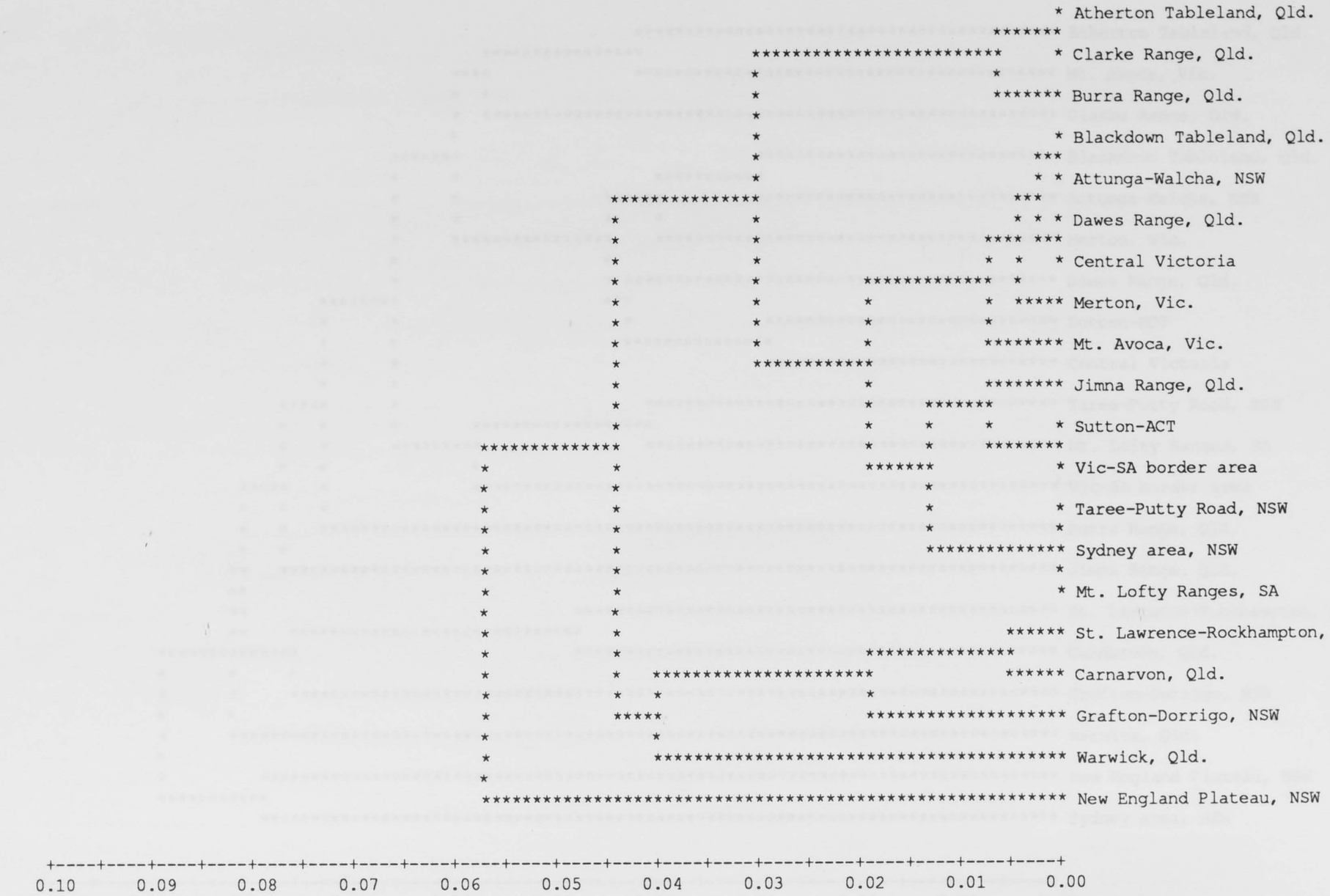


Figure 4.10 Dendrogram reduced by UPGMA from a matrix of Nei's (1978) genetic distance for *A. reguloides*.

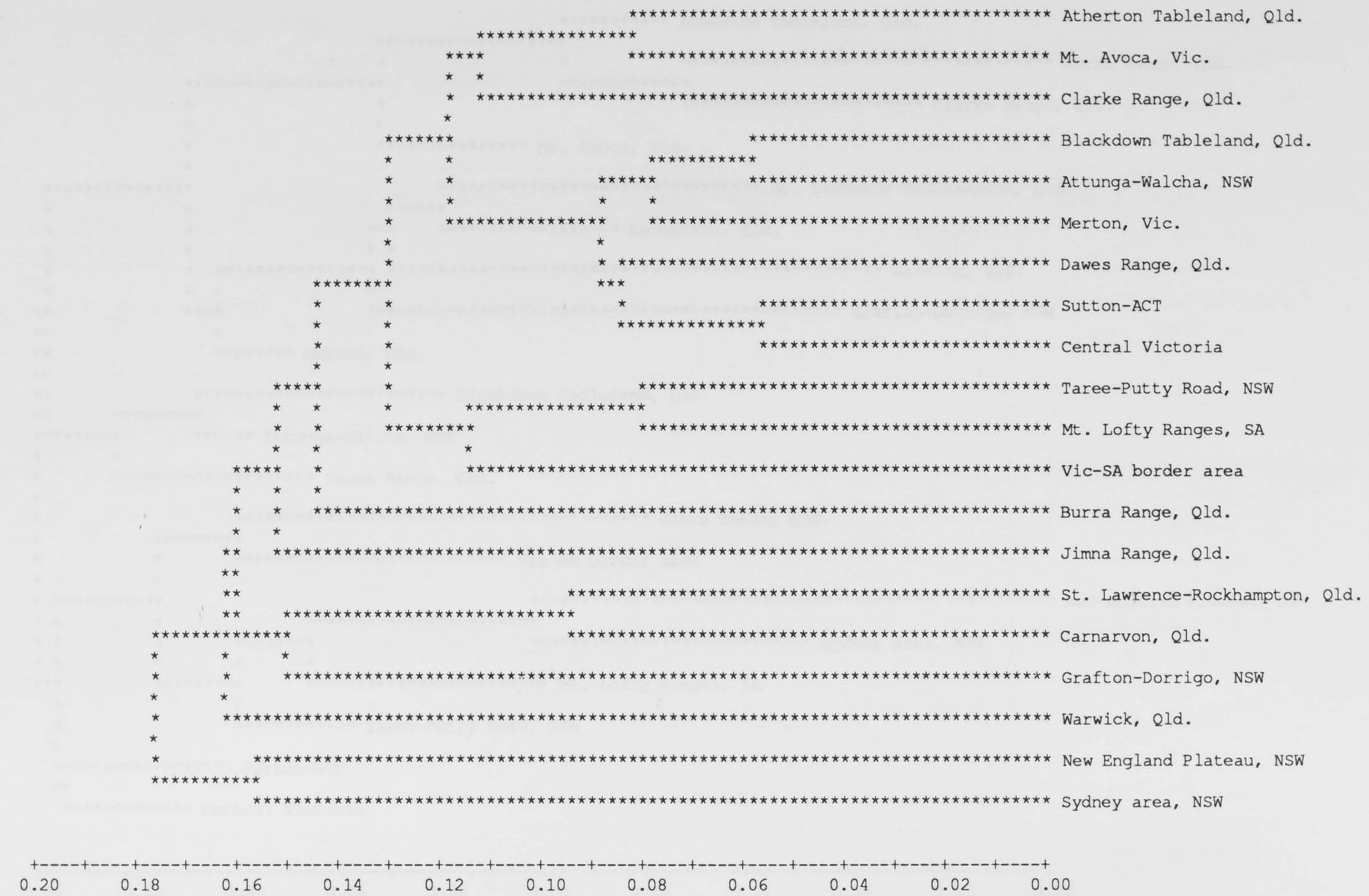


Figure 4.11 Dendrogram reduced by UPGMA from a matrix of Rogers' (1972) genetic distance for *A. reguloides*.

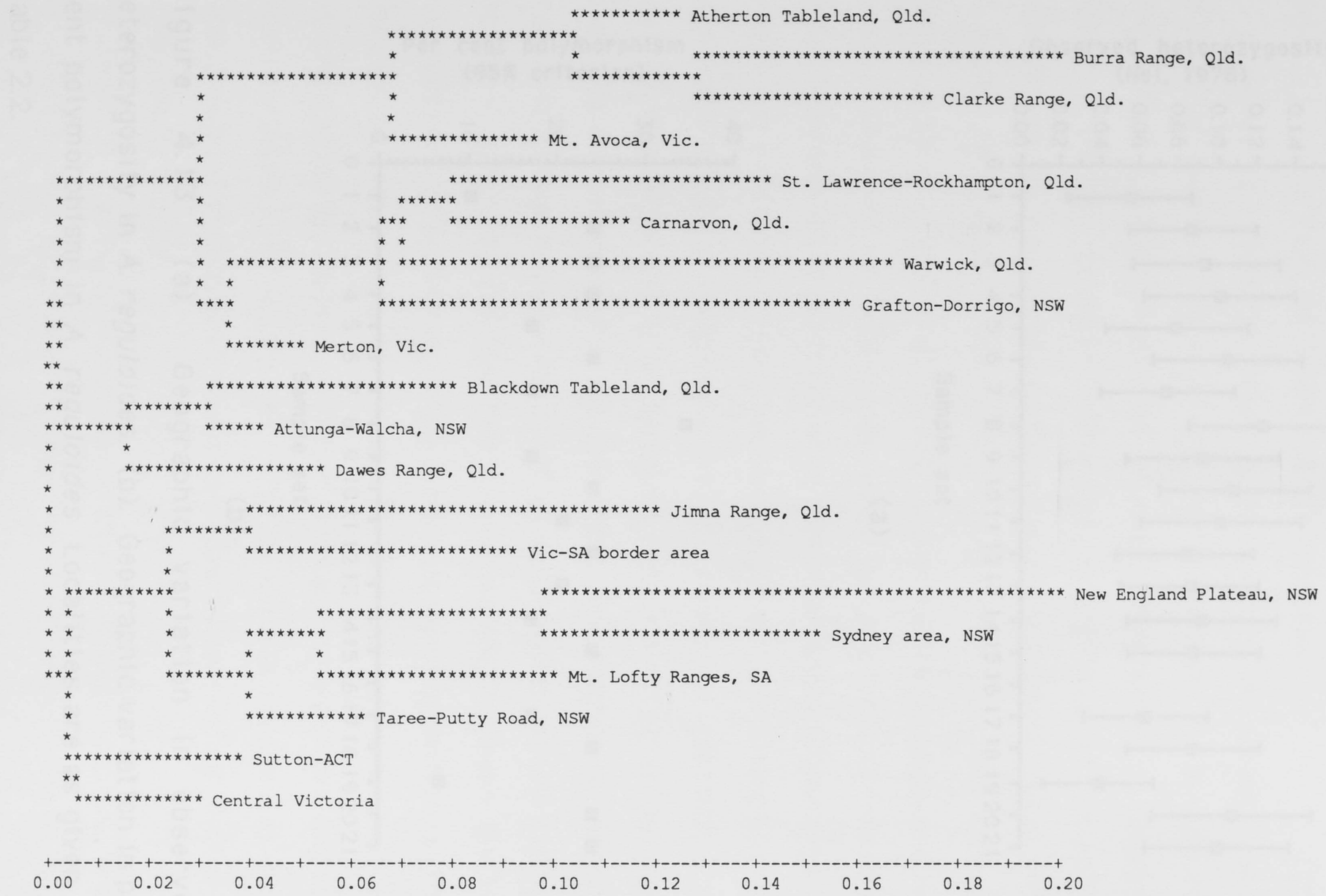
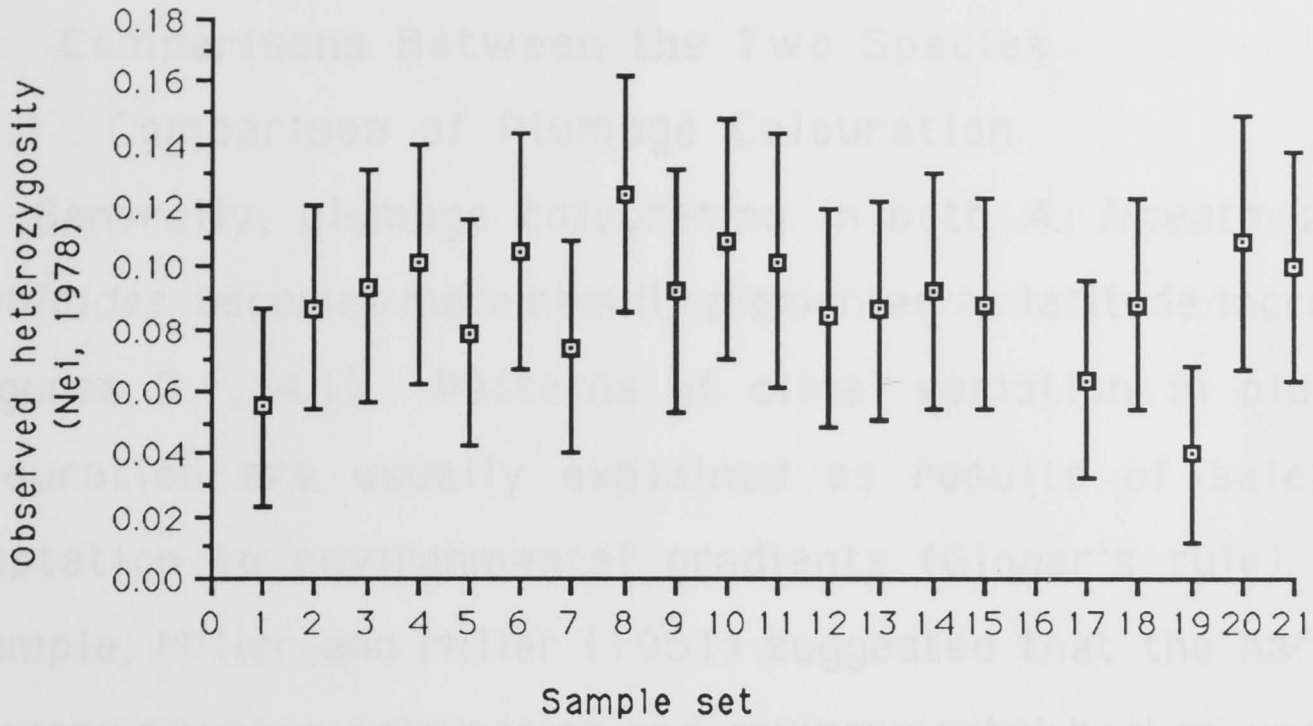
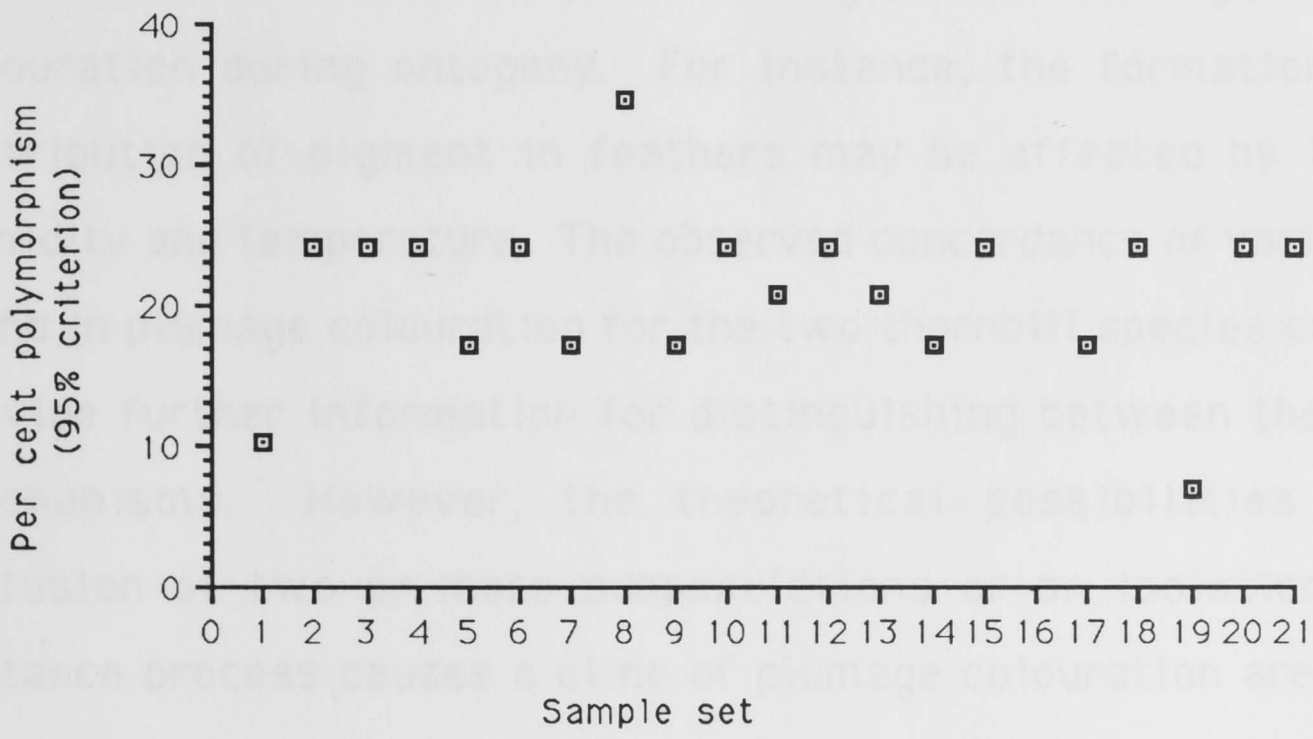


Figure 4.12 Dendrogram reduced by Distance Wagner analysis from a matrix of Rogers' (1972) genetic distance for *A. reguloides*.



(a)



(b)

Figure 4.13 (a). Geographic variation in observed heterozygosity in *A. reguloides*. (b). Geographic variation in per cent polymorphism in *A. reguloides* Localities are as given in Table 2.2.

CHAPTER 5 General Discussion

5.1 Comparisons Between the Two Species

5.1.1 Comparison of Plumage Colouration

Generally, plumage colouration in both *A. lineata* and *A reguloides* becomes more heavily pigmented as latitude increases (Figures 3.1, 4.1). Patterns of clinal variation in plumage colouration are usually explained as results of selective adaptation to environmental gradients (Gloger's rule). For example, Miller and Miller (1951) suggested that the harmony between plumage colouration and environmental background may reduce chances of being captured by predators. Another possibility is that climatic or other environmental factors mainly affect and determine the expression of genes controlling plumage colouration during ontogeny. For instance, the formation and distribution of pigment in feathers may be affected by light, humidity and temperature. The observed concordance of variation trend in plumage colouration for the two thornbill species cannot provide further information for distinguishing between the two mechanisms. However, the theoretical possibilities that diffusion of two or more subpopulations or an isolation-by-distance process causes a cline of plumage colouration are very small because it is less likely that so many bird species show similar clinal patterns of plumage colouration through diffusion or random processes.

Despite a general north-south cline in each species, great variation occurs in an area from Walcha to the Putty Road for *A. lineata*. Such variation has not been found for *A. reguloides* in this study. However, Ford and Simpson (1987) found a striking

variation in plumage colouration for *A. reguloides* in the McPherson Range. This difference may be due to the two different methods used in this study and in the study of Ford and Simpson (1987).

Two processes are more likely to be responsible for the great variation in plumage colouration for the two species. Firstly, the areas where great variation in plumage colouration were found may be ecotones where two or more components of environmental factors overlap. Great variation in plumage colouration is related to environmental diversity through either selective adaptation or environmentally controlled expression of genes for plumage colouration during ontogeny. The McPherson Range, where great variation in plumage colouration of *A. reguloides* was found by Ford and Simpson (1987), is within the range of the so-called McPherson-Macleay Overlap, an ecotone area suggested by Burbidge (1960). However, no evidence exists to indicate that the area from Walcha to the Putty Road, where great variation in plumage colouration of *A. lineata* was found, is an ecotone. Hence the hypothesis of ecotonal effects is less likely.

Secondly, increased variability in plumage colouration may indicate a past secondary intergradation. It can be hypothesized that each of the species might contract to a northern refuge and a southern refuge when climate and habitat became inhospitable. Following the subsequent breakdown of the geographic barrier between former refuges, vicariant differentiates extended their ranges and made secondary contact. Controversy exists as to which geographic barrier is most possible for some Australian bird species (e.g., Short et al., 1983; Ford, 1980, 1987). However, the fact that great variation for the two species occurs

in different areas can be most easily explained as the result of the different movement of contact zones, which is relevant to the different ecology and population structure of the two species. Hence, the observed great variation in plumage colouration can be accounted for by past secondary intergradations.

In summary, like most avian species, the two thornbill species follow Gloger's rule. It cannot be distinguished whether selective adaptation or environmentally affected ontogeny mainly causes the north-south clines in plumage colouration. Increased variability in plumage colouration could be due to either an ecotonal effect or a past secondary contact. Since no evidence indicates the existence of such an ecotone for at least one of the two species (*A. lineata*), the hypothesis of secondary intergradation seems to be more likely.

5.1.2 Comparison of Dimensional Characters

Sexual dimorphism in the six dimensional traits has been found in both species. However variation trends in both males and females are the same. Sexual differences are not affected by geographic locations.

In both species, dimensional characters generally express similar north-south clines for increasing trait values. The variation trends approximately follow Bergmann's rule. Temperature is usually supposed to be the selective force because large animals have low metabolic rates and are more adaptive to cold weather. As discussed in section 1.2.2, variation in dimensional characters usually shows high levels of heritability. Therefore, environmental effects through ontogeny seem to be less likely.

The similar patterns of geographic variation between sexes and among localities for both species may be due to the fact that they are congeneric species and share the similar distribution range. In other words, the two species may have similar biologies and react in a similar way to the same environmental gradients. Therefore, they display similar clines in dimensional traits.

Differences in patterns of geographic variation in dimensional traits exist between the two species. All six traits in *A. lineata* show significant clines whereas only 4 out of 6 do so in *A. reguloides*. Furthermore, the clines in the culmen length in *A. lineata* and the hindtoe length in *A. reguloides* show more obvious clines than clines of other traits in each species. This can be due to several reasons. First, clines in these two species are smooth. In this situation, statistic levels of significance can be less meaningful. Second, the body shape may vary geographically in different way in each species. Although body shape has been always considered to be a more reliable indicator of internal constitution of organisms than body size and several methods have been developed to measure body shape, controversy exists on the reliability of those methods (e.g., Somers, 1986; Rohlf and Bookstein, 1987; Sundberg, 1989). An analysis of body shape has not been conducted in this study. Third, the extent of clines may reflect different intensities of selection. However, the bill length may be important to *A. lineata* because it gleans in the canopy, whereas the hindtoe length may be important to *A. reguloides* because it mainly hops on the ground to forage. Usually, selection on a functionally important character should be stable. It is not clear whether the relative steepness of these

two clines reflects the stronger selection pressure of food or substratum on them.

In summary, both species show similar clines in most dimensional characters. This may indicate the similar response to the same environmental gradients. Differences in patterns of characters for the two species may be due to subtle clines, possible different variation in the body shape or different selection pressures on different traits between the two species.

5.1.3 Comparison of Allozymic Characters

The two species show very similar patterns of variation in allozymic features. Namely, they have no obvious abrupt or clinal allele variation, despite both possessing a considerable amount of alternative alleles and some unique alleles in some areas (e.g., allele D at the GPI locus for *A. lineata* and allele A at the GPI locus for *A. reguloides* in the Mt. Lofty Ranges). The distribution of variation in allele frequencies in both species is basically panmictic. Furthermore, the two species show very similar genetic structure in populations. Table 5.1 lists some multilocus descriptive values for both species. Though statistical comparisons were not performed, it is obvious that the values listed in the table are very close for the two species. The extensive similarity in genetic structure of these two populations may be due to their similar evolutionary time scale and demography.

Higher values of mean heterozygosity have been found at one or two localities for both species. Because of high heterogeneity in the allele frequency data and the limitation of sample sizes, statistical significance test was not carried out to check the significance of

difference in allele frequencies of any pair of localities (see section 3.1.3.1). However, higher heterozygosity may indicate the existence of a previous secondary intergradation within each of the two species.

In summary, both species show relatively panmictic patterns of variation in allozymes and high values of average heterozygosity in a region, that may indicate intergradation zones. They may have similar genetic structure and evolutionary history.

5.1.4 Overall Comparisons

In an effort to formulate a hypothesis of the evolutionary history of a species from observed patterns of geographic variation, historical and ecological factors must be distinguished. However, the interaction of both types of factors usually shape the evolution of a species. This is found to be the case for *A. lineata* and *A. reguloides*. General clines in plumage colouration and dimensional characters in both species are more plausibly due to ecological gradients, whereas, great variability in plumage and average heterozygosity may reflect the remnants of a historical event, possibly the last period of glacio-aridity of about 25000 to 15000 years BP.

The conclusion that both species have been shaped by similar selection or developmental influence of environmental gradients is directly derived from the concordance of their clines in plumage colouration and body size characters. In fact, many bird species show similar patterns of plumage colouration and body size characters (see section 1.2.2). Therefore, the parsimonious conclusion is that the environmental factors they share are

responsible because it is less likely that diffusion after a historical isolation or other random processes in so many species would produce congruent patterns of geographic variation in morphological characters.

The hypothesis that the two species underwent similar historical events is based on the concordance of the variability in plumage and average heterozygosity between the two species. There are some factors that act against this hypothesis. Firstly, striking variability in plumage colouration for *A. reguloides*, found by Ford and Simpson (1987), has not been found in this study. Secondly, the assumed intergradation zones for the two species are not concordant. However, due to the possible difference in ecology, population structure etc. between the two species, it is possible that their intergradation zones have moved at different rates. Thirdly, according to Barton and Hewitt (1985), variation in chromosomes, electrophoretic characters, morphology, behaviour, and mtDNA in most hybrid zones is often coincident. Among the three sets of characters studied in the present study, at least variation in dimensional characters is not coincident with that in plumage colouration (for *A. lineata*) and average heterozygosity. This, however, could be due to the possibilities that the dimensional differentiation acquiring during isolation was minor and that the environmental selection after rejoining of vicars was strong.

The evolutionary processes inferred from the congruence of patterns of geographic variation in the two thornbill species are consistent with those observed for many other Australian avian species (e.g., Short, et al., 1983; Ford, 1987). Namely, secondary contact has been suggested for many Australian bird species with

geographic variation. This indicates that geographic variation caused by historical events seems to be universal. For the two species in this study , contact zones have been decayed but the remnants are still detectable.

Furthermore, the process leading to intraspecific variation, as described for the two species, is very similar to the commonly recognized process leading to speciation. On studying the formation of biotas, theories of both panbiogeography and vicariance biogeography suppose that widely distributed ancestral species were separated by the development of geographic barriers into several isolated subpopulations within each population and finally those subpopulations evolved into new species (e.g., Cracraft, 1986). The difference between the two processes is that in the process leading to intraspecific variation different isolates prematurely rejoin before acquiring different sets of recognition signalling-systems whereas in the process leading to different species, the recontact has not occurred before complete reproductive isolation had been achieved.

In summary, the congruence of patterns of geographic variation in the three sets of characters between the two thornbill species suggests that both a historical event and environmental gradients shaped the present patterns of subpopulation distributions. This process is consistent with those supposed to lead to geographic variation in other Australian bird species and furthermore, similar to those believed to lead to many events of speciation.

5.2 Comparison with Other Species

The mean heterozygosity (Nei, 1978) and its standard errors in *A. lineata* (0.109, 0.041) and in *A. reguloides* (0.097, 0.036) are similar to those in some other *Acanthiza* species (the Mountain Thornbill, *A. katherina*: 0.109, 0.047; the Brown Thornbill, *A. pusilla*: 0.098, 0.035; and the Inland Thornbill, *A. apicalis*: 0.083, 0.032) but are higher than in the Tasmanian Thornbill, *A. ewingii* (0043, 0.026) (Norman, 1987). The generally similar levels of average heterozygosity may be due to the fact that congeneric species have a more similar evolutionary history.

The mean heterozygosity of *A. lineata* and *A. reguloides* is within the range of 30 well-studied avian species (0.007-0.147) listed by Barrowclough (1983), but is much higher than the average (0.053). Obviously, *A. lineata* and *A. reguloides*, like other *Acanthiza* species examined so far exhibit high levels of genetic heterogeneity.

As Barrowclough (1983) pointed out, specific comparisons of mean heterozygosity of any two avian taxa may not be meaningful because of large standard errors due to different sample sizes and the number and choice of loci examined. However, the comparison between *A. lineata* and *A. reguloides* is based on very similar sample sizes, number of loci and virtually the same loci (Tables 3.4, 4.4 and 5.1). For the comparison between *Acanthiza* species (*A. lineata* and *A. reguloides*) and other avian species, the average values and ranges of many other avian species are used. Therefore, these comparisons appear to valid.

The F_{st} values obtained for *A. lineata* based on the 17 local populations (0.160) and for *A. reguloides* based on the 20 local

populations (0.162) are extremely high compared with those listed by Barrowclough (1983), most of which were less than 0.06. This could be due to the relative lack of gene flow since both *Acanthiza* species are sedentary. But it is more possible that this is due to the fact that many alternative alleles in the *Acanthiza* species are only present in one or few localities (Tables 3.4 and 4.4) and that the number of localities in the present study (17 for *A. lineata* and 20 for *A. reguloides*) are relatively large. Therefore, higher observed F_{st} for *A. lineata* and *A. reguloides* could not be appropriately considered as an indicator of great variation among local populations.

The lack of significant patterns of allozymic variation among local populations in *A. lineata* and *A. reguloides* is consistent with that in some species studied in North America. For example, by comparing five populations of the Yellow-rumped Warbler (*Dendroica coronata*), Barrowclough (1980) found polymorphisms at 8 out of 32 gene loci, with five of these eight loci having three or four alleles. In spite of this amount of variation and the well distinguished plumage differences between the subspecies *D. c. audubone* and the nominate *coronato*, only one of the eight polymorphic loci showed a significant level of heterogeneity among the five localities and none of the allele frequencies at the eight loci varied clinally. Therefore, populations of the parental subspecies were not significantly different from one another, nor were there abrupt changes in allele frequency within the intergradation zone between them. Zink's (1986) study on the Fox Sparrow (*Passerella iliaca*) also showed similar results. Although the variation in morphological traits in the species is extensive among subspecies, he could not detect significant

geographic variation in alleles either among local populations or among subspecies. Therefore, it is usually supposed that high gene flow among avian populations offsets local variation due to drift and selective differences and causes low levels of differences among local populations.

In summary, levels of average heterozygosity for *A. lineata* and *A. reguloides* are generally similar to those for other *Acanthiza* species, and higher than those for most other passerine birds studied. Higher values of F_{st} for the two species may mainly be due to the large number of sample sets involved in the present study. The two *Acanthiza* species, as other well-studied bird species show no significant pattern of allozymic variation.

5.3 Discussion on Subspecific Taxonomy

Practically, avian subspecies have been given recognition when 75% or more of the individuals under consideration can be assigned to one or another phenotype based on morphological variation. However, the criterion itself and the use of subspecific status in avian taxonomy seems to be very incomplete and subjective. Firstly, only morphological characters are involved in assigning a taxon to the status of subspecies. The genetic basis of some morphological characters is poorly known (see section 1.2.2). In such a case, subspecific division may only reflect short-term plasticity of morphology caused by environmental factors. Secondly, much of the morphological variation in birds is clinal (Barrowclough, 1982). Whether to divide subspecies and how many subspecies to be divided often depend on whether the definer is a lumper or a splitter.

Consequently, many subspecies divisions seemed to be based on seemingly trivial differences and the delineation of the subspecific boundaries is arbitrary. Thirdly, concordance of clines in different (sets of) characters is often lacking. Which character is chosen to divide subspecies depends on the judgement of the taxonomist. Accordingly, the subspecies concept and its use in avian taxonomy have been seriously criticized. However, for the convenience of describing variation and offering gross view on variation, the concept is useful if the division of subspecies is more objective (e.g., Lanyon, 1982; Johnson, 1982).

In the cases of *A. lineata* and *A. reguloides*, as those in most Australian avian species, subspecies were defined on the basis of plumage colouration. Moreover, as the variation in plumage colouration is basically continuous, the views on the number of subspecies and their boundaries in the two *Acanthiza* species are different (Ford and Simpson, 1987; Mayr and Serventy, 1938; Mack, 1936). Therefore, the current subspecific division of *A. lineata* and *A. reguloides* seems to have little theoretical and practical value.

Although the Mt. Lofty area is more or less isolated from other areas, only one unique allele with a low frequency has been detected for each of the species. Moreover, distinct plumage colouration has not been found for the populations in this area. Therefore it seems that there is no further evidence to support the subspecies status for the populations in the Mt. Lofty Ranges.

From the observed geographic patterns of variation in the present study, it is suggested that each of the two species could be divided into two subspecies, with the proposed contact zones

acting as subspecies boundaries. For *A. lineata*, the Hunter Valley can be treated as its subspecies boundary. For *A. reguloides*, the McPherson Range can be treated as the boundary between its subspecies. For each species, the northern subspecies is bright in plumage colour and the southern subspecies is more brown-hued.

In summary, documented division of subspecies of both *A. lineata* and *A. reguloides* has not been supported by this study. Instead, two subspecies could be suggested for each of the two species.

5.4 Conclusions

In summary, the main conclusions to be drawn from the present study are as follows.

1). The general trend of variation in plumage colouration (expressed in colour index) for both *A. lineata* and *A. reguloides* follows Gloger's rule. Clines in plumage colouration are probably due to selection along environmental gradients. This study has not corroborated the patterns of variation in plumage colouration implied by the current subspecific division for both species.

2). The great variation in plumage colouration has been found approximately in the Hunter Valley for *A. lineata*, whereas no such variation has been found for *A. reguloides*, contrary to the great variability in plumage colouration for *A. reguloides* in the McPherson Range revealed by Ford and Simpson (1987). The zones where the observed abrupt variation in the plumage colouration occurs are congruent to those where the variation in the average heterozygosity is the highest.

3). Most of dimensional characters show clinal variation, with the trait values increasing from north to south. This may indicate that these characters are adaptive to some kind of environmental factors.

4). The genetic variability of both *A. lineata* and *A. reguloides* is high, when compared to the average genetic variability of other avian species. Despite the high amount of genetic variability, significant abrupt or clinal variation in allele frequencies has not been found in either *Acanthiza* species. This is consistent with the phenomena found in many avian species studied in North America. Variation in allozymic characters seems to be neutral.

5). Higher average heterozygosity (Nei, 1978) has been found at one or two particular localities for both *Acanthiza* species. This, together with the popularly recognized speculation on the development of the Australian environment and flora and fauna, the geographical concordance between the proposed intergradation zones of *A. lineata* and *A. reguloides* with the hybrid zones of other Australian avian species, and the stepped variation in plumage colouration revealed by this study and that of Ford and Simpson (1987), leads to a hypothesis that past secondary contact might have occurred. However, in the absence of significantly abrupt variation in allelic and dimensional characters, these contact zones must have decayed a great deal.

6). The different patterns of geographic variation in plumage colouration, the dimensional and allozymic characters for each of the two species seem to support the proposition that different sets of characters undergo the different evolutionary processes. The plumage and dimensional characters are more affected by the

environmental adaptation and the allozymes may be neutral and affected by gene flow, drift, effective population sizes and other demographic events.

7). The two *Acanthiza* species studied generally show the very similar patterns of geographic variation in dimensional characters and allozymes, and to some extent, plumage colouration. Given the fact that they are congeneric sympatric species, the two species must have reacted in a similar way to the same environmental gradients. On the other hand, the two species have different ecologies, such as foraging behaviour. Therefore, the similar patterns of geographic variation, especially great variability in plumage colouration and average heterozygosity, also suggest that the same historical events have shaped their evolution.

8). The considerable amount of genetic variability (average heterozygosity), the lack of abrupt and clinal allele variation and the conformance to Hardy-Weinberg equilibrium in the populations of the two species are generally similar to those documented for other avian species.

9). This study suggests a division of two subspecies for each of the species. The subspecies boundary could be located at the Hunter Valley for *A. lineata*, and at the McPherson Range for *A. reguloides*.

Table 5.1 Multilocus descriptive statistics in *A. lineata* and *A. reguloides*. Standard errors are in parentheses.

Descriptions	*A. lineata*	*A. reguloides*
Number of loci resolved	28	29
Number of polymorphic loci	10	11
Total alleles at polymorphic loci	48	43
Average number of alleles per locus per locality	1.4 (0.2)	1.4 (0.1)
Number of alleles per locus for the whole population	2.3 (0.4)	2.1 (0.3)
Average percent polymorphism per locality (95% criterion)	20.4	21.0
Percent polymorphism for the whole population	28.6	24.1
Average mean heterozygosity for localities	0.095 (0.040)	0.087 (0.036)
Mean heterozygosity for the whole population	0.109 (0.041)	0.097 (0.036)
Range of Nei's (1978) genetic distance	0.000-0.040	0.000-0.032
Range of Rogers' (1972) genetic distance	0.021-0.099	0.021-0.090
F_{st} among all localities	0.160	0.162

Appendix. A FORTRAN program for correlogram analysis of the six morphological characters of *A. lineata* (The program for *A. reguloides* is basically similar except for the different division of distance classes and the number of sample sets. See sections 2.1 and 2.4).

```
      PROGRAM CORRELOGRAM
      INTEGER N,MAX
      PARAMETER(N=18,MAX=2001)
      INTEGER  M(MAX,N),DUPX(N,N)
      REAL  H(N),V(N),DX(MAX,N,N),DY(N,N),ZP(MAX)
      REAL  CUL(N),TAR(N),HIN(N),WIN(N),TAI(N),WEI(N)
C
      ISEED=9999999
      DO 5 I=2,MAX
10    Q1=RAN(ISEED)
      Q2=Q1*(10000000)
      K1=INT(Q2)
      IF(K1 .LT. 1000000) GO TO 10
      IF((-1)**K1 .EQ. 1) K2=K1-1
        IF((-1)**K1 .EQ. -1) K2=K1+2
C
      NL=0
20    R1=RAN(K2)
      R2=R1*100.
      L=INT(R2)
      IF((L .GT. 0) .AND. (L .LT. N+1)) THEN
       NL=NL+1
       IF(NL .EQ. 1) THEN
        J=1
         M(I,J)=L
       ELSE IF(NL .GT. 1) THEN
        DO 15 I1=1,J
        IF(M(I,I1) .EQ. L) GO TO 20
15      CONTINUE
        J=J+1
         M(I,J)=L
        IF(J .EQ. N) GO TO 5
       END IF
      END IF
      GO TO 20
5     CONTINUE
C
      TCUL=0
      TTAR=0
      THIN=0
      TWIN=0
      TTAI=0
      TWEI=0
      DO 25 I2=1,N
       READ(5,2) NS,HL,VL,CULMEN,TARSUS,HINDTOE,WING,TAIL,
     $ WEIGHT
2       FORMAT(3X,I2,1X,F8.5,1X,F9.5,1X,5(F7.4,1X),F6.4)
      M(1,I2)=NS
```

```
      H(I2)=HL
      V(I2)=VL
      CUL(I2)=CULMEN
      TAR(I2)=TARSUS
      HIN(I2)=HINDTOE
      WIN(I2)=WING
      TAI(I2)=TAIL
      WEI(I2)=WEIGHT
      TCUL=TCUL+CUL(I2)
      TTAR=TTAR+TAR(I2)
      THIN=THIN+HIN(I2)
      TWIN=TWIN+WIN(I2)
      TTAI=TTAI+TAI(I2)
      TWEI=TWEI+WEI(I2)
25    CONTINUE
      CULB=TCUL/N
      TARB=TTAR/N
      HINB=THIN/N
      WINB=TWIN/N
      TAIB=TTAI/N
      WEIB=TWEI/N
C
      DO 35 I3=1,N
       A=90.00-H(I3)
       DO 45 J3=1,N
        IF((H(I3) .EQ. H(J3)) .AND. (V(I3) .EQ. V(J3))) THEN
        AB=0.
        GO TO 30
        END IF
         B=90.00- H(J3)
         C=ABS(V(I3)-V(J3))
         COSAB=COSD(A)*COSD(B)+SIND(A)*SIND(B)*COSD(C)
         AB=3.1415926*6367.4675*ACOSD(COSAB)/180
30       DY(I3,J3)=AB
45    CONTINUE
35    CONTINUE
C
      CALL SUB1(CUL,CULB,M,N,MAX,DX,DY,ZP,DUPX)
      CALL SUB1(TAR,TARB,M,N,MAX,DX,DY,ZP,DUPX)
      CALL SUB1(HIN,HINB,M,N,MAX,DX,DY,ZP,DUPX)
      CALL SUB1(WIN,WINB,M,N,MAX,DX,DY,ZP,DUPX)
      CALL SUB1(TAI,TAIB,M,N,MAX,DX,DY,ZP,DUPX)
      CALL SUB1(WEI,WEIB,M,N,MAX,DX,DY,ZP,DUPX)
      STOP
      END
C
      SUBROUTINE SUB1(CH,CHB,M,N,MAX,DX,DY,ZP,DUPX)
      DIMENSION CH(N),DX(MAX,N,N),DY(N,N),ZP(MAX)
      INTEGER M(MAX,N),DUPX(N,N)
      CALL SUB2(1, 300.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(2, 500.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(3, 700.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(4, 900.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(5,1100.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(6,1300.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(7,1500.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      CALL SUB2(8,1770.,ZP,CH,CHB,M,N,MAX,DX,DY,DUPX)
      RETURN
```

```
      END
C
      SUBROUTINE SUB2(NC,UPX,ZPC,CH,CHB,M,N,MAX,DX,DY,DUPX)
      DIMENSION CH(N),DX(MAX,N,N),DY(N,N),ZPC(MAX)
      INTEGER M(MAX,N),DUPX(N,N)
      DO 55 I4=1,N
       DO 65 J4=1,N
       IF(DY(I4,J4) .EQ. 0) DUPX(I4,J4)=0
       IF((DY(I4,J4) .GT. 0) .AND.
     $     (DY(I4,J4) .LT. UPX)) DUPX(I4,J4)=1
       IF(DY(I4,J4) .GT. UPX) DUPX(I4,J4)=0
65    CONTINUE
55    CONTINUE
C
      IP=0
      DO 75 I5=1,MAX
      S=0
      SM2=0
      SM4=0
      DO 85 I6=1,N
        S=S+((CH(M(I5,I6))-CHB)**2)/N
        SM2=SM2+((CH(M(I5,I6))-CHB)**2)/N
        SM4=SM4+((CH(M(I5,I6))-CHB)**4)/N
        DO 95 J6=1,N
           DX(I5,I6,J6)=(CH(M(I5,I6))-CHB)*(CH(M(I5,J6))-CHB)
95    CONTINUE
85    CONTINUE
      B2=SM4/(SM2)**2
C
      S0=0
      TS1=0
      S2=0
      SCHY=0
      DO 105 I7=1,N
       SYI=0
       SYJ=0
       DO 115 J7=1,N
         S0=S0+DUPX(I7,J7)
          TS1=TS1+((DUPX(I7,J7)+DUPX(J7,I7))**2)
          SCHY=SCHY+(DX(I5,I7,J7))*(DUPX(I7,J7))
         SYI=SYI+DUPX(I7,J7)
         SYJ=SYJ+DUPX(J7,I7)
115        CONTINUE
         S2=S2+(SYI+SYJ)**2
105   CONTINUE
       S1=TS1/2
C
      NB=1
      DO 125 I=N-3,N-1
      NB=NB*I
125   CONTINUE
C
      Z=SCHY/((S)*S0)
      ENZ=(-1.)/(N-1)
      ERZ=ENZ
      VNZ=((((N**2)*S1)-(N*S2)+(3*((S0)**2)))/
     $       ((S0**2)*((N**2)-1)))-(ENZ**2)
      VRZ=(N*((N**2-3*N+3)*S1-N*S2+3*S0**2)/(NB*S0**2))
```

```
     $      -(B2*((N**2-N)*S1-2*N*S2+6*S0**2)/(NB*S0**2))
    $     -(ERZ**2)
      TNZ=(Z-ENZ)/SQRT(VNZ)
      TRZ=(Z-ERZ)/SQRT(VRZ)
c     WRITE(6,*) 'ENZ=ERZ=',ENZ,'  ','TNZ=',TNZ,'    ','TRZ=',TRZ
      ZPC(15)=Z
      IF(ZPC(15) .GE. ZPC(1)) IP=IP+1
75    CONTINUE
      PI=IP-1
      P=PI/(MAX-1)
      IF(P .LT. 0.05) WRITE(6,*) NC,'  ','OBSERVED Z=',ZPC(1),
    $                  '  ','P(Z>=OBSERVED)=',P,'*'
      IF(P .GE. 0.05) WRITE(6,*) NC,'  ','OBSERVED Z=',ZPC(1),
    $                  '  ','P(Z>=OBSERVED)=',P
      RETURN
      END
```

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