Conilurine Rodent Evolution:

The role of ecology in modifying evolutionary consequences of environmental change

Thesis submitted by
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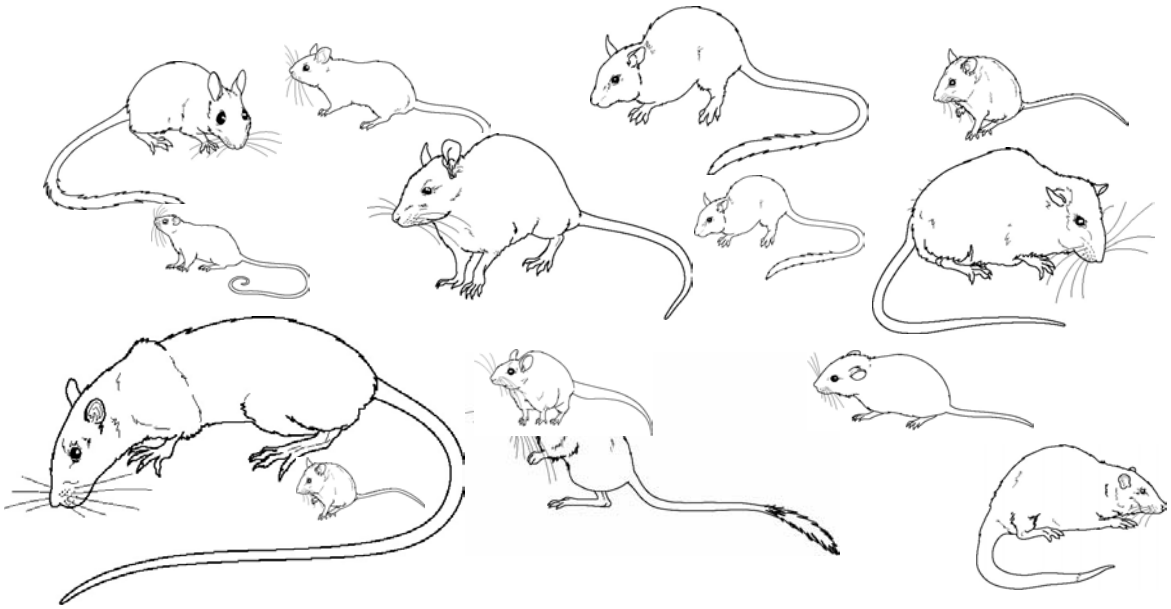
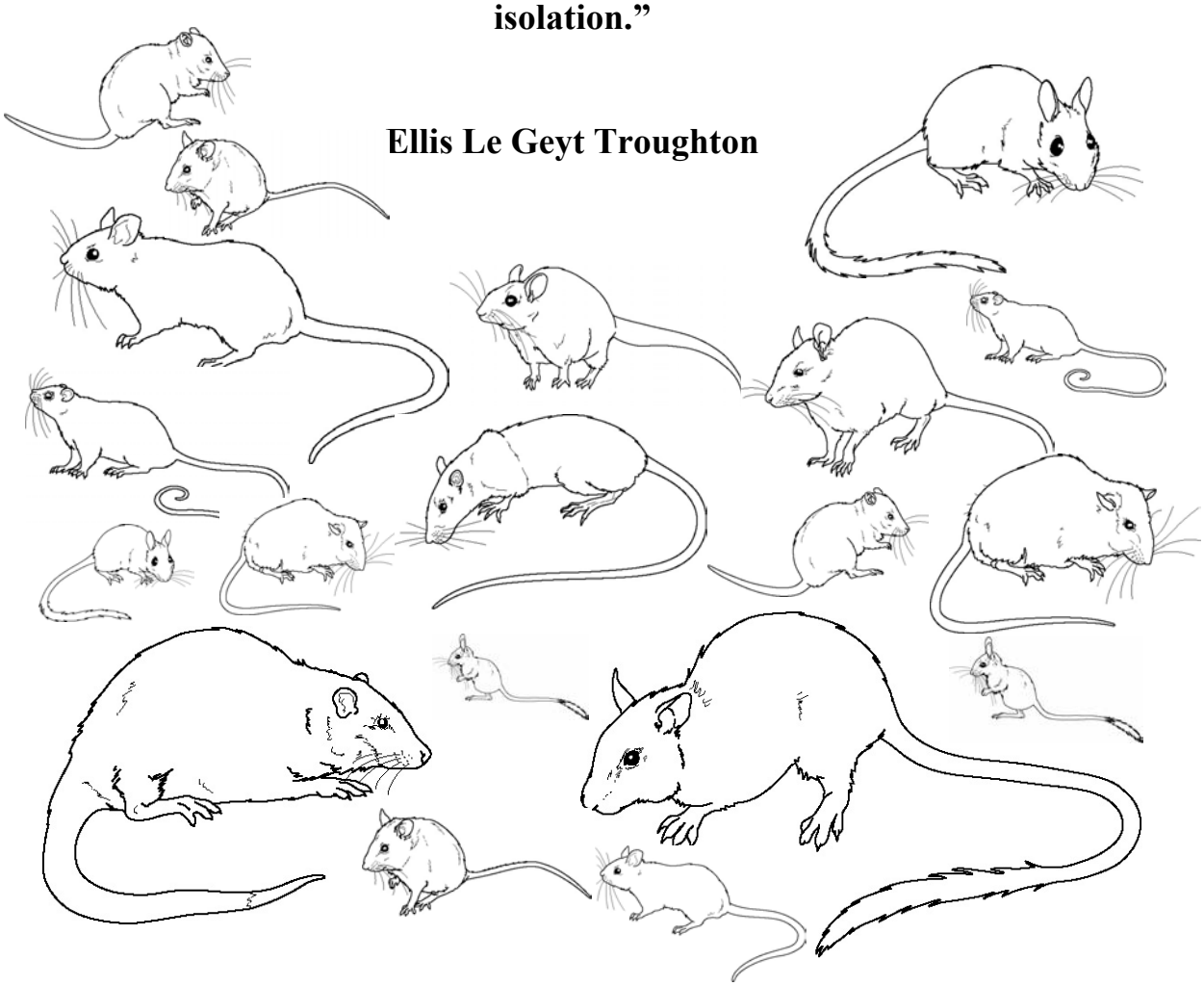


“Some of these exhibit the close similarity arising from their common ancestry abroad; others have evolved as peculiar types during prolonged isolation.”

Ellis Le Geyt Troughton



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Abstract

Conilurine rodents are the most speciose and ecologically diverse rodent group within Australia. Previous research has demonstrated that conilurines are members of a larger Australasian Murid tribe, the Hydromyini, and are generally thought to be an endemic Australian radiation with a fossil history dating to the mid Pliocene. However, monophyly of conilurines is not well established due to the distant placement of some genera in recent systematic studies. I investigated the evolution of conilurine rodents using a combination of molecular and ecological techniques. I examined phylogenetic relationships among all Australian rodent genera using the mitochondrial control region and a nuclear intron (DHFR 1). These results suggested that conilurines are monophyletic, and that unresolved generic relationships within the group are the product of an early savannah radiation. Building on the results of systematic studies, I examined patterns of speciation and population divergence in model species and species groups. Bioclimatic range modelling and some ecological studies were performed to aid interpretation of molecular evolution within these groups. The overall goal of this thesis is to provide a detailed account of some evolutionary processes affecting an interesting group for which such information is lacking, and to highlight the potential of conilurines for evolutionary study in an Australian context. A more specific goal is to investigate the effect that species ecology has on altering the evolutionary response of different species to the same environmental stimuli.

Molecular studies identified a number of taxonomic issues within the group. The genera *Notomys*, *Mastacomys* and *Pseudomys* formed a monophyletic group identified by previous studies. *Mastacomys* was placed within *Pseudomys*, with which it was recently synonymised, and *Notomys* was not clearly separable from *Pseudomys*. Both *Notomys* and *Mastacomys* should be recognised as genera. As a result, *Pseudomys* was split into several smaller genera. These included resurrection of the genus *Thetomys* for *P. gracilicaudatus* and *P. nanus*, and creation of a new genus *Phorolithomys* for the recently described pebble-mound mice. A further three groups are apparent within *Pseudomys*, but splitting of these at the generic level is contingent on further resolving the placement of several species among them. Some individuals previously described as *P. delicatulus* from north-western Australia

appear to be a morphologically cryptic species, and were placed outside the *P. delicatulus/ novaehollandiae* group in phylogenetic trees. The subspecies *P. delicatulus mimulus* has no genetic basis. *Pseudomys laborifex* is probably a subspecies of *P. johnsoni*, which is an unexpected result given the distribution of *P. laborifex* in the Kimberley, a region anticipated to contain the sib-taxon to the Top End *P. calabyi*.

Phylogeographic studies of variation in the mitochondrial control region revealed significant differences in the evolution of species groups as result of their differing ecologies. This contrast centres on the morphologically similar pebble-mound mice and delicate mouse group. These are the largest species groups within the current genus *Pseudomys*. Pebble-mound mice are characterised by the fact that they require pebbly habitat for breeding, and have therefore speciated in allopatry due to erosion and fragmentation of that habitat. Climatic change has also affected pebble-mound mice, and has driven recurrent extinction processes, in part re-enforced by the inability of pebble-mound mice to track climatic change across pebble-free habitats.

The delicate mouse group have distributions primarily determined by climate. This means they are periodically isolated and bottlenecked, but also experience secondary contact when conditions permit range expansion. A major finding was that there is no taxon corresponding to *Pseudomys pilligaensis,* and that individuals of both *P. delicatulus* and *P. novaehollandiae* have been included within that species. The genetic distinctness of *P. novaehollandiae* from *P. delicatulus* was no greater than that among individuals of *P. delicatulus*. Whether *P. delicatulus* and *P. novaehollandiae* represent sib-species or subspecies was not resolved, but evidence of clinal morphology and probable interbreeding suggests that they have not yet speciated. *Pseudomys hermannsburgensis*, an arid zone member of the delicate mice, showed little genetic variability, and no geographical structuring across a very large distribution. This result is consistent with ecological studies that show the species possesses morphological adaptations to aridity and exists in relatively mobile populations. A similar result was found for the co-distributed *P. desertor*.

Patterns of genetic structuring are not predictable based on crude habitat preference. It was thought *a priori* that *Zyzomys argurus* was a taxon with a similar habitat preference to pebble-mound mice and that it would also exhibit deep genetic structuring between allopatric populations found in rocky habitats across northern Australia. Previous isozyme studies did not find such a relationship, and this was confirmed by mtDNA sequencing. Unlike most other study species, there was no signal from the last glacial maximum, despite the fact that *Z. argurus* would not have been able to track climate change as many species did. A broad climatic tolerance and good dispersal ability seem to explain the lack of population structuring.

Amplification of nuclear copies of the mitochondrial control region hampered efforts to study the chestnut mice, *P. gracilicaudatus* and *P. nanus*. However, three clean control region sequences were obtained that apparently confirmed currently recognised species boundaries. Nuclear copies were also amplified from the pebble-mound mice, but primers were designed that prevented their hindrance of phylogeographic studies.

The characterisation of evolution among conilurine rodents presented here reveals that species ecology clearly accounts for many differences in patterns of population divergence and speciation. I suggest that ecological interpretation of evolutionary pattern can be extended to a general contrast between the entire conilurine assemblage and marsupials.

Acknowledgements

It is not hard to find those willing to travel the wilder parts of northern Australia, but it is often a struggle to find people who will embrace the reality of early mornings bashing through charcoal coated scrub just to scribe a few numbers and catch fleeting glimpses of small furry creatures. It is even more difficult to find people who will come back for more. Repeat offenders who provided invaluable help during field trips included Jane Antrobus, Zoe Baron, Mathu, Laura Inman, Ben Collins, Tabitha Lloyd, Allen McIllwee and Euan Ritchie. Others proved stoic enough to overcome both the hardships of fieldwork in remote locations and prolonged exposure to my rambling and sometimes (often?) demanding ways on long field trips- special thanks to Kathy Tsang, Dave Shevill, Chris Parker, Bob Wong, Daniel Keating and Kelli Gowland.

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Research detailed in this thesis has required constant high levels of funding. I again thank Chris and David for their efforts in securing and allowing me access to funding from the Australian Research Council and James Cook University Merit Research Grants scheme. Financial assistance was also provided by the Royal Zoological Society of New South Wales via an Ethel Mary Read scholarship, the Australian Geographic Society, a supplementary IRA grant and a Doctoral Merit Research Scheme grant awarded by School of Tropical Biology. I also received scholarship in the form of an Australian Postgraduate Award, and a completion scholarship from James Cook University, which is all that supports me as I write these acknowledgements. Research was carried out under ethics permits from the James Cook University (A547) and South Australian ethics committees (11/2002). Scientific permits: 7824, 9349 (Parks and Wildlife NT), N0/002424/99/SAA, F1/000391/00/SAA, F1/000391/02/SAA (Parks and Wildlife, QLD), 1444 (Natural Resources, QLD), permit M24574 and licence 92 (Environment and Heritage, SA), SF003492 (CALM, WA).

[1] and generally ignored

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Statement on Sources Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished work of others has been acknowledged in the text and a list of references is given.

_____ _____

Frederick David Ford

Terminology and nomenclature

Place names

Australian locations are referred to by the name in general Australian usage. For example "the Pilliga" refers to the Pilliga scrub of the western plains of northern New South Wales, "the Kimberley" to the Kimberley region of north-eastern Western Australia, and the "VRD" to the Victoria River District of the north-western Northern Territory. "New Guinea" refers to the entire island of New Guinea, not just the country of Papua-New Guinea. Important places frequently referred to in the text are identified on the map of Australia below.

Rodent nomenclature

Names of Australian rodent species and genera follow Mackenzie and Burbidge (2002). Native names given by Mackenzie and Burbidge (2002) are given at the first mention of the species' common name, e.g., delicate mouse (Molinipi; *P. delicatulus*). Following Watts and Baverstock (1994a) "Hydromyini", or hydromyine, refers collectively to all members of the previously defined tribes Conilurini, Hydromyini and Uromyini. "Conilurine" is used here to refer to genera previously included in the tribe Conilurini (*Conilurus, Leggadina, Leporillus, Mastacomys, Mesembriomys, Notomys, Pseudomys, Zyzomys*). The genera *Hydromys* and *Xeromys* are referred to as "water rats" and the genera *Uromys* and *Melomys* as "mosaic-tailed rats".

A number of taxonomic revisions are suggested in chapters 4-6. These are not formally proposed here, and this thesis does not constitute a published work under the Zoological Code of Nomenclature article 8.2 "Publication may be disclaimed". Therefore suggested names contained herein should not be considered a nomenclatural revision. Formal proposal of names will follow in appropriate journals, in some cases pending further research.

Other terms

Times and timescales are represented using geological nomenclature; million years ago- Ma, thousand years ago- ka. Time periods of relevance to this thesis are the Tertiary period (65Ma-~1.6Ma), which included the Miocene (22.5-5.5Ma) and Pliocene (5.5-~1.6Ma) epochs, and the Quaternary period (~1.8Ma- present), which consisted of the Pleistocene (~1.6Ma-10ka) and Holocene (10ka-present) epochs. The last glacial maximum is referred to as the LGM, and locally corresponds to the period 22-18ka. "Savannah" refers to those vegetation communities in northern Australia considered savannah by the Tropical Savannahs CRC, less the Gulf Plains and Mitchell Grass Downs, and includes grass-dominated monsoonal ecosystems, and those in which a grass-dominated understorey is present with varying degrees of tree cover, including woodland and open forest. Genetic

terms: base pair- bp; genomic DNA- gDNA; insertion-deletion mutation- indel; isolation-by-distance- IBD; Kishino-Hasegawa test- K-H test; mitochondrial DNA- mtDNA; polymerase chain reaction- PCR. “Sib-species” and “sib” are used to refer to recently diverged taxa that are each others closest relatives in phylogenetic reconstruction.

Chapter 1. General Introduction

1.1 Explaining the spectacular radiation

1.1.1 MICE, RATS AND MANY QUESTIONS

Rodents are a significant part of the Australian vertebrate fauna. Around 70 species in 13 genera are thought to have been present at the time of European colonisation, representing one quarter of the continent's mammals (Mackenzie and Burbidge 2002). Almost 50 species stem from a single late Tertiary colonisation that gave rise to the conilurine rodents (Lee et al. 1981), a radiation that has been described as "spectacular" (Ride 1970, Happold 1976). The ecology of some species is well researched (Cockburn 1980, Kemper 1990, Fox et al. 1994, Luo et al. 1994, Luo and Fox 1995, 1996, Read and Tweedie 1996, Smith et al. 1996, Smith and Quin 1996a, Haering and Fox 1997, Smith et al. 1997, Jerry et al. 1998), and Happold (1976) was fervent in her promotion of the group as an evolutionary model. However, although patterns of diversification (as species phylogenies) have been researched (Baverstock et al. 1981, Lidicker and Brylski 1987, Godthelp 1989, Watts et al. 1992), few studies have addressed intra-specific evolution in the group in detail (Jerry et al. 1998, Moro et al. 1998, Myroniuk 1998), and speciation processes are essentially unknown.

Many aspects of the conilurine group beg explanation. Their diversity has evolved over a short period (Baverstock 1984), often in the absence of any obvious geographical barriers. Systematic relationships are not resolved, particularly within the genus *Pseudomys* (Baverstock et al. 1981, Lidicker and Brylski 1987, Godthelp 1989, Watts et al. 1992), which contains almost half the conilurine species (Mackenzie and Burbidge 2002). There are few recognised sib-species (Lee et al. 1981). Conilurines have had great success in arid environments (Watts and Aslin 1981). However, although they probably evolved from New Guinean rainforest stock, they are rare or absent in wetter habitats in Australia (Lee et al. 1981, Baverstock 1984). The hopping mice (genus *Notomys*) are speciose (Mackenzie and Burbidge 2002), have greatly derived morphology (Watts and Aslin 1981), unique

reproductive physiology (Breed 1981, 1986) and have been accorded a very long history by some authors (e.g. Watts and Aslin 1981). However, these desert-living rodents are highly adapted to environments that are less than one million years old (Bowler 1982). It is not clear why the evolution of some characters, such as chromosomes (Baverstock et al. 1977b, Baverstock et al. 1983) and sperm morphology (Breed 1997), has been unusually conservative among all conilurine species, despite the marked ecological and morphological diversity of the group. With so many potential areas for investigation, this thesis focuses on building an empirical data set describing patterns of speciation and diversification, topics that until now could only be the subject of speculation. Essentially, I am trying to "get the ball rolling" by formulating evolutionary hypotheses for conilurine rodents based on evolutionary patterns within model species groups.

The empirical framework presented in this thesis is derived from nested molecular studies. These describe evolutionary patterns among Australian rodents at scales ranging from complete species phylogenies (chapter 4) to intra-specific population divergence (chapters 5-8). In addition to the studies detailed in this thesis, my research program involved significant bodies of work not reported here. In particular, I conducted ecological and behavioural studies that form important background for evolutionary studies of pebble-mound mice reported in chapter 5. Species ecology is a fundamental influence on evolution (Schliewen et al. 2001), and this is particularly so in Australia where events such as marine incursions or mountain building are rarely the cause of population isolation (Palaeogeographic Group 1990). Instead, climatic change has driven geographical isolation of populations (Keast 1961, Ford 1978, Nix 1982). This fact was central to the choice of species groups studied here. The two main groups, the pebble-mound mice and delicate mice, have fundamentally different ecologies despite very similar morphologies. One of the major questions tested here is whether the two groups differ in their evolutionary history despite having been subjected to the same climatic influences.

1.2 Focal questions

1.2.1 Systematic relationships

Various molecular and morphological methods have been applied to resolving the phylogeny of Australian rodents over the last twenty-five years (Baverstock et al. 1977b, Baverstock et al. 1981, Baverstock et al. 1983, Lidicker and Brylski 1987, Watts et al. 1992, Torrance 1997). Taxonomic studies before that time tended to suggest relationships and nominate species groups (Thomas 1910, Iredale and Troughton 1934, Tate 1951, Crabb 1977), and some speculation on ancestral characters was made. A review of systematic studies reveals moderately well resolved relationships between major Australian rodent lineages, but leaves many generic and species relationships unresolved. Australian rodents belong to at least three distinct lineages (Lee et al. 1981, Watts and Baverstock 1994a); 1) the tribe Hydromyini, 2) one species of *Pogonomys* and 3) *Rattus* species. Conilurine rodents, the focus of this thesis, are members of the Hydromyini (Watts et al. 1992). However, it is not clear from previous systematic studies whether conilurines are a monophyletic group (Baverstock et al. 1981, Lidicker and Brylski 1987, Watts et al.1992, Torrance 1997), despite the fact that all conilurine genera are endemic to Australia, and obviously closely related (Watts and Kemper 1989, Strahan 1995). Within the conilurine fauna, systematic relationships among some genera are well established, with the genera *Mastacomys*, *Notomys* and *Pseudomys* forming a strongly supported monophyletic assemblage (Baverstock et al. 1977b, Baverstock et al. 1981, Baverstock et al. 1983, Watts et al. 1992), and the tree rats *Conilurus* and *Mesembriomys* closely related to each other (Misonne 1969, Watts et al. 1992, Torrance 1997). Other genera are labile in their apparent relationships, and *Leggadina* has fallen outside the conilurine group in two separate studies (Baverstock et al. 1981, Watts et al.1992), despite historical synonymy with *Pseudomys*. *Pseudomys* is a genus of increasingly dubious status. Nearly half the conilurine rodents belong to *Pseudomys* (Mackenzie and Burbidge 2002), and recent molecular studies have shown that the long recognised genus *Mastacomys* consistently falls within the generic boundaries of *Pseudomys* (Baverstock et al. 1981, Watts et al. 1992, Torrance 1997). The

highly morphologically derived genus *Notomys* has also been included within *Pseudomys* in some analyses (Baverstock et al. 1981, Torrance 1997).

In the first results section of this thesis (chapter 4) I aimed to:

- Resolve whether conilurines are monophyletic with respect to the mosaic-tailed rats, which occur in both New Guinea and Australia
- Resolve phylogenetic relationships among Australian rodent genera (including non-conilurine genera)
- Establish species relationships within the currently defined genus *Pseudomys* with respect to *Mastacomys* and *Notomys*, and propose, where appropriate, new generic boundaries

1.2.2 SPECIATION STUDIES

Model groups

The major goal of this thesis was to describe patterns of divergence between closely related conilurine taxa, including populations, subspecies, sib-species and members of species groups. Such an aim is clearly unfeasible for the entire conilurine fauna. However, a prudent choice of model species groups allows general statements about the evolution of the entire group to be made. Conilurines are an ideal group for this kind of study, because they have a relatively short Australian history, and macro-evolutionary patterns are less likely to be masked by intermediate events than in the much older marsupial fauna. There are apparently few closely related conilurine species (Baverstock et al. 1981, Lee et al. 1981), and of those that are, few are still distributed throughout their natural range (Smith and Quin 1996b), so a further aim of systematic studies was to identify groups of closely related species that may not have been previously identified. Two groups within the genus *Pseudomys* were thought *a priori* to be ideal, and systematic studies (chapter 4) confirmed the monophyly of each. The pebble-mound mice and *Pseudomys delicatulus* and its close relatives (the "delicate mice") are both groups of five currently recognised species. They

are widely distributed (fig. 1.1), subjecting them to processes influencing many other conilurine species.

Pebble-mound mice and delicate mice are the smallest Australian rodents, with the average weight of most species between 10 and 15 grams. They are morphologically very similar, and where species of the two groups occur in sympatry, it is only in the last twenty years or so that a taxonomic distinction has been made between them (Kitchener 1980, 1985, Kitchener and Humphries 1986, 1987, Van Dyck 1991). The delicate mice are important in the context of Australian rodent biogeography, because they include a south-eastern Australian member, *P. novaehollandiae*, and a central Australian member, *P. hermannsburgensis*, that are still found at locations scattered throughout most of their known historical and pre-historical distributions (Breed 1995a, Kemper 1995). Delicate mice and pebble-mound mice also complement each other as study groups because the delicate mice exhibit a preference for widespread lowland habitats (Braithwaite and Covacevich 1995, Breed 1995a, Fox 1995a, Kemper 1995, Watts 1995a), and are thus widely distributed, with members of the group being parapatric and sympatric, while species of pebble-mound mice have allopatric distributions on rocky uplands (Kerle 1995a, Start and Kitchener 1995, Woinarski et al. 1995, Van Dyck and Birch 1996).

There are questions regarding the taxonomic status of regional populations currently described as *P. delicatulus* (Breed 2000), and Lee (1995) regarded establishing the taxonomy and systematics of the delicate mice as a priority, particularly the species status of *P. pilligaensis*. There have also been numerous discoveries of new populations of pebble-mound mice scattered between the locations from which species descriptions were originally made. There is thus a real need for population-level sampling of all small *Pseudomys* species to establish phylogenetic relationships and species boundaries. This type of study allows powerful inferences to be drawn regarding the processes leading to divergence of those species, particularly where differences are apparent between pebble-mound mice and delicate mice, morphologically similar species that fundamentally differ in their distributional patterns as a result of their habitat preference.

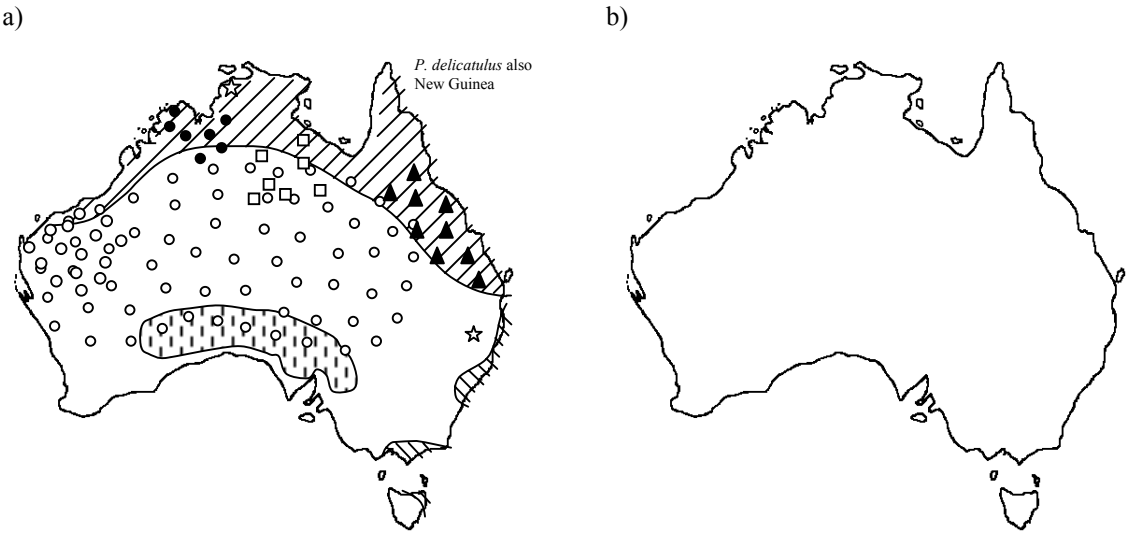


Figure 1.1. Distribution of pebble-mound mice and delicate mice
a) Pebble-mound mice: *P. calabyi* ☆ ; *P. chapmani* ○ ; *P. johnsoni* □ ; *P. laborifex* ● ; *P. patrius* ▲
b) Delicate mice: *P. bolami* ⓘ ; *P. delicatulus* ⊘ ; *P. hermannsburgensis* ○ ; *P. novaehollandiae* ⊘ ; *P. pilligaensis* ☆ .

Other conilurines

Body size is a basic species characteristic that influences many aspects of ecology including vagility, diet and population dynamics (Begon et al. 1996). It is therefore desirable to examine how differences in body size may influence species-specific evolution in response to changing environment. In Australia, the influence of body size is unfortunately most obvious in the disappearance of "critical weight range" animals (35-5500g) following European occupation (Burbidge and Mackenzie 1989). Larger conilurines were severely affected, particularly in the arid zone. Critical weight range savannah rodents have fared reasonably well. Although not large among the savannah rodents, the chestnut mice are roughly three to five times the size of delicate and pebble-mound mice (30-100g). They are generalist herbivore/omnivores that occur in a range of habitats (Luo et al. 1994, Fox 1995b, Robinson 1995). The eastern chestnut mouse, *P. gracilicaudatus*, is distributed along the east coast, as well as in north-eastern savannahs (Fox 1995b). The western chestnut mouse, *P. nanus*, was formerly distributed through arid areas along the west coast,

but has since contracted to northern areas of its distribution in the Kimberley and Top End (Robinson 1995). The chestnut mice are superficially similar to the desert mouse, *P. desertor*, an ecologically analogous species with a range now known to encompass most of the arid zone (Kutt et al. in press). The chestnut mice and *P. desertor* have distributions that are very similar to those of *P. delicatulus* and *P. hermannsburgensis*, and to some extent *P. novaehollandiae*, and therefore provide a suitable test of the effect body size may have on divergence among widespread species.

To complement the chestnut mice and *P. desertor* as an analogue of the delicate mice, it is desirable to identify a group with relatively larger body size that exhibits similar distribution patterns to pebble-mound mice, and a similar specialisation to rocky habitats. No such group exists, but a single species, *Zyzomys argurus*, is distributed across northern Australia, and is generally restricted to rocky habitats that provide it with suitable nesting locations (Fleming 1995, Trainor et al. 2000). Its distribution is similar to the combined distribution of the five species of pebble-mound mice, and populations are allopatric in the same regions. *Zyzomys argurus* is thus an interesting comparison as a larger-sized (30-50g), specialised, taxon to compare with the larger-sized, widespread, chestnut mice. The distribution of one species, *Z. argurus*, across a range that includes five allopatric species of pebble-mound mice also allowed comparison of geographical structuring of clades in response to body size, and assessment of the validity of taxonomic rank assigned to populations with equivalent geographical distributions and habitat preference.

The specific aims of speciation studies were:

- For the pebble-mound mice, delicate mice, chestnut mice, *Pseudomys desertor* and *Zyzomys argurus* establish, where possible:
    a. Evolutionary relationships between populations
    b. The number of species
    c. Probable causes of observed patterns of diversification

The synthesis of these studies allowed general inferences to be made regarding broader patterns of conilurine evolution. Specific modifications to those patterns may be key to understanding the role ecology has played in speciation processes in late Tertiary and Quaternary Australia.

1.2.3 Ecology and diversification: evolution played out on different stages

The two major species groups discussed here, the pebble-mound mice and delicate mice, are excellent models for testing the notion that ecology may produce different evolutionary relationships among co-distributed species groups with different ecologies. One under-emphasised aspect of biogeography in northern Australia is the large fluctuations in coastline throughout the Pleistocene. The present day coast is an atypical situation, yet it is the basis for discussion of areas of endemism (Keast 1961, Cracraft 1982). Many species have the opportunity to exploit areas to the north of Australia when sea levels are lowered (section 2.3.3), while some cannot exploit the entire area bounded by the current coastline. For instance, the pebble-mound mice are apparently restricted to hilly or pebbly landscapes, while delicate mice are not particularly constrained by topography (see above). The present day 200m asl contour is a rough proxy for hilly landscapes, and therefore the maximum possible extent of the distribution of pebble-mound mice in the last 20ky. The 150m bsl contour is a proxy for the maximum extent of the distribution of more generalist delicate mice, a pattern re-iterated throughout the Pleistocene due to changing sea level (fig. 1.2; Chappell and Shackleton 1986, Haq et al. 1987). Climate will have been an important influence on both groups, but only the delicate mice have the capacity to fully trace climatic changes across the landscape. Thus, there are delicate mice in southern New Guinea (Flannery 1995), but no pebble-mound mice. Many regions occupied by pebble-mound mice are ancient, stable, landscapes, and a lack of recent mountain building means that the extent of suitable habitat for pebble-mound mice has probably only decreased in area since rodents colonised Australia (Palaeogeographic Group 1990, Twidale and Campbell 1995).

Figure 1.2. Approximate maximum distributional limits of pebble-mound mice and delicate mice.
Inner line 200m asl contour (from BIOCLIM data), which roughly equates to maximum distribution of pebble-mound mice in northern Australia. Outer line current 150m bsl contour/shoreline during LGM (Palaeogeographic Group 1990, Yokoyama et al. 2001) that encloses maximum distribution of delicate mice (which also includes areas above 200m).

Distributions, areas of endemism and speciation

A major confounding factor in any analysis of speciation is a limited ability to extrapolate past events from current species distributions (Endler 1982). Distributions of Australian vertebrate species are generally fragmented into a number of reasonably well-defined areas of endemism (Heatwole 1987, Cranston and Naumann 1991), and sib-taxa are often restricted to complementary areas of endemism isolated by arid corridors (Keast 1961, Ford 1978). However, the isolation of several major areas of endemism has been periodic throughout the late Pleistocene, and initial segregation of populations was probably a

moderately recent event (mid? Pleistocene) (Bowler 1982, Archer and Fox 1984). Timescales involved in isolation of such populations have often not been sufficient to allow speciation according to many authors (Smith-White 1982, Avise et al. 1998, Knowles 2001). This fact is highlighted among rodents by Watts and Aslin's (1981) assertion that the sib-species *Pseudomys albocinereus* and *P. apodemoides* were probably isolated by rising sea levels following the LGM, 16-18Ka. This biogeographical scenario is probably correct, but speciation over an 18ky period simply due to allopatry is highly improbable among conilurine rodents, which, unlike native *Rattus*, do not seem pre-disposed to processes such as chromosomal re-arrangements (Baverstock et al. 1977b,c,d) that could lead to rapid speciation.

I explored some of these issues by focussing on the northern savannah conilurine fauna. This is the richest rodent community in Australia, and is partitioned into three major allopatric refuge areas. Relatively recent arid barriers in the form of the Great Sandy Desert and Carpentarian Barrier separate the major refugia. By virtue of the fact that part of the Carpentarian Barrier is the Gulf of Carpentaria itself, this barrier is only a periodic isolating mechanism for most species, as the floor of the Gulf is exposed when sea levels fall below 50m bsl (section 2.3.3; Chivas et al. 2001). The Great Sandy Desert is probably a more severe long-term barrier. Most rodent distributions are affected by these barriers, yet current taxonomy suggests very different relationships between allopatric populations among the different groups. This provided an excellent opportunity to contrast events leading to speciation and more recent events dictating species distributions.

1.3 Reconstructing evolutionary pattern

To address the aims of speciation studies, population sampling was required across the distribution of the species involved. This allowed construction of molecular phylogenies that traced an evolutionary continuum of divergence from populations through to species groups. Previously, intra-specific taxa such as subspecies have been based solely on morphological variation, which is often subject to natural selection or random effects such as drift, and may reflect little of the evolutionary history of the taxa involved (Thorpe et al.

1995, Salzburger et al. 2002a). Population genetics can determine the level of genetic divergence or gene flow among populations (Goldstein et al. 1995, Hartl and Clark 1997, Parker et al. 1998), but DNA sequencing of neutral markers can further reveal the evolutionary relationships between populations (i.e. the genetic divergence of populations through time) in the absence of effects due to natural selection (Avise et al. 1987, Bermingham and Moritz 1998). MtDNA has become the primary tool of such investigations, because it does not recombine, and therefore preserves historical signal (Avise et al. 1987, Templeton et al. 1995). There is an enthusiastic literature dedicated to exploring ways of utilising the new types of information that can be gleaned from DNA sequencing methods, and the study of intra-specific mtDNA phylogenies has become known as phylogeography (Fitch 1995, Harvey et al. 1995, Templeton 1998).

Trees based on mtDNA essentially identify patterns of cladogenesis rather than speciation. This is due to two factors; 1) in many biologists view, including mine, speciation is the result of permanent cessation of gene flow between previously interbreeding populations (Briddle and Jiggins 2000, Coyne and Orr 1998) and 2) mitochondrial DNA cannot record gene exchange due to its matrilineal pattern of inheritance (Avise et al. 1997). Thus, nuclear introns are also examined here to corroborate findings of mtDNA analysis. Should obvious divergence at nuclear loci occur between mtDNA taxa in geographical proximity, than a strong case for specific difference can be made. Such was the case with the original description of *Pseudomys bolami*, a member of the delicate mice. Fixed differences at isozyme loci between nearby populations of *P. bolami* and *P. hermannsburgensis* were the basis for the re-description of the sub-species as a full species (Kitchener et al. 1984). The field of phylogeography relies on the fact that intra-specific taxa are commonly encountered in mitochondrial studies, and determining the species status of some mtDNA taxa identified in this thesis is impossible based on results presented here. Details of these taxa are provided in results chapters, in particular chapter 6. As a result of the ambiguity of these results, the contentious field of species definitions is for the most part ignored in favour of a more general interpretation of patterns of the cladogenesis, which are unambiguous, and suitably highlight the potential differences in modes of speciation between study groups.

Analysis of intra-specific genetic variation can rely on simple phylogenetic methods. Evolutionary relationships among populations can be examined using normal tree building procedures, and molecular subspecies and their geographical distributions easily identified. However, new techniques have been developed that attempt to remove some of the speculation involved in interpretation of phylogenetic trees. Nested clade analysis is a technique developed by Templeton (1993) and Templeton et al. (1995) that compares the geographical distribution of clades at a series of nested levels. The exact nested contingency test of Templeton and Sing (1993) allows a non-subjective estimation of whether significant departures from the expected pattern of genetic relatedness occur over given distances (i.e. departures from panmixia due to restricted gene flow and geographical association of haplotypes). Further exploration of the cause of restricted gene flow is provided by comparison of the geographical distribution of haplotypes within the nested clade to the distribution of the entire clade. An inference key (Templeton et al. 1995, Templeton 1998) allows the probable cause of restricted gene flow to be determined where geographical sampling of populations is adequate. This method has been criticised, in particular the inference key, as it does not allow an estimation of error, or give any indication of how much better the explanation provided by the key is than are alternative interpretations (Knowles and Madison 2002). Nevertheless, nested clade analysis provides an additional interpretive tool, and much of the doubt surrounding the validity of answers provided by the inference key can be removed by taking into account other information such as palaeoclimatic data. Nested clade analysis was used to explore the spatial and temporal relationships of populations of six species in chapters 5-7.

Palaeoclimatic history, comparative patterns in co-distributed species, and climatic distribution modelling were additional lines of evidence used to aid interpretation of species evolution from patterns of molecular evolution. Distribution modelling using BIOCLIM (Nix 1986) aimed to elucidate what critical factors determine the current distribution of some species examined in chapters 5-7, and by inference, what the effect of known changes in past climates or geology might have been.

1.4 Thesis structure

Section1. *Background to Australian rodent evolution*.
Includes chapters 1-3 detailing the known evolutionary history of Australian rodents and the methods used in this thesis.

Section 2. *Systematic framework for Australian rodent biogeography*.
Includes chapter 4, which presents new phylogenetic studies and a synthesis of past taxonomic and systematic work.

Section 3. *Phylogeographic case studies of conilurine rodents*.
Includes chapters 5-8 describing and attempting to explain patterns of molecular divergence evident in model rodent groups outlined above.

Section 4. *Ecology, evolution and conilurine rodents*.
Includes chapter 9, which gives an overview of patterns of conilurine evolution at all taxonomic scales. The effect that ecology has had on patterns of evolution is explored, and the relationships of rodent evolution to that of marsupials is discussed in terms of large-scale processes framed against the background of new neutral models of macroecology.

Chapter 2. Murid rodents in Australia

CHAPTER SUMMARY

In this chapter I review evidence that Australian rodents are the result of a series of recent invasions. Most are members of the tribe Hydromyini, which includes three groups (the conilurines, mosaic-tailed rats and water rats), but two non-hydromyine rodent genera occur in Australia; *Pogonomys* and *Rattus*. The conilurine rodents are the oldest and most diverse rodent lineage within Australia, with a probable history on the continent of five million years. Other groups invaded more recently, some making use of land connections with New Guinea during the Pleistocene, while others may have arrived in the late Pliocene. The complex evolutionary history of landscapes and faunas of the Australasian region makes determining phylogenetic relationships difficult. Even the monophyly of the conilurine lineage is questionable on the basis of systematic studies, despite the endemism of the group within Australia. Irrespective of the relationships of conilurines to other groups, it is only conilurine genera that show extensive speciation within Australia. Species diversity of conilurines is centred on the tropical savannahs and central deserts. This contrasts with the mesic preferences of most Australian vertebrate groups, including other rodent lineages. The history of savannah and arid zone habitats suggests that the diversity of savannah conilurines is the result of an ancestral radiation, which is now relict, and arid zone faunas are a recently derived sub-set of savannah faunas. There is a marked lack of conilurines in wetter habitats, and this is unlikely to be the result of marsupial competition. Rather, the history of conilurines may have been constrained to some extent by an initial success in seasonally arid savannah habitats, which allowed further success in desert environments as they developed, but precluded invasion of tropical rainforests.

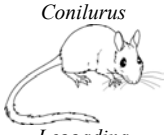
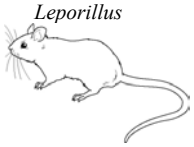
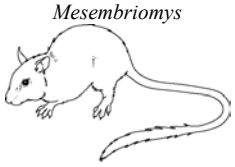
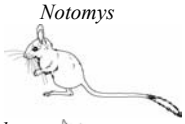
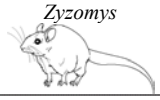
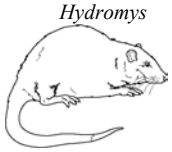
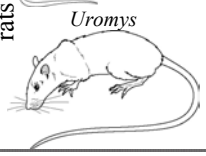
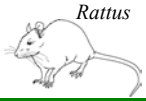
2.1 The modern fauna

2.1.1 The largest Australian family

Mckenzie and Burbidge (2002) recognised 69 species of native Australian rodent, all within the family Muridae, sub-family Murinae. This represents 27% of the native mammal fauna. Australian rodents range in size from the delicate mouse (*Pseudomys delicatulus*) that may reach maturity at 6g (Braithwaite and Covacevich 1995), to the large rats *Hydromys chrysogaster* (avg. 755g, but up to 1275g) and *Mesembriomys gouldii* (avg. 728g) (Watts and Aslin 1981, Strahan 1995). Rodents inhabit every Australian environment, and physiological adaptations to specialised lifestyles are evident in a number of taxa, notably the aquatic water rats (*Hydromys*) and desert-living hopping mice (*Notomys*) (Watts and Aslin 1981). Five distinct groups are recognisable within the Australian rodents (table 2.1), and have been ascribed familial, sub-familial or tribal status in the past (Simpson 1961, Watts and Aslin 1981, Lidicker and Brylski 1987). The current classification in Australian literature unites three groups, the endemic Australian genera (previously the tribe Conilurini (Dahl 1897, Watts and Aslin 1981) and subfamily Pseudomyinae (Simpson 1945)), the water rats (previously the tribe Hydromyini (Watts and Aslin 1981) and subfamily Hydromyinae (Thomas 1896)) and the mosaic-tailed rats (previously the tribe Uromyini (Watts and Aslin 1981)) in a single tribe, the Hydromyini, within the subfamily Murinae (Watts and Baverstock 1994b). The water rats and mosaic-tailed rats also occur in New Guinea, where they are far more diverse than in Australia (Flannery 1995). A single species of prehensile-tailed rat (*Pogonomys* sp.) and eight species of *Rattus* also occur in Australia (Mackenzie and Burbidge 2002). Combined, these groups constitute the most speciose family of Australian mammals. In this thesis the Australian endemic rodent genera are still described as conilurines (Dahl 1897), as they form a long recognised natural grouping that appears to have systematic support (chapter 4). Other hydromyine groups are referred to simply as mosaic-tailed rats and water rats. The Hydromyini and *Pogonomys* sp. have been described as "old endemics" in recognition of an older Australasian origin than species of *Rattus*, which have been labelled "new endemics" (Tate 1951, Watts and Aslin 1981).

Table 2.1. Native rodents of Australia.

Illustrations courtesy of Alice Whetherell. Notes from Watts and Aslin (1981) and Strahan (1995).

	Genus	N# spp. (extinct)	Distribution and habitat notes
Conilurines	*Conilurus* 	2 (1)	Nth and E Australia (1 sp. also southern New Guinea) Woodlands. Arboreal nesting rats that also forage on the ground. 100 - 240g
	Leggadina 	2	Nth Australia Dry ridges, grasslands, arid interior. 15 - 25g
	Leporillus 	2 (1)	SW and Sth Australia Built familial stick nests across much of the southern arid zone. Now one extant species confined to offshore islands. ~100 - 450g
	Mesembriomys 	2	Nth Australia Tropical Woodlands. Scansorial rats that favour *Pandanus*, other fruits and plant material. 200 - 880g
	Notomys 	10 (5)	Predominantly Sth and Central Australia Arid areas. Some populations may irrupt during favourable seasons. Highly adapted to arid conditions. 25 - 100g
	Pseudomys *ys* 	26 (2?) 1 *Mast*.	Australia (1 sp. also New Guinea) Wide range of ecologies. Predominantly savannah, semi-arid, arid areas and heath. 6 - 145g
	Zyzomys 	5	Nth Australia Nest in rock crevices. Monsoonal rainforest pockets, savannah and arid areas. 25 - 185g
Water rats	*Hydromys* 	4	New Guinea (1 sp. Australia) Permanent watercourses. Preys on crustaceans and is the most diurnal Australian rodent. 340 - 1275g
	Xeromys 	1	Nth Australia and New Guinea Mangroves. Constructs mud houses that protect against high tides. 32 - 55g
Mosaic-tailed rats	*Melomys* 	~14	New Guinea (3 spp. Nth Australian mainland, 3? endemic. 1 sp. offshore) Rainforest and surrounding habitat. Agile climbers, forage in canopy and on ground. 25-125g
	Uromys 	3	New Guinea (2 spp. NE Australia, 1 endemic) Rainforest. Formidable omnivores. 170 - 890g
	Pogonomys 	4 or 5	New Guinea (1 sp. NE Australia, may be endemic) Rainforest. Arboreal forager very rarely encountered in Australia. 40 - 80g
	Rattus 	>50	Mainly SE Asia (8 spp. Australian mainland, 6 endemic, 2 also New Guinea. 1 sp. offshore Australia probably introduced). 25 - 280g

2.1.2 DISCOVERY AND DESCRIPTION

Early explorers of the Australian interior often noted rodent species such as *Leporillus apicalis* and *Conilurus albipes,* both now extinct (Mitchell 1839, Sturt 1849, Smith and Quin 1996b). It is an unfortunate fact that pastoralism and invasion of feral species far outpaced scientific endeavours in central Australia. In regions such as western New South Wales the rodent fauna was once thought to be meagre, but fossil evidence has revealed the presence of may taxa not historically recorded from the region (Dickman 1994, Ellis 1995, Dickman et al. 2000). Ten conilurine species recognised by Mackenzie and Burbidge (2002) are known only from limited material. Lack of museum material, ecological, and distribution information for some of these species makes accurate assessment of their taxonomic status difficult (Watts and Aslin 1981).

Following the initial phase of discovery and description of Australian rodents Gould (1863) recognised 27 species of Australian rodent in three genera; 1) *Hapalotis*, which included species now placed in *Conilurus*, *Mesembriomys*, *Notomys* and *Pseudomys*, 2) *Hydromys* and 3) *Mus*, which included species now in *Melomys*, *Rattus* and *Pseudomys*. These were predominantly described on the basis of external characters, but 13 of the 16 conilurine species recognised by Gould (1863) are still regarded as valid. Specimens of a single species, *Pseudomys australis*, were included in both *Mus* and *Hapalotis* (table 2.2). Gould (1863) did not recognise the previously described genera *Conilurus* Ogilby 1838 (see Mahoney (1982) for nomenclatural comments on *Conylurus* Ogilby 1837), *Notomys* Lesson 1842, or *Pseudomys* Gray 1832, although he gave an account of species belonging to those genera, including the generic types. In the late nineteenth and early twentieth century Thomas (1882, 1889, 1906b, a, Thomas and Dollman 1909, Thomas 1910, 1921) was active in describing new genera and species of Australian rodent, and collectors such as Stalker were visiting remote locations that yielded many new species (Thomas 1906b, Thomas and Dollman 1909). In the wake of Thomas' work, Troughton reviewed the status of many taxa, and also described a number of new species (Troughton 1923, 1932, Iredale and Troughton 1934, Troughton 1936, 1937). Tate (1951) also conducted a major taxonomic review of Australasian rodents. Among the conilurine rodents (the "*Pseudomys*

group" of Troughton (1941) and the "Australian genera" of Tate (1951)) the large-bodied genera *Leporillus* and *Mesembriomys* were considered by Iredale and Troughton (1934), and Tate (1951), to consist of the same species recognised today, with the addition of *L. jonesi*, now regarded as an insular (and only surviving) form of *L. conditor*. Iredale and Troughton (1934) and Ellermann (1941) recognised two species of *Conilurus* regarded as synonymous with *C. penicillatus* by Tate (1951) and all subsequent authors, but the taxonomy of that genus has been relatively stable. *Zyzomys* has also seen little taxonomic revision since the inclusion of *Laomys* Thomas 1909 as a sub-genus (Tate 1951), now a junior generic synonym (Kitchener 1989), although two new species within the previously recognised *Z. woodwardi* were described by Kitchener (1989). The taxonomy of *Notomys* species has been relatively stable, although there has been confusion between several species in the past (Mahoney 1969, 1982) and the description of several synonymous forms. *Notomys cervinus* was placed in a separate genus by Waite (1900) and Iredale and Troughton (1934), but was retained in *Notomys* by Brazenor (1934) and all subsequent authors (table 2.2).

The remaining conilurine genera have a more complicated taxonomic history, which is still not resolved. Thomas (1910) united a large number of relatively non-descript species within *Pseudomys* Gray 1832 and described four sub-genera, *Gyomys*, *Leggadina*, *Pseudomys*, and *Thetomys*. Troughton (1932) still regarded these as sub-genera, but Iredale and Troughton (1934) elevated Thomas' four sub-genera to full genera (table 2.3), a development that Troughton (1937) felt was in keeping with Thomas' (1910) implicit opinion of the level of difference between the groups. Ellermann (1941) regarded *Thetomys* and a sub-genus of *Pseudomys*, but did not regard *Leggadina* or *Gyomys* as closely related to that genus, suggesting a closer affinity of *Gyomys* with *Rattus* and *Leggadina* with *Mus*. Tate (1951) regarded *Gyomys*, *Pseudomys* and *Thetomys* as sub-genera of *Pseudomys*, while he separated *Leggadina* as a full genus, in which he regarded "*Leggadina delicatula*" to be particularly divergent from *Pseudomys* (table 2.2). *Pseudomys delicatulus* is now regarded as a good *Pseudomys* species (Mackenzie and Burbidge 2002), despite the generic distinction of *Leggadina* (Watts et al. 1976).

Within the *Pseudomys* group of genera Iredale and Troughton (1934), Ellermann (1941) and Tate (1951) recognised roughly the same species, although some new species were described in the interim, and assignment to genera/sub-genera differed slightly (table 2.3). Discrepancies included Tate (1951) regarding Troughton's (1936) *Leggadina berneyi* as *Pseudomys (Gyomys) berneyi*. This mistake is perplexing, as a large accessory M1 cusp typifies the species, which is now synonymous with *Leggadina forresti* (Troughton 1936). *Gyomys* was distinguished from *Leggadina* by the lack of such a cusp. Tate presumably did not view the type, although it is listed on page 191 as "studied and photographed". He apparently followed Ellermann (1941), who listed *Gyomys berneyi* without explanation, and without viewing the type. Troughton (1973) still regarded the species as a *Leggadina*.

Iredale and Troughton (1934) placed the damaged type of *P. fieldi* in *Leggadina*. Troughton (1937) reviewed the type of *P. fieldi* and placed it within *Pseudomys*, but Ellermann (1941) still regarded it as a species of *Leggadina*. Tate (1951) included *P. fieldi* in *Thetomys*. All these authors placed *P. praeconis* in *Thetomys*. Despite inconsistent and mutually exclusive generic assignment in the past, *Pseudomys fieldi* and *P. praeconis* are now considered to represent a single species (Morris and Robinson 1995). All authors before Ride (1970) recognised multiple synonyms of *Leggadina forresti, P. australis* and *P. nanus*. Many of these species were described because Troughton and earlier authors exhibited a general reluctance to accept very large species distributions, which are actually typical of central Australian rodent species. The recognition and description of many synonymous forms by these authors was therefore the result of the limited nature of reference material, recognition of insular forms as full species rather than local phenotypes, and geographically disparate collecting locations.

Table 2.2. A brief taxonomic history of conilurine rodent genera.

For each author: the first line gives the number of species recognised in each genus or sub-genus, the second line the number of those species still recognised, and the genus/genera to which they are assigned. Where a genus was recognised as a sub-genus, the senior generic name is indicated in abbreviated form, e.g. (sub-*Pseud*) indicates that Tate (1950) recognised *Gyomys* Thomas 1910 as a sub-genus of *Pseudomys* Gray 1832. "-" indicates that a genus was not recognised as containing conilurine species. The number of species currently assigned to each genus (Mackenzie and Burbidge 2002) is shown to aid interpretation of earlier treatments.

Author	*Mus* Linnaeus 1758	*Leggadina* Thomas 1910	*Gyomys* Thomas 1910	*Thetomys* Thomas 1910	*Pseudomys* Gray 1832	*Conilurus* Ogilby 1838	*Leporillus* Thomas 1906	*Mesembriomys* Palmer 1906	*Hapalotis* Lichtenstein 1829	*Zyzomys* Thomas 1909	*Laomys* Thomas 1909	*Mastacomys* Thomas 1882	*Ascopharynx* Waite 1900	*Notomys* Lesson 1842
Gould (1863)	10 1 *Melomys* 6 *Pseudomys* 4 *Rattus*	-	-	-	-	-	-	-	13 2 *Conilurus* 2 *Leporillus* 1 *Mesembriomys* 3 *Notomys* 1 *Pseudomys*	-	-	-		-
Iredale & Troughton (1934)	-	7 1 *Leggadina* 4 *Pseudomys*	5 5 *Pseudomys*	5 3 *Pseudomys*	6 3 *Pseudomys*	4 2 *Conilurus*	3 2 *Leporillus*	2 2 *Mesembriomys*	-	1 1 *Zyzomys*	2 2 *Zyzomys*	2 1 *Mastacomys*	1 1 *Notomys*	8 6 *Notomys*
Ellermann (1941)	-	7 1 *Leggadina* 4 *Pseudomys*	8 1 *Leggadina* 7 *Pseudomys*	(sub-*Pseud*):5 3 *Pseudomys*	6 3 *Pseudomys*	4 2 *Conilurus*	3 2 *Leporillus*	2 2 *Mesembriomys*	-	1 1 *Zyzomys*	2 2 *Zyzomys*	2 1 *Mastacomys*	-	10 7 *Notomys*
Tate (1951)	-	6 1 *Leggadina* 4 *Pseudomys*	(sub-*Pseud*): 9 1 *Leggadina* 8 *Pseudomys*	(sub-*Pseud*): 6 4 *Pseudomys*	(sub-*Pseud*): 7 4 *Pseudomys*	2 2 *Conilurus*	3 2 *Leporillus*	2 2 *Mesembriomys*	-	1 1 *Zyzomys*	(sub-Zyz): 2 2 *Zyzomys*	2 1 *Mastacomys*	-	12 8 *Notomys*
Ride (1970)	-	-	-	-	17 1 *Leggadina* 15 *Pseudomys*	2 2 *Conilurus*	2 2 *Leporillus*	2 2 *Mesembriomys*	-	3 3 *Zyzomys*	-	1 1 *Mastacomys*	-	9 9 *Notomys*
Watts & Aslin (1981)	-	2 2 *Leggadina*	-	-	18 17 *Pseudomys*	2 2 *Conilurus*	2 2 *Leporillus*	2 2 *Mesembriomys*	-	3 3 *Zyzomys*	-	1 1 *Mastacomys*	-	10 10 *Notomys*
Mackenzie & Burbidge (2002)	-	**2**	-	-	**26**	**2**	**2**	**2**	-	**5**	-	**1**	**-**	**10**
Other generic synonyms					*Paraleporillus*[1] Martinez and Lidicker 1971			*Ammomys* Thomas 1906						*Thylacomys* Waite 1897 *Podanomalus* Waite 1898

1 *Paraleporillus stirtoni* (Martinez and Lidicker 1971) was described from a fossil, and has never been applied to modern specimens of *P. australis*, with which it is considered synonymous.

Nineteen seventy and 1976 saw two major developments in the taxonomy of small conilurines. Ride (1970) published a volume in which rodent taxonomy was based on the unpublished work of J. Mahoney. Thomas' (1910) sub-genera were recombined in a single genus, *Pseudomys*, which contained 17 species, two of which have since been synonymised as *P. fieldi*, one was *Leggadina forresti*, and the remainder of which are still regarded as valid (table 2.3; Mackenzie and Burbidge 2002). Sub-generic rankings were abandoned due to lack of clear-cut characters separating different groups, as evidenced by the plasticity of previous generic arrangements (table 2.3). Watts (1976) and Watts and Aslin (1981) modified Ride's classification by recognising *Leggadina forresti*, and added *L. lakedownensis* to that genus (Baverstock et al. 1976, Watts 1976). This basic classification is that accepted by Strahan (1995) and Mackenzie and Burbidge (2002), and in general usage today. Several authors have suggested that *Pseudomys* could, or should, be split into multiple genera (Baverstock et al. 1981, Godthelp 1989), but identifying valid species groups within the monophyletic complex has proved difficult (Watts et al. 1992).

2.1.3 Relationships within the Hydromyini

Despite the diversity of morphology and ecology evident among hydromyine rodents they are an extremely conservative group in some respects. All species have only four inguinal teats (Thomas 1910). Sperm morphology is highly derived compared to *Rattus*, but is conserved across all genera, with isolated departures from the ancestral form found only in some species of *Pseudomys* and *Notomys* (Breed 1997). The karyotype is subject to minor re-arrangement in some species, with no significant modification to the presumed ancestral type except in the genus *Zyzomys* (Baverstock et al. 1981, Baverstock et al. 1983). Dentition does vary between hydromyine lineages. Water rats are distinctive in that the molar row has been reduced to two basined molars (Tate 1951). Conilurines and mosaic-tailed rats possess three molars that have more typical murid cusps (Ellermann 1941), although there is a marked reduction in the size of upper labial cusps of conilurines when compared to *Mus* and *Rattus* (Simpson 1961). Crabb (1977) described complex patterns of dental evolution among Australian rodents, and noted cases of convergence and parallel evolution in dental form. The fact that some characters vary widely among species, while

others are highly conserved, has made identifying phylogenetically informative synapomorphic characters among conilurines difficult. Consequently, relationships between many taxa are still unresolved.

Table 2.3. Generic and sub-generic assignments of *Pseudomys* and *Leggadina* species.
Current taxonomy is given first (Mackenzie and Burbidge 2002), including previous synonyms (small type). Where past taxonomy differs from Mackenzie and Burbidge (2002), either by full generic distinction, or by recognising synonymous forms as distinct species, the genus (or sub-genus) name is given in black. Where previous treatments do not differ generic or sub-generic names (if recognised) are given in grey.

Mackenzie and Burbidge (2002)	Iredale and Troughton (1934)	Ellermann (1941)	Tate (1951)	Ride (1970)
Leggadina forresti	*Leggadina*	*Leggadina*	*Leggadina*	*Pseudomys*
messorius	*Leggadina*- species	*Leggadina*- species	*Leggadina*- species	-
waitei	*Leggadina*- species	*Leggadina*- species	*Leggadina*- species	-
berneyi	-	*Gyomys*- species	*Gyomys*- species	-
L. lakedownensis	-	-	-	-
Pseudomys albocinereus	*Gyomys*	*Gyomys*	*Gyomys*	*Pseudomys*
P. apodemoides	*Gyomys*	*Gyomys*	*Gyomys*	*Pseudomys*
(*P. glaucus?*)				
P. australis	*Pseudomys*	*Pseudomys*	*Pseudomys*	*Pseudomys*
greyii	-	-	-	-
lineolatus	-	-	*Pseudomys*- species	-
murinus	-	-	-	-
auritus	*Pseudomys*- species	*Pseudomys*- species	*Pseudomys*- species	-
minnie	*Pseudomys*- species	*Pseudomys*- species	*Pseudomys*- spp. *P. aus.*	-
rawlinnae	*Pseudomys*- species	*Pseudomys*- species	*Pseudomys*- species	-
P. bolami	*Leggadina*- ssp. *P. herm.*	*Leggadina*- ssp. *P. herm.*	*Leggadina*- ssp. *P. herm.*	*(Pseudomys)*
P. calabyi	-	-	-	-
P. chapmani	-	-	-	-
P. delicatulus	*Leggadina*	*Leggadina*	*Leggadina*	*Pseudomys*
pumilus	-	*Gyomys*- species	*Gyomys*- species	-
P. desertor	*Gyomys*	*Gyomys*	*Gyomys*	*Pseudomys*
P. fieldi	*Leggadina*	*Leggadina*	*Thetomys*	*Pseudomys*
praeconis	*Thetomys*- species	*Thetomys*- species	*Thetomys*- species	*Pseudomys*- species
P. fumeus	-	*Gyomys*	*Gyomys*	*Pseudomys*
P. glaucus	*Gyomys*	*Gyomys*	*Gyomys*	-
P. gouldii	*Thetomys*	*Thetomys*	*Thetomys*	*Pseudomys*
P. gracilicaudatus	*Thetomys*	*Thetomys*	*Thetomys*	*Pseudomys*
P. hermannsburgensis	*Leggadina*	*Leggadina*	*Leggadina*	*Pseudomys*
P. higginsi	*Pseudomys*	*Pseudomys*	*Pseudomys*	*Pseudomys*
leucopus	-	-	-	-
P. johnsoni	-	-	-	-
P. laborifex	-	-	-	-
P. nanus	*Thetomys*	*Thetomys*	*Thetomys*	*Pseudomys*
ferculinus	*Thetomys*- species	*Thetomys*- species	*Thetomys*- species	-
P. novaehollandiae	*Gyomys*	*Gyomys*	*Gyomys*	*Pseudomys*
P. occidentalis	-	-	*Gyomys*	*Pseudomys*
P. oralis	*Pseudomys*- ssp. *P. aus.*	*Pseudomys*- ssp. *P. aus.*	*Pseudomys*	*Pseudomys*
P. patrius	*Leggadina*	*Leggadina*	*Leggadina*	-
P. pilligaensis	-	-	-	-
P. shortridgei	*Pseudomys*	*Pseudomys*	*Pseudomys*	*Pseudomys*
P. sp. (2: 1-Vic, 1-QLD)	-	-	-	-

Watts and Aslin (1981) presented a synthesis of morphological and early molecular work (fig. 2.1). Following Baverstock et al. (1977b) *Rattus* was regarded as an outgroup to all other Australian rodents, which agreed with earlier notions that Australian rodents were derived from a *Rattus*-like ancestor (Tate 1951, Watts 1974). *Pogonomys* was separated as a sister group to the remaining taxa, which were combined within the subfamily Hydromyinae. The Hydromyinae (as used by Watts and Aslin 1981 and Lee et al. 1981) subsumed the older sub-families Pseudomyinae (Simpson 1961) and Hydromyinae (Thomas 1896), and recognised their close affinity with the mosaic-tailed rat genera, *Uromys* and *Melomys,* which had previously been allied with *Rattus* (e.g. Tate 1951, Ride 1970). Watts and Aslin (1981) grouped the genera of "mice" *Mastacomys*, *Leggadina* and *Pseudomys*, and speculated that the genus *Leporillus* may be related to the group. The close relationship they postulated between *Leggadina* and *Pseudomys* reflected previous inclusion of *Leggadina* species within *Pseudomys* (Thomas 1910, Ride 1970) and the inclusion of *Pseudomys delicatulus* and *P. hermannsburgensis* within the genus *Leggadina* (Troughton 1941, Tate 1951). The tree rat genera *Mesembriomys* and *Conilurus* are clearly related based on general characters and dental morphology (Tate 1951, Misonne 1969), and the rock rats, *Zyzomys*, were nominated by Watts and Aslin (1981) as their sister taxon. The highly derived morphology that characterises the genus *Notomys* was thought by Watts and Aslin (1981) to be the result of 15 million years of independent evolution (fig. 2.1). Watts and Aslin (1981) outlined broad generic relationships, but there was little resolution of those relationships, and no phylogenetic treatment at the species level, although species groups were nominated within the genera *Pseudomys* and *Notomys*. Timescales proposed by Watts and Aslin (1981) are far in excess of those now regarded as relevant to Australian rodent evolution (see section 2.2), and the early divergence times given in their tree pre-date the origin of the murine sub-family (Jacobs and Downs 1994).

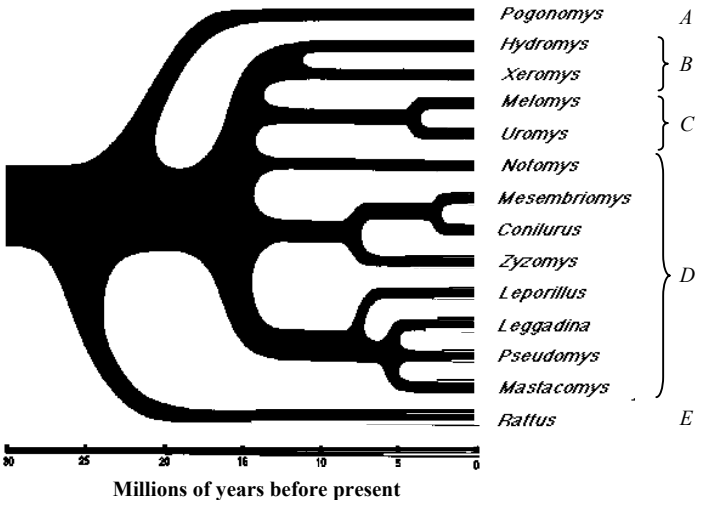


Figure 2.1. Relationships among Australian rodent genera (Watts and Aslin 1981).
Tree modified from Watts and Aslin (1981). A, *Pogonomys*; B, water rats; C, mosaic-tailed rats; D, conilurines; E, *Rattus*.

A number of molecular and morphological studies have attempted to clarify the uncertainty inherent in Watts and Aslin's tree (Baverstock et al. 1981, Baverstock et al. 1983, Lidicker and Brylski 1987, Watts et al. 1992, Torrance 1997). The consistency of some generic relationships among these studies is sufficient to establish relationships between some of Australia's endemic rodent genera, but the relationships of New Guinean hydromyines are still problematic (Donnellan 1989, Watts and Baverstock 1994a).

Pseudomys *group*

The most convincing clade within the Australian conilurines consists of the morphologically unremarkable *Pseudomys* and *Mastacomys*, and the most morphologically derived genus, *Notomys*. Ellermann (1941) foreshadowed this relationship by suggesting that *Notomys* and *Mastacomys* may have been derived from *Pseudomys*, but the first clear indication of monophyly of this group was provided by Baverstock et al. (1983) who identified a reciprocal translocation between chromosomes 1 and 2, shared by the three genera. The rarity of reciprocal translocations and the correlation of chromosomal location

inspired the statement that this relationship was "unquestionable" (Baverstock et al. 1983 pg. 114) Further support for the close relationship is indicated by sequencing of mitochondrial DNA (Torrance 1997), isozyme electrophoresis (Baverstock et al. 1981), penis morphology of many species (Lidicker and Brylski 1987), and microcomplement fixation (Watts et al. 1992). Dental characters are shared between these three genera (Crabb 1977), although independent evolutionary trends occur within each, and Crabb (1977) felt that although *Mastacomys* and *Pseudomys* were closely related, the singular morphology of *Notomys* warranted its separation as an independent lineage. *Mastacomys* is a monotypic genus with very distinctive broad molars (Misonne 1969) that has repeatedly fallen within *Pseudomys* (as currently defined) in phylogenetic analysis (Baverstock et al. 1981, Watts et al. 1992, Torrance 1997), and Watts and Baverstock (1992) formally recommended the sinking of *Mastacomys* within *Pseudomys*. *Notomys* has sometimes fallen within the genus *Pseudomys* in systematic analysis (e.g. Torrance 1997). The *Notomys*/*Pseudomys* lineage represents a clade to the undoubted exclusion of tree rats, *Leggadina* and New Guinean genera (Baverstock et al. 1983). Their relationship to *Leporillus* and *Zyzomys* has not been firmly established.

Tree rats

The tree rats, genera *Conilurus* and *Mesembriomys*, form a second credible grouping in accordance with general morphology, skull morphology and dentition (Tate 1951, Misonne 1969, Crabb 1977, Watts and Aslin 1981), penis morphology (Lidicker and Brylski 1987), mitochondrial DNA sequencing (Torrance 1997), isozymes (Baverstock et al. 1981) and microcomplement fixation (Watts et al. 1992). There is some support for the inclusion of *Leporillus* in this clade, as a sister taxon to *Conilurus*/*Mesembriomys*. Watts and Aslin (1981) placed *Leporillus* as a sister to *Pseudomys*/*Mastacomys*, and Baverstock et al. (1981) found *Leporillus* isozymes to group within *Pseudomys* in some analyses. *Leporillus* has only two internal cusps on its first two upper molars, while tree rats have three, and Crabb (1977) considered tree rats distinct from *Leporillus* on that basis. However, Misonne (1969) considered *Leporillus* dentition to be a close derivative of that of *Mesembriomys*. Lidicker and Brylski (1987), Watts et al. (1992) and Torrance (1997) all found a close

relationship to tree rats. Chromosomal evidence is not available to assess the presence of the *Notomys/Pseudomys* reciprocal translocation in *Leporillus*.

Leggadina *and* Zyzomys

The remaining conilurine genera are more difficult to place. *Leggadina* and *Zyzomys* form inconsistent relationships with conilurine and New Guinean genera in systematic studies. Unlike the conservative chromosomal evolution of most hydromyines, *Zyzomys* has a highly derived karyotype (Baverstock et al. 1977b). Its skull morphology is similar to the tree rats (Misonne 1969), and like them it has three internal cusps on its first two upper molars (Tate 1951, Crabb 1977). Other studies (Baverstock et al. 1981, Lidicker and Brylski 1987) indicated a closer relationship to *Pseudomys*, although the major reorganisations of its karyotype make it unclear whether it possesses a *Notomys/Pseudomys* chromosomal translocation (Baverstock et al. 1983). Mitochondrial cytochrome b sequences suggested that *Zyzomys* and *Leggadina* form a sister clade to *Notomys* and *Pseudomys* (Torrance 1997), although there was little bootstrap support for this relationship. *Leggadina* resembles members of *Pseudomys* in general morphology and penis morphology (Lidicker and Brylski 1987), but microcomplement fixation indicated a basal position to the entire hydromyine tree (Watts et al. 1992), and isozyme studies provided a similar result (Baverstock et al. 1981).

Conilurines

Conilurines do not always form a monophyletic clade excluding New Guinean genera in systematic studies. Watts et al. (1992) and Baverstock et al. (1981) presented trees in which conilurines were not monophyletic. Similarly, Lidicker and Brylski's (1987) analysis of penis morphology did not segregate conilurines from mosaic-tailed rats, nor did the result of Torrance's (1997) mitochondrial sequencing. However, conilurines are easily made a monophyletic clade in Baverstock et al's (1981) study of isozymes if the position of *Leggadina* is ignored. *Leggadina* is also problematic in Watts et al's (1992) tree, but the mosaic-tailed rats, *Zyzomys* and tree rats further confound the issue by forming a sister-

clade to *Notomys/Pseudomys*, although relationships within this clade were unresolved. Lidicker and Brylski (1987) concluded that the conilurines were a monophyletic tribe despite their analysis showing several New Guinean genera within the conilurines. This seems justified given that their analysis split the *Notomys/Pseudomys* clade into two divergent branches separated by members of *Melomys* and several other New Guinean genera. Penis morphology is therefore probably not a useful marker for discerning higher-level relationships, a view supported by Watts et al. (1992). Tate (1951) and Troughton (1941) both thought that conilurines were a distinct assemblage, although Tate (1951) speculated that *Lorentzimys,* which is not particularly closely related (Donnellan 1989), might be a member of the group. Baverstock et al. (1983) proposed a model of chromosome evolution within the Hydromyini that entailed an early divergence of mosaic-tailed rats and conilurines from water rats, and a subsequent splitting of the conilurines from mosaic-tailed rats.

Established relationships

The discussion above noted few well established relationships. This thesis investigates diversification of conilurine rodents, yet it is still not clear whether conilurines are a monophyletic clade. Within the conilurines there is support for a monophyletic clade containing the genera *Mastacomys*, *Notomys* and *Pseudomys* and another containing *Conilurus* and *Mesembriomys*. The genus *Leporillus* is probably related to *Conilurus/Mesembriomys* clade, but it is not clear where the affinities of *Leggadina* or *Zyzomys* lie (fig. 2.2). Relationships are not problematic within most conilurine genera, as they mostly contain only two species. However, within *Pseudomys* there is a history of taxonomic unrest (section 2.1.2), and few species relationships are known with any confidence. Species relationships within this genus are discussed in detail in chapter 4.

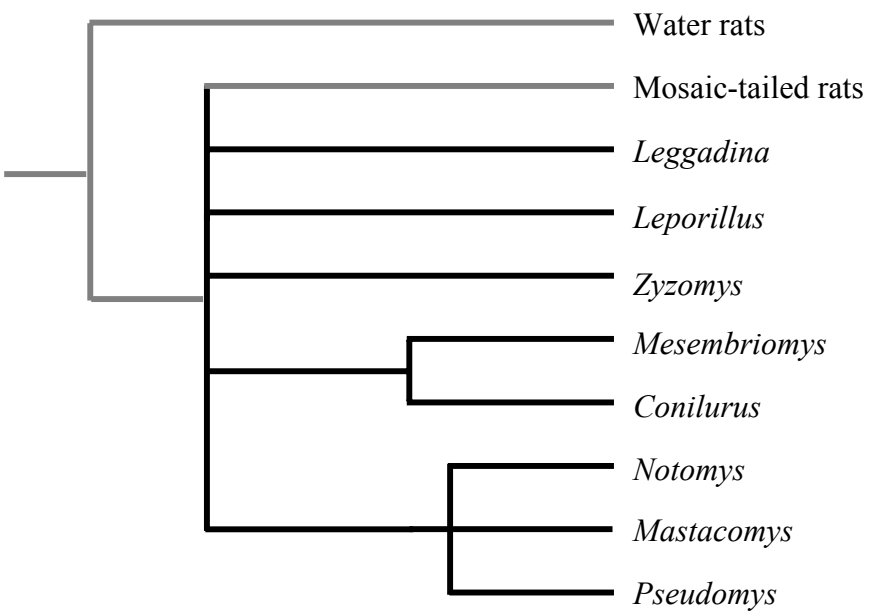


Figure 2.2. Relationships among Australian hydromyine rodents.
Relationship shown are those with good support from previous systematic studies. Grey branches highlight taxa shared with New Guinea (Water rats (Australian genera *Hydromys* and *Xeromys*) and Mosaic-tailed rats (Australian genera *Melomys* and *Uromys*)). Black branches are conilurine genera.

2.2 Origins and fossil history of Australian rodents

2.2.1 Origins and Relatives

The origin of the hydromyine lineage is obscure. Based on microcomplement studies that could not identify any sister-taxon among the Australasian and South-east Asian species they examined, Watts and Baverstock (1994b) speculated that the Hydromyini are an early branching of the South-east Asian murine radiation, and probably arrived in Australasia in the late Miocene 6-8Ma. Aplin (accepted) supports this date based on fossil evidence and biogeographical reconstructions. *Rattus* is often used as an outgroup in systematic studies (e.g. Baverstock et al. 1981, Baverstock et al. 1983, Lidicker and Brylski 1987), but more for convenience sake than a conviction that *Rattus* is the sister taxon of the Hydromyini. The genus *Pogonomys* is a member of a similarly sister-less group of New Guinean rodents (Watts and Baverstock 1994a, Watts and Baverstock 1994b). Many authors have felt that

the ancestor of Australian rodents was *Rattus*-like (Watts 1974, Hand 1984), or a *Rattus*-like form possessing a cricetid two-cusped dental pattern (Tate 1951), although Simpson (1961) proposed a purely cricetine ancestor. These authors were referring to conilurine rodents, as at that time water rats and mosaic-tailed rats were considered distantly related to conilurines. Some authors noted New Guinean or South-east Asian species that possess characters they thought were ancestral to the conilurine rodents (Lidicker 1968, Misonne 1969), including the genera *Anisomys* and *Lenothrix*. Misonne (1969) followed Tate (1951) in including the New Guinean genus *Lorentzimys* as a member of his Australian Pseudomyinae. The close relationship of some Australian and New Guinean genera has been confirmed by chromosomal, isozyme and microcomplement fixation studies (Baverstock et al. 1977b, Baverstock et al. 1981, Baverstock et al. 1983, Watts et al. 1992), but other New Guinean rodents are relatively distantly related to the Hydromyini. Fossil rodents are known from eastern Indonesia, but none appear ancestral to the Hydromyini (Flannery 1989).

The fact that the sister group of the Hydromyini is proving difficult to find is not surprising. A chaotic geological and biogeographical history in the late Tertiary (Hall 1998, Holloway and Hall 1998) has produced a series of rapid murine sub-radiations in South-east Asia, including those of New Guinea and Australia (Musser and Carlton 1993, Watts and Baverstock 1994a, Watts and Baverstock 1994b). The formation of modern New Guinea was a particularly dynamic process of land accretion, upheaval, fragmentation, and unification (Polhemus and Polhemus 1998). The land units that comprise modern New Guinea have probably been sites of repeated processes of radiation and extinction (Heads 2002), and although Watts et al. (1995) favour an origin of the Hydromyini west of Wallace's line, the evolutionary history of New Guinean faunas could mask any phylogenetic signal from a New Guinean sister clade.

In contrast, the origin of the conilurine lineage appears to have been a relatively simple event, resulting from a single invasion of a stable continent (Dahl 1897, Simpson 1961, Lidicker 1968, Happold 1976), although the uncertainty of systematic relationships among the hydromyine rodents (Watts and Baverstock 1994a) provides a caveat. The fact that

mosaic-tailed rats and water rats are predominantly New Guinean would suggest that colonisation of Australia took place via part of what today is the island of New Guinea (Watts and Baverstock 1994b, Strahan 1995, Aplin accepted). This differs from the ideas of Flannery (1989), Godthelp (1999) and (Hand 1984) who suggested that rodents entered Australia directly from Indonesia, possibly from the drier islands of Timor or Flores. This unlikely scenario would suggest that water rats and mosaic-tailed rats originated in Australia and later spread to New Guinea. Lowered sea levels in the late Miocene (Chappell and Shackleton 1986, Haq et al. 1987) probably resulted in a land connection between Australia and parts of New Guinea that could have facilitated an invasion of Australia from New Guinea. Marsupial interchange between Australia and New Guinea has been dated to this time (Aplin et al. 1993, Krajewski et al. 1993). Waif dispersal from New Guinea could have occurred at any time. Multiple invasions of Australia by New Guinean mosaic-tailed rats, *Pogonomys*, and *Rattus* occurred during the Pleistocene via land bridges across Torres Straight (Simpson 1961, Watts and Aslin 1981, Taylor et al. 1983). Two conilurine species (*Conilurus penicillatus* and *Pseudomys delicatulus*) invaded New Guinea (Flannery 1995). They each have a New Guinean distribution restricted to the Trans-Fly plains, an area that is essentially the northern extension of Australian habitats cut off by rising sea level at the end of the LGM (Flannery 1995).

2.2.2 FOSSIL HISTORY

Tertiary fossil history

Dating the entry of rodents to Australia would ideally rely on the appearance of rodent fossils at a discrete time interval in a widespread, continuous, fossil history. Unfortunately, the Tertiary fossil record of Australia is poor and comprised of scattered faunas representing relatively short time periods that are often difficult to date (Rich et al. 1982, Archer et al. 1989). The earliest reliably dated Australian fossil rodents are found in northern and central Australian deposits dated between 3 and 4 Ma (table 2.4). There is an apparently well-defined age of 4+/- 0.12 Ma for the rodent-bearing Bluff Downs local fauna in northeast Queensland (Archer and Wade 1976). However, Aplin (accepted)

considers rodent bearing deposits older than 3.4Ma to be either unreliably dated, or not to have been suitably examined to establish their rodent content. Rodent fossils are not present in any Miocene deposits, even in faunas that contain rodent-sized marsupials (Archer et al. 1989, Tedford and Wells 1990, Archer et al. 1999), so an early Pliocene or latest Miocene colonisation is strongly suggested by the fossil record. Most Tertiary rodent fossils are obviously conilurine, although Godthelp (1999) suggested a Dendromurine-like fossil from Riversleigh is distinct from all modern Australian rodents, and Aplin (accepted) has recovered a distinctively basined water rat tooth from a 2.8Ma+ deposit on Barrow Island.

The Rackham's roost small mammal fauna from Riversleigh in northwest Queensland has been speculatively dated at 4-5Ma (Archer et al. 1991, Godthelp 1999). However, this is a biostratigraphic date, and Aplin (accepted) is not confident of a date older than 3Ma. Rackham's roost contains thirteen rodent species in three conilurine genera; *Leggadina*, *Pseudomys* and *Zyzomys*. On the basis of the diversity of rodents in this deposit Godthelp (1999) proposed a Miocene rodent colonisation of Australia. However, given the essentially un-dated nature of the deposit, caution dictates that it be regarded as a "Pliocene" fauna, with no greater significance in resolving origins of the conilurines until a firm dating method is applied to the site. The fact that rodents were apparently absent from the Hamilton area of southern Australia in the early Pliocene (Hand 1984) suggests a post-Miocene origin. However, habitat surrounding the Hamilton deposits at the time of deposition was generally a mixture of wet forest types (Macphail 1996) that may have precluded conilurine rodents solely on the basis of ecological preference (section 2.3.2).

Fossil deposits from the late Pliocene onward are generally rich in rodent fossils, although there is a dearth of early Pleistocene material. Many sites have been assigned an age relating roughly to the Plio/Pleistocene boundary (Rich et al. 1982). However, most have not been appropriately dated and may be much more recent. Two dated sites of note from the late Pliocene are Dog Rocks (2.03-2.48Ma; Whitelaw 1989), which contains two *Pseudomys* species from southern Victoria (table 2.4), and Fisherman's Cliff on the lower Murray River. The latter (2.42-2.87Ma; Whitelaw 1991) contains a diverse rodent fauna (Marshall 1973, Crabb 1977) consisting of seven conilurine species in five genera, although

Crabb's *Leggadina idjibudoo* is probably a *Pseudomys* species, as he included *P. hermannsburgensis* and *P. delicatulus* within *Leggadina*. Other species present resembled *Leporillus apicalis*, *Notomys, Pseudomys occidentalis* and *P. albocinereus*. The presence of putative modern species in the deposit (Crabb 1977) is probably an indication of comparison to modern forms rather than of a static fauna in the region. No *Conilurus* or *Mesembriomys* fossils have been recorded from the Pliocene or early Pleistocene, despite obviously deep phylogenetic division between them and other conilurines (section 2.1.3).

The first appearance of the genera *Hydromys* and *Rattus* may be recorded in the two million year old Floraville deposit of northwest Queensland (Rich et al. 1982), although this Plio/Pleistocene age is based only on biostratigraphic evidence. Marshall (1973) recorded *Rattus* from Fisherman's Cliff, however Crabb (1977) did not. The fossil record of Australian *Rattus* is certainly older than the 40ka quoted by Watts and Aslin (1981), and Godthelp (1999) favoured a 2Ma origin of Australian *Rattus*. However, he suggested that the original lineage has been superseded by New Guinean species. Aplin (accepted) does not consider there to be any relevant evidence regarding the date of entry by *Rattus*. A possible mechanism for colonisation around 2Ma would have been provided by a drop in sea level due to the first formation of the northern polar ice cap (Chappell and Shackleton 1986, Haq et al. 1987).

Table 2.4. Pliocene rodent faunas of Australia
Data from Aplin (accepted), Marshall (1973), Rich et al. (1982), Whitelaw (1989, 1991), Godthelp (1999).

Site	Location	Age	Rodents present
Parwan	S Vic	4.06-4.2a	Rodent
Bluff Downs	NE QLD	3-4Ma	Unidentified conilurine (1)
Kanunka	NE SA	3.4Ma	Muridae
Riversleigh	NW QLD	3+Ma	*Leggadina* (2 spp.)
			Pseudomys (7 spp.)
			Zyzomys (1 sp.)
			"Dendromurine" (1 sp.)
			Unidentified (2)
Barrow Island	NW WA	2.8-3.6Ma	*Pseudomys* (1sp.)
			Zyzomys
			New genera (2)
Chinchilla	SE QLD	2-3 Ma	*Pseudomys vandycki*
Fisherman's Cliff	NW Vic	2.42-2.87Ma	*Arujaomys*
			Leggadina idjibudoo[1]
			Leporillus apicalis
			Notomys (1 spp)
			Pseudomys (2 spp.)
			P. albocinereus
			P. occidentalis
			Murinae
Dog Rocks	S Vic	2.03-2.48Ma	*Pseudomys* (2 spp.)
Floraville	NW QLD	~2?Ma	*Pseudomys* spp.
			Rattus sp.
Malkuni	NE SA	~2Ma	Rodent

1 Probably a *Pseudomys* species.

Quaternary Australian Rodents

Abundant rodent fossils are found in Pleistocene and Holocene deposits. Nearly all rodents in these deposits are modern species (table 2.5). Most Pleistocene and Holocene sites are cave deposits from owl roosts (Wakefield 1972), or lacustrine and fluvial deposits in central Australia (Tedford and Wells 1990). Quaternary fossil deposits reveal wholesale changes in the distribution of rodent and marsupial species in response to natural climatic fluctuations, and more recently as a result of European colonisation. Both features are of relevance to this thesis.

The co-occurrence of many species in late Pleistocene deposits has been labelled as "disharmonious" (Lundelius 1983, Tedford and Wells 1990) due to the fact that the species in question are now geographically separated and appear to have fundamentally different ecological preferences. This highlights the importance of species ecology in dictating different responses to changing environmental conditions, thereby modifying the potential

evolutionary consequences of environmental change for different species. The presence of koalas (*Phascolarctos cinereus*) on the Nullarbor Plain during the past 20ky indicates caution is required when discussing barriers and biogeographic pattern (Lundelius 1983). The Nullarbor Plain is currently one of the most arid regions of Australia and is considered a major barrier between many taxa, including the rodent sib-species *Pseudomys albocinereus* and *P. apodemoides*. The Nullarbor is a significant long-term barrier to plants (White 1994), but some "forest" mammals were able to exploit the region quite recently, which must be taken into account when discussing allopatric populations. Comment on natural biogeographic pattern is also made difficult by post-colonisation range reductions (Smith and Quin 1996b). In fact several of Lundelius' own "disharmonious" allopatric species were not naturally allopatric. Notable among the rodents in this regard is *P. desertor*, which is still sympatric with the rufous bettong (*Aepyprymnus rufescens*), but was not known to be so until very recently. Recent range reductions of many rodent taxa are recorded in sub-fossil deposits in which bones of locally extinct species are found in association with those of introduced *Mus musculus*, *Rattus rattus* or *Oryctolagus cuniculus* and in radio-carbon dated late Holocene deposits (Archer and Baynes 1972, Baynes 1984, Ellis 1995). Some conilurine species have disappeared from tens of thousands of square kilometres (Smith and Quin 1996b).

Table 2.5. Some Quaternary rodent fossil deposits.
(P) Pleistocene only. (H) Holocene only. * Extinct; # no longer present in region of deposit

Site	Location	Age	Rodents present
Pyramid Cave (Wakefield 1972)	NE Victoria	~>20ka (P) and <10ka (H)	*Rattus fuscipes* *R. lutreolus* *Hydromys chrysogaster* (P) *Melomys cervinipes* (P)# *Conilurus albipes** *Mastacomys fuscus* *Pseudomys fumeus* *P. higginsi* (P)# *P. novaehollandiae* *P. oralis* (H)#
Devil's Lair (Baynes et al. 1975)	SW Western Australia	12-20ka	*Rattus fuscipes* *Hydromys chrysogaster* *Notomys sp.** *Pseudomys albocinereus* *P. fieldi*# *P. occidentalis*# *P. shortridgei*#
Texas Caves (Archer 1978)	SE Queensland	Late? Pleistocene	*Rattus fuscipes?* *R. lutreolus?* *Conilurus albipes** *Pseudomys gracilicaudatus* *P. desertor*# *P. novaehollandiae* *P. oralis*# *P.* sp.
Mootwingee (Ellis 1995) (pers. comm.)	W New South Wales	Post-colonisation	*Leporillus conditor*# *Mus domesticus* *Notomys cervinus*# *N. longicaudatus** *Pseudomys australis*# *P. hermannsburgensis* *P. bolami*# *Rattus villosissimus*#
Wombeyan (Ride 1960)	SE New South Wales	Pleistocene	*Mastacomys fuscus* *Pseudomys australis*# *P. fumeus*#
Wilgie Mia (Baynes 1984)	CW Western Australia	1ka-present	*Leporillus apicalis** *L. conditor*# *Notomys* sp. *N. longicaudatus** *Pseudomys nanus*# *P. fieldi*# *P. hermannsburgensis* *Rattus tunneyi*
Victoria Cave (Wells et al. 1984)	S South Australia	<150ka?	*Conilurus* cf. *albipes** *Mastacomys fuscus*# *Pseudomys albocinereus*[1] *P. australis*# *P.* cf. *fumeus*# *Rattus* sp.

1. *P. apodemoides*?

2.3 Evolution of the Australian biota

2.3.1 Climate and Vegetation

Overview

Extensive deserts and arid landforms that currently typify Australia are atypical when compared to past conditions (Ayliffe et al. 1998). As late as the early-mid Miocene, much of Australia supported rainforests of Gondwanan origin (Christophel and Greenwood 1989, White 1994). Throughout the mid-late Tertiary and Quaternary, climatic events have caused the gradual and accelerating loss of rainforests and their replacement by xeric communities (Bowler 1982, Christophel and Greenwood 1989, Flower and Kennett 1994, Martin 1998). Deflection of ocean currents by drifting continents and events such as the closure of the isthmus of Panama intensified latitudinal sea and air temperature gradients, altering pressure gradients and forcing the sub-equatorial high-pressure belt northward (Bowler 1982). Movement of the high-pressure belt caused increasingly seasonal weather patterns and produced strong, dry, westerly winds that resulted in a continuous reduction in precipitation across Australia (Bowler 1982). Superimposed on these longer-term processes were shorter Milankovitch cycles acting on time scales of 19Ky, 40Ky and 100Ky that alter insolation and were responsible for severe cold periods and glaciations during the Pleistocene (Imbrie et al. 1989, Webb and Bartlein 1992). Australia's low relief and latitudinal position minimised the direct impact of Pleistocene glacial ice (Twidale and Campbell 1995). However, the intensely arid conditions and strong winds that accompanied large scale glaciations of the Pleistocene saw the formation of Australia's arid landscapes, with dune fields, gibbers and playa lakes becoming commonplace only in the last 500ky (Bowler 1982, Krieg et al. 1990, Clarke 1994, Hesse 1994, Macphail 1997, Zheng et al. 1998, Pell et al. 2000, Bowler et al. 2001).

Vegetation of the Late Tertiary

By the latest Miocene (~6Ma) Australia was beginning to resemble the continent of today. In general the climate was still wetter, but moisture and temperature gradients were similar to today (Macphail 1997). Rainforests had long since disappeared from central and western Australia and much of Queensland, and open vegetation with xeric components was present (Lange 1978, Truswell and Harris 1982, Christophel and Greenwood 1989, Martin and McMinn 1994). The northeast of Australia supported a habitat mosaic of rainforest and sclerophyll forest, although of different composition and in different proportion to today (Christophel and Greenwood 1989, Martin and McMinn 1993). South-eastern Australia supported extensive rainforest and sclerophyll forests in which *Eucalyptus* was increasingly common (Truswell and Harris 1982, Christophel and Greenwood 1989, Macphail et al. 1995, Macphail 1996, Martin 1997, Greenwood et al. 2000). The early Pliocene was relatively warmer and wetter than the cold period that ended the late Miocene (Galloway and Kemp 1981). Rainforests of the east expanded slightly during the early-mid Pliocene (Martin 1991), but grasses continued to increase throughout the interior and areas such as the Eyre Peninsula, far western New South Wales and western Queensland were home to woodland and savannah communities (Truswell and Harris 1982, Christophel and Greenwood 1989, Martin 1997). The vegetation of the current arid zone and its margins contained a richer mosaic of vegetation than today, including developing xeric communities and relict forest and rainforest communities (Benbow et al. 1995, Martin 1998). Grasses had been increasing in abundance since the Eocene, but it was not until the Pliocene that woodlands and savannah woodlands became prevalent, being replaced by grasslands and shrubland during the Pleistocene (Christophel and Greenwood 1989, Martin 1998). Savannahs of the Pliocene experienced severe winter aridity, with a summer wet season, causing extensive leaching of soil nutrients and reactivation of drainages of the interior that were dormant during the late Miocene (Bowler 1982, Palaeogeographic Group 1990).

By the late Pliocene cooler conditions had returned as the northern polar ice cap formed (Shackleton et al. 1984). This heralded the beginning of increasingly severe climatic cycles.

By the end of the Pliocene, around 1.6 Ma, the current distribution of Australian vegetation had been roughly established, with sclerophyll forests restricted to continental margins in the east and southwest, and rainforests reduced to isolates in the east (Christophel and Greenwood 1989). During the Pleistocene these boundaries fluctuated markedly on local scales in concert with glacial cycles, and cyclical altitudinal changes in vegetation are well documented in eastern Australia and Tasmania (Hopkins et al. 1993, Kershaw 1994, Macphail et al. 1995, Nanson and Price 1998). The major changes in Australian vegetation over the past million years have been related to the formation of the current arid zone, with sand, gibber and salinity replacing woodlands and savannah, and the development of hardy chenopod and spinifex communities capable of enduring these conditions (Bowler 1982, Christophel and Greenwood 1989, Martin 1998). The extent of the arid zone increased markedly during glacial maxima, and evidence of aridity reaching the present continental margins is found in the form of aeolian dune fields of desert origin in western Victoria, southern South Australia and the Kimberley (Jennings 1975). Very recent changes in Pleistocene and Holocene vegetation, and the observed aridity of the present inter-glacial compared to equivalent periods earlier in the Pleistocene, have been attributed to the impact of human arrival during the last glacial cycle (Kershaw and Nanson 1993, Ayliffe et al. 1998, Luly 2001).

Modern Bioregions

Despite the atypical nature of the modern environment, Nix (1982) has shown that the broad distributions of modern plant taxa conform to current environmental conditions, and are not an artefact of past climate. Using only modern thermal and moisture regimes as an indication of plant growing conditions Nix (1986) was able to demonstrate that the biogeographic subregions usually recognised within Australia (Archer and Fox 1984, Cranston and Naumann 1991) emerge from analysis (fig. 2.3). These basic subregions, the Bassian, Torresian and Eyrean are referred to throughout the remainder of this thesis. Each is further sub-divided, and many modifications of the basic scheme have been proposed for different groups of organisms (Burbidge 1960, Kikkawa and Pearse 1969, Cranston and Naumann 1991). However, rodents conform reasonably well to the scheme shown in figure

2.3, and divisions within subregions are the basis of further discussion below and in chapter 9.

The biogeographic subregions essentially identify areas containing suites of endemic plants and animals with distributions restricted to; forest and woodland communities of the northern and eastern tropics (Torresian), temperate forests and woodlands of the southeast and southwest (Bassian) and the arid zone (Eyrean). Endemic species or subspecies found within subregions often have sib-taxa in another subregion. Among rodents the Torresian delicate mouse (*Pseudomys delicatulus*), Bassian New Holland mouse (*P. novaehollandiae*) and Eyrean sandy inland mouse (*P. hermannsburgensis*) have been cited as a classic bioregional species complex (Baverstock 1982), and are all members of the delicate mice, a major study group for this thesis. Overlap of biotas occurs at the boundaries of subregions, best documented in the MacPherson-Macleay (Archer and Fox 1984).

Figure 2.3. Biogeographic subregions of Australia.
Torresian ; Bassian ; Eyrean
Cross-hatching between Torresian and Bassian bioregions is the MacPherson-Macleay overlap.

Living on the outer

The major biogeographic subregions reflect the overall biogeographic pattern of the Australian biota. There is a recently developed, relatively homogeneous, arid interior surrounded by a non-arid periphery (Heatwole 1987). The periphery consists of much older and more heterogeneous ecosystems. This is reflected in the fact that species distributions in the Torresian and Bassian zones are often fragmented or restricted when compared to broad distributions of many central Australian species (Archer and Fox 1984, Heatwole 1987). Many areas of endemism have been described that identify repeated patterns of range fragmentation occurring in habitats that surround the arid zone, generally as a result of increasing aridity (fig. 2.4; Keast 1961, Cracraft 1982, Crisp et al. 2001). The relevance of areas of endemism to understanding evolutionary pattern, particularly in northern and central Australia, is discussed briefly in chapter 9. The main basis for that discussion is the critical examination of the effect two major barriers in northern Australia, the Carpentarian Barrier and the Great Sandy Desert, have had on the distributions and divergence of conilurine rodents (section 2.3.3).

Figure 2.4. Some areas of endemism around the Australian continental margin.
Areas modified from Cracraft (1982). Areas in central Australia are not shown.

2.3.2 Recent history of Australian vertebrates

"Non-forest animals"

Northward-drifting Tertiary Australia carried a cargo of largely Gondwanan temperate rainforest plants and animals (Archer et al. 1991). These groups underwent major radiations, and the mid Miocene marsupial fauna of Australia was possibly more diverse than at any other time (Archer et al. 1999). As the continent entered lower latitudes the endemic fauna was faced with widespread loss of its rainforest habitat, which retreated to the cooler south and wetter continental margins (section 2.3.1). Fossil faunas of arboreal marsupials in central Australia are replaced by progressively more terrestrial forms throughout mid-late Miocene deposits (Hope 1982), and by 8Ma large terrestrial birds and

mammals dominate the central Australian Alcoota deposit, collectively termed "non-forest animals" by Hope (1982). The transition of the fauna from forest to non-forest animals is well illustrated by the genus *Burramys*. These pygmy possums were found throughout Miocene rainforests of central Australia, and are common in Miocene deposits from Riversleigh (Archer et al. 1989). Their distribution can be traced through late Tertiary fossil deposits, first becoming restricted to the south-east (Macphail 1996), and then to relictual pockets of rainforest of the eastern highlands (Ride 1960, Archer et al. 1991). Others, like the ektopodontids, simply disappeared (Pledge 1982).

Forest animals

Although many native species adapted successfully to the increasingly arid and open centre of Australia, communities of the arid zone are significantly less diverse than those of surrounding habitats. There are twice as many bird species found in tropical and eastern Australia than there are in central Australia (Pianka and Schall 1981, Schodde 1999). Bats share this pattern of diversity, and like birds, are more diverse in the tropics than in temperate forests (Pianka and Schall 1981). Marsupials are more diverse in forested eastern Australia than in the tropics and arid Australia is clearly depauperate compared to both regions (Pianka and Schall 1981). Partitioning of habitats is a fundamental process leading to species diversity (Begon et al. 1996), and forests, particularly rainforests, provide far greater opportunity for niche partitioning than do the simple, widespread, nutrient-deprived, communities of arid Australia (Morton and James 1987, Schodde 1999). Structural diversity is therefore probably the primary factor dictating the diversity of most vertebrate groups. However, the history of a group will indelibly mark its future evolution, and the arboreal legacy of Gondwanan rainforests should not be underestimated in dictating a predisposition towards forest habitats (Smith-White 1982).

Partitioners

Lizards are a vertebrate group that has been particularly successful in the arid zone (Pianka and Schall 1981). Lizards effectively partition simple desert habitats such as spinifex, with

a single spinifex plant representing multiple feeding niches (Morton and James 1987). Mammals or birds could not partition habitat at that level, and Morton and James (1987) further propose that the abundance of termites in arid Australia has provided a specialised feeding niche that lizards are better adapted to exploit than other vertebrates. Australian desert lizards have long been noted as remarkably diverse when compared to those of North America, while other vertebrates appear to be less successful in comparison to that continent (Morton et al. 1994). The uncertainty of rainfall and general unpredictability of habitats in the Australian arid zone may have favoured lizards over other groups because they were capable of withstanding longer periods without food than endotherms and were advantaged by open spaces in which to bask (Schall and Pianka 1978, Morton and James 1987).

Environmental and systematic aspects of rodent diversity

Like other vertebrate groups, rodents exhibit high diversity in tropical forests. However, this diversity is attributable to multiple recent invasions from New Guinean rainforests (Lee et al. 1981), and the origin and distribution of endemic species diversity lies elsewhere (Watts and Aslin 1981). The distribution of conilurine species diversity (fig. 2.5) reveals the group to be non-forest animals. The fact that the bulk of Australian rodents are non-forest taxa draws an immediate contrast to marsupials and birds (above). Structural arguments regarding the relatively low diversity of marsupials and birds in the arid zone should equally apply to rodents, indicating that other factors are influencing the diversity of the conilurine lineage.

Watts and Aslin (1981) noted that Australian rodents were "strongly adapted to drier habitats" (pg. 19), and a preference for dry conditions is immediately apparent from the distribution of conilurine species diversity in relation to a soil moisture index (fig. 2.5). Unlike *Rattus* species, water rats and mosaic-tailed rats, diversity of conilurines is greatest in the arid zone and the seasonally arid northern savannahs. Species diversity is low in the moist southeast, where forests are common, and conilurines are completely absent from closed forests of the northeast. Species diversity in the arid zone is unlike that of northern

savannahs in that the bulk of species belong to the genera *Pseudomys* or *Notomys*. In contrast, generic diversity in savannahs is high, while species diversity within most genera is low.

The mosaic-tailed rats and *Pogonomys* sp. are recent descendents of New Guinean rainforest stock (Lee et al. 1981, Watts and Baverstock 1994a), and are still basically confined to that habitat, although *Melomys burtoni* is found in tropical grasslands, and *Melomys cervinipes* may occur outside rainforest in the southern parts of its range (Kerle 1995c, Redhead 1995). If species diversity of New Guinean rodent groups occurring in Australia is compared to a soil moisture index (fig. 2.5), a clear preference for the wettest available habitats is evident. This is especially true of the aquatic genera *Hydromys* and *Xeromys*. Species of *Rattus* also exhibit a marked preference for forest habitats and wetter areas of the continent (fig. 2.5), although *Rattus villosissimus* occurs in moist refugia of the inland and occupies large tracts of typically arid habitat when conditions are suitable (Newsome and Corbett 1975), and *Rattus tunneyi* may once have been very widespread throughout arid Australia, but has now disappeared from most of the continent (Watts and Aslin 1981).

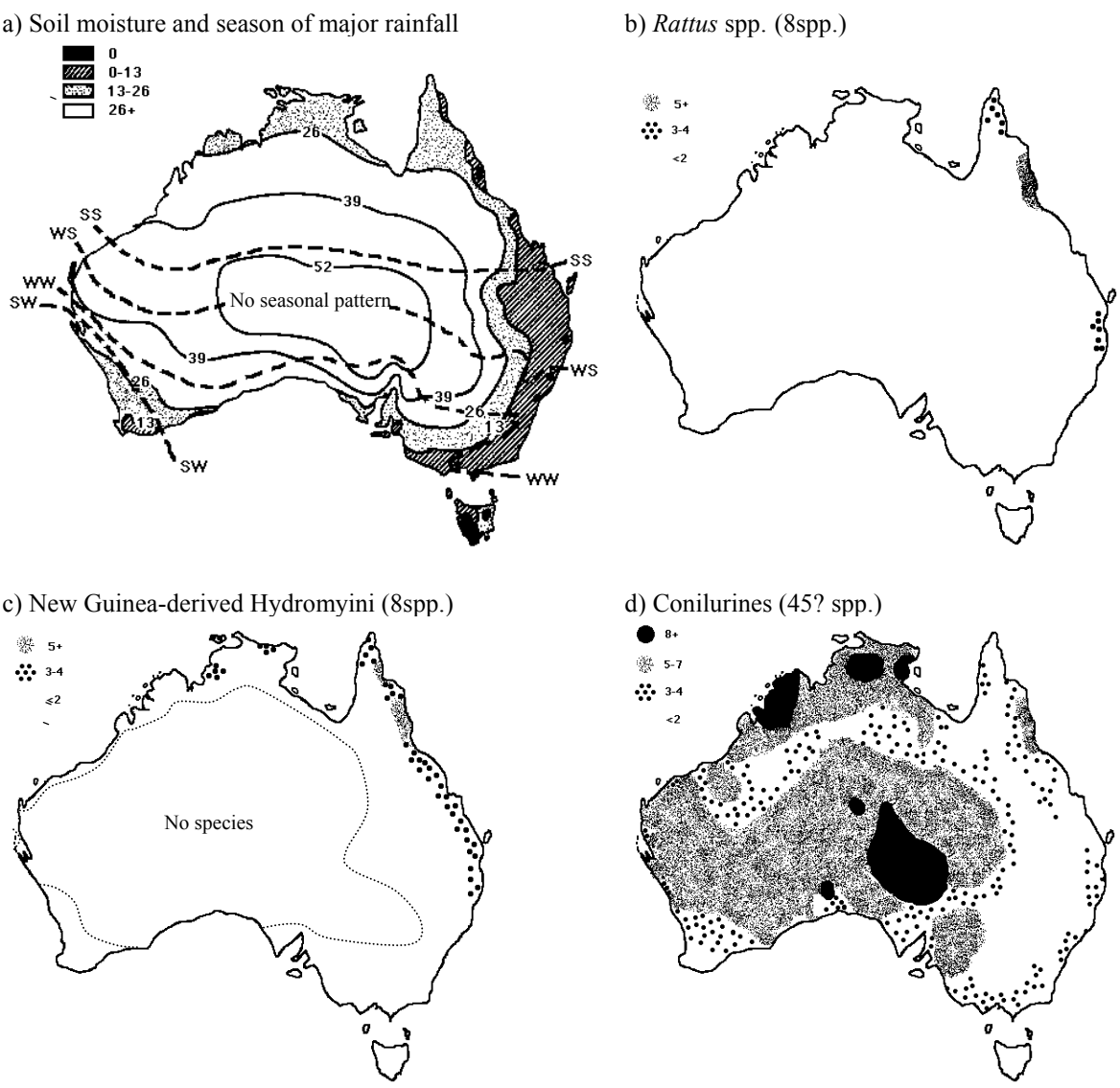


Figure 2.5. Distribution of Australian rodents in relation to moisture regime.
Soil moisture index describes the balance between evaporation and precipitation, represented here as the number of weeks per year in which evaporation is greater than precipitation (from Nix 1982). Seasonality of rainfall is represented by isohyets SS (strongly summer), WS (weakly summer), WW (weakly winter) and SW (strongly winter) (Nanson and Price 1998). Rodent distributions are based on Strahan (1995), Western Australian Museum Faunabase (http://www.museum.wa.gov.au/faunabase), field data, and personal communications from J. Woinarski, J. Cole, S. Van Dyck and A. Kutt.

2.3.3 Relict Savannah diversity

Eucalypt-dominated monsoonal habitats with a grassy understorey, collectively referred to here as tropical savannahs, are the only Australian habitats with a richer conilurine fauna than the arid zone (fig. 2.5). Under the auspices of "savannah" I also include the restricted monsoonal rainforest pockets inhabited by large *Zyzomys*, although they are included more as a matter of geographical convenience than genuine similarity. The savannahs are subject to highly seasonal rainfall, and have a negative water budget (evaporation>precipitation) for up to six months of the year (fig. 2.5). Those of the Kimberley and Top End contain two genera and several species of different sized mice, two genera of large and medium-sized tree rats, and specialised species such as rock-rats, a hopping mouse and pebble-mound mice (Woinarski 2000). I propose this indicates the diversity of savannahs is ancestral, as it is only in savannahs that such ecologically and systematically diverse conilurine communities are found. Early fossil faunas are also from savannah habitats (Godthelp 1999), and the Pliocene spread of savannah conditions probably coincided with the arrival or diversification of conilurine rodents (section 2.3.2). The fate of these ancestral conilurine communities during the Pleistocene has paralleled the earlier fate of forest marsupial and bird communities, in that they have become restricted to the continental margin. The spread of arid conditions has forced savannahs northwards, where they now form three major refuge areas in the Pilbara, Kimberley-Top End and Cape York (fig. 2.6). Keast (1961) termed these the Hammersly, northwest and northeast refuges and recognised them as having a marked effect on divergence of bird races and species. The Pilbara actually lies within the arid zone proper, but by virtue of its gorges provides microclimates that harbour savannah species such as *Mesembriomys macrurus* (Morton et al. 1996).

As noted above, savannah faunas are partitioned into three large refuge areas. The Great Sandy Desert and Carpentarian Barrier are responsible for this partitioning. Each is a significant departure from the vegetation, topography and climate of the adjacent savannahs (below and fig. 2.6). The effect of these two barriers on the rodent fauna of northern Australia is a major focus of speciation studies in this thesis. Both barriers cause disjunction of many vertebrate distributions. However, both are typified by relatively recent

Pleistocene aridity and landforms (Bowler 1976, Wyrwoll et al. 1986, Kershaw and Nanson 1993), so attributing a role in speciation to them on the basis of current species distributions is potentially controversial (Avise et al. 1998). A detailed reconstruction of the evolution of these barriers, and their probable relationship to animal distributions, is therefore required before discussing their possible influence on rodent diversification.

History of the savannah belt

The landforms of the savannah regions have been stable for very long time periods, and are some of the best-preserved ancient landscapes in the world (Twidale and Campbell 1995). Recent geomorphological evolution of the Great Sandy Desert and Carpentarian Barrier is discussed below. The two major aspects of Pleistocene environmental change in northern Australia are a cyclic pattern of effective precipitation (Bowler et al. 2001, De Dekker 2001), and large fluctuations in the extent of land in response to changing sea level (Yokoyama et al. 2001). These two factors do not interact in a straightforward manner. Although the Holocene is a warm interglacial period and is wetter than the cold LGM, cooler temperatures during glacial cycles can actually result in increased effective precipitation due to reduced evaporation (Ayliffe et al. 1998, Bowler et al. 2001), and some evidence suggests that greater precipitation may also have occurred (Nott et al. 1996). This means that for plants that can tolerate cooler temperatures the extent of suitable conditions may actually expand considerably to the north during parts of glacial cycles. Migration of plant species into, and out of, local areas is recorded over short time scales in the Kimberley (Wallis 2001). However, during glacial maxima, bands of habitats move northward in response to arid conditions, and the eucalypt woodlands currently found in the Top End were postulated by van der Kaars (1991) to be found 800km further north than today during the LGM (fig. 2.6).

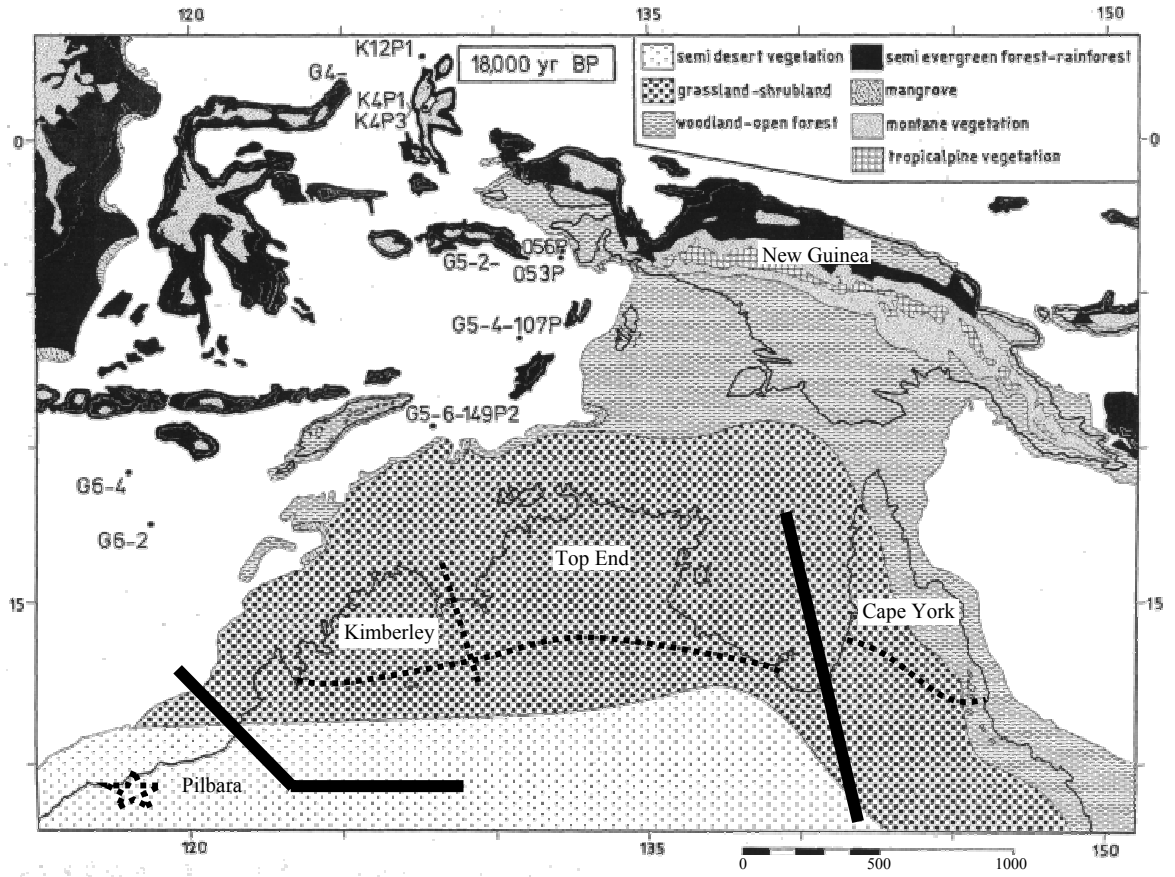


Figure 2.6. Vegetation of Australasia during the LGM.
Modified from van der Kaars (1991). Modern savannah regions and New Guinea are labelled. Major barriers shown as thick lines, Great Sandy Desert in the west, Carpentarian in the east. Dotted lines are boundaries of modern savannah regions. Dotted line between the Kimberley and Top End indicates the Ord arid intrusion, which is a barrier sub-dividing those regions during glacial periods, and which currently affects some species. Numbered dots offshore indicate location of pollen-bearing deep-sea cores.

Van der Kaars (1991) suggested that the vegetation currently typical of arid Australia extended over 200km off the north coast of the Top End during the LGM, essentially rendering the entire region that now contains savannah refuges uninhabitable to the savannah fauna. At times other than the LGM, habitats would have been found further south, still on the exposed Arafura Plain. This was a tentative reconstruction based on very few sources of data, mostly pollen from three deep-sea cores to the northwest of Australia (van der Kaars 1991), and I am confident that it is too severe an interpretation of habitat change. Unless there were extreme cases of micro-climatic influence, species such as the large rock-rats (*Zyzomys*), which are associated with monsoonal rainforest in sandstone gorges, would have gone extinct. The Australian eucalypt-associated fauna (savannah fauna

here) would have primarily migrated to New Guinea, and occupied a significant portion of that island, yet only two (of a possible 17) savannah conilurines are now found in eucalypt habitats of southern New Guinea. I am aware of no fossil evidence that suggests most of the fauna was present in southern New Guinea in recent times. Flannery (1989) suggested that woodland and savannah habitats were present between the two islands, and Nix and Kalama (1972) proposed woodland and open forest. However, allowing for the speculative nature of van der Kaars (1991) map, two features are of great importance. 1) Large areas to the north of Australia historically provided suitable habitats for species currently confined by high sea level, 2) many areas currently occupied by species on the mainland would not have been suitable for them in the recent past. The interaction of the formation of the Carpentarian Barrier and Great Sandy Desert with these changes in habitat creates a complex situation in which to interpret population divergence that is not suggested by the contemporary distributions of species.

Geological formation of barriers

The Carpentarian Barrier and Great Sandy Desert were both inundated by Cretaceous seas, an event that occurred long before murid rodents evolved, but one that indicates a long-term history of low elevation and topography (Palaeogeographic Group 1990). Beard (1974) described the Great Sandy Desert as "a monotonous expanse of gently undulating country" (pg. 30) and (Twidale 1956) regarded the Carpentarian plains (which are comprised of the Gulf Plains and Mitchell Grass Downs) as an excellent example of a peneplain- an area completely flattened by erosion. Both areas have been gently up-warped since the Cretaceous marine incursion (Twidale 1956, Beard and Webb 1974, Coventry et al. 1980), a process that may be ongoing in the region of the Selwyn Upwarp in the Carpentarian plains (Twidale 1966). However local topographic relief is minimal, and Twidale described the "Selwyn Range" as a "figment of some cartographer's imagination" (pg. 491). Both the Carpentarian plains and the region of the Great Sandy Desert were subject to processes of deep weathering during the Miocene and Pliocene, which produced lateritic surfaces still evident in many areas (Twidale 1956, Beard and Webb 1974, Coventry et al. 1980, Twidale and Campbell 1995).

While the Pliocene history of the two regions is similar, the Pleistocene saw active drainage and fluvial action in the Great Sandy Desert cease (Beard and Webb 1974, Palaeogeographic Group 1990). Fluvial erosion has been ongoing on the Carpentarian plains, and resulted in the current peneplain following stripping of the lateritic surface (Twidale 1956, Coventry et al. 1980). The current level of the plain was probably achieved only in the last 1Ma (Coventry et al. 1985). The cessation of fluvial action in the Great Sandy Desert was followed by predominantly aeolian activity that has seen the formation and spread of a large sand sheet. The sand sheet has been worked into longitudinal dunes running ESE-WNW that form the northwest portion of a massive whorl of desert dunes around the centre of the continent (Wasson 1986). The dunes are oriented according to the dominant wind direction during the late Pleistocene (Jennings 1975, Wasson 1986), when the fields were active, but they are now stabilised by *Acacia*-dominated vegetation (Wyrwoll et al. 1986). The age of the Great Sandy Desert dune fields has not been established, but those in the northeast are older than 230ka (Bowler et al. 2001). No dune fields in Australia have been dated as older than 700ka (Wasson 1986, Gardner et al. 1987). It seems reasonable to assume an age of around 500ka for a significant inland barrier to animal movement through the region. However, aeolian dunes continuous with the main Great Sandy Desert at the mouth of the Fitzroy River, in the west Kimberley, have been dated to around the time of the LGM or early Holocene (Jennings 1975), so contact between the Kimberley and Pilbara may have been possible along the coastal strip much more recently than 500ka.

Vegetation history of barriers

The history of vegetation in the northwest is poorly known. On the basis of a single sea-core Martin and McMinn (1994) suggested that *Casuarina* forests dominated the late Miocene vegetation of the region, and these were progressively replaced by grasslands through the Plio-Pleistocene with no dominance by eucalypts at any time. If true, these results would deny the evidence of a previously continuous savannah fauna across northern Australia. However, they are probably wrong, as van der Kaars (1991) recorded abundant

eucalypt pollen in sea-cores off the northwest of Australia until 190ka, when cycles of grass-dominated vegetation appear in the record. It appears that up until the mid Pleistocene there may have been contact of savannah habitats between the Kimberley and Pilbara. But in the late Pleistocene conditions became arid, periodically very arid, and dune fields and unsuitable habitats isolated the Pilbara (Wende et al. 1997, Bowler et al. 2001). This was not the case in the Carpentarian Barrier. The development of the semi-arid, almost tree-less vegetation that dominates the Carpentarian plains is unknown, but is probably largely irrelevant to the contact of Cape York and Top End faunas. Exposure of the Arafura Plain and Carpentarian basin during long periods of the Pleistocene has meant that the current isolation of Cape York due to the Gulf of Carpentaria and inland plains is a comparatively infrequent situation (Torgensen et al. 1983, Yokoyama et al. 2001). In the last 100ka the Gulf bed was exposed periodically between 80ka and 40ka and was continuously exposed from 40ka until 9.7ka, when the last marine transgression occurred (Chivas et al. 2001). At times when the basin is empty a large lake, Lake Carpentaria, fills much of the south-eastern part of the present day Gulf (Torgensen et al. 1983, Chivas et al. 2001). Grasslands and swamps are common around the lake, but eucalypt-dominated vegetation would almost certainly be present in the north (Torgensen et al. 1988, van der Kaars 1991). The most obvious effect of land exposure to the north of Australia has been the re-current exchange of animals with New Guinea, but it is possible that such exposure has been a major factor in maintaining the cohesion of the savannah fauna in the face of the current dissected distribution of such habitats.

2.3.4 Speciation in novel environments: origins of arid zone species diversity

The centre of Australia has long been viewed as a sink. In the report of mammals collected on the Horn Expedition Spencer wrote “Following extinction in the central area of the greater part of its marsupial fauna, there ensued migration of such forms as could live in an arid region…”(Spencer 1896; pg. 7). Theories regarding the adaptation of Australia’s fauna to the arid zone usually invoke the idea of reinvasion and speciation from surrounding habitats. Schodde (1999) unambiguously described the pattern of diversification within Australian birds as grading “from rainforest source to desert sink” (pg. 314). On theoretical

grounds Greenslade (1982) demonstrated that under a modified r-K selection model, diversification of species once they had adapted to the arid zone was unlikely due to prevailing selection regimes, and concluded that the source of the arid zone biota was likely to be the semi-arid zone to the north, a pattern cited by Ride (1970) in regards to the macropod genera *Onychogalea* and *Lagorchestes*.

Christophel and Greenwood (1989) provide a more inspiring appraisal of Australia's drier habitats by describing rainforests as being "surrounded by a sea of evolutionary novelty" (pg. 107) in recognition of the fact that loss of forests was not simply a process of attrition. Archer and Fox (1984) also suggested that central Australian taxa had gained the "opportunity to undergo rapid and divergent evolution" (pg. 14). This was certainly true for plants. Despite the impression of Australia as an entirely green continent for the bulk of the Tertiary, xeromorphic plants may have been present in Australia since early in the period due to generally poor soils and locally drier conditions (Smith-White 1982, Truswell and Harris 1982). The expansion of aridity resulted in further adaptation of these xeric plants, and eventually the evolution of new desert genera, and a suite of new habitats (Truswell and Harris 1982). Endemic xeromorphic plants are now one of three major components of Australia's flora, and deserts are dominated by such species (Keast 1981). The fauna reacted to aridity in similar fashion. The displacement of arboreal taxa from central Australia represents the end of a long period of forest mammals (Archer et al. 1999). However, they were replaced by animals more suited to open spaces such as grazing kangaroos, which underwent a large radiation in the Pliocene as grasses and woodland became commonplace (Cooke and Kear 1999). Hope (1982) identified the greater part of the Pliocene, between around 4Ma and the beginning of the Pleistocene as the key period in the evolution of Australia's modern fauna. However, the general impression of the evolution of Australia's fauna is one centred on forests and rainforests, and that, as Smith-White (1982) stated in summarising contributions to a volume on evolution of arid zone species "deserts themselves have rarely been centres of evolutionary episodes of speciation" (pg. 378). Most of Australia is therefore viewed as a sink consisting of arid habitat of recent origin, and unlikely to be a venue for speciation. Baverstock (1982) extended this general model of Australian biotic evolution to include the rodents, citing the

closely related *Pseudomys delicatulus*, *P. novaehollandiae* and arid zone *P. hermannsburgensis* as an example of rodent invasion of the arid zone. However, Baverstock (1982) acknowledged that the diversification of arid-adapted *Notomys* was possibly centred on the arid zone.

The notion that animals disappeared from central Australia, were restricted to near-coastal habitats, and then re-invaded the centre, in some ways does not correspond well to the actual development of the modern arid zone. The loss of forest was a gradual process, and large treed areas persisted in inland Australia long after the bulk of forest had retracted south-eastward (Truswell and Harris 1982, Christophel and Greenwood 1989, Benbow et al. 1995), just as aquatic crocodiles and flamingos persisted in central Australia well into the Pleistocene due to significant run-off from the eastern highlands (Tedford and Wells 1990). Patches of remnant vegetation still persist in moist havens of the central ranges, containing species with extremely restricted distributions and affinities to species or populations found in coastal regions (Morton et al. 1996). Koalas still occur along riparian strips of red-gum forest surrounded by completely unsuitable environments in southwest Queensland (Martin and Handasyde 1995). The semi-arid zone and savannahs are also not just bands of habitat that surround the margins of the contemporary arid zone, they resemble the habitats that preceded arid conditions in central Australia (section 2.3.1). If the semi-arid zone or savannahs are likely sources of arid zone species, it is a far simpler model to invoke *in situ* adaptation to increasing aridity than extinction followed by re-invasion of habitats that were formerly occupied. This model of faunal evolution would produce the same pattern of mesic-arid sib-taxa as would re-invasion, and is therefore untestable, but seems a more plausible hypothesis for the origin of at least some arid zone species.

Arid zone species presumably have an origin relating to the development of the arid zone. Yet development of true desert is a very recent phenomenon, probably beginning in earnest around 1Ma (Bowler 1976) and not completed until the final arid phase of the Pleistocene 22-16ka years ago (Bowler 1982, Krieg et al. 1990, Clarke 1994, Hesse 1994, Zheng et al. 1998, Pell et al. 2000). 700ky is a particularly short time scale on which to contemplate

macroevolutionary patterns, and is considered by some authors too brief to reasonably permit speciation events (Avise et al. 1998, Knowles 2001). In fact Smith-White (1982) and Nix (1982) regard the Pleistocene in Australia as a time of range reduction and movement, but not diversification. Avise (1998) concluded that the Pleistocene may have initiated processes that will result in speciation, but time periods of two million years are generally required to produce species. In north-eastern Australia the contraction of rainforests to high altitude refugia during the Pleistocene has been proposed as a mechanism of sib-taxon divergence, but Moritz et al. (2000) have applied molecular clocks to show divergence of some taxa occurred up to seven million years ago, despite clear mechanisms for divergence during the Pleistocene. The evolution of conilurine diversity in the arid zone is therefore of interest due to the unusual rapidity with which it must have occurred, particularly within *Notomys*, and because the arid zone rodent fauna is significantly more species rich than that in forests, which are the proposed sources of avian and marsupial arid zone diversity.

Mesic enigma

The lack of conilurine rodents in forest habitats is the greatest enigma of the group's evolution, particularly given their probable Mio/Pliocene New Guinean rainforest heritage. *Conilurus albipes* was once found in woodlands of the southeast, but it is only *Pseudomys* species that frequent forests. There are only five species of *Pseudomys* that may be found in forest environments in the southeast, and of these, only *P. fumeus, P. higginsi* and *P. oralis* are true forest taxa (Stoddart and Challis 1993, Smith and Quin 1996a, Ford et al. 2003). *Pseudomys gracilicaudatus* and *P. novaehollandiae* are species that frequent heaths that may be found as a sub-stratum in forests (Kemper 1990, Luo and Fox 1996, Wilson and Bradtke 1999), and *P. fumeus* is usually dependent on a heathy understorey (Ford et al. 2003). None of these species are now sympatric in forests, and all occur at low density (Watts and Aslin 1981). The lack of conilurines in forest habitats has been speculatively attributed to competition from marsupials (Lee et al. 1981), but typical rodent niches are not, and have not been, occupied by marsupial species (Archer et al. 1999). Possums might compete with omnivorous tree rats for food and nesting hollows, but species of both are

sympatric in the north. In general, rodent-sized marsupials are insectivores, while conilurine rodents tend to be omnivorous, with a tendency towards granivory and herbivory (Watts 1977, Murray et al. 1999). The distinctive dentition of rodents allows exploitation of gnawing niches unavailable to most other groups (Watts and Aslin 1981). A simpler explanation for the lack of forest conilurines lies in a chance ancestral radiation in savannah environments that established a fundamental preference for drier environments. Flannery (1989) proposed a similar scenario whereby Australian rodents were derived from dry South-east Asian habitats. However that does not account for clear systematic relationships with New Guinean rodents (Watts and Baverstock 1994a, Watts and Baverstock 1994b). The general absence of conilurines from forests will probably remain a matter for speculation.

Chapter 3. Materials and Methods

METHODS SUMMARY

It is difficult to unravel processes of speciation that probably pre-date the modern distributions of species by a million years or more. A simple approach was adopted in this thesis, the generation or review of multiple lines of corroborative evidence derived from palaeoecology, ecology, ecological biogeography, taxonomy, and molecular phylogenetics. This approach was used to allow objective evaluation of the accuracy of hypotheses based on molecular methods and associated analyses. Any method of evolutionary reconstruction or interpretation of molecular data can reasonably be questioned (Zharkikh and Li 1992, Wakeley 1996, Farris et al. 2001, Knowles and Madison 2002), and I felt that over-analysing data in an attempt to achieve apparent statistical rigour could be counter-productive. Molecular phylogenetic trees form the basis for understanding the relationships between species and populations. These trees were produced using standard methods of Kimura two-parameter distance and maximum-likelihood analyses. Bootstrap testing, topology tests and saturation tests provided evaluations of the internal support for molecular trees. Nested clade analysis was performed as a supplementary method of examining molecular sequence data. Sampling was not designed with this process in mind, and samples sizes were often too small for successful use of the method. Ecological analyses attempted to characterise the distributions of species and critical limiting factors determining those distributions. This involved bioclimatic distribution modelling for several species, and in the case of pebble-mound mice, detailed ecological studies that will be reported elsewhere.

3.1 Molecular methods

3.1.1 Sampling and extraction of DNA

Entire cellular DNA was obtained from field-collected biopsies, samples provided by other researchers, and museum collections. Field biopsies were tail-tip or ear punches immediately preserved in 100% ethanol after collection. The source and nature of all samples involved in a particular study is noted in the relevant sections of chapters 4-8. DNA was extracted using phenol:chloroform extractions modified from Hoelzel (1992). Tissue samples were minced and placed in 300µl of lysis buffer (40mM Tris ph 8.0, 2mM EDTA, 0.2 NaCl, 1% SDS) and 1µl Proteinase K (20mg/ml) then incubated for several hours at 55°C. Two digestions were performed with 300µl phenol:chloroform:IAA, followed by a single digestion with chloroform:IAA. DNA was precipitated overnight at 4°C in 2.5 volume of 100% ethanol and 0.1 volume 3M NaAC, then centrifuged to pellet the DNA at 10,000rpm for 10 minutes. The pellet was washed in 800µl 70% ethanol and stored in nuclease-free water at 4°C for immediate use, or -20°C for longer-term use.

3.1.2 Amplification and sequencing of DNA

Mitochondrial control region

Approximately 400bp of mitochondrial DNA was amplified from the 5' end of the control region, between the tRNA Proline and central conserved domain (block CSB-D (Southern et al. 1988)). Primers used were MT 15996L (Campbell et al. 1995) and MT16502H (M. Elphinstone, pers. comm.). The amplified segment includes a hyper-variable region that had been successfully employed in studies of Australian rodents by Campbell et al. (1995) and Jerry et al. (1998). Amplification reactions were run on an MJ Research PTC 200 Peltier thermal cycler. PCR reaction volumes were; 1µl of DNA template from a 1:10 dilution of DNA derived from phenol:chloroform extractions, 2µl of $MgCL_2$ (25mM), 2µl of Qiagen™ PCR buffer solution, 0.1µl (5 units) QIAGEN Taq DNA polymerase, 0.5µl of each primer (10pm/µl), 0.4µl dNTP, and 13.5µl of nuclease-free water. Cycling conditions

were 94°C for 1min, 30 cycles of (94°C for 10sec, 55°C for 10sec, 75°C for 1min) followed by 75°C for five minutes.

Nuclear copies of mitochondrial DNA

Nuclear copies of the control region were identified from eight *Pseudomys* species. I designed the primers FFMTH1 (5'-GTTTTATGGGCCCGGAGCG) and FFMTH2 (5'-TGGTGGGCGGGTTGTTG) based on alignment of true *Pseudomys* control region sequence to prohibit amplification of the nuclear copy identified from pebble-mound mice (section 5.3.1). Only results from the longer strand amplified by FFMTH1 are reported, and this primer was located 15bp internally from the heavy strand primer MT16502H. The primers NCMTH1 (5'- TCCCAA(G/T)TT(G/T)AATGTTCCCGGAGCA) and NCMTH2 (5'- AGTTTCACAGAAGATGGTAGAAG) were designed from sequence of nuclear copy obtained from *P. calabyi* and *P. patrius* and specifically amplified the nuclear copy of the control region that was excluded by FFMTH1 and FFMTH2.

Purification of control region PCR product

PCR products were purified by isopropanol precipitation (Hoelzel 1992). PCR reactions were combined with 120µl 70% isopropanol and left at room temperature for 15 minutes before pelleting the PCR product by centrifuging at 11,000rpm for 20 minutes. After removal of the supernatant, the pellet was washed in 500µl of 70% isopropanol and re-spun at 11, 000rpm for 13 minutes. After drying, the pellet was re-suspended in 20µl of nuclease free water.

Nuclear introns

Sixty intron primer pairs designed by Lyons et al. (1997) were screened on DNA derived from *Pseudomys patrius*. To allow twenty primer-pairs to be screened simultaneously, semi-optimal touchdown cycling protocols and reaction conditions were designed based on optimal conditions provided by Martin Elphinstone (Southern Cross University). ACTC,

ARAF1, CHAT, COL3A1, DHFR and MYH2 produced PCR product, and were then screened under optimal conditions on DNA from *P. delicatulus*, *P. hermannsburgensis*, *P. laborifex*, *P. patrius* and *Zyzomys argurus*. ARAF, CHAT and COL3A1 produced extremely variable patterns of multiple banding between species and genera. ACTC, DHFR1 and MYH2 produced a single band of similar size in all species, and the products were sequenced to assess variability of each marker. For those loci where results are reported in this thesis reaction volumes and cycling conditions for PCR were:

Dihydrofolate reductase (DHFR) intron 1; reaction volumes as for control region; cycling conditions 94^{o}C for 1min, 30 cycles of (94^{o}C for 15sec, 57^{o}C for 20sec, 75^{o}C for 1min) followed by 75^{o}C for five minutes.

A-raf (ARAF) intron 1; reaction volumes as for control region, except for 1µl of $MgCL_2$ (25mM) and 14.5µl of nuclease-free water; cycling conditions 94^{o}C for 1min, 30 cycles of (94^{o}C for 15sec, 51^{o}C for 20sec, 75^{o}C for 1min) followed by 75^{o}C for five minutes.

Further PCR of ACTC, DHFR1 and MYH2 identified multiple bands that were absent from *P. patrius* or superimposed on the original check gels. Intron PCR products of ARAF, ACTC, CHAT DHFR1, and MYH2 were therefore run on 1.5% agarose 1x TAE gels at 80v to separate bands. PCR bands were excised and cleaned using a commercial QIAquik® gel extraction kit. Identification of the correct intron sequence was attempted by alignment against sequences of the intron downloaded from Genbank and by BLAST searches of Genbank. For most PCR products BLAST searches resulted in ambiguous results where gene segments from other chromosomal locations or loci were amplified, possibly due to transposable elements. Pure exon sequences were also commonly amplified. In cases where intron sequence was identified, that band was used as a standard to aid subsequent PCR cleanups.

Sequencing of control region and intron PCR product

Approximately 400ng of purified PCR product was prepared for sequencing using commercial BigDye™ Terminator sequence preparation kits. Sequencing reactions were

purified by ethanol precipitation (Crouse and Amorese 1989). The reaction mix was added to 2µl 3M NaAC, 47.5µl 100% ethanol and 2.5µl 100% isopropanol, vortexed, and left on ice for fifteen minutes. Reactions were centrifuged at 11,000rpm for 20 minutes, rinsed with 500µl 70% ethanol, and re-spun at 11,000rpm for 15 minutes, then dried. Samples were forwarded to Griffith University Molecular Biology Facility where they were read on an Applied Biosystems 377 DNA Sequencing System.

3.1.3 Analysis of sequence data

Sequence editing

Electropherograms of DNA sequence were examined and edited individually before preliminary alignment in Se-Al v2.0a11(Rambaut 2002). Visual alignment of sequences was straightforward between related taxa. Aligned sequences were exported to PAUP* 4.0b10 (Swofford 1998) and a distance tree was produced using a neighbour-joining algorithm based on a Kimura two-parameter distance matrix. Groups of taxa with similar sequence identified by distance analysis were then aligned in Se-Al. Electropherograms of each taxon were examined in the context of the aligned sequence of the most similar taxa, and, where available, the complimentary sequence of the same taxon.

Ambiguities in sequences were resolved where the following criteria were met:

- all taxa with similar sequence shared the same unambiguous base, as did members of at least three other related groups identified by distance analysis, and that base was evident in the ambiguous electropherogram signal of the taxon in question, or
- the reverse sequence of the taxon in question had a resolved base at that site, and that base matched all other members of the closely related taxa

Ambiguities were recorded where:

- forward and reverse sequences differed in a resolved base at a given site (unless that site was conserved across all sequenced Australian rodent taxa)

- forward (and reverse sequences if available) showed ambiguous electropherogram signals at a given site that differed from the base evident in related taxa at that site.

All sequences were then re-aligned in Se-Al. Intron data sets were readily aligned without the aid of automatic alignment, however, control region sequences were first aligned using Clustal X (Thompson et al. 1994), and then imported into Se-Al for final alignment. Aligned sequences were tested for substitution saturation using the index of Xia et al. (2003) in the program DAMBE (Xia 2000, Xia and Xie 2001).

Tree building

Aligned sequences were exported to PAUP* for analysis. Distance trees formed by neighbour-joining of Kimura two-parameter matrices (Kimura 1980) and maximum-likelihood trees (Felsenstein 1981) were the primary basis for analysis. Maximum-likelihood tree topologies were initially found using an exhaustive search and mutational parameters of sequence data determined by Modeltest v3.04 (Posada and Crandall 1998). Bootstrapping of distance and likelihood trees was based on 100 replicates using a full heuristic search algorithm. In some instances there was justification for further analysis. Bootstrapped parsimony trees were run with gaps treated as a fifth base to account for indels. Some further manipulation of sequence alignments was undertaken to examine specific questions, as detailed in relevant chapters. Maximum-likelihood tree topologies were compared under varying topological constraints in PAUP* using two-tailed Kishino-Hasegawa tests (Kishino and Hasegawa 1989) employing a RELL distribution and 1000 bootstrap replicates.

Nested clade analysis

The program TCS 1.13 (Clement et al. 2000) was used to create nested clades from sequence alignment files. Nested clade files based on TCS clades were written according the protocol detailed in the program documentation for GeoDis v2.0 (Posada 2000), and were analysed using that program. Decimal latitude and longitude were used, and outgroup

probabilities were not defined. Details of taxa subjected to this analysis are provided in chapters 5, 6 and 7.

Molecular clocks

Some readers will consider the fact that I have not applied a molecular clock to my data a glaring omission. It was not, there is; 1) no way of calibrating a molecular clock for the control region of conilurine rodents, which is a very specific case of molecular evolution that cannot rely on any other mutational rate calculation (Larizza et al. 2002) and 2) dating the divergence of haplotypes will not resolve the date of important cladogenic events, particularly in the late Pleistocene, because the coalescence of haplotypes will predate events such as range fragmentation by a considerable margin. This is because divergence of haplotypes within clades is not generally related to the cladogenic event (see for example section 5.4.3), moreover, the dynamics of mtDNA evolution suggest that mtDNA molecular clocks may not be applicable to relatively recent events (Hoelzer et al. 1998).

3.2 Ecological methods

3.2.1 Ecology of pebble-mound mice

Outcomes of behavioural and ecological studies of pebble-mound mice are briefly reported in chapter 5. These studies incorporated almost 80 000 trap nights and covered the region between southeast Queensland and the Kimberley. Radiotelemetry and observational studies were also conducted. The aims of ecological studies were to establish the importance and function of pebble mounds, determine habitat preference and distribution of pebble-mound mice at local, regional and continental scales, and to describe the population dynamics, vagility and breeding behaviour of *P. patrius*.

3.2.2 BIOCLIM DISTRIBUTION MODELLING

Bioclimatic modelling of the distribution of selected clades was undertaken to investigate the relationship between potential climatic tolerances of a species and the realised distribution of species. This was of particular interest in the pebble-mound mice, as a central premise of this thesis is that the distribution of pebble-mound mice is constrained by both topographic and climatic factors, whereas delicate mice are not particularly constrained by topography. Models were run by Emily Bolitho, JCU, using 14 climatic layers (table 3.1) in BIOCLIM (Nix 1986). Only 14 climatic layers were used due to the continental scale of questions being addressed, and the relatively few locations used in modelling. Although an extensive location database was available for delicate mice and some other species, it was decided to only use those locations from which mice had been sequenced due to differences between reported identification of specimens and their molecular identity. The limited locations from which pebble-mound mice were sequenced often represented the known distribution of the species involved. A nine-second digital elevation model (AUSLIG 1997) was used in modelling climatic profiles.

The small number of geographical locations meant that distribution modelling performed here is generally a conservative estimate of the potential distribution of most clades. However, both genetic analysis and BIOCLIM are best performed on a representative sampling of a species distribution. Thus, by attempting to sample the entire geographical range of most species, a reasonable sampling of that species climatic envelope was achieved. This was particularly so in the relatively flat landscapes of northern Australia where topography is a less important influence on climatic shape than in the southeast, where only one species was modelled (*P. novaehollandiae*) based on the majority of known locations for the species.

Table 3.1. Climatic layers used for distribution modelling in BIOCLIM.

Layer	Description
Annual mean temperature	Mean weekly temperature
Isothermality	Mean diurnal temperature range/annual temperature range
Temperature seasonality	Standard deviation of mean weekly temperature
Max temp of warmest period	
Min temp of coolest period	
Temperature annual range	
Annual precipitation	
Precipitation seasonality	Standard deviation of weekly precipitation
Precipitation of wettest quarter	
Precipitation of driest quarter	
Precipitation of warmest quarter	
Precipitation of coldest quarter	
Annual mean radiation	Mean weakly radiation
Radiation seasonality	Standard deviation of weekly radiation

Chapter 4. Species-level phylogeny of Australian rodents

CHAPTER SUMMARY

Results are presented here from two genetic loci in an attempt to reconcile differences among previous phylogenetic studies of conilurine rodents. The majority of Australian rodent species were sequenced, and all but one extant conilurine rodent were involved in the studies. Most relationships supported by mitochondrial control region and the nuclear DHFR intron 1 locus agree with a synthesis of previous results. Marginal support for monophyly of the conilurine rodents was found, but repeated patterns of radiation within Australasian and Australian rodents makes resolution of generic and sub-tribal relationships difficult. It is concluded that conilurines are probably monophyletic, and should be considered so until proof against their monophyly is forthcoming. Support was found for the monophyly of all conilurine genera except *Pseudomys*, which included *Mastacomys*, and was not clearly separated from *Notomys* in any analysis. The genera *Mastacomys* and *Notomys* are retained, and at least three further genera are recognised within the *Pseudomys* group in order to reconcile the recognition of *Mastacomys* and *Notomys* with the remaining diversity of the current genus *Pseudomys*. Support was not found for some unusual results from past studies, including the placement of *Leporillus* within *Pseudomys*, or the basal placement of *Leggadina* within the Hydromyini.

4.1 Chapter background and aims

A robust phylogeny can provide a cornerstone for evolutionary studies. Unfortunately the phylogeny of Australian rodents is poorly known (section 2.1.3). To fully resolve the relationships of Australian rodents it would be necessary to investigate large numbers of species from New Guinea in addition to those occurring in Australia (Donnellan 1989, Watts and Baverstock 1994a, Watts and Baverstock 1994b, Menzies 1996, Torrance 1997). However, conilurine rodents are the focus of this thesis, and it is possible to concentrate on this group in an exclusively Australian context. This chapter attempted to reconcile results

from previous phylogenetic studies of Australian species, including water rats, mosaic-tailed rats and conilurines, and adds two new phylogenies derived from DNA sequencing of the mitochondrial control region and a nuclear intron. An ideal outcome of these studies would have been a fully resolved species phylogeny. However, the realities of systematic studies are generally far from ideal and studies in this chapter had three major aims:

1) Identify closely related species groups suitable for studies of speciation
2) Determine whether conilurine rodents are monophyletic to the exclusion of mosaic-tailed rats
3) Examine the integrity of the genus *Pseudomys* and, if appropriate, recommend intra- or inter-generic divisions that are consistent with the biological and molecular diversity of species within the genus, and the taxonomic status of equivalent conilurine groups

4.2 Tissue samples, sequencing and analysis

Tissue was obtained from all extant species of conilurine rodent recognised by Mackenzie and Burbidge (2002) except *Notomys aquilo* (table 4.1). Museum samples provided by the Australian Biological Tissue Collection of the South Australian Museum formed the bulk of species for this study. Field biopsies were also collected for many species. Where multiple tissue samples were held for a single species they were used to test the consistency of sequence obtained from different geographical locations. Few major discrepancies were found between different tissue samples, and where these were identified, they were the product of miss-labelled tissues or miss-identified museum vouchers. A single sequence was therefore used to represent each species in the final analysis. The tissue from the closest sampled location to the type locality of each species was chosen to ensure the correct identity of species included in the phylogeny. Liver samples where preferentially used to minimise the risk of incorrect sequences due to amplification of nuclear copies of mitochondrial DNA (e.g. sections 5.3.1 and 8.3.1). Sequences included in analysis of the control region in this chapter do not exhibit characteristics that identified the presence of nuclear copies in some species (chapters 5 and 8).

Relationships within *Pseudomys* are a focus of this chapter and were re-examined independently of broader conilurine species phylogenies (section 4.4.3). Control region data were re-analysed to better evaluate relationships within *Pseudomys*. To reduce possible ambiguities in alignment only *Notomys mitchelli*, *Mastacomys fuscus* and those species currently assigned to *Pseudomys* were included in analysis. To minimise the possible effect of site saturation 99 haplotype sequences from nine species of delicate mice and pebble-mound mice were aligned. Any sites that exhibited intraspecific variation involving bases present at that site in related species were recorded as ambiguities. A consensus sequence for each species of pebble-mound mouse and delicate mouse was then aligned against the *Pseudomys* data set. Invariable sites were excluded from analysis. Sites that were variable within more than one species of delicate mouse or pebble-mound mouse were excluded, unless highly conserved among other species. Sites that exhibited high levels of variation that did not correspond to known phylogenetic patterns were also excluded. For example, at a site where "A" was present in most species, if a "G" was present in comparatively unrelated species such as *P. higginsi*, *P. desertor*, *P. chapmani* and *N. mitchelli*.

Table 4.1. Tissue type and source.

Extant: Extinct. Details of samples used in species trees given first, comparative samples second (see text). All conilurine species recognised by Mackenzie and Burbidge (2002) are shown. ABTC- Australian biological tissue bank; GB- Genbank; NTMU- Northern Territory Museum; SAMAM- South Australian Museum; SCU- Southern Cross University; WAMM- Western Australian Museum

Species	Site	Type	Source/voucher
conilurines			
Conilurus albipes			
Conilurus penicillatus	Mitchell Plat., WA, (Kakadu)	Liver, (ear)	(ABTC) WAMM 21963, Field biopsy
Leggadina forresti	Unknown, (Various)	Liver, (gDNA)	(ABTC) WAMM 17031, SCU
Leggadina lakedownensis	Mitchell Plateau, WA (various)	Liver, (gDNA)	GB AF084248 Field biopsy (A. Kutt), SCU
Leporillus apicalis			
Leporillus conditor	Franklin Island, SA	Liver	ABTC 13331
Mastacomys fuscus	Kosciuszko NP, NSW	Liver	(ABTC) SAMAM 10928
Mesembriomys gouldii	Mitchell Plat., WA, (Mareeba)	Liver, (ear)	(ABTC) WAMM 21995, Field biopsy (Ford)
Mesembriomys macrurus	Mitchell Plateau, WA	Liver	(ABTC)
Notomys alexis	Curtin Springs, NT	Liver	(ABTC) SAMAM 17468
Notomys amplus			
Notomys aquilo			
Notomys cervinus	Sandringham Station, QLD	Liver	(ABTC) SAMAM 16886
Notomys fuscus	Unknown	Liver	(ABTC) SAMAM 17508
Notomys longicaudatus			
Notomys macrotis			
Notomys mitchelli	Konettra Downs, SA	Liver	(ABTC) SAMAM 18497
Notomys mordax			
Notomys sp.			
Pseudomys albocinereus	Wanneroo, WA	Liver	(ABTC) WAMM 18856
Pseudomys apodemoides	Lucindadale, SA	Liver	(ABTC) SAMAM 19345
Pseudomys australis	Giddi Giddina ck., SA	Liver	(ABTC) SAMAM 17428
Pseudomys bolami	Whyalla, SA	Liver	(ABTC) SAMAM 18279
Pseudomys calabyi	Kakadu, NT, (Litchfield NP)	Ear, (Liver)	Field biopsy (M. Watson)
Pseudomys chapmani	Woodstock, WA, (Various)	Liver	WAMM 29440, (See ch.5)
Pseudomys delicatulus	Patonga, NT, (Various)	Liver, (Various)	ABTC 8034, (See ch.6)
Pseudomys desertor	Alice Springs, NT, (Various)	Liver, (Various)	ABTC 8143, (See ch.7)
Pseudomys fieldi	Shark Bay, WA	Liver	ABTC 8093
Pseudomys fumeus	Yarrangobilly, NSW, (Various)	Ear, (Ear, Tails)	Field biop. (Ford), (Field biops. Ford/Banks)
Pseudomys glaucus			
Pseudomys gouldii			
Pseudomys gracilicaudatus	Blackbraes NP, QLD, (Various)	Liver, (Various)	Biopsy (Ford/P. Williams), (See ch.7)
Pseudomys hermannsburgensis	Curtin Springs, NT, (Various)	Ear, (Various)	SAMAM 12570, (See ch.6)
Pseudomys higginsi	Captive Bred	Liver	ABTC 8139
Pseudomys johnsoni	Kurundi Station, NT, (Various)	Tail, (Various)	Field biop.(Ford), (See ch.5)
Pseudomys laborifex	Mitchell Plat., WA, (Various)	Liver, (Various)	WAMM 21764, (See ch.5)
Pseudomys nanus	Mitchell Plat., WA, (Various)	Liver, (Various)	(ABTC) WAMM 21625, (See ch.7)
Pseudomys novaehollandiae	Anglesea, VIC, (Various)	Ear, (Various)	Field biopsy (B. Wilson), (See ch.6)
Pseudomys occidentalis	Berdering, WA (Berdering)	Liver	ABTC 8042, (ABTC)
Pseudomys oralis	Carai, NSW, (Billilimbra)	Liver	Biopsy (SCU), (Biopsy SCU)
Pseudomys patrius	Hidden Valley, QLD	Liver	Biopsy (Ford)
Pseudomys pilligaensis	Pilliga, NSW, (Binnaway)	Liver	ABTC 18120, (Field biopsies H. Tokushima)
Pseudomys shortridgei	Unknown	Liver	ABTC 8145
Pseudomys sp.			
Zyzomys argurus	Pellew Is., NT, (Various)	Liver, (Various)	(ABTC) NTMU 1423, (See ch. 7)
Zyzomys maini	Nourlangie Rck, NT, (Various)	Liver, (Various)	(ABTC) 7899, (ABTC, Field Biop. Watson)
Zyzomys palatalis	Woologorang, NT (via TWP)	Ear	Biopsy (Ford)
Zyzomys pedunculatus	MacDonnell R., NT	Ear	ABTC 65820
Zyzomys woodwardi	Mitchell Plateau, WA	Liver	(ABTC) WAMM 21550
"New Guinean" hydromyines			
Melomys burtoni	Hidden Valley, QLD	Tail	Field biopsy (Ford)
Melomys cervinipes	Paluma, QLD	Tail	Field biopsy (Ford)
Uromys caudimaculatus	Paluma, QLD	Tail	Field biopsy (Ford)
Uromys hadrourus	Thornton Peak, QLD	Sequence	GB U17184
Xeromys myoides	Ramingining, NT	Liver	(ABTC) NTMU 4744
Murinae			
Rattus tunneyi	Drysdale River, WA	Tail	Field biopsy (Ford)

4.3 Species phylogenies

4.3.1 Mitochondrial control region

Sequencing of the mitochondrial control region was primarily undertaken to allow phylogeographic studies of closely related taxa (chapters 5-8). However, alignment of control region sequences was relatively straightforward between divergent taxa such as *Uromys*, *Leggadina* and *Pseudomys*. It might be expected that relationships at deeper taxonomic levels would be unresolved due to substitution saturation at variable sites, and the tree would therefore be useful for identifying closely related species and little else. However, one of the major aims of this chapter is to identify sib-species and species complexes suitable for biogeographic study, and the control region is an excellent marker for that purpose. Approximately 360bp of control region sequence was unambiguously amplified from at least one member of each Australian genus except *Hydromys* (appendix i). Nearly all extant conilurines were sequenced. However, *Notomys aquilo* tissue was not available, multiple nuclear copies of the mitochondrion confounded attempts to sequence *Pseudomys fumeus,* and PCR of *Zyzomys pedunculatus* was unsuccessful due to unidentified inhibitors. Alignment of hydromyine taxa against *Rattus* and *Mus* was possible, but unreliable. Trees presented therefore include *Xeromys myoides* as an outgroup to four species and two genera of the Australian mosaic-tailed rats and 36 conilurine species, including 22 *Pseudomys* species.

Bootstrapped Kimura two-parameter distance and maximum-likelihood analyses were performed as detailed in chapter 3. Analysis was performed on 362bp of aligned sequence, with 111 variable sites, 79 of which were parsimony informative. Likelihood analysis was based on the mutational model determined by Modeltest (GTR+I+G model: (A 0.3481; C 0.0661; G 0.2002; T 0.3856) Nst=6 Rmat=(A-C 1.2845; A-G 6.3413; A-T 1.3815; C-G 0.7802; C-T 3.7810; G-T 1.000) Rates=gamma Shape=0.7685). The distance tree (fig. 4.1; minimum evolution score: 1.80196) and likelihood tree (fig. 4.2; likelihood score: -ln 3496.53342) both supported similar relationships between terminal taxa, but, as expected, there was little support for relationships at deeper taxonomic levels, and the trees differed

markedly in their topologies. Enforcing the distance tree topology on the likelihood tree resulted in a tree that was not significantly different to the best tree (-ln 3519.35212; K-H test p= 0.084). In fact, the only re-arrangement of generic relationships that resulted in a significantly worse supported tree topology for the maximum likelihood tree was the exclusion of *Mastacomys* from *Pseudomys* (likelihood score: -ln 3542.7346; K-H test p< 0.000). Saturation tests (Xia et al. 2003) with assumed proportion of invariant sites set at 0, 0.3 and 0.6 indicated little effect of saturation between aligned sequences (p<0.000).

Despite the lack of rigorous support for tree topologies in constraint tests, strong bootstrap support is provided for the monophyly of all genera except *Pseudomys* and *Notomys* (figs. 4.1 and 4.2). There is also support for the monophyly of a group containing the genera *Conilurus*, *Leporillus* and *Mesembriomys*. The mosaic-tailed rat genera *Melomys* and *Uromys* are monophyletic in the distance tree, but not in the likelihood tree. The five species of pebble-mound mice (*Pseudomys calabyi, P. chapmani, P. johnsoni, P. laborifex* and *P. patrius*) are a very strongly supported clade. Three species of *Notomys* (*N. alexis* and *N. fuscus* and *N. mitchelli*) are strongly supported as a monophyletic group, but *N. cervinus* does not group with them. Enforcing monophyly on *Notomys* barely changes support for the likelihood tree (likelihood score: –ln 3498.78935; K-H test p= 0.742). Aside from the pebble-mound mice, there are few well-supported groupings within *Pseudomys*. The only other clade of more than two species to gain bootstrap support is the delicate mice. *Pseudomys delicatulus*, *P. novaehollandiae* and *P. pilligaensis* are a monophyletic group with 76% bootstrap support, and *P. bolami* and *P. hermannsburgensis* are united with this clade in both trees, but have marginal bootstrap support (49% in distance analysis). The sib-species *P. albocinereus* and *P. apodemoides*, *P. higginsi* and *P. fieldi* and, *P. nanus* and *P. gracilicaudatus*, are all well supported.

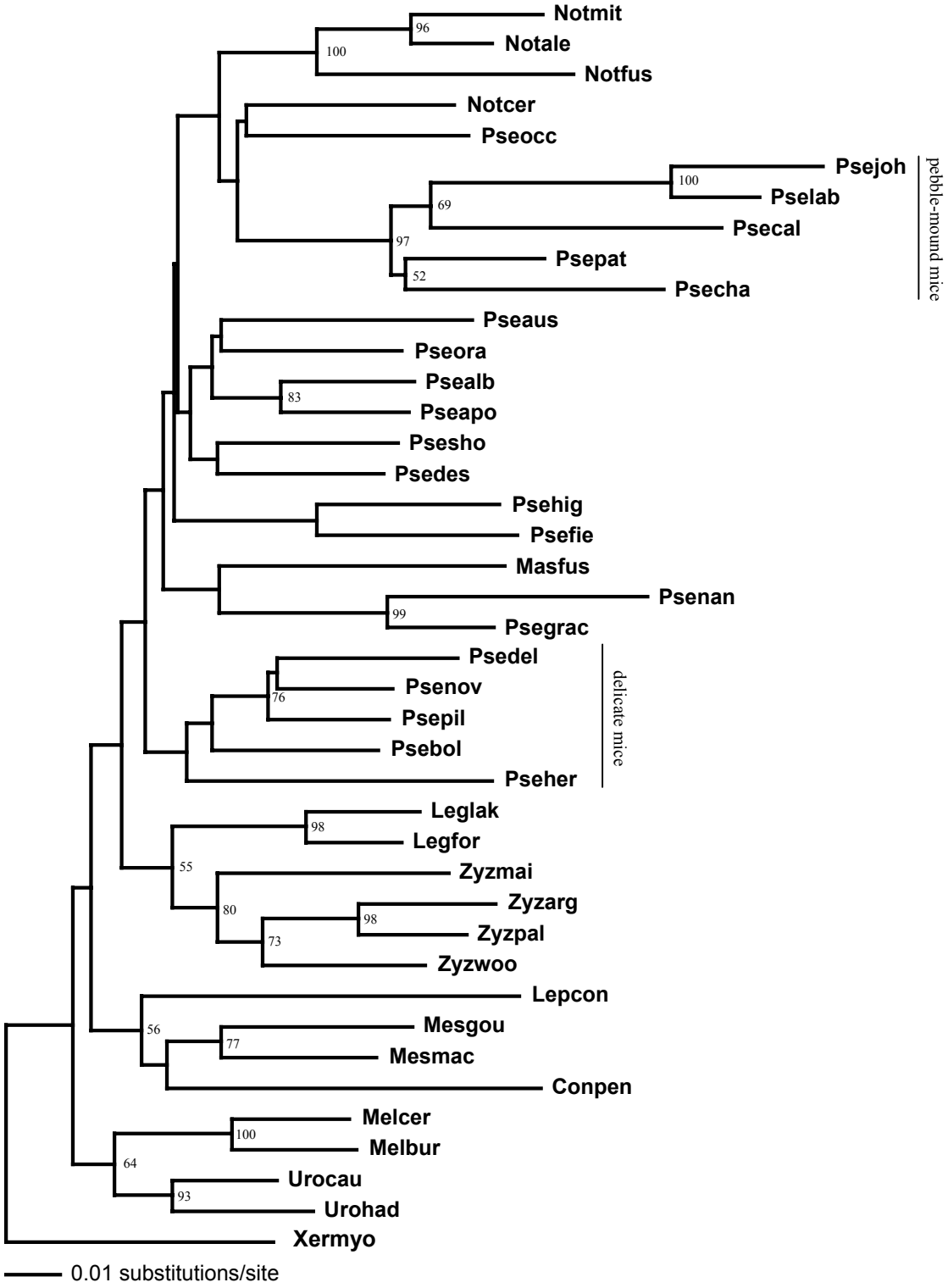


Figure 4.1. Mitochondrial control region sequence divergence among Australian rodents.
Tree produced by neighbour-joining of Kimura two-parameter distance matrix of 362bp sequences. Outgroup *Xeromys myoides*. Node labels= bootstrap support (>50%) based on 100 replicates.

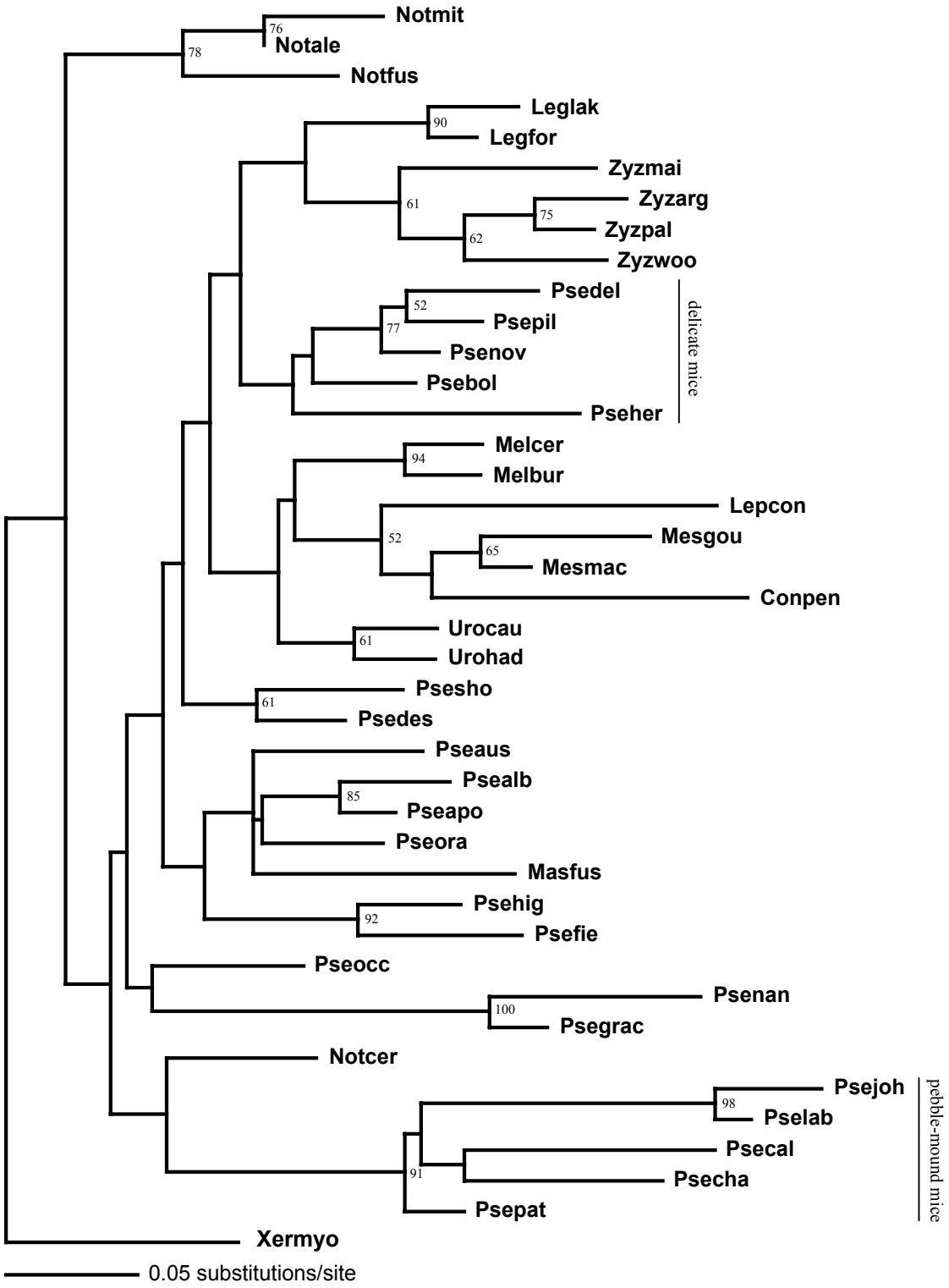


Figure 4.2. Maximum-likelihood tree of Australian rodent control region sequence.
Maximum-likelihood tree based on parameters derived from Modeltest for 362bp of sequence. Outgroup *Xeromys myoides*. Node labels= bootstrap support (>50%) based on 100 replicates.

4.3.2 DHFR INTRON 1

Screening of introns (section 3.1.3) identified the Dihydrofolate reductase (DHFR) intron 1 as a marker that seemed to be evolving at a rate appropriate for resolving generic and sub-generic relationships among Australian rodents. Heterozygosity at the locus was not a problem for sequence interpretation, as homozygotes appear to differ by only a few bases (see chapters 5 and 6). Sequences of 398bp were readily aligned among Australian Old Endemic rodents, and aligned less comfortably against *Rattus tunneyi* sequence (appendix i). Alignment against an incomplete *Mus musculus* sequence downloaded from Genbank was possible, but the *Mus* sequence was obviously more divergent from Australian rodents than was *Rattus*. As with the control region, several taxa proved problematic to sequence. *Notomys cervinus* was a taxon of particular interest due to its alliance with *Pseudomys occidentalis* in control region phylogenies. However, repeated attempts to sequence the species yielded what was interpreted to be a transposable element, but not DHFR intron sequence. *Zyzomys pedunculatus* and *Z. palatalis* also yielded short products that were not DHFR intron sequence. *Pseudomys australis* did not produce PCR product, despite multiple attempts and two re-extractions of gDNA. *Xeromys myoides* yielded only 350bp of clean sequence. However, the final 40bp of the DHFR sequence was highly conserved across the mosaic-tailed rats and tree-rats, allowing an estimation of *Xeromys* sequence to be used in alignment that contained only a single ambiguous site. Likewise *P. gracilicaudatus* did not produce clean product after bp 345, however, clean sequence from the very closely related *P. nanus* and the relatively conserved nature of the final portion of sequence allowed a probable sequence to be inserted. The causes of these problems were not clear, but given an abundance of transposable elements and multiple banding patterns produced by PCR, results for the DHFR intron require further investigation before publication and should be interpreted as preliminary results only.

Despite these problems, the Kimura two-parameter distance tree and maximum-likelihood tree based on DHFR intron 1 sequences are highly concordant with those based on the control region, differing in the fact that they tend to better support relationships at deeper levels of the phylogeny due to a slower rate of mutation. Sequences differed from the

control region in that only 129 sites (of 398) were variable among Australian rodents and *Rattus tunneyi*, and only 49 of those were parsimony-informative due to a long branch-length to *Rattus*. No effect of saturation is evident in sequences ($p<0.000$). Trees were rooted on *Rattus tunneyi*, and the use of *Xeromys myoides* as an outgroup to the conilurines and mosaic-tailed rats in control region analysis was supported by both the distance tree (fig. 4.3; minimum evolution score: 0.44631) and maximum-likelihood tree (fig. 4.4; likelihood score: –ln 1580.77449; substitution model: TrN+I (A 0.2146; C 0.2947; G 0.2821; T 0.2086) Nst=6 Rmat=(A-C 1.0000; A-G 3.0258; A-T 1.0000; C-G 1.0000; C-T 3.5696; G-T 1.0000) Rates=equal). Topologies of the two trees were very similar, the major differences being the lack of structure within the genus *Pseudomys* in the maximum-likelihood tree, and the separation of conilurines from mosaic-tailed rats in the maximum-likelihood tree.

The monophyly of *Notomys*, *Mastacomys* and *Pseudomys* was suggested by control region distance trees (but not statistically supported), and gained strong bootstrap support in both DHFR intron 1 trees. Bootstrapping of the distance tree, but not the maximum-likelihood tree, supported monophyly of a clade containing the genera *Conilurus*, *Leporillus* and *Mesembriomys*. *Mesembriomys* and *Conilurus* were allied in both trees, but this relationship was only given bootstrap support in the distance tree. The mosaic-tailed rats were strongly supported as a monophyletic clade in both trees. Three *Notomys* species produced identical DHFR sequences and form a clade with *P. occidentalis* within *Pseudomys*. The two species of *Zyzomys* included in the tree formed a monophyletic clade, and there was moderate bootstrap support for a sib-taxon relationship to the *Notomys-Pseudomys* group. *Pseudomys* was the only genus not supported as a monophyletic clade, and members of *Pseudomys* formed a polytomy with the genera *Mastacomys* and *Notomys*. Species groups within *Pseudomys* supported by control region analysis were also present in DHFR intron 1 trees. The delicate mice, pebble-mound mice, *P. albocinereus* and *P. apodemoides, P. fieldi* and *higginsi, P. nanus* and *P. gracilicaudatus, P. desertor* and *P. shortridgei* all form groups in trees, and most had moderately strong bootstrap support. *Pseudomys fieldi* and *higginsi* were supported at 49% in bootstrapping of the distance tree.

Pseudomys fumeus was not included in control region trees but is supported at 77% as a sib-taxon to *P. albocinereus* and *P. apodemoides*.

Several indels in DHFR intron 1 sequences appeared to be phylogenetically informative. Parsimony analysis was performed to account for sequence gaps by treating them as a "fifth state". A bootstrapped parsimony tree yielded a similar result to maximum likelihood, but tended to better support some relationships (tree length: 191; CI: 0.7853, CI excluding uninformative characters: 0.5638). *Pseudomys fieldi* and *P. higginsi* were allied (62%) and *Pseudomys oralis* was allied with *P. desertor/shortridgei* (65%). The undoubted sib-taxa *P. gracilicaudatus* and *P. nanus* that were not supported by maximum-likelihood bootstrapping were supported as a monophyletic lineage (92%). However parsimony also allied *Mastacomys fuscus* with the delicate mice (82%), and *Xeromys myoides* with *Uromys caudimaculatus* to the exclusion of *Melomys* on the basis of partially overlapping (analogous) deletions. Stronger support was generally indicated by parsimony analysis for relationships supported by maximum-likelihood, notably the delicate mice, which share two unique (homologous) deletions when compared to other conilurines and were supported as a monophyletic group at 95%. The parsimony tree is not presented as its topology is identical to the likelihood tree.

Specific tests of tree topology were conducted on the maximum-likelihood tree to examine past results regarding the placement of some taxa. A tree in which *Leggadina* was basal to the water rats, mosaic-tailed rats and conilurines was not significantly less supported than the best tree found (likelihood score: -ln 1584.4326; K-H test p= 0.598). A tree that included *Leporillus conditor* within *Pseudomys* was obviously less well supported (likelihood score – ln 1609.2554), but this difference was marginally non-significant in a Kinisharo-Hasigawa test (p= 0.072), although a Shimodaira-Hasegawa test indicated that the tree was significantly worse (p= 0.048). Trees that enforced monophyly on *Pseudomys* were not significantly different to the best tree (likelihood score: 1592.17203; K-H test p= 0.281), whether *Mastacomys* was included within *Pseudomys* or not (likelihood score of 1587.22806 with *Mastacomys* included).

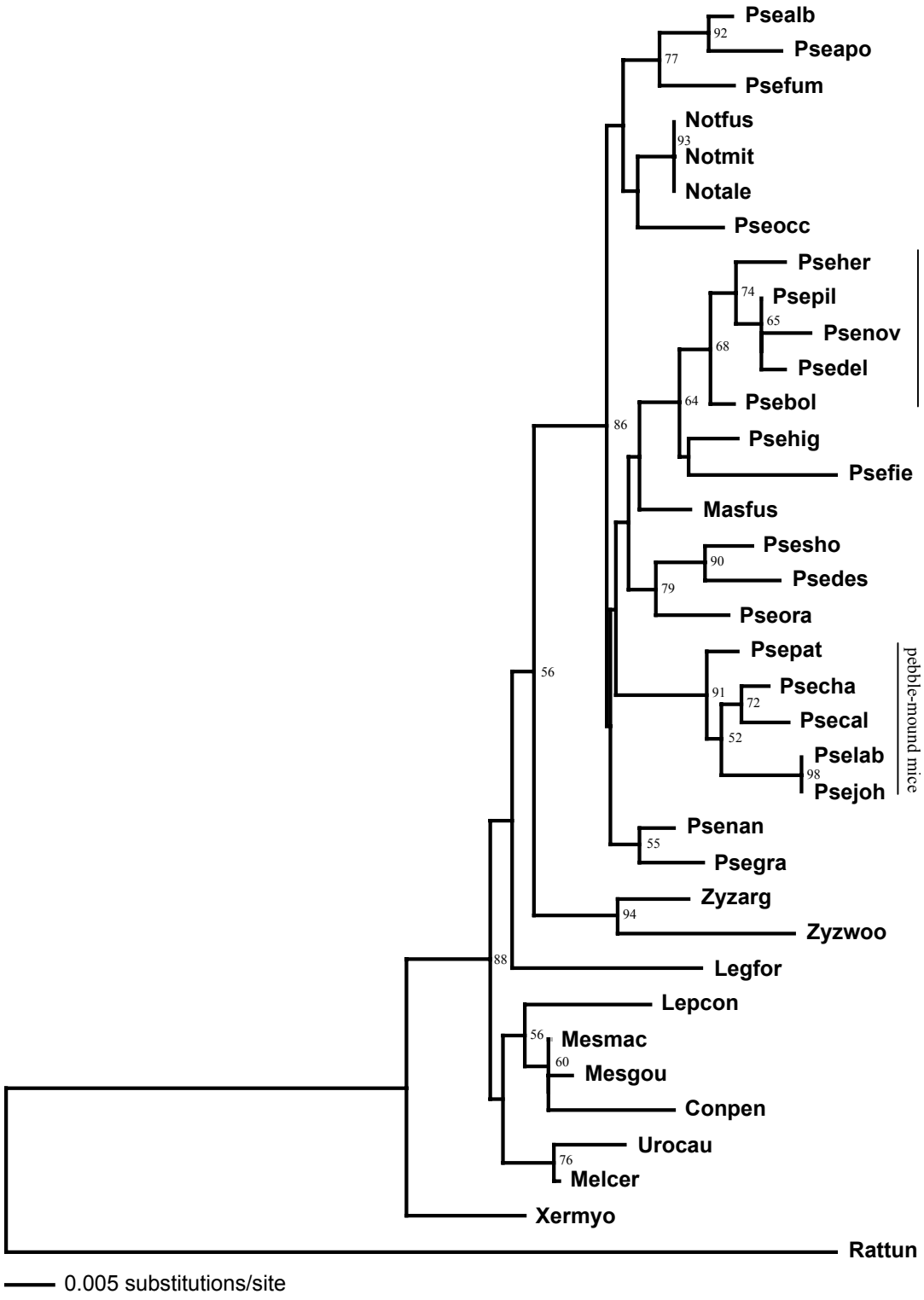


Figure 4.3. DHFR intron 1 sequence divergence among Australian rodents.
Tree produced by neighbour-joining of Kimura two-parameter distance matrix of 398bp sequences. Outgroup *Rattus tunneyi*. Nodes labels= bootstrap support (>50%) based on 100 replicates.

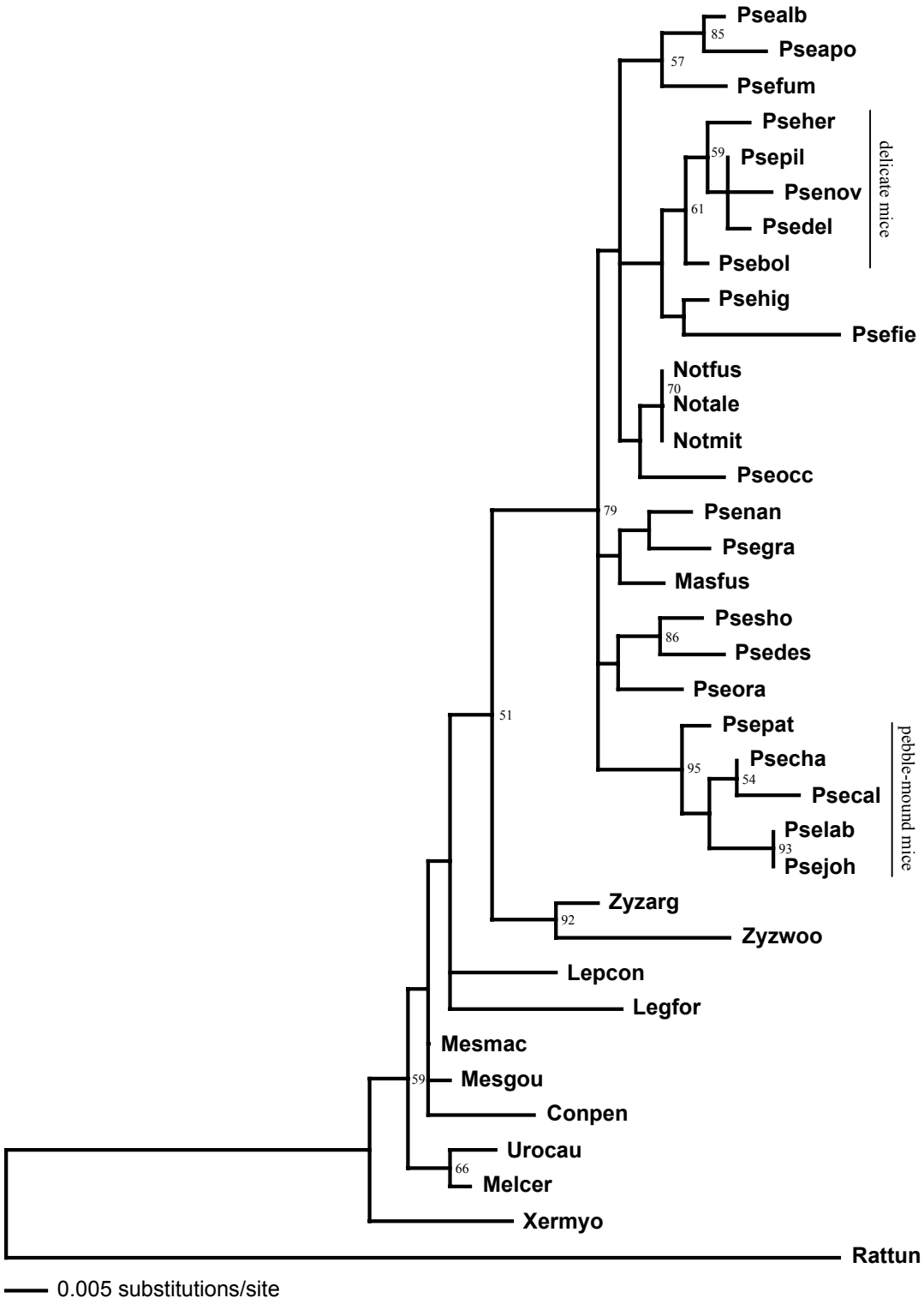


Figure 4.4. Maximum-likelihood tree of Australian rodent DHFR intron 1 sequence.
Maximum-likelihood tree based on parameters derived from Modeltest for 398bp of sequence. Outgroup *Rattus tunneyi*. Node labels= bootstrap support (>50%) based on 100 replicates.

4.4 A realistic phylogeny: a biogeographical framework

The results presented here accord well with previous work, in particular microcomplement fixation (Watts et al. 1992). Australian Old Endemic rodents (the Hydromyini) form a coherent, closely related clade when compared to *Rattus*. Within this group, water rats seem to be basal, and the conilurine clade is closely related to the mosaic-tailed rats. Many species relationships supported by phylogenies based on DNA sequencing are also expected based on previous research, and the independent production of these results should place those relationships beyond doubt. Other results, such as the possible inclusion of *Notomys* within *Pseudomys* (as currently defined) are less expected, but are consistent with some previous findings.

4.4.1 Conilurine monophyly: are patterns of endemism indicative of phylogeny?

Analysis of control region and DHFR intron 1 sequences separated the Australian endemic conilurine rodents from the New Guinea-derived mosaic-tailed rats *Uromys* and *Melomys*, but there was no statistical support for this relationship. Conilurine rodents are obviously closely related to mosaic-tailed rats, and the fact that topology tests indicated that *Leggadina* could be placed in a basal position to all hydromyine rodents without significantly altering support for the DHFR tree is indicative of a probable series of radiations at deeper levels of the phylogeny, as is the lack of saturation revealed by Xia et al. (2003) tests. However, there was a separation of conilurine rodents from the *Uromys/Melomys* clade in all bar the DHFR intron 1 distance tree. Penis morphology (Lidicker and Brylski 1987) and microcomplement fixation (Watts et al. 1992) studies were unable to separate several conilurine genera from mosaic-tailed rats, and cytochrome-b sequencing results included many New Guinean genera amongst the Australian endemics (Torrance 1997), although that tree was affected by saturation at deeper levels (S. Donnellan pers. comm.). On the basis of these results it would seem that the conilurines are not strongly supported as a monophyletic lineage, and (Watts and Baverstock 1994b) synonymised the tribes Conilurini, Hydromyini and Uromyini into a single tribe, the Hydromyini, on the basis of microcomplement fixation results.

The possibility that the conilurines are not monophyletic is biogeographically perplexing given the strongly endemic nature of both the conilurines and New Guinean genera. It is the genera *Mesembriomys*, *Leporillus* and *Conilurus* that most often associate with the mosaic-tailed rats, although the labile position of *Leggadina* in phylogenies is enigmatic. Therefore, a simple biogeographic scenario of the evolution of the conilurines may involve an early invasion from New Guinea that gave rise to *Notomys/Pseudomys*, *Zyzomys* and *Leggadina*, followed by a more recent invasion by tree rats from New Guinea. The fossil record does not preclude such a scenario (Godthelp 1999). However, the weight of evidence still seems to suggest that the conilurines are a monophyletic assemblage, and that difficulties in resolving their relationship to New Guinean genera stem from virtually simultaneous processes of divergence from New Guinean stock and radiation within Australia. Separation of New Guinean and Australian stocks, either by rising Pliocene sea levels or waif dispersal from New Guinea (section 2.2.1) would, in terms of DNA sequence analysis, be contemporaneous with an early Pliocene generic radiation of rodents within Australia.

Conclusion

Lack of synapomorphic characters defining the conilurine clade, and a shallow basal divergence from New Guinean stock when compared to deep generic divisions within the conilurines has lead to difficulties in establishing their monophyly. However, conilurines are probably a true monophyletic group, closely allied to the mosaic-tailed rats and more distantly to the water rats. This result is suggested by molecular studies presented here based on two genetic loci and by karyotypic studies. However, there is no definitive proof of conilurine monophyly.

4.4.2 GENERIC RELATIONSHIPS

Conilurine rodents represent a true radiation. The ecological diversity of genera and plasticity of topologies at deep nodes of the conilurine tree, in the absence of saturation, support this fact. This makes resolving some generic relationships difficult. The tree rat genera *Conilurus* and *Mesembriomys* and the stick nest rats (*Leporillus*), form a monophyletic group, a result suggested by previous research (Lidicker and Brylski 1987, Watts et al. 1992, Torrance 1997) and strongly supported here. *Mastacomys fuscus, Notomys* and *Pseudomys* form a large and complex monophyletic assemblage in which generic boundaries are ill defined (Baverstock et al. 1977b, Baverstock et al. 1981, Baverstock et al. 1983, Godthelp 1989, Watts et al. 1992). *Zyzomys* species form a well-supported monophyletic genus that may be a sister-taxon to either *Leggadina* or *Notomys*/*Pseudomys* on the basis of results presented here.

Since Baverstock et al. (1976) and Watts (1976) re-defined *Leggadina* only Lidicker and Brylski (1987) have, erroneously, questioned the species composition of the genus. *Leggadina forresti* and *L. lakedownensis* are the only two known species, and have repeatedly been shown to be divergent from *Pseudomys* species (Baverstock et al. 1977b, Baverstock et al. 1981, Baverstock et al. 1983, Watts et al. 1992, Torrance 1997). This result is strongly supported by this thesis. However, *Leggadina* has been enigmatic in its placement in phylogenies. Baverstock et al. (1981) and Watts et al. (1992) both found *Leggadina* to lie outside the conilurine lineage. However, DNA sequencing at mitochondrial (control region, this study: cyt-b, Torrance (1997)) and nuclear loci (DHFR intron 1, this study) shows *Leggadina* to be a conilurine genus. Statistical support for that relationship in sequencing trees is not strong, but there is a strong morphological argument against a stronger affinity with water rats or basal hydromyine position (Troughton 1941, Tate 1951, Watts and Aslin 1981), and no reason to doubt the expected placement of *Leggadina* within the conilurine clade. The genus *Zyzomys*, which is a sister-taxon to *Leggadina* in the control region trees, possesses such a highly derived karyotype that Baverstock et al. (1977b) and Baverstock et al. (1983) were reluctant to ally the genus with any particular group on the basis of chromosomal homologies. However, this thesis, penis

morphology (Lidicker and Brylski 1987), isozyme (Baverstock et al. 1981) and microcomplement fixation (Watts et al. 1992) studies all indicate that *Zyzomys* is a conilurine genus.

Affinities of the genus *Leporillus* have also been a matter of speculation since isozyme analysis placed the genus within *Pseudomys* (Baverstock et al. 1981). Watts and Aslin (1981) also suggested an affinity with *Pseudomys* and *Leggadina*. No study of the karyotype of *Leporillus* has been published, and the probable absence of the *Notomys*/*Pseudomys* reciprocal translocation between chromosomes 1 and 2 has therefore not been established. Both genetic loci studied in this thesis show a close relationship to the tree rats, *Conilurus* and *Mesembriomys*. These genera differ from *Leporillus* in the number of internal cusps on the M1 and M2 molars, a character that has formed the basis for the presumed affinity of *Conilurus* with *Mesembriomys* and *Zyzomys* (3 cusps), and *Leporillus* with *Pseudomys* (2 cusps)(Crabb 1977, Tate 1951). *Conilurus* and *Leporillus* are, however, superficially similar genera. Both have been described as "rabbit rats" because of their size, large ears and rounded body form (Troughton 1923, Watts and Aslin 1981). There has been confusion over the identity of specimens referred to (*Conilurus*) *albipes* and (*Leporillus*) *apicalis* by early explorers (Mahoney 1982), and Ogilby (1838) described some individuals of *Conilurus albipes* as *Conilurus constructor* in the mistaken belief that it was *C. albipes* not *L. apicalis* that built stick nests. Microcomplement fixation (Watts et al. 1992) and penis morphology (Lidicker and Brylski 1987) support the close relationship of *Leporillus*, *Conilurus* and *Mesembriomys*.

4.4.3 Species relationships

Species relationships within most conilurine genera are straightforward; there are only two species in *Conilurus*, *Leggadina*, *Leporillus* and *Mesembriomys*. Relationships among the five species of *Zyzomys* cannot be resolved on the basis of results presented in this thesis. However, it would appear that the recently split species *Z. maini*, *Z. palatalis* and *Z. woodwardi* (Kitchener 1989) do represent true species, and that *Z. palatalis* may be more closely related to *Z. argurus* than to *Z. maini* and *Z. woodwardi* with which it was

synonymous until 1989. *Zyzomys pedunculatus* must be included in analyses before firm conclusions regarding inter-specific relationships within *Zyzomys* are made.

Some authors have speculated that *Pseudomys* might be a "taxonomic wastebin" that includes a collection of species united solely by lack of derived characters (Baverstock et al. 1981, Watts and Aslin 1981, Godthelp 1989). Others have recognised all species as closely related, but have split the genus into multiple genera (Iredale and Troughton 1934, Tate 1951). As a result, there is a wealth of information regarding relationships between *Pseudomys* species. However, published phylogenies have not fully sampled the genus, and those species that have been examined are often not consistent between studies. Results of DNA sequencing presented here have confirmed that 23 species currently referred to the genus *Pseudomys* form a closely related but complex group. *Mastacomys fuscus* is confirmed here as a member of *Pseudomys* if the current definition of the genus is accepted. However, the inclusion of, or very close relationship with, *Notomys* species in mitochondrial and nuclear phylogenies, penis morphology (Lidicker and Brylski 1987) and one method of isozyme analysis (Baverstock et al. 1981) increases the number of species within the "genus" to over thirty. This result indicates that at the very least, sub-genera need to be re-described, and in all probability generic divisions should be recognised within the complex. It is also clear that association of species regarded as members of *Pseudomys* is not simply a result of retention of ancestral characters. With the exclusion of *Leggadina* species historically regarded as members of *Pseudomys*, all remaining species of *Pseudomys*, *Notomys* and *Mastacomys* form a monophyletic derived lineage, the "*Pseudomys* group", a relationship strongly supported by sequence analysis (Torrance 1997, this thesis), karyotypic (Baverstock et al. 1977b, Baverstock et al. 1983) and microcomplement fixation (Watts et al. 1992) studies. There is enough consistency between past studies of *Pseudomys* species relationships and the current study to deal explicitly with the question of how to split *Pseudomys* into sensible genera. These should be consistent with the level of taxonomic division between other conilurine genera, and in my opinion they should also identify natural groups of species that share unique or characteristic biological attributes.

The hopping mice

Species belonging to the genus *Notomys* possess some of the most derived characteristics of any Australian rodents. Their gross morphology and locomotion is distinctive; elongate ears and feet, long brush-tipped tail and a bipedal gait at high speed (Watts and Aslin 1981). *Notomys* skulls are distinctive, in particular the structure of the zygomatic plate and arch (Watts and Aslin 1981). Reproductive tracts of *N. alexis*, *N. aquilo*, *N. mitchelli* and *N. fuscus* are highly divergent, not only from other conilurines, but from other mammals (Breed 1981, 1985, 1986). *Notomys alexis* and *N. fuscus* are some of the few hydromyine species in which the structure of the sperm significantly diverges from the ancestral form (Breed 1997). Watts and Aslin (1981) considered *Notomys* so distinctive as to be a clade of equal age to the divergence of mosaic-tailed rats and all other Australian rodents, and thought 15My of evolution had led to the modern hopping mouse morphology. Does it make sense then, that sequencing studies presented here suggest *Notomys* is of no greater evolutionary age than several species groups within the genus *Pseudomys*, and that hopping mice are derived from an ancestral "*Pseudomys*" species, not basal hydromyine stock?

Recent publications have considered that Watts and Aslin's (1981) time line is significantly too long, and predates the origin of hydromyine rodents (Aplin accepted, Watts et al. 1994). The oldest *Notomys*-like fossils are late Pliocene in age, and are teeth that appear ancestral to the modern dentition of *Notomys* species (Crabb 1977, Godthelp 1999). No information on morphology of the early *Notomys* lineage is available. *Notomys* is clearly allied with *Pseudomys* and *Mastacomys* to the exclusion of all other rodents (Baverstock et al. 1977b, Baverstock et al. 1983). Baverstock et al's (1981) isozyme study show *Notomys* to form a clade within *Pseudomys* in distance analyses, and the distance to *Notomys* from many *Pseudomys* species was less than between other *Pseudomys* species in their Wagner tree analysis. The idea that *Notomys* evolved from *Pseudomys* is also not without precedent. Ellermann (1941) proposed that *Mastacomys* and *Notomys* evolved from *Pseudomys* on the basis of gross morphology, and, barring internal morphological peculiarities of some *Notomys* species, the hopping mice are essentially "*Pseudomys*" that hop and have big ears and feet. There is a strong evolutionary argument for the evolution of *Notomys* from a

"*Pseudomys*" ancestor (section 9.2.3). However, the genus is distinct, and could be considered a very closely related monophyletic sib-taxon to *Pseudomys* on the basis of topology tests and the results of Watts et al. (1992) and Baverstock et al. (1981).

At least two well-defined clades are identifiable within *Notomys*. The *Notomys alexis/mitchelli/fuscus* clade is evident as a very closely related assemblage in both control region and DHFR intron 1 trees. This clade is that which possesses highly derived reproductive tracts (Breed 1981, 1985, 1986), and on this basis also includes *N. aquilo* (W. Breed pers. comm.). This clade matches Watts and Aslin's (1981) "group 3", and probably includes the remaining member of that group *N. mordax*. *Notomys cervinus* more closely resembles *Pseudomys* species in its reproductive tract than it does the *N. alexis* clade (Breed 1985, 1986), a state considered ancestral within *Notomys* by Breed (1986). Mitochondrial control region does not yield a monophyletic *Notomys* due to the similarity of *N. cervinus* sequence to that of *P. occidentalis*, although the inclusion of extinct *Notomys* species in analysis would no doubt separate *N. cervinus* from *Pseudomys occidentalis*. Skull and dental characters (Tate 1951), the lack of a throat gland (Watts and Aslin 1981), karyotype (Baverstock et al. 1977b, Baverstock et al. 1977d), isozymes (Baverstock et al. 1981) and microcomplement fixation (Watts et al. 1992) all indicate *N. cervinus* is quite distinct from the *N. alexis* clade, and Waite (1900) placed it in a separate genus, primarily on the basis that it lacked a throat gland. Watts and Aslin (1981) ally *N. macrotis* with *N. cervinus*. *Notomys longicaudatus* also lacks the derived reproductive tract of the *N. alexis* clade (Breed 1989b) but was placed in a separate group with *N. amplus* and *Notomys* sp. by Watts and Aslin (1981), based mostly on body size. The lack of derived reproductive characters cannot be taken as an indication of the monophyly of *N. cervinus* and *N. longicaudatus*, and their relationship is unresolved. The placement of *N. mordax* close to *N. cervinus* by Watts and Aslin (1981) is followed here.

"Pseudomys"

Figure 4.5 presents relationships of *Pseudomys* and *Notomys* species taken from all relevant systematic studies. Some interpretation of results is necessary to produce the figures, primarily due to the splitting of *Pseudomys* species by other genera (4.5c), and inconsistent results between analytical methods in the same paper that the authors did not attempt to reconcile (4.5a and d). Trees were formed by uniting the closest related *Pseudomys* species, and retaining clades indicated by the original source. The number of species included in each study varies, and some inconsistencies between studies are probably related to missing taxa that are intermediate between those that were included. A single *Notomys* species is shown in each figure as it is assumed that the genus is monophyletic, but is a lineage within, or very closely related to, the currently defined genus *Pseudomys*. Kimura two-parameter and parsimony trees derived from analysis of the reduced alignment of 118bp (outlined in section 4.2 (all sites variable, 80 parsimony-informative)) are shown in figure 4.6 and are very similar to past results and relationships presented in more comprehensive species phylogenies.

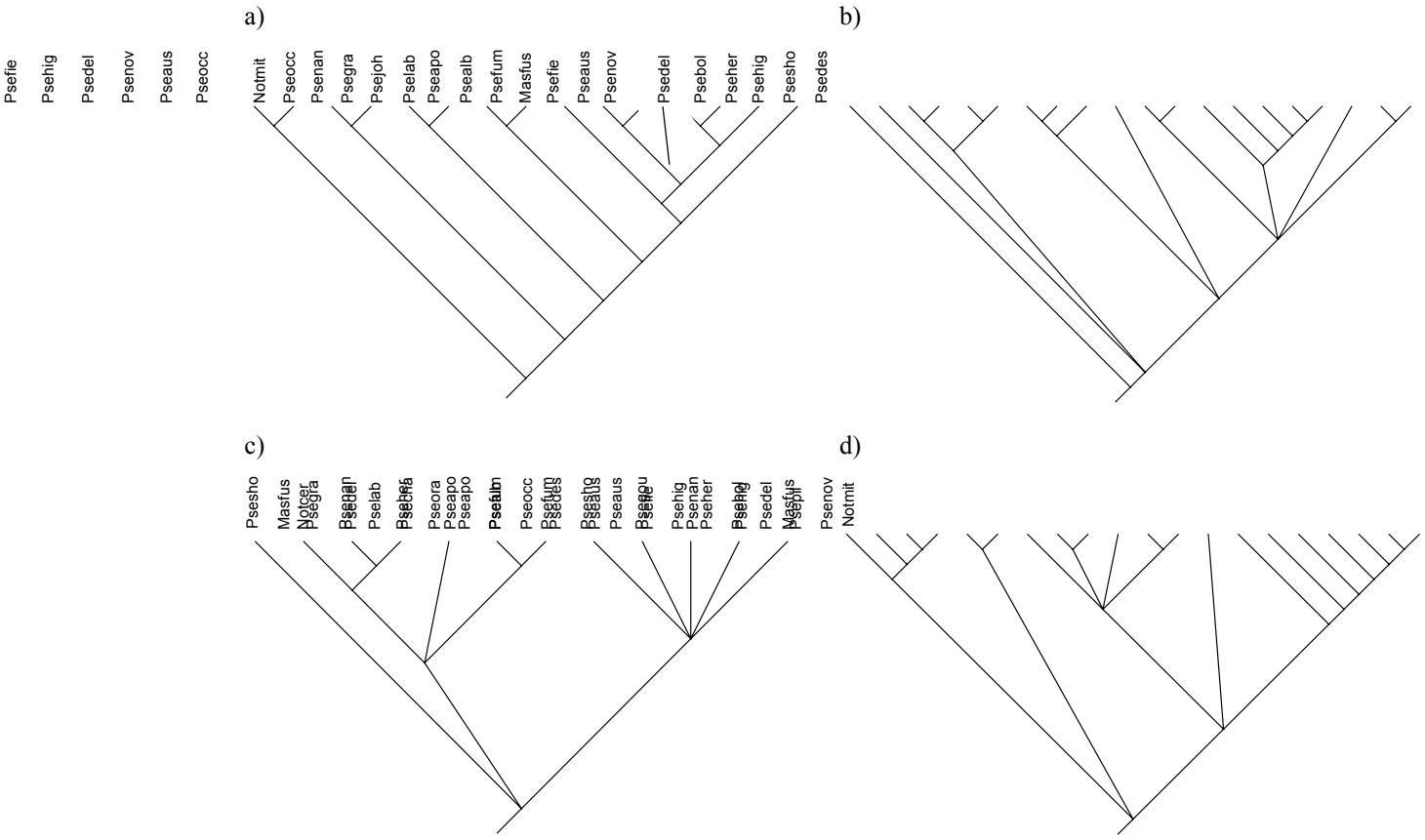


Figure 4.5. Systematic relationships among *Pseudomys* group rodents
a) Isozyme electrophoresis (phenogram only) (Baverstock et al. 1981). b) Microcomplement fixation (Watts et al. 1992). c) Penis morphology (Lidicker and Brylski 1987). d) Cytochrome-b sequencing (Torrance 1997).

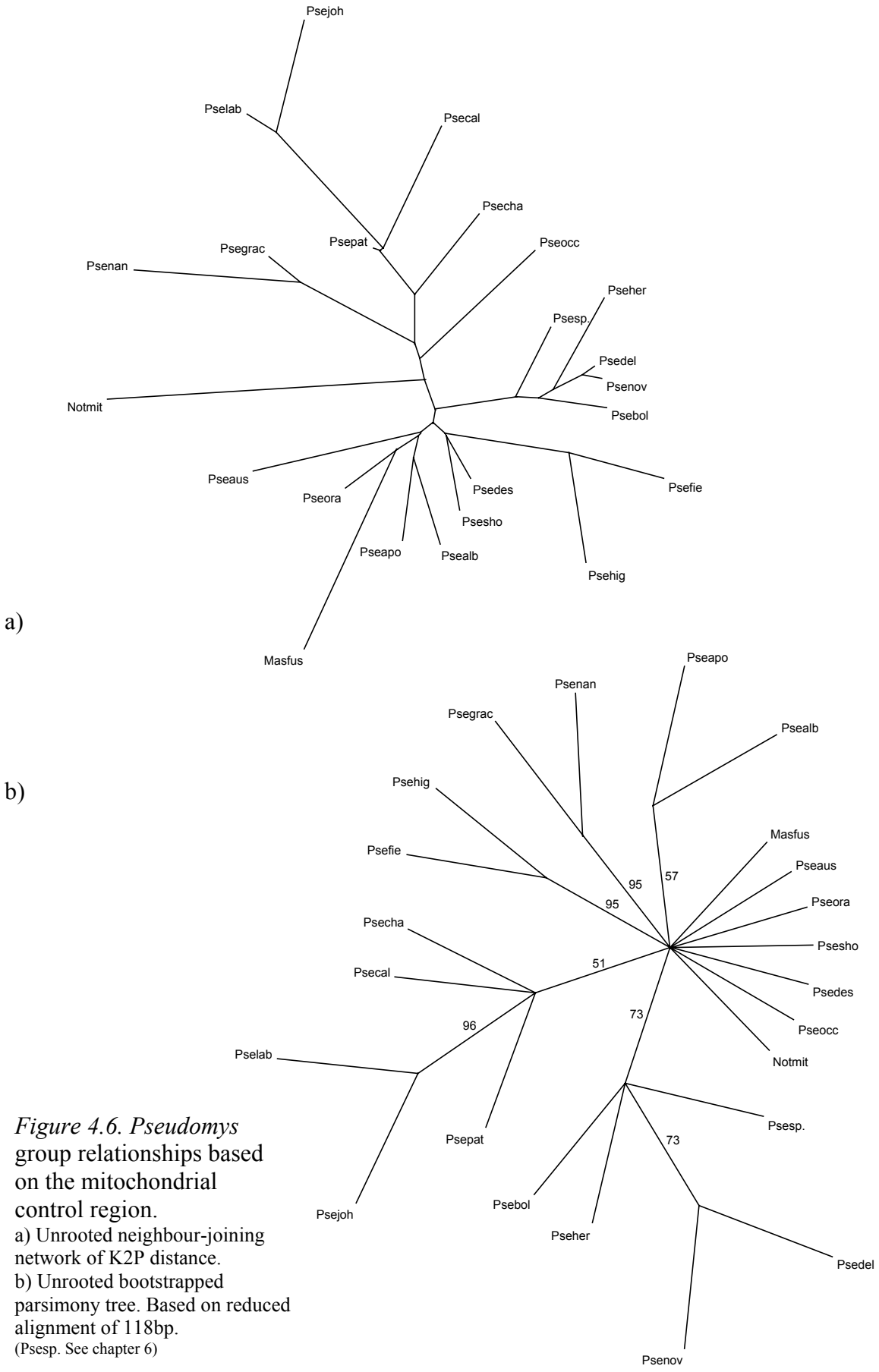


Figure 4.6. Pseudomys group relationships based on the mitochondrial control region.
a) Unrooted neighbour-joining network of K2P distance.
b) Unrooted bootstrapped parsimony tree. Based on reduced alignment of 118bp.
(Psesp. See chapter 6)

Genera within Pseudomys

Four clades within "*Pseudomys*" are clearly divergent from the bulk of other *Pseudomys* species:

1) Mastacomys fuscus

Mastacomys Thomas 1882 has long been regarded as a monotypic genus on the basis of highly divergent dentition (Iredale and Troughton 1934, Tate 1951, Ride 1970, Watts and Aslin 1981), and continues to be regarded as such (Strahan 1995, Mackenzie and Burbidge 2002) despite clearly and consistently falling within *Pseudomys* in systematic studies (Baverstock 1981, Lidicker and Brylski 1987, Watts et al. 1992). Baverstock et al. (1981) retained *Mastacomys*, but were reluctant to split *Pseudomys* to recognise genera of equivalent standing because of the inconsistency of relationships between other species and the creation of many smaller genera. Watts et al. (1992) formally included *Mastacomys* within *Pseudomys*, but this nomenclature has not been widely adopted. *Mastacomys* has dentition that separates it from all other *Pseudomys* species, is not particularly closely related to any *Pseudomys* species, and should be recognised as a genus.

2) Pseudomys gracilicaudatus, Pseudomys nanus (and *P. gouldii*?)

The chestnut mice were previously regarded as members of *Thetomys* either as a sub-genus (Thomas 1910) or genus (Iredale and Troughton 1934). That name is available, and should be applied to *(P). nanus* (the type of *Thetomys*) and *(P.) gracilicaudatus*. Watts and Aslin (1981) allied the chestnut mice with *P. desertor* and *P. shortridgei*, but Troughton (1932) and Tate (1951) concluded that there were conspicuous differences between *P. nanus* and *P. desertor* and placed them in different sub-genera. Chestnut mice have a shorter gestation period than other *Pseudomys* species. *Pseudomys nanus* has a gestation period of only 22-24 days, which is significantly shorter than that of any other hydromyine (Taylor and Horner 1972), although the taxonomic importance of this is doubtful. *Pseudomys gracilicaudatus* and *P. nanus* share a pseudogene not detected in any other conilurine

rodent (chapter 8). Skulls of the extinct species *P. gouldii* closely resemble those of the chestnut mice (Watts and Aslin 1981), and *P. gouldii* was included in *Thetomys* by all authors (Thomas 1910, Iredale and Troughton 1934, Ellermann 1941, Tate 1951). However, although not closely related to *P. nanus* and *P. gracilicaudatus*, *P. higginsi* and *P. fieldi* were also included in *Thetomys* (Tate 1951), and the tentative inclusion of *P. gouldii* in *Thetomys* here is solely based on the greater similarity of its skull to the chestnut mice than other species. Crabb (1977) considered the chestnut mice a distinct group within *Pseudomys* on the basis of dental characters, but considered *P. gouldii* close to *P. australis*. However, Crabb (1977) based *P. gouldii* largely on specimens of *P. australis* (A. Baynes in Aplin accepted).

3) Pseudomys occidentalis

Pseudomys occidentalis shows no consistent alliance with any *Pseudomys* species. The status of this species is unclear, as sequencing results and microcomplement fixation (Watts et al. 1992) show it to be divergent from other *Pseudomys*, and it may constitute a genus at the level of division proposed here. Historically the species has been allied with the *P. albocinereus* group in *Gyomys* (Tate 1951), and Watts and Aslin (1981) included it in their "group 3" that also included the *P. albocinereus* group. Microcomplement studies place the species close to *Notomys*, and basal to *Pseudomys* (Watts et al. 1992). The species associated most strongly with *Notomys* in trees presented here, but tree manipulations of control region and DHFR intron 1 data that enforced monophyly on *Notomys* allied *P. occidentalis* with the *P. albocinereus* group, and this may indicate a deep relationship with that lineage.

4) Pseudomys calabyi, Pseudomys chapmani, Pseudomys johnsoni, Pseudomys laborifex, Pseudomys patrius

As expected based on the occurrence of the unique, shared, behaviour of pebble-mound building in these species they are strongly supported as a monophyletic clade in control region and DHFR intron 1 sequencing. The biogeographic history of the clade indicates

that it is old (chapter 5) and the genetic distance encompassed within the group is equivalent to that within much of the remainder of *Pseudomys* (fig. 4.6). A pseudogene present in all species of pebble-mound mice is unique to the group, and is highly divergent from true mitochondrial sequence, indicating an old origin and isolation of the pebble-mound mouse lineage when compared to other *Pseudomys*. The only studies that have included pebble-mound mice (Watts et al. 1992, Torrance 1997) have shown labile or distant relationships to other *Pseudomys* species (Torrance's (1997) "*P. johnsoni*" and "*P. calabyi*" were based on miss-identified tissue samples (S. Donnellan pers. comm.)). Sequencing of the ZPc gene by Christine Swann, Adelaide University, has also identified the group as divergent from all other *Pseudomys* in the possession of a protein-changing mutation not found in any other murid rodent (C. Swann pers. comm.). Therefore, pebble-mound mice are characterised by a unique behaviour, which indicates a strong biological argument for recognition of the group as a distinct genus, and this distinction is matched by divergence in molecular studies. The name *Phorolithomys* derived from the Greek for "stone carrying mice" will be proposed for this group.

The remaining members of *Pseudomys* tend to group reasonably closely in systematic analyses, and are shown at the bottom of figure 4.6 (a). Several strongly supported species groups are apparent (below). Inter-relationships among these groups are more problematic than the separation of the chestnut mice and pebble-mound mice from them, because the distances between groups are not so great, and some species show labile affinities. However, several genera could be recognised within this group, but this will probably rely on further study due to the unresolved relationships of some taxa.

Pseudomys australis

Pseudomys australis is the type species of the genus. There is a great deal of taxonomic confusion over specimens referred to the species, and it is not clear what the natural distribution or taxonomic composition of the species is (or was) (Watts and Aslin 1981, Brandle et al. 1999). In studies presented here it is difficult to determine any clear relationships of the species because DHFR intron 1 sequence was not obtained. In several

studies the species groups closely with the delicate mice and *P. fieldi/higginsi* (Baverstock et al. 1981, Watts et al. 1992, Torrance 1997), although it sometimes groups, more distantly, with the *P. albocinereus* group in different analyses performed by those authors. It is proposed here that in its most limited form, the generic name *Pseudomys* should be applied to the delicate mice, *P. fieldi/higginsi* and *P. australis* (including any forms that eventually are regarded as closely related or synonymous with *P. australis*). However, this must carry the proviso that there is only slightly stronger support for the alliance of *P. australis* with the delicate mouse group than there is for its association with other species such as *P. albocinereus* (control region tree here, some cytochrome-b trees (Torrance 1997)).

Delicate mice

All methods of analysis in this thesis support the monophyly of *P. bolami*, *P. delicatulus*, *P. hermannsburgensis*, *P. novaehollandiae* and *P. pilligaensis*. Although not an unexpected result, this group has not been fully dealt with in previous studies. Watts et al. (1992) found that *P. bolami*, *P. delicatulus*, *P. hermannsburgensis* and *P. novaehollandiae* were a monophyletic assemblage. Lidicker and Brylski (1987) only examined the penis morphology of *P. delicatulus* and *P. hermannsburgensis*, which they considered to be species of *Leggadina*. Although not included in their analysis, they regarded *P. novaehollandiae* to be a member of a separate genus, *Gyomys*. The isozyme study of Baverstock et al. (1981) showed *P. delicatulus* and *P. novaehollandiae* as sib-species, but *P. hermannsburgensis* was somewhat distant in both their distance and Wagner trees, although it is clear that Baverstock (1982) regards these three species as a closely related group. When describing *P. pilligaensis* Fox and Briscoe (1980) and Briscoe et al. (1981) also assumed that *P. hermannsburgensis*, *P. delicatulus* and *P. novaehollandiae* were a closely related species group. Control region and DHFR intron 1 sequencing has confirmed the suspected alliance of all five of these species (see chapter 6 for a taxonomic and systematic review of this group).

Pseudomys fieldi (*including* P. praeconis) *and* Pseudomys higginsi

These two species are given strong support as sibs in analyses presented here. Watts et al. (1992) show *P. fieldi* (as *praeconis*) to be allied with *P. australis*, while they place *P. higginsi* closer to *P. desertor/shortridgei*. However there is little difference in distances they report between these taxa, and the relationships they show are not supported by the same authors earlier studies of isozymes (Baverstock et al. 1981), which agree with the relationships suggested here. Cytochrome-b sequencing supports the sib-species relationship of *P. higginsi* and *P. fieldi* (Torrance 1997). Troughton (1941) and Tate (1951) regarded *P. higginsi* and *P.* (*praeconis*) as members of different genera or subgenera, but also split the synonymous *P. fieldi* and *P. praeconis*. *Pseudomys fieldi* and *P. higginsi* consistently group with the delicate mice in systematic studies (Baverstock et al. 1981, Watts et al. 1992, Torrance 1997).

Pseudomys desertor, P. shortridgei *(and* P. oralis)

The desert mouse (*P. desertor*) is allied here with the heath rat (*P. shortridgei*). The close relationship of *P. desertor* and *P. shortridgei* is supported by microcomplement fixation (Watts et al. 1992) and cytochrome b sequencing (Torrance 1997). Lidicker and Brylski (1987) did not include *P. desertor* in their analysis, and the two species have radically different sperm morphology (Breed 1997). Crabb (1977) considered them distinct within *Pseudomys* and closely related to each other. Troughton (1941) and Tate (1951) separated the species in different subgenera/genera. Baverstock et al. (1981) did not examine *P. desertor* isozymes. The further alliance of the *P. desertor/shortridgei* clade with *P. oralis* in DHFR intron 1 studies accords with skull similarities (Watts and Aslin 1981), but differs from dental characteristics (Crabb 1977). *Pseudomys oralis* has not been included in any published phylogenies, and Watts and Aslin (1981), like Tate (1951), considered it to be more closely related to *P. australis*, as it was originally described as a subspecies of that species (Thomas 1921). *Pseudomys oralis* is therefore only tentatively allied with *P. desertor* and *P. shortridgei*. Barring an aberrant result in isozyme studies, the *P. desertor/shortridgei* group tends to ally with the *P. australis*/*fieldi*/delicate mouse group,

but at a level deep enough to constitute recognition as a separate group at either the sub-generic or generic level. The name *Lasiomys* derived from the Greek for "hairy mice" is appropriate for mice in this group, which have a grizzled, shaggy, appearance. The name appears to be a disused synonym of *Lophuromys* Peters 1874, and should be available.

Pseudomys apodemoides, P. albocinereus, (P. glaucus), P. fumeus

Pseudomys albocinereus and *P. apodemoides* are a well-recognised sib-species pair, strongly supported by trees presented in this thesis. The further alliance of these species with *P. fumeus* is indicated in DHFR intron 1 trees, a relationship supported by general morphology (Watts and Aslin 1981), microcomplement fixation (Watts et al. 1992), penis morphology (Lidicker and Brylski 1987), some cytochrome–b trees (Torrance 1997) and dentition (Crabb 1977). *Pseudomys fumeus* is one of four species (*Leggadina forresti*, *Leporillus apicalis* and *P. shortridgei* are the other three) that were placed in unusual phylogenetic positions by isozyme studies (Baverstock et al. 1981) when compared to other studies. Isozyme analysis distanced *P. fumeus* from most *Pseudomys* species, and it was particularly isolated from *P. albocinereus* and *P. apodemoides*. All were regarded by Tate (1951) to be members of the sub-genus *Gyomys*, and although control region sequence is not available for *P. fumeus*, the relationship with *P. apodemoides/albocinereus* is reasonably strongly supported. This gains further support from the occurrence of communal breeding in all three species, despite the atypical conditions under which this occurs in *P. fumeus* (Woods and Ford 2000). The extinct *P. glaucus* is probably a member of this group, as it has been referred to *P. albocinereus* (Cockburn 1995b), although Burbidge and Mackenzie (2002) recognise it as a distinct species. Despite biogeographical concerns about the synonomy of *P. albocinereus* and *P. glaucus*, *P. glaucus* was probably more closely related to *P. albocinereus/apodemoides* than is *P. fumeus*. The Tertiary fossil species *Pseudomys vandycki* resembles *P. albocinereus* (Godthelp 1989). This group is closely related to the *P. australis* and *P. desertor* groups. The group could be regarded as a genus. However, *P. novaehollandiae* is the type of the previous generic name used to describe these species. The name *Calotrichomys* derived from the Greek for "beautiful-haired mice" would be appropriate for this group, as most members are named after the

colour or complexion of their fur, and are noted for their beautiful appearance and demeanour.

4.5 Synthesis

The definition and taxonomic rank of species groups within *Pseudomys* is a perennial problem of Australian taxonomy, and with the sinking of the former tribe Conilurini within the tribe Hydromyini there is now considerably less scope for ascribing evolutionary significance to deeper level relationships within the group. In my view, the conilurine rodents should be considered monophyletic at least at the sub-tribe level until conclusive evidence *against* their monophyly is presented (fig. 4.7). The composition and taxonomic rank of the Hydromyini is a topic beyond the scope if this thesis. Irrespective of the status of the conilurines, the genera *Notomys*, *Pseudomys* and *Mastacomys* clearly constitute a large well-defined group deserving of some recognition, possibly as a sub-tribe, although this would re-elevate the conilurines to tribal status. I will simply refer to them as the *Pseudomys* group (fig. 4.6.1). The *Conilurus/Leporillus/Mesembriomys* clade (tree rat/*Leporillus* clade) would appear to be of the same status and deserving of the same rank, while the affinities of *Zyzomys* and *Leggadina* are unclear, but they are clearly conilurine. Genera outside the *Pseudomys* group are all well supported as monophyletic units (fig. 4.7).

Relationships within the *Pseudomys* group are still not fully resolved, despite great effort having been expended on defining species groups. This is probably the result of rapid radiation of early *Pseudomys*-like rodents, and ongoing processes of speciation and extinction with radical changes in environment over the last few million years. In this regard, my explanation for the observed pattern of relationships differs markedly from Lee et al. (1981) who proposed an early radiation of the conilurine rodents followed by long periods of stasis, leading to isolated clades such as *Mastacomys*. Whether *Pseudomys* should be so broadly defined as to include such distinct forms as *Mastacomys*, which shows no particular affinity to any other *Pseudomys* species, is even more pertinent in light of DNA sequencing of mitochondrial and nuclear loci presented here, which shows that "*Pseudomys"* contains a number of divergent evolutionary lineages characterised by unique

adaptations including the pebble-mound mice and possibly *Notomys*. The most logical approach is to erect multiple genera that reflect the diverse clades within the group.

A glance at the distance trees presented here (figs. 4.1, 4.3 and 4.6 (a)) illustrates the problem faced by all authors who have attempted to unite species at this level; the basal node of the *Pseudomys* group is only marginally deeper than is the basal node of most clades within the group. There is also a lack of closely related species that leaves several isolated species in parsimony and likelihood analyses (figs. 4.2, 4.4 and 4.6 (b)). This is reflected in the morphology of species within the group, and was the motivation for Ride (1970) to disregard intra-generic taxonomy. The fact that defining multiple genera within *Pseudomys* may lead to genera containing only one or two species has been argued to potentially make the process counter-productive (Baverstock et al. 1981). However, the historical recognition of *Mastacomys* as a distinct genus, and the fact that *Conilurus*, *Leporillus*, *Mesembriomys* and *Leggadina* contain only two species negates that argument.

Within the *Pseudomys* group the previously defined genera *Notomys* and *Mastacomys* should be recognised (fig. 4.7). The pebble-mound mice and chestnut mice are as isolated, or more so, from other *Pseudomys* species as are *Notomys* and *Mastacomys*, and should be recognised as genera. A large genus *Pseudomys* is retained by the recognition of the *P. australis/P. fieldi*/delicate mouse complex as a genus. The closely related *P. desertor/ shortridgei/oralis*? clade should probably be regarded a sub-genus, or as is currently the case, members of *Pseudomys*, until more evidence confirms groupings suggested here. *Pseudomys albocinereus, P. apodemoides, P. fumeus* and *P. glaucus* are considered to probably be a good genus with a fossil history of at least three million years. However, as with the last group, retention in *Pseudomys* seems an equally valid interpretation until more data is presented. The relationships of *P. occidentalis* are unclear, but may lie with the *P. albocinereus* group.

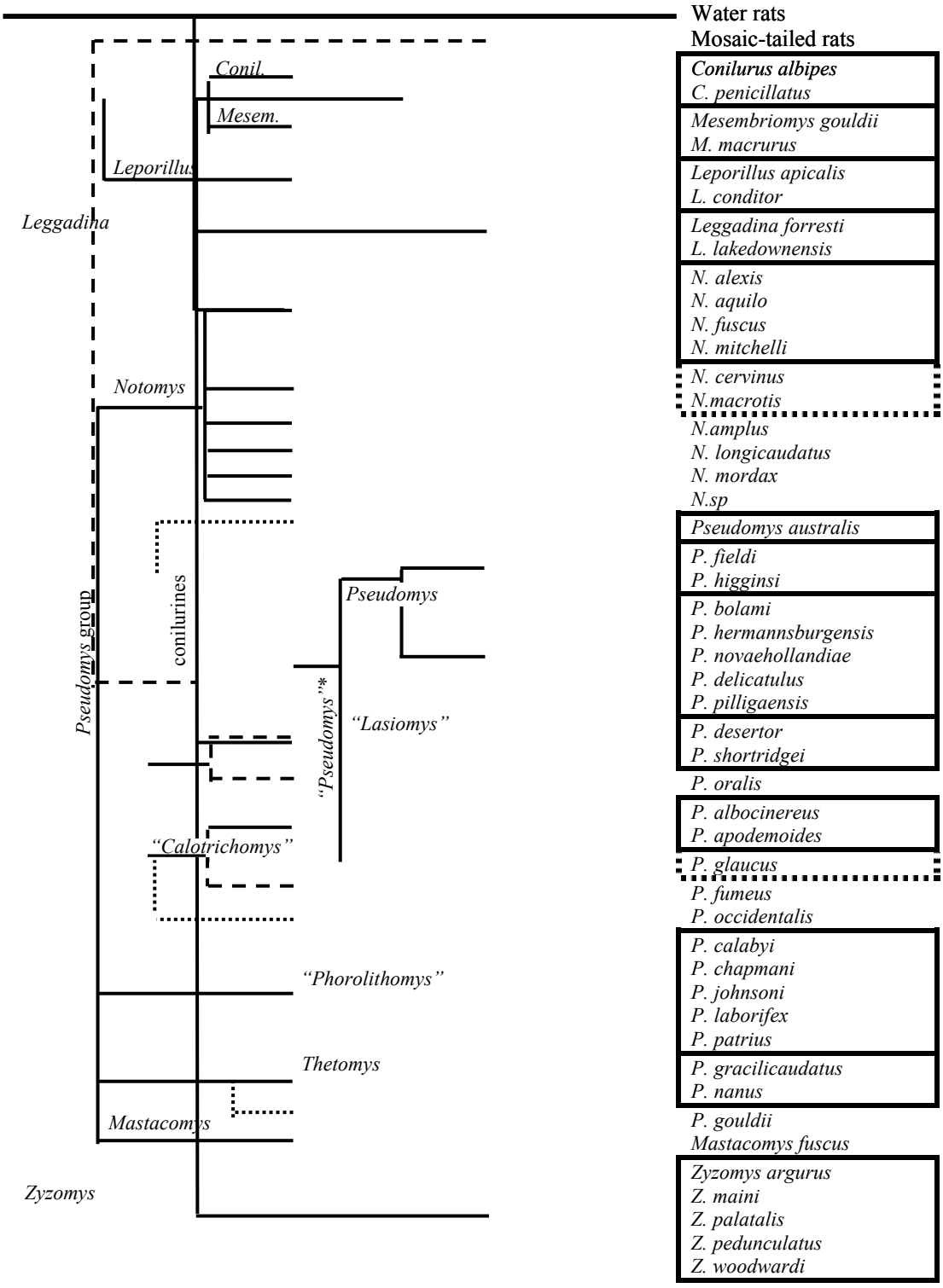


Figure 4.7. Synthesis of phylogenetic relationships between conilurine rodents.
Solid boxes denote strongly supported species groups (= genera except in *Notomys* and *Pseudomys*). Solid lines are well-supported relationships, dashed lines moderately supported relationships, dotted lines are more speculative. *Alternative (and largest justifiable) definition of the genus *Pseudomys*.

Chapter 5. Evolution of distributions and species boundaries within the pebble-mound mice

CHAPTER SUMMARY

Population divergence among five currently recognised species of pebble-mound mice is investigated using the mitochondrial control region and DHFR intron 1. An unexpected finding was that *P. johnsoni* of central Australia and *P. laborifex* of the Kimberley and VRD are probably subspecies, and form the most distinct clade within the group. Evolution of the group has not been a straightforward process of isolation. Rather, there has been a dynamic interaction between geological, topographical and climatic changes. Current distributions of species are dictated by topographical and geographical constraints, in particular the Great Sandy Desert and the Carpentarian plains. Range shifts have probably altered geographical relationships among species, as current distributions are unlikely to reflect patterns at the time of speciation events. Tertiary lateritic surfaces, now degraded, are implicated in allowing contact until the mid Pleistocene? between populations of pebble-mound mice now isolated by pebble-free habitats. Both *P. johnsoni* and *P. patrius* have undergone recent range expansion following the LGM. Population extinction and bottlenecking, probably during the LGM, lead to the formation of three distinct mtDNA lineages within *P. johnsoni*, one of which equates to the described Kimberley form *P. laborifex*.

5.1 Ngadji and its relatives

***Pseudomys calabyi* Kitchener and Humphries 1987, *Pseudomys chapmani* Kitchener 1980, *Pseudomys johnsoni* Kitchener 1985, *Pseudomys laborifex* Kitchener and Humphries 1986, *Pseudomys patrius* Thomas and Dollman 1909**

5.1.1 Pebble-mound mice by name…

There are five recently described or rediscovered species of small *Pseudomys* known as pebble-mound mice. They are patchily distributed across northern Australia (fig. 5.3), and construct unique pebble structures in and around their nesting burrows (section 5.1.2). New populations are still frequently discovered, and the known distributions of pebble-mound species are far broader than shown in Strahan (1995). Common names of the pebble-mound mice reflect their distributions. The western pebble-mound mouse (*P. chapmani*) is found in Western Australia in the Pilbara, Hamersley Ranges, western uplands of the Little Sandy Desert, and the Gascoyne and Murchison Ranges (Start et al. 2000). Start et al. (2000) adopted the nomenclature recommended by Braithwaite et al. (1995) in calling *P. chapmani* Ngadji, the name used by the Panyjima people of the Hamersley Ranges. The central pebble-mound mouse (Ilyema; *P. johnsoni*) is found in the Davenport and Murchison Ranges of the Northern Territory, and in rocky ranges and desert country bordering the Barkly Tableland (Kerle 1995a, J. Cole pers. comm.). It is known from owl pellets and miss-identified specimens of *P. hermannsburgensis* from rocky ridges in the Mittibah-Alexandrina area in the central Barkly Tableland (Thomas 1906b, Kerle 1995a). Populations of mice from the Mt Isa-Cloncurry region of western Queensland were initially thought to be *P. johnsoni* by Van Dyck (pers. comm.), but he later (Van Dyck and Birch 1996, Van Dyck 1997) regarded them as an undescribed species. Populations near the Gulf of Carpentaria are of uncertain status, but may be *P. johnsoni* (S. Donnellan pers. comm.). The Kimberley mouse (Tataroo; *P. laborifex*) is distributed throughout the north and east of the Kimberley (Kitchener 1995a). Newly discovered populations of pebble-mound mouse in the western Northern Territory and Gardner Range in the northwest Tanami Desert, Western Australia, have been identified as *P. laborifex* by J. Woinarski (pers. comm.) and

N. Cooper (pers. comm.). The Kakadu pebble-mound mouse (Pinti; *P. calabyi*) was thought to be restricted to Stage III of Kakadu National Park, but has recently been discovered over a wider area of the Top End both inside and outside the boundaries of Kakadu (Field data, J. Griffith pers. comm., G. O'Neil pers. comm., M. Watson pers. comm.). The Queensland, or eastern, pebble-mound mouse (*P. patrius*) is found between the Great Dividing Range and the Queensland coast from Gympie to Cairns (Van Dyck and Birch 1996, S. Burnett pers. comm., field data).

All species are small, with the average weight of *P. chapmani*, *P. johnsoni* and *P. laborifex* around 12g (Kitchener 1985, 1995a, Start et al. 2000) and *P. calabyi* and *P. patrius* slightly heavier at 14-15g (Woinarski et al. 1995, Cermak 1999). Field observations and the above sources indicate that all species share a rich orange-brown upper pelage tinted by dark emergent guard hairs and a white belly that contrasts strikingly with the upper coat on the flanks and chin. The thin tail may be pink, pink-grey or grey, and may have faint white bands. In some older animals the tail may become quite fat (up to 4mm diameter) along its entire length. In general appearance pebble-mound mice resemble *P. delicatulus* and *P. hermannsburgensis*, which are sympatric with pebble-mound mice in many areas of northern Australia.

Remote habitats and cryptic morphology have ensured that pebble-mound mice remained unknown to science across most of their distribution until very recently. Pebble-mounds, however, have not gone altogether unnoticed. Although Watts and Aslin (1981) cite Spencer (1896) as noting the presence of pebble-mounds attributed to *P. hermannsburgensis* in the Horn Expedition Report, W.B Stalker, the true source of the observation they cite (reported in Thomas 1906) did describe pebble-mounds from the eastern Northern Territory as early as 1905. Yet it was not until 1980 when Kitchener (1980) described *P. chapmani* from pebble-mounds in the Pilbara region that the use of both "pebble-mound mouse" and "sandy inland mouse" to describe *P. hermannsburgensis* (e.g. Ride 1970, Watts and Aslin 1981) was questioned. Dunlop and Pound (1981) tested Kitchener's notion that the synonymy of *P. hermannsburgensis* and *P. chapmani* was responsible for the dual nomenclature of *P. hermannsburgensis.* The co-occurrence of *P.*

chapmani and pebble mounds which they inhabited, but widespread distribution of *P. hermannsburgensis* in the absence of mounds finally extricated *P. chapmani* from the delicate mouse group, and established it as the first recognised species of pebble-mound mouse. The description of *P. johnsoni* as a pebble-mound mouse followed (Kitchener 1985), and gave a species name to the builder of mounds described eighty years earlier by Stalker. *Pseudomys laborifex* and *P. calabyi* (as *P. laborifex calabyi*) were initially described only as small *Pseudomys* (Kitchener and Humphries 1986, 1987), and were more recently recognised as pebble-mound mice (Woinarski 1992, MacGuire unpublished, Woinarski pers. comm.).

The earliest description of a species of mouse from pebble mounds actually dates from 1909. Stalker noted mounds at the northeast Queensland site where he captured specimens that Thomas and Dollman (1909) later described as "*Mus patrius"*. Although later authors recognised *Pseudomys patrius* (Tate 1951, Briscoe et al. 1981), and Troughton (1941) and Finlayson (1942) reported two further individuals of *"Leggadina patria"* from near the Queensland coast, the species was not regarded as a pebble-mound mouse, and was synonymised with *P. delicatulus* by Ride (1970). This meant that the 1991 capture of a *Pseudomys* species from a pebble mound south of Charters Towers was somewhat of a revelation to modern biology (Van Dyck 1991). The specimen proved to be *P. patrius*, and the first described species of pebble-mound mouse was recognised as such 82 years later. A survey over the next four years revealed a distribution stretching over 1,000km of latitude in eastern Queensland (Van Dyck and Birch 1996).

5.1.2 Ecology of pebble-mound mice

Extensive studies of pebble-mound mouse ecology were conducted in conjunction with molecular studies presented here. They will be reported in detail elsewhere. Ecological studies aimed to describe habitat preference of all species except *P. chapmani*, which has been the subject of some ecological studies due to conflict with mining operations in the Pilbara (Anstee 1996, Anstee et al. 1997). Variation in mound architecture was of interest because a role has been proposed for pebble-mounds in mate choice (Cermak 1999), and

mounds could therefore impact directly on speciation processes. Population dynamics and movement of *Pseudomys patrius* in Hidden Valley, northeast Queensland, were studied over a four-year period to aid interpretation of genetic results. A brief summary of results is presented here to establish the contrast between the ecologies of pebble-mound mice and delicate mice.

Mound architecture, usage and function

Behavioural observations and variation in mound architecture suggest that pebble mounds primarily perform a defensive function. Mounds are built in and around nesting burrows (fig. 5.1). Burrow systems can be quite complex, and have multiple entrances. Total weight of pebble accumulations may be over 50kg, but mounds of that size are likely to be decades old. Pebbles are gathered from the surrounding ground surface to construct mounds. Pebbles are used to block burrow entrances upon entering and leaving the nest. This behaviour, and the fact that mice use pebbles to block damage to the burrow system establishes the primary role of pebbles to be one of defence. There is no evidence of a role in insulation, or in sexual selection by either sex.

Variation in pebble mound architecture revealed no species-level preference for a particular design of mound, rather all pebble mounds consist of the same types of structures (fig. 5.1), but these vary in number and size between mounds. Variation tends to reflect the local ecological conditions of a population, age of the mound, and the immediate situation in which a mound is built. For example, in Hidden Valley mounds tend to consist of two or three volcano-shaped pebble ramparts around burrow entrances, at least one of which is usually comprehensively blocked. Larger mounds incorporate other structures such as piles of pebble with no obvious function, and pebble "stuffing" along cracks in tree roots or rocks (fig. 5.1). "Stuffing" is used to block apparent routes of entry to the burrow system under the mound. In other regions where trees and rock outcrop are not common, pebble mounds tend to be more sprawling structures with sheets of pebbles covering the ground. However, almost as much variation is generally found within large populations than among populations from across northern Australia, and identical mound design can be found in

populations of mice in the Tanami Desert (desert, *P. johnsoni*), Bungle-Bungle ranges (sub-desert, *P. laborifex*), Kakadu National Park (tall-grass savannah, *P. calabyi*) and Hidden Valley (heathy savannah, *P. patrius*). Mounds are therefore unlikely to play any direct role in speciation. However, females of *P. patrius* did not breed without pebble mounds, and most bred in more than one mound. Segregation of litters in different mounds may be important in preventing siblicide in this species, which, to maximise reproductive success over a short lifetime must maxime offspring survival. The need for (multiple?) pebble mounds makes them a critical limiting resource, and has important ramifications for habitat choice, population dynamics and site fidelity.

Habitat preference

Pebble-mound mice require pebbles and are therefore restricted to rocky, erosional landscapes. Aggradational landforms are not inhabited, unless they are pebbly, and immediately adjacent to erosional landforms. Habitat preference of *P. calabyi*, *P. johnsoni*, *P. laborifex* and *P. patrius* are very similar. All these species, and *P. chapmani*, inhabit rocky, rolling, hilly landscapes (field data, Woinarski 1992, Anstee et al. 1997, Start et al. 2000). Steeper slopes tend to be avoided, and where steep slopes are part of the local topographic sequence, pebble-mound mice tend to be located on waxing upper slopes of moderate or gentle incline, or on waning foot slopes of moderate or gentle incline. A grassy understorey with a sparse shrub layer is the most common vegetation association, although "grass" may be *Themeda triandra*, other pasture species, *Sorghum* spp. or spinifex. *Eucalyptus* species tend to form the dominant upper stratum, even in arid areas were *Acacia* is the regionally dominant vegetation, although in some desert sites, and in the Pilbara, trees are often scattered (Start et al. 2000, field data).

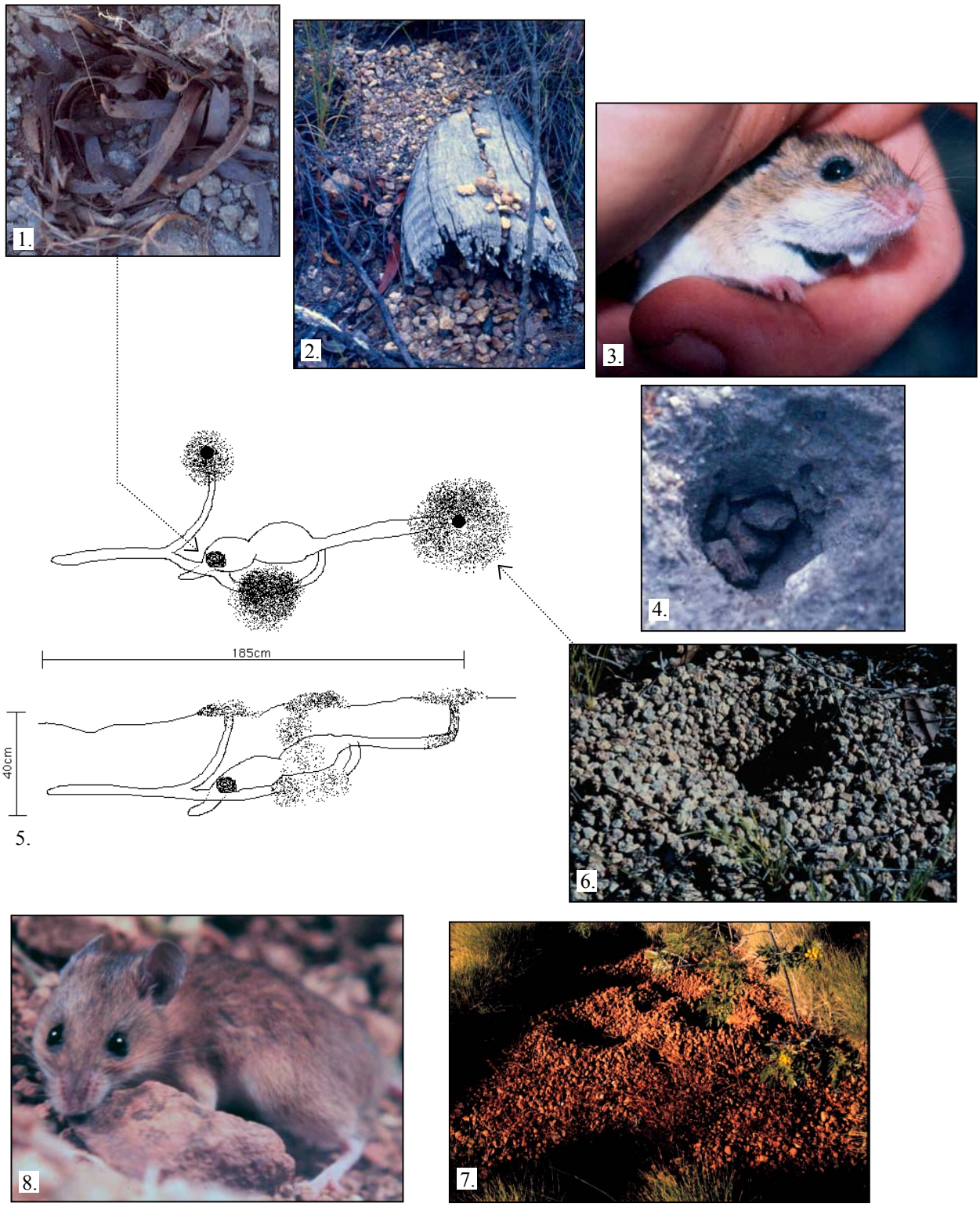


Figure 5.1. Images from the life of pebble-mound mice.
1. Underground nest- *P. patrius*, 2. Mound built around fallen log in bulldozer mullock (note "stuffing" along crack in log)- *P. patrius*, 3. Radio-collared *P. patrius*, 4. Pebble blockage in mound-less burrow entrance, 5. Diagram of excavated mound and burrow system, 6. Mound at burrow entrance- *P. patrius*, 7. Large "carpet" mound- *P. johnsoni*, 8. *P. patrius* dragging large pebble during mound construction.

Vagility

Captures of pebble-mound mice in Hidden Valley were largely restricted to sites at which mounds were located. However extensive inter-site movement occurs over distances of 1-3km within homogeneous habitats, particularly among reproductive males. Movements were never recorded over steeper ranges that isolated populations 2-3km apart. This is presumably because all recorded long-range movements were by males searching for mates, and males are unlikely to leave habitats in which females are located. Females require mounds for breeding and are reluctant to leave sites that contain them.

Population dynamics

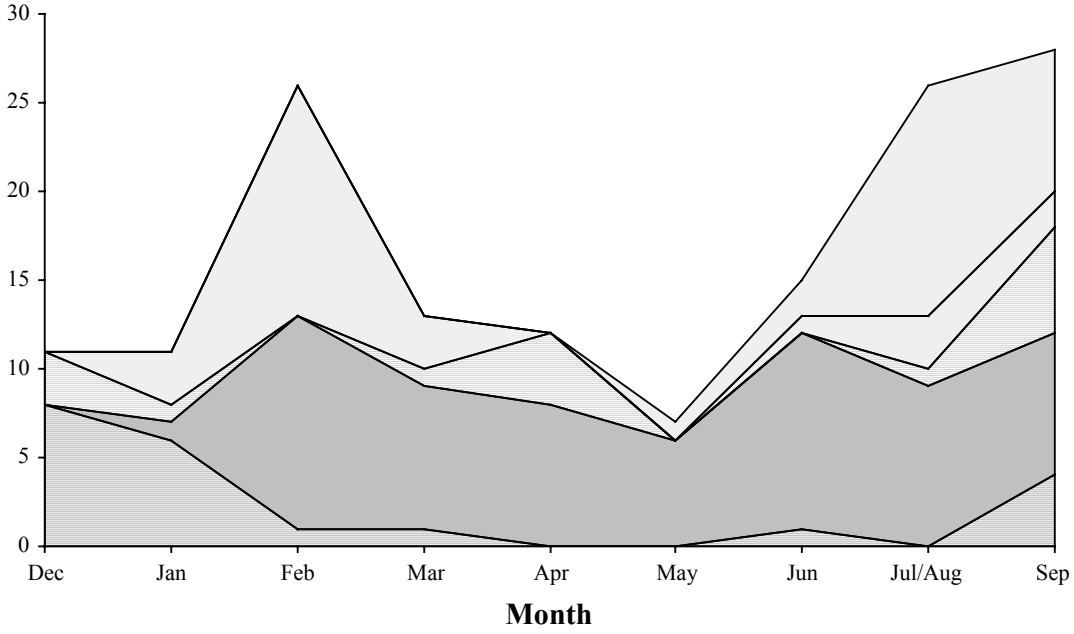
Almost ten years of trapping data in Hidden Valley by Cermak (1999) and myself has recorded a consistent pattern of population cycling, with no major irruptions, and a seasonal cycle reflecting an annual lifecycle (adult life expectancy ≤1year, significantly less for males) (fig. 5.2). Start et al. (2000) speculate that *P. chapmani* is a boom-bust species, but do not present data of any major population increases, and population irruptions of several orders of magnitude such as occur in delicate mice species (section 6.1.2) have not been recorded. Their suggestion that mounds may be a limiting resource suppressing population booms is almost certainly correct based on my results. Following fire *Pseudomys patrius* does not undergo major population booms. In fact, fire has no detectable effect on population dynamics, even immediately post-fire (fig. 5.2). Start et al. (2000) also noted that fire did not seem to have a significant effect on populations of *P. chapmani*. Fire is a major factor affecting population dynamics of *P. delicatulus*, *P. hermannsburgensis* and *P. novaehollandiae* (section 6.1.2). The relatively stable population dynamics of *P. patrius*, and probably all pebble-mound mice, are attributable to the fact that mound sites are very long-term stable resources. Good mounds last for decades, possibly centuries, and are inhabited by successive generations of short-lived mice. Female mice are therefore highly philopatric, and breeding populations of mice are not mobile. Availability of mounds may restrict breeding potential, but as a trade-off, most pebble-mound mouse populations appear to be very stable in the long-term, and I have followed up records of "*P. delicatulus*" that were later referred to *P. patrius*, and found the populations in exactly the same locations as

thirty years previously. Location and size of populations of delicate mice species are often not stable on a monthly basis, let alone longer time-scales (section 6.1.2).

5.2 Geographical sampling for molecular studies

Tissue samples of pebble-mound mice were derived from field studies and museum collections (table 5.1). Pebble-mound mice occur in 19 bioregions identified by Thackway and Creswell (1995). The difficulty of adequately sampling the expanding known ranges of all species meant that the primary aim of sampling was to gain tissue from each of these bioregions. This was achieved (fig. 5.3). *Pseudomys chapmani* samples were obtained from only three sites, but these represent the geographical limits of its range for which samples were available. Only two sites were sampled for *P. calabyi*, but the species has only been recorded from a handful of sites, all within 50km of one or other of the sites sampled here. Reasonably comprehensive sampling of the known distributions of *P. johnsoni*, *P. laborifex* and *P. patrius* was achieved.

a)



b)

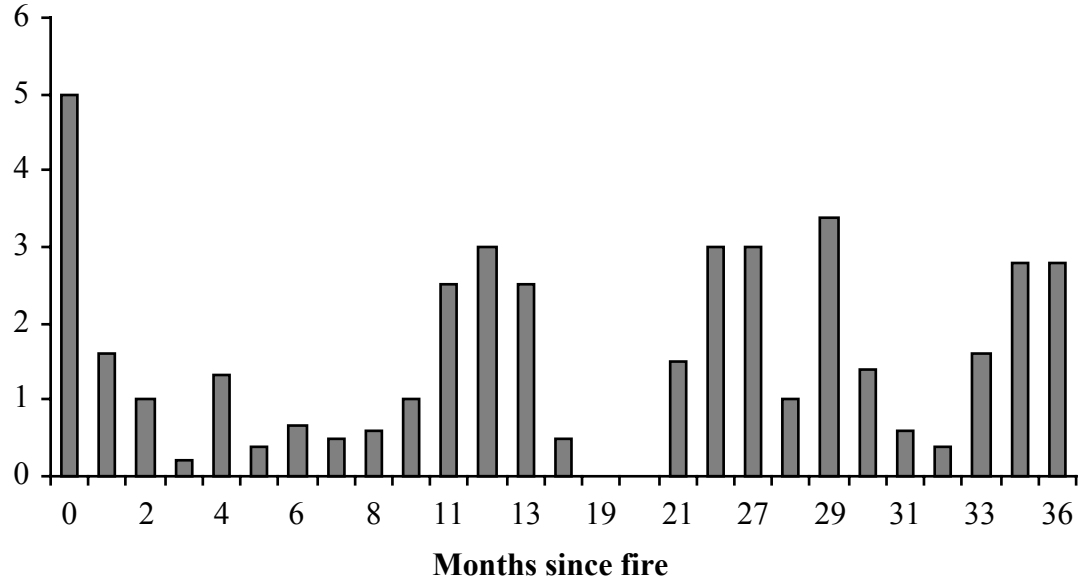


Figure 5.2. Aspects of population dynamics of *Pseudomys patrius*.
a) Typical population cycling of *P. patrius* in Hidden Valley. Graph shows period between lowest (pre-breeding) population until maximum population (post juvenile recruitment) for eight 40 trap grids trapped for 3 nights per session (9500 trap nights total). Female non-breeding ; female breeding ; male non-breeding ; male breeding ; sub-adult .
b) Effects of fire on pebble-mound mouse populations. Sample size is not shown, but a least squares regression model indicates only season has a significant effect on population size ($p<0.000$).

Table 5.1. Sampling locations and tissue sources for pebble-mound mice.
(ABTC)- Australian biological tissue collection with museum voucher; ABTC- no voucher; NTMU- Northern Territory Museum; QMJM- Queensland Museum; WAMM- Western Australian Museum. N= sample size. Sequence ID's are used to identify individuals from a particular site in results trees.

Site	Lat.	Long.	N	Type	Source/voucher	ID's
P. calabyi						
1. Kakadu	-12.9767	132.5220	3	Tail	Field biopsies (M. Watson)	c1-3
2. Litchfield	-13.4100	130.8972	3	Liver	NTMU 4323,4324, ABTC 29492	c5-7
P. chapmani						
3. ~LSD	-22.4833	122.3000	1	Liver	WAMM 40577	ch3
4. LSD	-23.8794	120.5033	1	Liver	WAMM 44989	ch4
5. Woodstock	-21.6958	119.0542	6	Liver	(ABTC) WAMM 29440, 29441,29447,29449,32761, 34297	ch1,2,5,6,7
P. johnsoni						
6. Helen Springs	-18.4978	133.8738	3	Tail	Field biopsies (Ford)	j1,5,6
7. Kurundi	-20.4667	134.4500	2	Liver/Tail	Field biopsy (Ford), (ABTC)	j8,15
8. Moronan	-21.1667	140.9000	3	Liver	(ABTC) QMJM 10866, 10867,10868	j10,11,13
9. Muckaty	-18.6274	133.9458	1	Tail	Field biopsy (Ford)	j16
P. laborifex						
10. Gardner R.	-19.3334	128.8667	4	Liver	ABTC 66290,66291,66292, 66293	l6,7,11, 12
11. Granny Soak	-19.1292	128.8919	1	Liver	(ABTC) WAMM 51934	l8
12. Gregory NP	-15.4917	131.3003	1	Tail	Field biopsy (Ford)	l18
13. Mitchell Plateau	-14.8222	125.8417	5	Liver	WAMM 21764,21895, 21962, ABTC 8095,8136, 8172	l2,3,9,13,14 ,15
14. Mount Sanford	-17.3705	130.8048	3	Liver	ABTC 30349,30350,30351	l4,5,10
15. Purnululu	-17.3996	128.2717	1	Liver	Biopsy (Ford) lodged in WAM	l1
16. Victoria R.	-15.6170	131.1280	1	Liver	ABTC 61616	l23
17. Wickham R.	-16.9576	130.4539	7	Liver	(ABTC) NTMU 4897,4898, 4899,4900,4901,4902,4903	l16,17, 19,20-22
P. patrius						
18. Burra Range	-20.5674	145.0967	5	Tail	Field biopsies (Ford)	p6,12, 13,18,37
19. Goondicum	-24.8670	151.4330	1	Tail	Field biopsy (Ford)	p62
20. Hidden Valley	-19.0028	146.0705	10	Liver/Tail	Field biopsies (Ford), QMJM 10865	p1,4,10, 38-40,46, 47,51, 86
21. Many Peaks	-24.5667	151.3000	1	Liver	QMJM 11940	p134
22. Marodian	-25.9662	152.0900	10	Liver/Tail	Field biopsies (Ford), QMJM 11271	p7,20-27
23. Minnerva Hills	-24.0995	148.0429	6	Tail	Field biopsies (Ford)	p8,14,28-31
24. Mount Britton	-21.3948	148.5229	2	Tail	Field biopsies (Ford)	p59,60
25. Mount Moffat	-25.0333	147.8833	1	Liver	QMJM 12735	p135
26. Mount Pollux	-22.4764	147.8705	6	Tail	Field biopsies (Ford)	p11,15, 16,17,33,34

Figure 5.3. Distribution of pebble-mound mice and sampling locations.
Distributions based on Start *et al*. (2000), Van Dyck and Birch (1996), field observations and sampling localities. Numbers refer to population numbers given in table 5.1. *P. calabyi* ——— ; *P. chapmani*····· ; *P. johnsoni* – – – ; *P. laborifex*····· ; *P. patrius* — — . Tissue was available for Bauhinia Downs☆) but good sequence was not obtained. The population has been identified as both *P. johnsoni* and *P. laborifex*.

5.3 Molecular divergence among pebble-mound mouse populations

5.3.1 MITOCHONDRIAL CONTROL REGION

Nuclear pseudogenes

Sequences obtained from tail tips of *Pseudomys patrius* consistently exhibited multiple peaks at single nucleotide locations (fig. 5.4). These peaks often made sequences too noisy to be interpretable. There was an obvious similarity between sequences from different individuals in both the primary signal, assumed to be the true mitochondrial sequence, and

the background “noise”, assumed to be nuclear copies of the mitochondrial sequence. Cleaner sequences were obtained from mitochondrial-rich liver tissue, in which the greater relative abundance of mtDNA allowed preferential amplification of true mitochondrial sequence (fig. 5.4). Sampling of populations of other pebble-mound mice, *P. calabyi, P. johnsoni* and *P. laborifex*, confirmed the constant presence of multiple sequences when DNA was amplified from tail tips. Two specimens of *P. calabyi* from Kakadu National Park yielded what appeared to be almost clean copies of the sequence discernable as background noise in other species, as did one specimen of *P. patrius* from the Burra Range. The sequence was significantly more divergent from conilurine control region sequences than was *Mus musculus* sequence. This was the result of mutations at sites that are highly conserved in the true control region of all murid rodents and lagomorphs. There was also no difference between the assumed nuclear sequence from the Burra Range and Kakadu, which indicated conservatism in mutation expected of nuclear sequence, but not mitochondrial control region (Lopez et al. 1994, Greenwood and Pääbo 1999, Mirol et al. 2000).

The fact that assumed nuclear sequences from the Burra Range and Kakadu were identical, and differed from all pebble-mound species at a number of conserved sites, made the design of primers to exclude the nuclear copy straightforward. Despite the use of control region-specific primers, it proved necessary to perform gel clean-ups on sequence from *P. calabyi* tail-tips due to the presence of another, much smaller and unrelated PCR product. Primers were also designed to preferentially amplify the pseudogene. It was hoped to be able to use the pseudogene as a phylogenetic marker, as its divergence from the true control region sequence was quite marked, but in all species of pebble-mound mice it was identical, suggesting an old origin. However, sequencing of *Leggadina forresti*, *Notomys alexis*, *N. cervinus*, *Pseudomys albocinereus*, *Rattus tunneyi*, and *Xeromys myoides* failed to reveal the presence of the nuclear sequence.

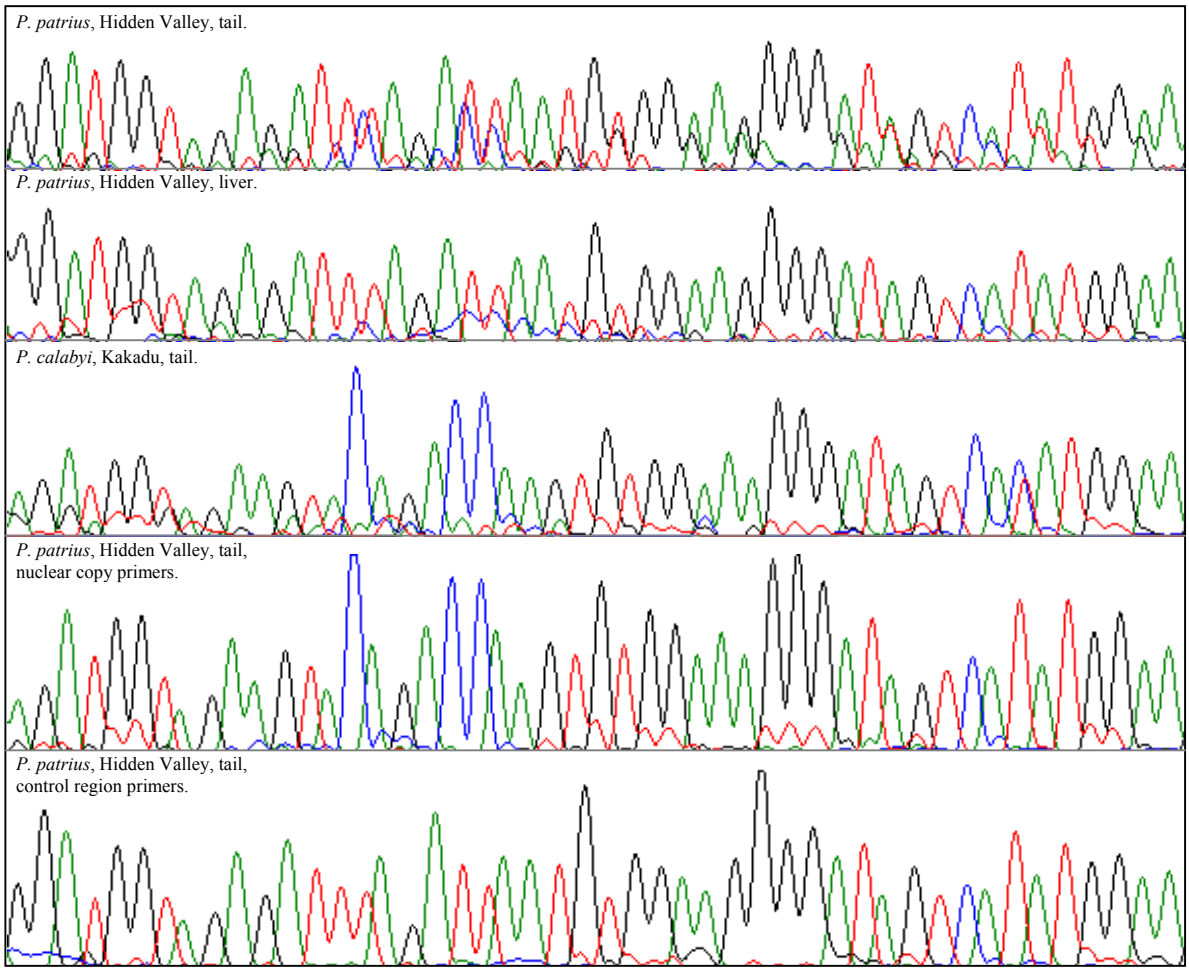


Figure 5.4. Nuclear copy and true mitochondrial sequence from pebble-mound mice. Forty-seven bp are shown that exemplify the nature of a pseudogene isolated from pebble-mound mice. Green- A; blue- C; black- G; red- T. The top three sequences were obtained using general murid primers. The bottom two sequences were obtained using primers designed by the author. Note in particular the three "C" peaks that dominate nuclear copy amplified from both *P. calabyi* and *P. patrius.* In sequence from tail these peaks are of similar amplitude to true control region sequence, in sequence derived from liver the peaks are reduced to background noise, and using pseudogene-excluding primers they are not present. Several other sites show a similar pattern. Electropherogram signal strength is shown to scale, and as expected, is significantly stronger in "true" sequences.

Control region

Four major clades of pebble-mound mouse control region haplotypes are present across northern Australia (figs. 5.5 and 5.6). Aligned sequences were shorter than for other study groups (chapters 7-9) and for the general conilurine phylogeny (chapter 4) because of the use of pseudogene-excluding primers. Sequences were 336bp in length, including 71 variable sites (65 parsimony-informative). Each clade is separated from the others by

significant Kimura two-parameter distance in a well-resolved bootstrapped tree (fig. 5.5; minimum evolution score: 0.38351). Haplotypes from individuals of *Pseudomys calabyi*, *P. chapmani* and *P. patrius* cluster into tight groupings conforming to current species definitions. All remaining haplotypes belong to a single lineage that incorporates individuals currently described as *P. johnsoni*, *P. laborifex* and *Pseudomys* sp. of Van Dyck and Birch (1996). This lineage also includes new populations of mice from the VRD that have been assigned to *P. laborifex*. Within this clade three distinct lineages are identified in distance analysis; *P. johnsoni* and *Pseudomys* sp. from the central Northern Territory and western Queensland, *P. laborifex* from the Kimberley as described by Kitchener and Humphries (1986) (populations 10, 13 and 15 in fig. 5.3), and new populations of "*P. laborifex*" identified from the VRD (populations 11, 12, 14, 16, 17). Relationships identified by distance analysis are upheld by a bootstrapped maximum-likelihood tree (fig. 5.6; likelihood score: -ln 1134.53589; substitution model: TIM+I+G (A 0.3294; C 0.0646; G 0.1989; T 0.4071) Nst=6 Rmat=(A-C 1.0000; A-G 9.1980; A-T 0.3403; C-G 0.3403; C-T 4.0186; G-T 1.000) Rates=gamma Shape=1.8345). *Pseudomys calabyi*, *P. chapmani* and *P. johnsoni/laborifex* are all supported at above 98%. Within the *P. johnsoni/laborifex* clade the three internal clades identified by distance analysis are apparent, but only the VRD and *P. johnsoni* clades have bootstrap support. The likelihood tree taken from PAUP* placed the *P. laborifex* (Kimberley) clade basal to "*P. johnsoni*" and "*P. laborifex* (VRD)", but not as a monophyletic clade. However monophyly is enforced in figure 5.6 because long branch lengths to other species, combined with few mutational differences within the *P. johnsoni/laborifex* clade creates doubt about the basal placement of this clade, as does the likely manner of its evolution (section 5.4.3). The trees were of the same length.

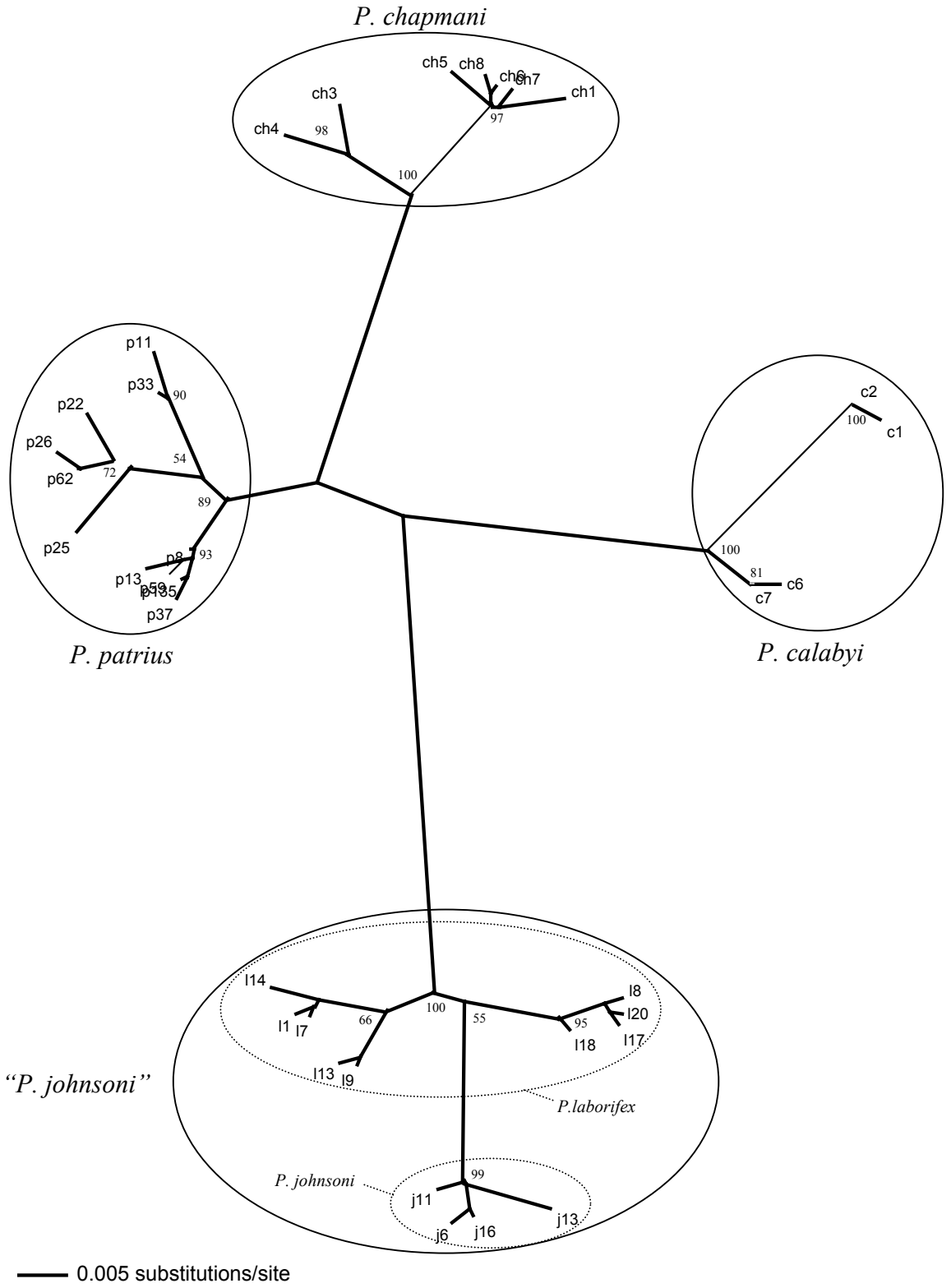


Figure 5.5. Genetic distance between 35 pebble-mound mouse control region haplotypes. Unrooted neighbour-joining tree of Kimura two-parameter distance estimates for 36 haplotypes of 336bp isolated from 87 individuals. Taxon labels correspond to one individual that carried a particular haplotype. Species boundaries proposed in this thesis are shown as solid circles, currently described species boundaries are shown as broken circles. Node labels= bootstrap support for major nodes based on 100 replicates.

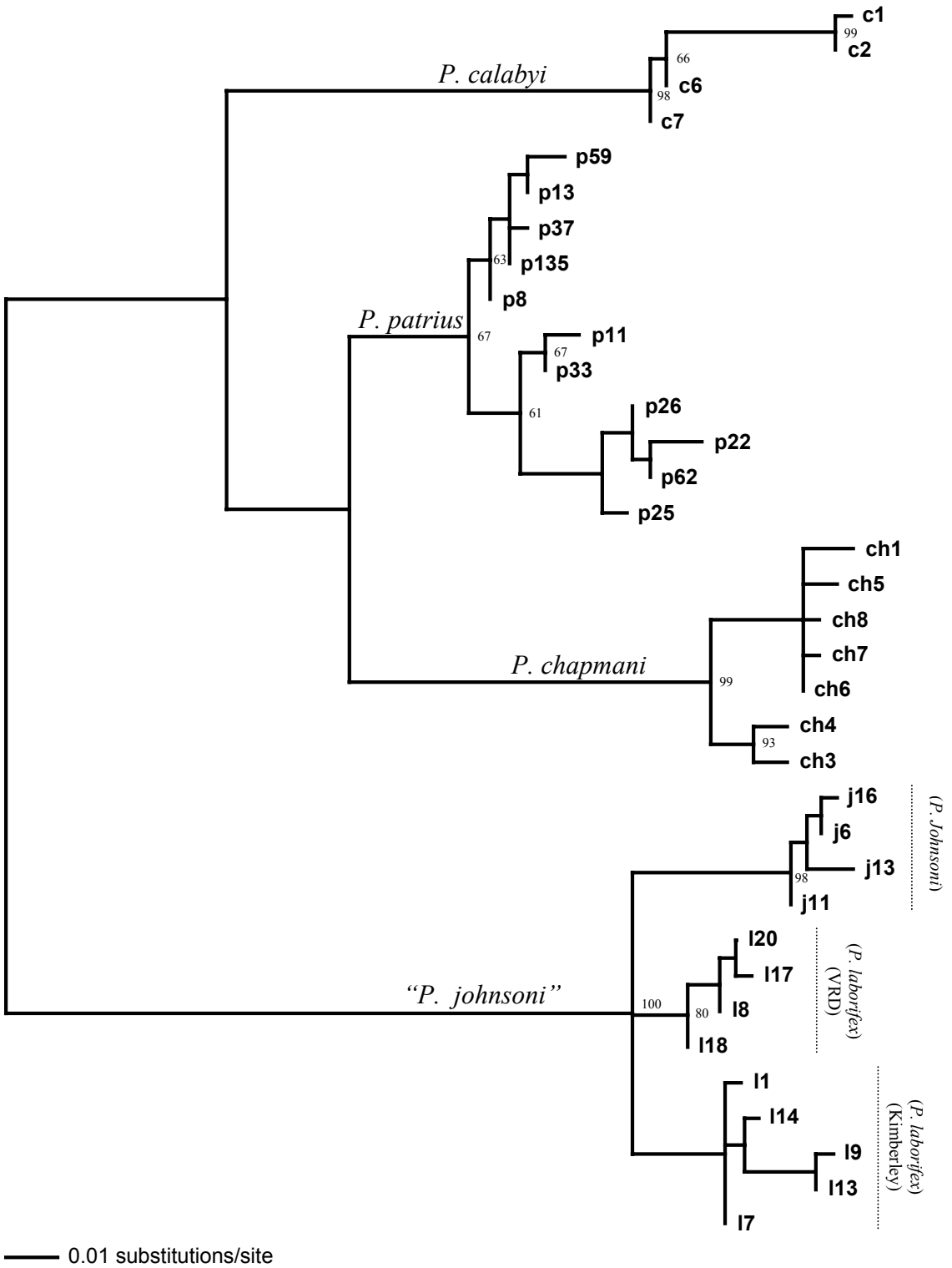


Figure 5.6. Maximum-likelihood tree of pebble-mound mouse control region haplotypes. Maximum-likelihood tree for 35 haplotypes based on parameters derived from Modeltest for 336bp of sequence isolated from 87 individuals. Taxon labels correspond to one individual that carried a particular haplotype. Tree is midpoint rooted; monophyly is enforced for *P. laborifex* (Kimberley). Node labels= bootstrap support (>50%) based on 100 replicates.

5.3.2 NUCLEAR INTRONS

In species-level phylogenies using the DHFR intron 1 locus the pebble-mound mice exhibited sufficient genetic diversity to warrant further use of the marker to examine relationships within the group. Heterozygosity at the locus was low among sequenced individuals, and only a few bases appeared to differ between homozygotes (fig. 5.7). The DHFR locus confirms results suggested by control region analysis. Individuals belonging to *P. calabyi*, *P. chapmani* and *P. patrius* are slightly divergent from each other (one or two bp differences), whereas individuals belonging to *P. johnsoni* and *P. laborifex* show little variation at the DHFR intron 1 locus across an extensive geographical range. Three fixed mutations and two deletions (totalling 4bp) separate the *P. johnsoni/laborifex* lineage from the other pebble-mound mice (fig. 5.7). Phylogenetic inferences beyond this are unreliable due to the limited number of variable sites. However, *P. calabyi* and *P. chapmani* share two fixed mutations that are not present in *P. patrius*.

One individual of *P. laborifex* yielded a unique DHFR intron 1 sequence, more similar to that of *P. patrius* than other species, and lacking the deletions that characterise the *P. johnsoni/laborifex* clade. Unfortunately the origin of the specimen is not clear, and it may have been derived from captive stock that had the opportunity for cross breeding with *P. calabyi*. Two other alternatives are possible; the specimen is a wild hybrid and was collected from the northern margins of the distribution of *P. laborifex*, close to the known distribution of *P. calabyi*, or the sequence derived from that individual was true DHFR intron 1 sequence, and the other nine individuals yielded a very similar but incorrect product. The latter case is unlikely as all sequences were carefully separated during gel clean-ups, and no signs of other product or background signal were apparent in sequences. Because of these issues, sequence from the individual in question is not shown in figure 5.7, but the origin of the animal and the possibility of natural crossbreeding or confounding genetic products are under investigation.

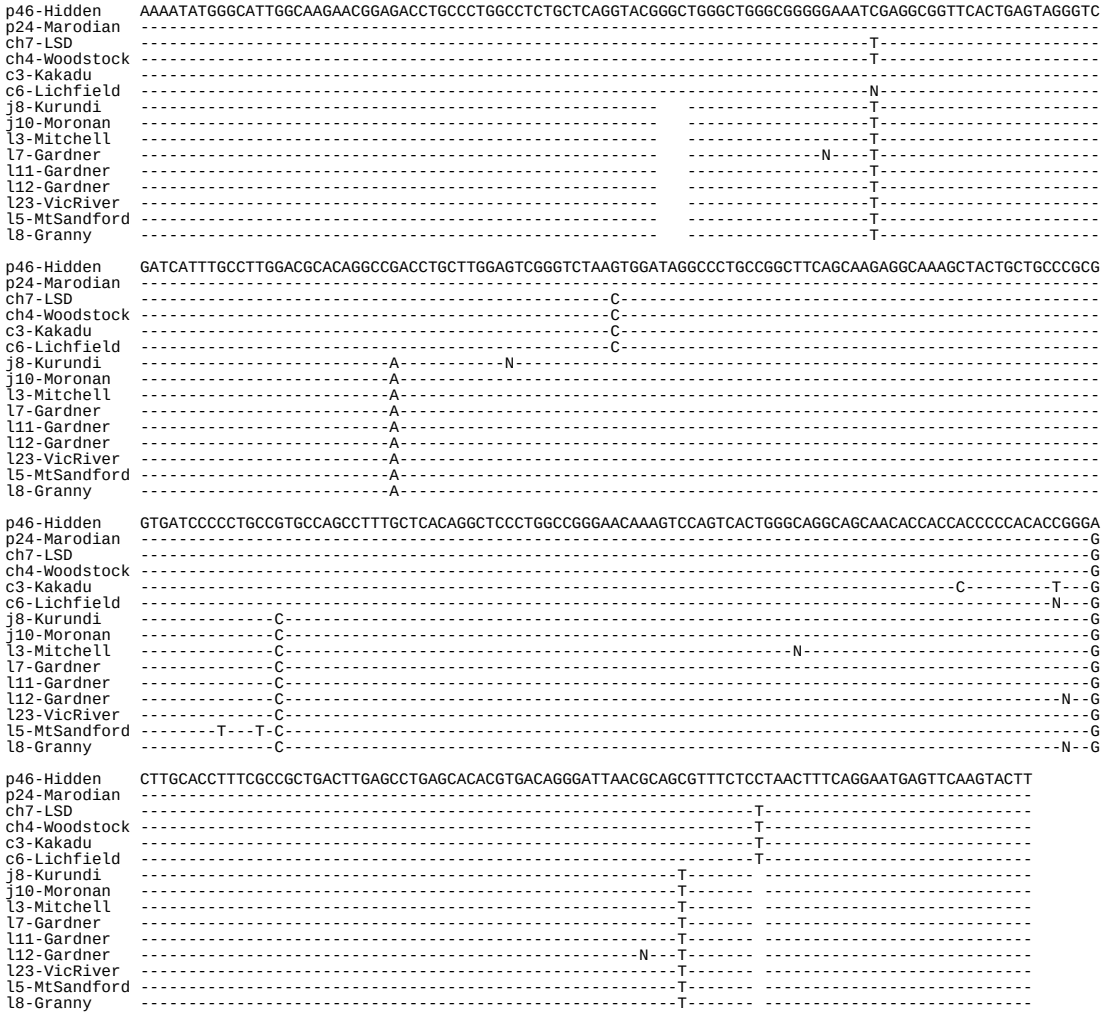


Figure 5.7. Alignment of DHFR intron 1 sequence from fifteen pebble-mound mice.
Codes represent a single individual and its site of origin. cx- *P. calabyi*; chx- *P. chapmani*; jx- *P. johnsoni*; lx- *P. laborifex*; px- *P. patrius*. N is recorded for ambiguous sites in heterozygote individuals.

5.4 Evolution of the pebble-mound mice

5.4.1 How many species of pebble-mound mice are there?

On the basis of results presented in section 5.3 there is probably only four species of pebble-mound mice; *P. calabyi*, *P. chapmani*, *P. johnsoni* and *P. patrius*. This conclusion is based on the relatively small genetic distances separating control region clades within the *P. johnsoni/laborifex* clade, the probable very recent origin of these clades (section 5.4.3),

and the fact that populations of *P. laborifex* sampled in this study do not form a clade to the exclusion of *P. johnsoni*. Lack of DHFR variation also indicates a recent shared ancestry of the three taxa in the *P. johnsoni* clade. *Pseudomys johnsoni*, *P. laborifex* (Kimberley) and *P. laborifex* (VRD) could be viewed as subspecies, and the names *P. johnsoni johnsoni*, *P. j. laborifex* (Kimberley lineage) and *P. j. kitcheneri* (VRD) will be proposed. The naming of a form after Daryl Kitchener is appropriate, as he was primary author of the description or re-description of five taxa studied in this thesis, including *P. johnsoni* and *P. laborifex*. Maximum inter-clade distances within the *P. johnsoni* lineage (6.64% "johnsoni" vs. Kimberley and 5.31% "johnsoni" vs. VRD) are less than almost all minimum interspecies differences between other pebble-mound mice (minimum distance between two individuals of other species 6% *P. chapmani* vs. *P. patrius*), and average pair-wise differences are significantly less than those between other species (table 5.1). However, other species of pebble-mound mice have far more isolated geographical distributions when compared to each other than do members of the *P. johnsoni* clade, and relative mtDNA distances are possibly not a useful indicator of species boundaries within *P. johnsoni*.

Table 5.2. Inter-taxon control region divergence among pebble-mound mice.
Distances based on average pair-wise Kimura two-parameter distances. Species shown at upper left, proposed subspecies lower right. Based on 336bp sequences. "*P. johnsoni*" in species comparisons refers to all members of the *P. johnsoni/laborifex* lineage, in subspecies comparisons "*P. johnsoni*" refers only to the currently recognised taxon.

	P. calabyi	*P. chapmani*	*P. patrius*	*P. johnsoni*	*P. laborifex* (Kimberley)	*P. laborifex* (VRD)
P. calabyi	-					
P. chapmani	10.55%	-				
P. patrius	8.15%	7.16%	-			
P. johnsoni	11.91%	12.97%	10.39%	-		
			P. laborifex (Kimberley)	4.84%	-	
			P. laborifex (VRD)	4.09%	2.86%	-

5.4.2 GEOGRAPHICAL DISTRIBUTION OF PEBBLE-MOUND MOUSE CLADES AND SPECIES

The known distributions of the four major pebble-mound mouse control region clades are all allopatric (fig. 5.8). Figure 5.9 shows the distribution of three major soil types. Known occurrences of pebble-mound mice map almost perfectly onto the distribution of "shallow rocky soils". "Cracking clays" and "deep sand" typify areas in which pebble-mound mice are conspicuously absent. The combination of these maps and known distributions of pebble-mound mice suggests that the limits of the distribution of most species are probably already known. However, in my opinion there is little indication that *P. calabyi* should not be found throughout much of Arnhem Land, and the presence of *P. johnsoni* on the Gulf Fall indicates that the two species could be in contact throughout the mid-south of the Top End (fig. 5.8). The distribution of *P. patrius* possibly includes large areas of Cape York from which it has not yet been recorded. Geographical isolation of *P. chapmani* in the Pilbara/Gascoyne and *P. patrius* in the eastern highlands of Queensland is probably real, as they are isolated by deep sands of the Great Sandy Desert (*P. chapmani*) and cracking clays of the Carpentarian plains (*P. patrius*) (fig. 5.9). The geographical separation of *P. calabyi* from *P. johnsoni* (including *P. laborifex*) is doubtful. Two hundred kilometres currently separates the closest known populations of these two species, Depot Creek (*P. calabyi*) and Gregory NP north ("*P. laborifex" (VRD)*). The landscape between these sites is generally rocky hills, although the Daly River basin bisects the gap between the taxa, and may prove to be an isolating barrier. Within the *P. johnsoni/laborifex* clade the known distribution of "*P. johnsoni*" is separated from "*P. laborifex*" (Kimberley) and "*P. laborifex*" (VRD) by the dune fields of the northern Tanami, but is probably more continuous than currently known. Unlike the situation with *P. chapmani* and *P. patrius,* soils between these areas are not in unsuitable groups, and the intervening gap in figure 5.9 is benign compared to the deep sands and cracking clays. The two clades of "*P. laborifex*" are largely restricted to the east or west of the WA-NT border region, but are almost certainly in contact in the Gardner Range (less than 20km between known sites) in the northwest Tanami Desert.

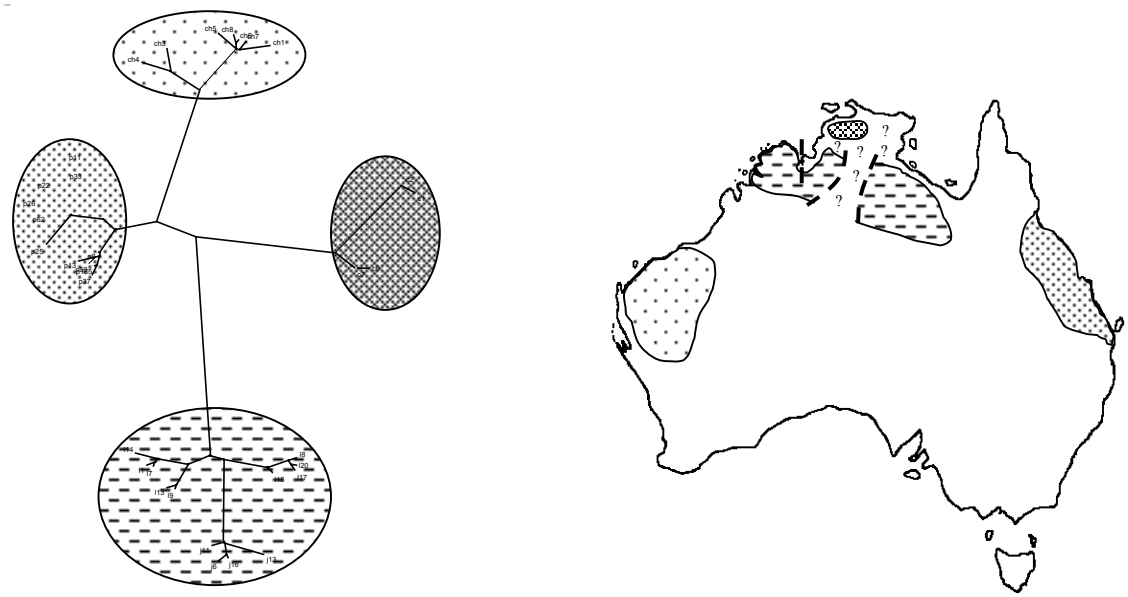


Figure 5.8. Geographical distribution of pebble-mound mouse control region clades. *P. calabyi* ; *P. chapmani* ; *P. johnsoni* ; *P. patrius* . Dashed lines indicate rough boundaries between major intra-specific clades of "*P. johnsoni*".

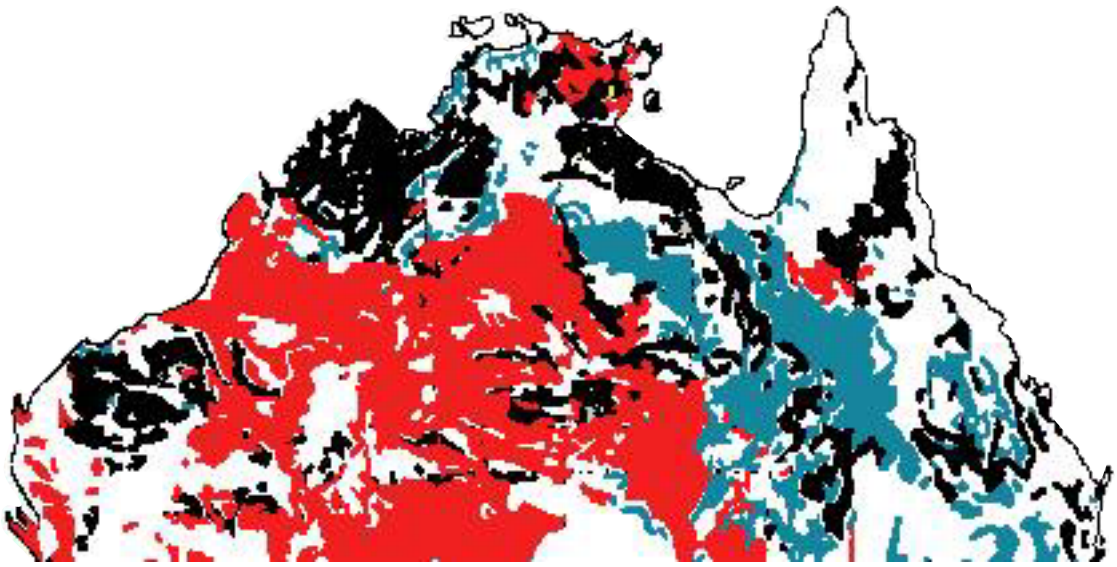


Figure 5.9. Major soil type and the distribution of pebble-mound mice.
Soils shown black are "shallow stony" soils associated with pebble-mound mouse populations. Deep sands- red; cracking clays- blue. Maps based on Taylor and Eggleton (2001).

5.4.3 POPULATION DIVERGENCE AMONG PEBBLE-MOUND MICE

One of the obvious features of the evolution of pebble-mound mice that begs explanation is how *P. patrius* and *P. chapmani* were able to colonise the areas they now inhabit, given that there are substantial rock-free barriers between them and other pebble-mound mice. In both cases, the underlying topography has an origin as a flat plain pre-dating the entry of rodents to Australia by millions of years (section 2.3.3), so how did these species of rocky hills cross such areas? I believe the answer cannot lie in a change of habitat preference or one-off migration event. The behavioural compulsion displayed by pebble-mound mice to build pebble mounds is too strong a limiting factor, and the distances between pebble-free habitats are too great. Obviously this must not have always been the case. In lieu of the rugged, rocky, ranges that typify many parts of the distribution of pebble-mound mice, I propose that eroding lateritic surfaces such as once covered the Mitchell Grass Downs and areas of the present day Great Sandy Desert may have provided an avenue for continuous habitation by pebble-mound mice (Twidale 1956, Beard and Webb 1974, Coventry et al. 1980).

The degradation of the rock-like lateritic surfaces in the Mitchell Grass Downs is almost complete, with a few remnants found near Kynuna and Winton (Learmonth and Learmonth 1971). These hills harbour the only populations of the rock-dwelling *Zyzomys argurus* found in western Queensland (QLD museum records). Equivalent lateritic surfaces occur to the east of the Mitchell Grass Downs, which are likely to be part of the same deeply-weathered Tertiary surface, although establishing the relative stratigraphy of such surfaces is difficult (Beard and Webb 1974, Twidale and Campbell 1995, Twidale and Bourne 2001). Much larger lateritic plateaux remain in the Great Sandy Desert because fluvial erosion, such as stripped the Mitchell Grass Downs surface, ceased in the early-mid? Pleistocene (Beard and Webb 1974, Palaeogeographic Group 1990). Lateritic surfaces are often utilised by pebble-mound mice, particularly *P. johnsoni*, because ferricrete pebbles produced by the degrading surface are ideal for constructing pebble-mounds. They are preferred to quartzite pebbles derived from the parent rock along the edge of the Tanami Desert (field data). The timing of degradation of lateritic surfaces is difficult to establish. However, rates of planation at the eastern margin of the Mitchell Grass Downs suggest that 20 metres of material has been lost in the last million years (Coventry et al. 1985). In

central Australia some lateritic mesas used by *P. johnsoni* stand only 10m above the surrounding sand plain, and the Kynuna-Winton Hills are often in the range 20-30m above the plains. Rocky contact was probably possible between eastern and western Queensland until at least the early Pleistocene. Similar dates are probably relevant to the Great Sandy Desert, although the underlying topography of the region has been partly covered by the modern sand sheet (section 2.3.3), and a more complex interaction of aridity, sand, and degradation of laterites may have been responsible for isolation of that region.

The most intriguing feature of pebble-mound mouse relationships is the juxtaposition of the *P. johnsoni* clade in the Kimberley and VRD. A sib-taxon relationship is expected between the Top End and these regions. This is true of *Leggadina lakedownensis* populations (Moro et al. 1998), and at least sixteen sub-specific or specific sib-taxa among birds (Ford 1978). *Pseudomys calabyi* and *P. laborifex* have been cited as an example of such a relationship (Woinarski 1992). However, DNA sequences indicate that they are not sibs. The fact that the (probably/nearly) parapatric *P. calabyi* and *P. johnsoni* are more genetically distinct than *P. calabyi*, *P. chapmani* and *P. patrius* are from each other indicates that evolution of pebble-mound mice in northern and central Australia has not been a simple process of isolation in response to topographic/geological change, and suggests a role for more dynamic processes attributable to climatic change.

To investigate the origin of isolation and intraspecific evolution of *P. patrius*, and to reconcile the geographical distribution of *P. johnsoni* with the environmental history of northern and central Australia, nested clade analysis (section 1.3 and 3.1.3) was performed on these two taxa. A bioregional sampling strategy of *P. calabyi* and *P. chapmani* tissues (section 5.2) means that little can be said of the phylogeographic structure within these species, although the distance between *P. calabyi* haplotypes from Litchfield and Kakadu National Parks appears to be high given the relatively small distance between them (200km), and suggests that with the discovery of further populations of this species a detailed phylogeographic study is warranted.

Pseudomys johnsoni

The nested clade diagram (fig. 5.10) shows tight clustering of haplotypes belonging to the major clades within *P. johnsoni*. There is strong geographical association of related haplotypes. However, clades 1-5, 1-6 and 1-20 are all found in the Gardner Range, despite the fact that clade 1-20 is in a different 2- and 3-step clade to the other two (because *"P. laborifex"* (Kimberley) and *"P. laborifex"* (VRD) are both found in the Gardner Range. Exact contingency tests revealed significant effects in clades 4-1 (χ^2 15; p= 0.009) and 4-2 (χ^2 16; p= 0.000) of the nested haplotype network. These represent a rejection of the null hypothesis of no geographical association of haplotypes. Significant effects detected in clades 4-1 and 4-2 equated to contiguous range expansion across the known distribution of the species, which is evident from the lack of genetic diversity across large areas. The lack of geographical sampling in areas between Helen Springs and the VRD creates some ambiguity in results regarding the relationship between these areas, as it is not clear whether mice are absent from the region. Extensive dune systems are found in the northern Tanami Desert between the VRD and Helen Springs, but areas to the north of the Tanami are likely to contain populations of pebble-mound mice that reduce the geographical distance between the clades. At a higher level, evidence of fragmentation operating between major clades was suggested by a significant result for the entire cladogram (χ^2 23; p= 0.000). However, the clade diagram does not identify the major clades within *P. johnsoni*, so it is not clear what the relevance of this result is. Fragmentation or bottlenecking leading to the three major clades of *P. johnsoni* is, however, an obvious interpretation of the distance tree and haplotype network that warrants further investigation when the full distributions of species are known.

The direction of the initial range expansion in this species is somewhat speculative. The distinctness of the *P. johnsoni* clade when compared to the remaining pebble-mound mice would suggest that the lineage did not originate in the Kimberley or VRD, or it would presumably be more similar to *P. calabyi*. However, there is an unavoidable circularity in this argument. In an effort to test the reliability of accepted area relationships and evolutionary patterns the unexpected relationship between *P. johnsoni* and *P. calabyi* cannot be taken as evidence of an origin elsewhere. Possible fragmentation of the species'

range is more straightforward. Many species share a sub-specific division on either side of the Ord arid intrusion that separates the Kimberley and VRD (Ford 1978). This indicates a surprising susceptibility of *P. johnsoni* to increased aridity given the arid conditions now inhabited by the species. Bottlenecks that apparently severely reduced genetic diversity in the Kimberley and VRD clades probably indicate a northwards contraction of these populations to the Mitchell Plateau and far northern VRD during the LGM, when evidence of heightened aridity is found through much of the central Kimberley (Jennings 1975, Wende et al. 1997). To the east it is likely that the species range contracted northward to the Gulf Fall, which would have been the least arid part of the current eastern distribution of *P. johnsoni*. However, the Davenport Ranges, which are a noted refuge area (Morton et al. 1996), or the Mount Isa Inlier, are possible sites of the bottleneck that gave rise to "*P. johnsoni"*. Subsequent range expansion has lead to few haplotypes in any clade, differing by only a few mutations, being distributed over distances of 1000km or more. It is therefore likely the bottleneck of each clade involved only a single refuge area.

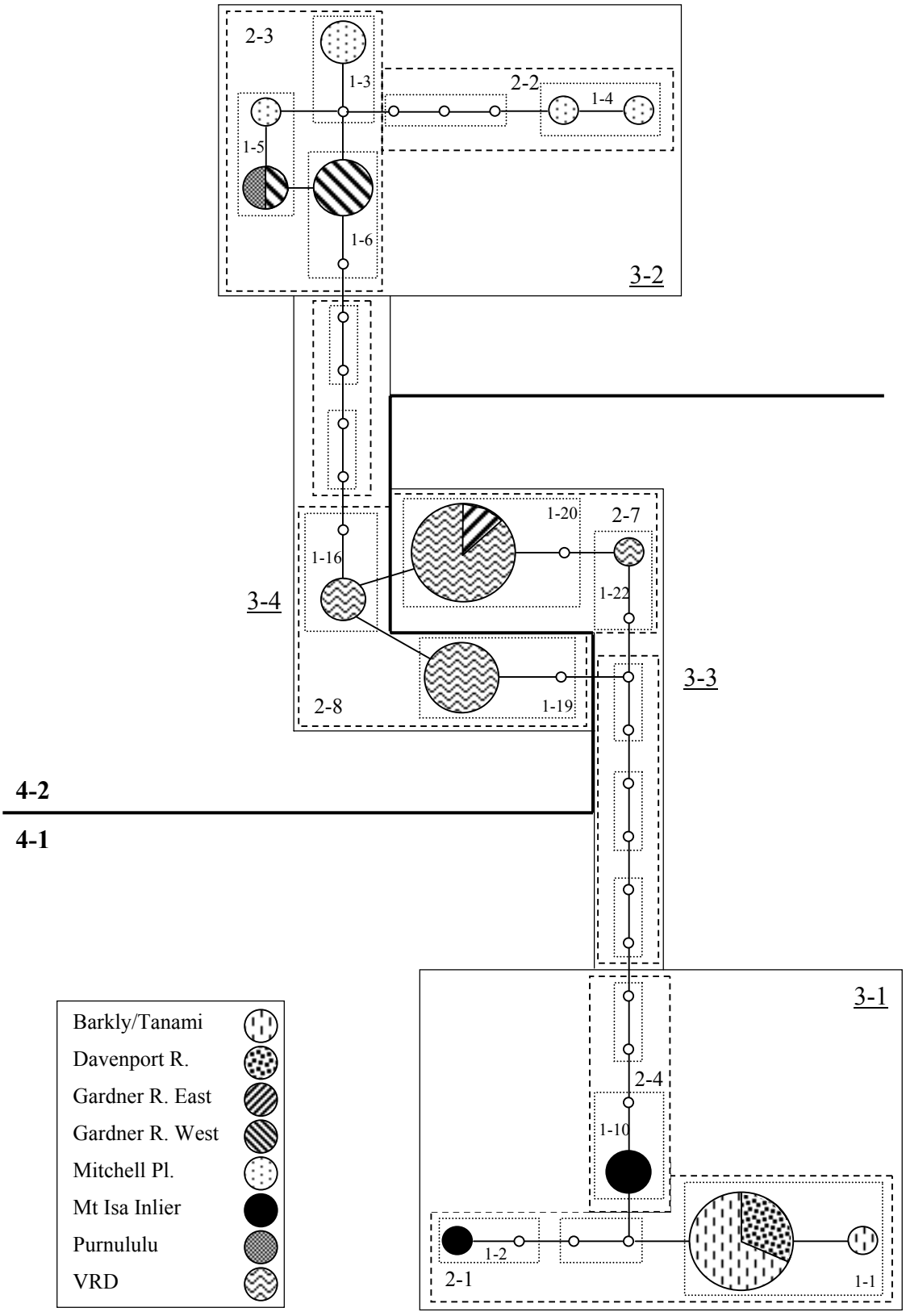


Figure 5.10. Nested haplotype network of *Pseudomys johnsoni* control region haplotypes. Large circles represent sequenced haplotypes (size= n). Small circles are unrecorded mutational steps. Shading as per key (may represent multiple sites within a region). Significant clades: 4-1 RE-C, 4-2 RE-C, Total cladogram PF.

Topographic constraints on the distribution of Pseudomys johnsoni

Given that *P. johnsoni* inhabits environments ranging from deserts of the northern arid zone to tropical savannah it is of interest what is constraining, or has constrained, the expansion of the species' distribution. Bioclimatic modelling based on the limited site data available shows that the southern distribution limit of the species may be climatically controlled (fig. 5.11). However, the eastern and western limits are the result of topographic and geological influence. Ideal climatic conditions for the species exist in areas of the Great Sandy Desert and on the clay plains of the Mitchell Grass Downs.

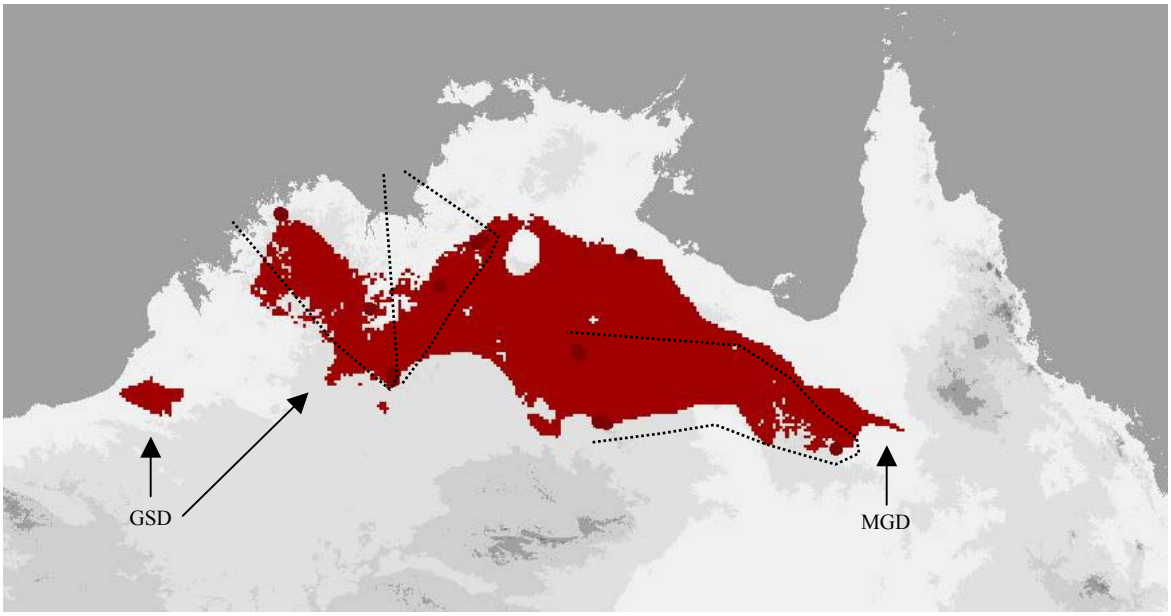


Figure 5.11. BIOCLIM prediction of the distribution of *Pseudomys johnsoni*.
Dotted lines separate control region clades. Arrows show climatic prediction of occurrence in the Great Sandy Desert and the Mitchell Grass Downs. Red shading is predicted range based on climate. Dots represent populations used in modelling. The population near the Gulf of Carpentaria was not successfully sequenced in this thesis beyond identification to species level. Shading represents 250m asl contours.

Pseudomys patrius

Eleven haplotypes were isolated from 42 *P. patrius* individuals. Two shallow clades were apparent in distance analysis, and nested clade analysis also identifies these two clades (fig. 5.12). One is restricted to sites in southeast Queensland. This clade appears to have had a static distribution with evidence of isolation by distance suggested by nested clade analysis (clade 2-2; χ^2 9; p= 0.030). Compared to the restricted south-eastern clade, there is only marginally more genetic variability across the remainder of the species distribution, which includes areas stretching over 1000km from the sandstone belt in south-central Queensland to areas north of Hidden Valley in north Queensland. A single population at Marodian, in the south-eastern clade, contained three haplotypes separated by up to seven mutations. Populations in the north tend to contain only one or two closely related haplotypes, and these are shared between sites hundreds of kilometres apart. Such a pattern is indicative of range expansion through much of eastern Queensland, and that is the result yielded by nested clade analysis (clade 1-4; χ^2 20.417; p= 0.046: clade 3-1; χ^2 29;p= 0.000) (fig. 5.12). The origin of the two major clades is possibly previous fragmentation of the species' distribution, although nested clade analysis could not distinguish between fragmentation and contemporary IBD, but did detect a significant geographical association of haplotypes for the entire cladogram (χ^2 20.1; p= 0.043).

The fact that *P. patrius* is highly mobile over evolutionary timescales is of interest given the habitat fidelity shown by the species on a local scale in Hidden Valley. This is particularly so given that mitochondrial movements are the result of female movement, and females are highly philopatric. The fact that the species could (re)colonise such a large area is attributable to the reasonably continuous nature of rocky ranges in the eastern highlands. The widespread distribution of haplotypes also suggests that the contemporary distribution of the species is probably fairly continuous throughout suitable habitats of the highlands.

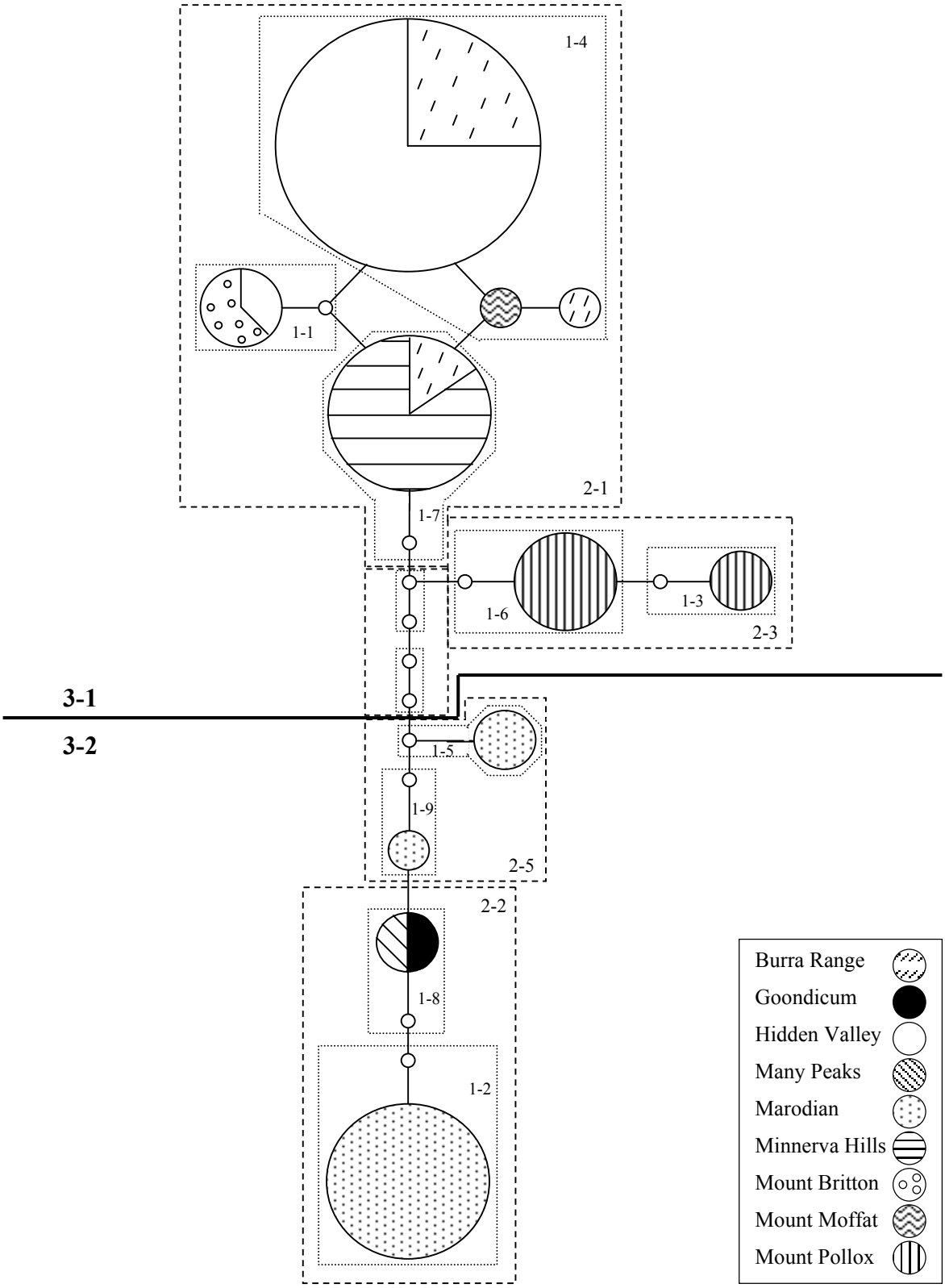


Figure 5.12. Nested haplotype network of *Pseudomys patrius* control region haplotypes. Large circles represent sequenced haplotypes (size= n). Small circles are unrecorded mutational steps. Shading as per key. Significant clades: 1-4 RE-C, 2-2 IBD or LDD, 3-1 RE-C, Total cladogram IBD or PF (see text for full explanation).

Low intra-specific genetic diversity of *P. patrius* compared to long inter-species genetic distances suggests that population extinction has been an important process in this species, just as in *P. johnsoni*. Northern parts of the species' current distribution have always been topographically suitable for the species, and a role for climatic change is again implicated in the evolution of *P. patrius*. Aridity is the likely cause of population extinctions through central Queensland. The extent of arid conditions during the LGM reached at least to the Townsville region where hopping mouse fossils have been found dating from the LGM (Aplin pers. comm.). Much of the area currently occupied by *P. patrius* would have been desert 20ka. *Pseudomys patrius* is currently sympatric with the eastern-most populations of *P. desertor*, which is also presumably indicative of a recent history of aridity at those sites. Thus, the apparently stable south-eastern populations of *P. patrius* affected by IBD, and not range expansion, are probably in a region not affected by aridity. Re-invasion of much of the species' range has probably occurred only in the last 16ky or so. Populations closer to the central Queensland coast recorded by Troughton (1941) and Finlayson (1942) could prove of interest, as they may not have been affected by aridity.

5.4.4 SPECULATIVE HISTORY OF THE PEBBLE-MOUND MICE

The current distributions of pebble-mound mice do not seem to reflect the probable history of speciation in the group. Isolation of *P. patrius* is an obvious cause of speciation, although the mechanism may not be degradation of Tertiary land surfaces as proposed here. Isolation of *P. chapmani* in the Pilbara by the Great Sandy Desert is also probably the cause of divergence of that species, however, it is not likely that either of these species diverged from *P. johnsoni*, which now occupies habitats to the east of the Great Sandy Desert and west of the Carpentarian plains. Nor is it likely that *P. calabyi* has diverged from *P. johnsoni*, despite the near parapatric known distributions of the two species, and the usual sib-taxon relationship between the regions they inhabit. A model of evolution in this group must therefore allow for probable contact between *P. chapmani*, *P. calabyi* and *P. patrius* more recently than the divergence of the *P. johnsoni* clade from them.

A possible scenario (fig. 5.13) would therefore involve: A widespread savannah species that inhabited rocky habitats across northern Australia during the Pliocene. At some point divergence of the *P. johnsoni* lineage occurred. I would suggest that this occurred in central Australia, possibly due to early development of arid conditions leading to a bottleneck in the Davenport Ranges, or some other random event that allowed adaptation of the clade to developing arid conditions in central Australia. A single lineage still occupied the Pilbara, Kimberley-Top End and Queensland, just as many savannah taxa still do. Contact with Queensland and the Pilbara may have been long-term across lateritic surfaces, or possibly could have been via a dispersal event across a temporary connection. The continuous range of the Pilbara-Queensland lineage became fragmented during the early and mid Pleistocene (via degradation of lateritic surfaces?) and the formation of the Great Sandy Desert. By this stage the four species lineages were formed, and the *P. calabyi* lineage was probably distributed throughout the Kimberley-Top End. The replacement of the *P. calabyi* lineage by *P. johnsoni* in the Kimberley presumably occurred because the *P. johnsoni* lineage is better adapted to arid conditions. The Kimberley has been subjected to periods of aridity that caused local extinction of plants currently typical of the region (Wallis 2001). This event may have been correlated with the first major loss of eucalypts from the region around 190ka (van der Kaars 1991). The savannah adapted *P. calabyi* type that presumably occupied the Kimberley would be limited in its ability to react to increasing aridity because the northward movement of habitats during glacial maxima involved exploitation of the pebble-free exposed sea bed. This lead to the extinction of the savannah clade and its replacement by *P. johnsoni,* and to the geographical juxtaposition of *P. johnsoni* between the more closely related taxa *P. chapmani, P. calabyi* and *P. patrius*. The expanded range of *P. johnsoni* was subsequently fragmented by aridity during the LGM, an event that has lead to three molecular subspecies of limited genetic diversity. Populations of *P. patrius* also went extinct across much of its current distribution, and the species has expanded its range through areas of central Queensland that were desert during the LGM. The expansion of both these species' ranges has been restricted by the Carpentarian plains, and the western limit of *P. johnsoni* is constrained by the Great Sandy Desert. The recent history of *P. chapmani* probably involves similar patterns of range contraction and expansion, limited by the Great Sandy Desert.

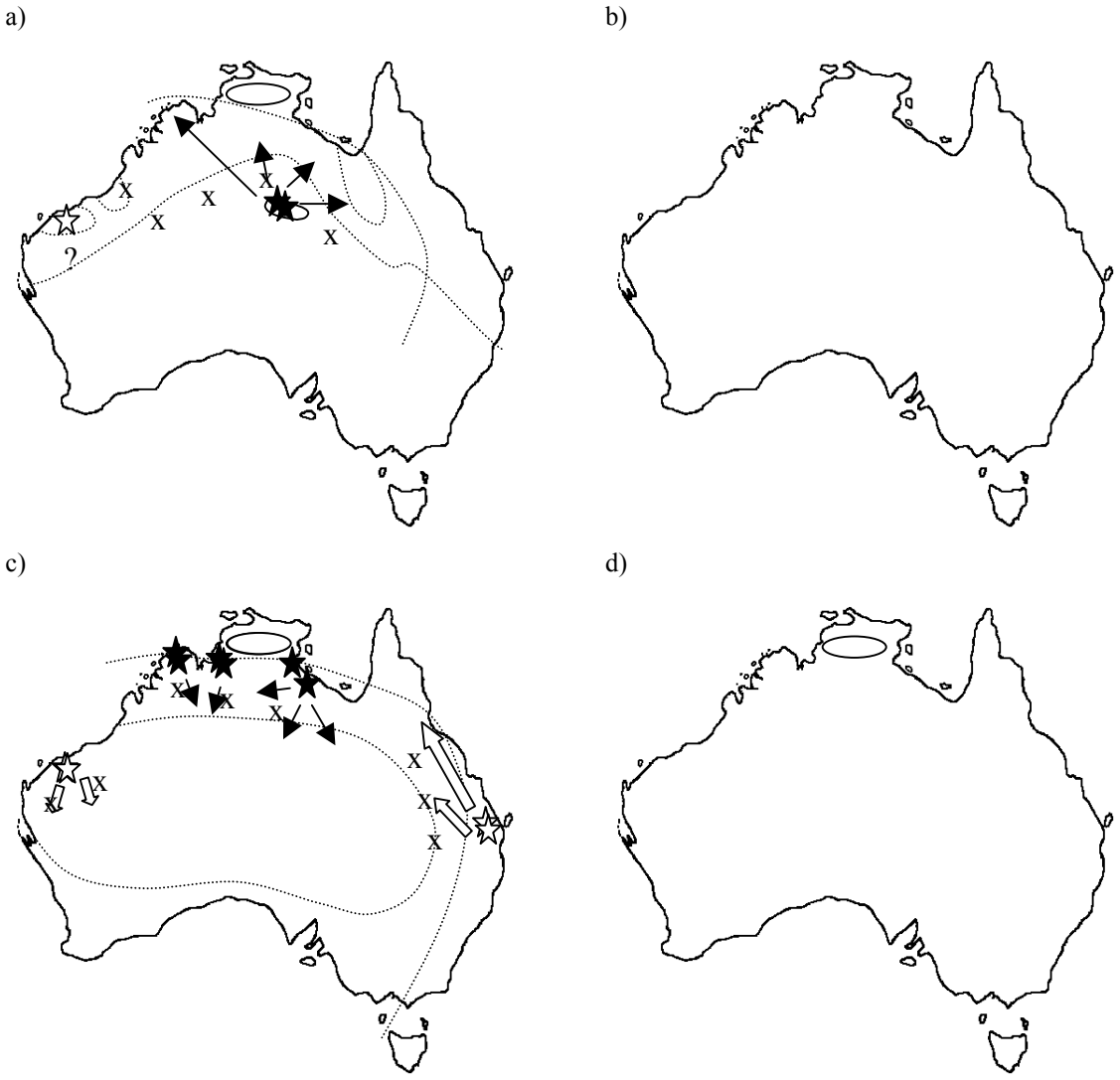


Figure 5.13. Speculative history of the pebble-mound mice.
a) Disruption of the range of an ancestral savannah species? leading to the formation of the *P. johnsoni* clade. Contact between eastern Queensland and elsewhere continues or post-dates this event. B) Pleistocene arid cycle 190ka? caused the northward range expansion of the more arid-adapted *P. johnsoni* lineage and replacement of the presumed ancestral savannah form in the northern Kimberley. The savannah form could not move northwards because no rocky landscapes were available to the north. The evolution of the Great Sandy Desert isolated the Pilbara, and *P. chapmani* may have experienced a bottleneck at this time. C) During the LGM populations of *P. johnsoni* experienced severe bottlenecks in the north Kimberley, VRD and somewhere to the east, probably the Gulf Fall. Populations of *P. patrius* in central Queensland became extinct, and the species contracted to less arid areas (of the southeast?). *Pseudomys chapmani* presumably experienced a similar bottleneck to these species. *Pseudomys calabyi* was probably relatively unaffected. Since the LGM *P. johnsoni* and *P. patrius* have expanded their ranges. Limit of non-arid pebbly habitat ····· ; Stable habitat (*P. calabyi*) ⬭ ; Bottlenecked population- *P. chapmani-patrius* clade ☆ *P. johnsoni* clade ★ ; Range expansion- "savannah" clade ⇨ ; *P. johnsoni* "arid" clade → ; X extinction.

5.5 Summary

-Pebble-mounds are primarily defensive structures.

-Pebble mound architecture varies greatly, with variation not particularly related to genetic or climatic influences, but strongly influenced by the immediate situation in which a mound is constructed

-Mounds have not played a role in speciation

-Pebble-mound mice inhabit erosional landscapes capable of producing pebbles, and are generally found in the upper elements of the local topographic sequence

-Populations of pebble-mound mice are typified by long-term site fidelity, small size and stable (non-irruptive) population dynamics due to the limiting influence of pebble mounds

-All pebble-mound mice share a unique nuclear copy of their mitochondrial control region sequence

-There are probably only four species of pebble-mound mice, *P. calabyi*, *P. chapmani*, *P. johnsoni* and *P. patrius*. *Pseudomys johnsoni* includes *Pseudomys* sp. of Van Dyck and Birch (1996), and *P. laborifex.*

-The current distributions of pebble-mound mice are not indicative of speciation processes, and are the result of dynamic interactions between landscape and climate.

-Divergence of pebble-mound species has involved geographical isolation as a result of:

    -loss of suitable pebbly habitat in the Great Sandy Desert and Carpentarian plains

    -changing climate? in central Australia

-A reliance on limited topographic situations has constrained the ability of pebble-mound mice to react to climate change in:

    -the Kimberley, where the presumed ancestral "savannah" form has been supplanted by an "arid" form as aridity spread northwards

    -the eastern and western limits of *P. johnsoni's* distribution, where unsuitable landscapes have prevented range expansion such as occurred in the Kimberley.

    -the eastern limit of *P. chapmani*'s distribution

    -the western limit of *P. patrius*' distribution

-Intra-specific evolution of *P. patrius* and *P. johnsoni* is typified by population extinction leading to:

-fragmentation of *P. johnsoni* into three distinct molecular subspecies

-recent re-invasion of much of eastern Queensland by *P. patrius*

Chapter 6. Evolution of distributions and species boundaries within the delicate mice

CHAPTER SUMMARY

Patterns of divergence among populations of delicate mice reveal very different evolutionary relationships than suggested by the current taxonomy of the group. Only two currently described species, *P. hermannsburgensis* and *P. bolami*, are monophyletic. Individuals described as *P. pilligaensis* do not form a taxon of any rank, as the currently recognised species contains members of *P. delicatulus* and *P. novaehollandiae*, rendering those two species paraphyletic. Further paraphyly of *P. novaehollandiae* results from the description of some individuals carrying *P. novaehollandiae* mitochondria as *P. delicatulus*. A morphologically cryptic species is reported from the arid north-western parts of the distribution of the currently recognised *P. delicatulus*, making *P. delicatulus* polyphyletic. It is proposed that true *P. delicatulus* populations from the north Kimberley and Top End constitute a subspecies, as do those from Queensland, and that these subspecies are of roughly equivalent status to the currently described species *P. novaehollandiae*. North-south clinal variation in morphology seems to characterise the eastern distribution of *P. delicatulus* and *P. novaehollandiae*, and where these two clades are in secondary contact in the Pilliga and southeast Queensland, their morphology is identical. The distributions of all species and clades are primarily under climatic control. Speciation and taxon divergence within the group mirrors patterns in the general biota, with a widespread, genetically homogeneous central Australian taxon, and a fragmented group of taxa around the periphery of the continent. The level of divergence of each taxon from its nearest relatives is correlated with the degree of aridity of the environments they inhabit, implicating extinction, range movements and bottlenecks in the evolution of species within delicate mice.

6.1 Molinipi and its relatives

***Pseudomys delicatulus* (Gould 1842), *Pseudomys hermannsburgensis* (Waite 1896), *Pseudomys novaehollandiae* (Waterhouse 1843), *Pseudomys bolami* Troughton 1932 and *Pseudomys pilligaensis* Fox and Briscoe 1980**

6.1.1 DELICATE MICE

The five named species referred to here as delicate mice have a continent-wide distribution, with the exception of southwest Tasmania and far south-western Western Australia (fig. 6. 1). The three widespread members of the group have distributions that largely conform to the Torresian, Bassian and Eyrean subregions (chapter 2). The delicate mouse (Molinipi; *Pseudomys delicatulus*) is distributed in suitable habitats throughout the Torresian subregion from the northern Pilbara to southeast Queensland (Braithwaite and Covacevich 1995). The sandy inland mouse (Mingkiri; *P. hermannsburgensis*) occupies the arid interior (Breed 1995a). *Pseudomys hermannsburgensis* is sympatric with *P. delicatulus* in the southern Kimberley and western Queensland (S. Van Dyck pers. comm., A. Kutt pers. comm., field data). Scattered populations of the New Holland mouse (Pookila; *P. novaehollandiae*) are found on the south-eastern Australian mainland from southeast Queensland to central Victoria, and in north-eastern Tasmania (Kemper 1995, Van Dyck 1998). Bolam's mouse (Poonta; *Pseudomys bolami*) occupies areas immediately inland from the Great Australian Bight, including the Nullarbor Plain. *Pseudomys bolami* and *P. hermannsburgensis* are sympatric across the northern areas of the formers distribution (Kitchener et al. 1984). The Pilliga mouse (*P. pilligaensis*) is restricted to the Pilliga Scrub of inland northern New South Wales (Fox 1995a).

Delicate mice have been considered close relatives of the pebble-mound mice on the basis of their extremely similar morphology (Kitchener et al. 1984, Kitchener 1985, Kitchener and Humphries 1986, Kitchener 1995a), although the two groups are not particularly closely related based on molecular data (chapter 4). The pelage of *P. bolami, P. delicatulus* and *P. hermannsburgensis* is almost identical to that of pebble-mound mice, being white below, and orange-brown above, with protruding black guard hairs (Watts and Aslin 1981,

Braithwaite and Covacevich 1995, Breed 1995a, Watts 1995a). Weights of these species range between 9 and 15g (Watts and Aslin 1981, Braithwaite and Covacevich 1995, Breed 1995a, Watts 1995a). *Pseudomys novaehollandiae* is grey-brown above and off-white below and is slightly heavier, particularly in the south of its range where weights reach 25g (Pye 1991, Kemper 1995).

Unlike the short history of pebble-mound mice in the literature, the delicate mice have a long history of description, re-assignment and rediscovery. *Pseudomys novaehollandiae* was among the earlier rodent species noted from Australia (Gould 1863) but was thought to be extinct until its rediscovery on the New South Wales coast in 1968 (Mahoney and Marlow 1968). *Pseudomys delicatulus*, *P. hermannsburgensis* and *P. novaehollandiae* were originally described as species of *Mus* in the 1800s but included by Thomas (1910) in his expanded genus *Pseudomys*. *Pseudomys delicatulus* and *P. hermannsburgnesis* were considered members of the sub-genus *Leggadina*. Thomas split the delicate mice into two sub-genera by describing his sub-genus *Pseudomys* (*Gyomys*) based on (*Mus*) *novaehollandiae.* Iredale and Troughton (1934) elevated these sub-genera to genera. Troughton (1936) also split individuals now recognised as a single species, *P. delicatulus,* into separate genera by describing some individuals from southeast Queensland as the pigmy mouse, *G. pumilus.* Ride (1970) subsumed *Leggadina* and *Gyomys* within *Pseudomys* and did not recognise sub-genera. Aside from *P. bolami* and *P. pilligaensis* Ride (1970) recognised currently accepted species boundaries by synonymising *P. pumilus* and *P. delicatulus. Pseudomys bolami* was described as a subspecies of *P. hermannsburgensis* by Troughton (1932), and was elevated to species status by Kitchener et al. (1984). Specimens eventually described as *Pseudomys pilligaensis* were thought to be an inland population of *P. novaehollandiae* when first captured in 1975 (Fox 1995a), but subsequent studies of morphology and enzymes concluded that they represented a distinct species. Lee (1995) stressed the need for further examination of the taxonomic status of *P. pilligaensis*, which he felt was not suitably established to formulate conservation priorities.

6.1.2 ECOLOGY OF THE DELICATE MICE

All delicate mice show a general preference for sandy substrates (Watts and Aslin 1981). *Pseudomys delicatulus* and *P. novaehollandiae* are found in habitats ranging from stabilised coastal dunes to inland eucalypt forests and woodlands (Keith and Calaby 1968, Fox and Fox 1978, Kemper 1990, Braithwaite and Brady 1993, Braithwaite and Covacevich 1995, Kemper 1995, Haering and Fox 1997, Lock and Wilson 1999). *Pseudomys bolami* and *P. hermannsburgensis* inhabit dunes and plains of the arid zone and its southern periphery and each has been recorded from a variety of vegetation associations (Kitchener et al. 1984, Robinson et al. 1988, McKenzie et al. 1993, Breed 1995a, Moseby and Read 1998, Letnic 2002). Where these species are sympatric, *P. bolami* shows a preference for lower loamy areas, and *P. hermannsburgensis* is found on higher sandy dunes (Dell and How 1988, McKenzie et al. 1992). *Pseudomys pilligaensis* is found in *Eucalyptus* and *Callitris* associations on sandy plains (Fox and Briscoe 1980, Fox 1995a). The diet of all species is somewhat opportunistic and omnivorous, with a tendency towards granivory, particularly in *P. bolami* and *P. delicatulus* (Cockburn 1980, Braithwaite and Brady 1993, Murray et al. 1999, Wilson and Bradtke 1999). *Pseudomys* species often show a strong summer and spring preference for seed, and this has been linked to habitat preference in several species (Cockburn 1978, 1981b, Ford et al. 2003). Habitat selection in *P. delicatulus* and *P. novaehollandiae* has likewise been linked to a preference for floristically rich successional stages that produce suitable food, in particular nutritious seeds (Cockburn 1980, Braithwaite and Brady 1993). Seed accounted for 90% of the summer diet of *P. novaehollandiae* in one study in New South Wales (Thomson 1980). However, Wilson and Bradtke (1999) recorded only 14% seed in the diet of *P. novaehollandiae* from southern Victorian sites. They suggested that their result indicates dietary flexibility allowing exploitation of available resources across a variety of habitats.

Pseudomys delicatulus, *P. novaehollandiae* and *P. pilligaensis* exhibit marked population fluctuations following fire (Kemper 1990, Braithwaite and Brady 1993, C. Eddie pers. comm., H. Tokushima pers. comm.). Braithwaite and Brady (1993) recorded a population increase of *P. delicatulus* from less than 10 to more than 100 individuals between October

and January following a fire. Tokushima (H. Tokushima pers. comm.) recorded an even more dramatic irruption of mice in the Pilliga, his population growing from a few individuals to several hundred. Trap saturation meant the full extant of the irruption could not be determined. Similar patterns have been observed in *P. delicatulus* in central Queensland (C. Eddie pers. comm., N. Thurgate pers. comm.). Rodents in central Australia are noted for spectacular population irruptions (Newsome and Corbett 1975), although the use of the term "irruption" by Predavec (1994) to describe population fluctuations in a population of *P. hermannsburgensis* is potentially misleading, as he recorded increases of only 20 or so individuals on his study grids. Recruitment of this level could be the result of only five or six females breeding successfully, and increases of this magnitude are to be expected in many *Pseudomys* populations. Where Predavac's populations differ from pebble-mound mice is in their mobility, particularly of breeding females. Individuals of *P. hermannsburgensis* move very large distances (16km+) (Dickman et al. 1995), and "populations" of *P. bolami*, *P. delicatulus* and *P. hermannsburgensis* seem to be highly mobile over short time frames, deserting an area occupied in the previous month, or exhibiting extremely high rates of individual turnover at a given site (Predavec 1994, Moseby and Read 1998, C. Eddie pers. comm., A. Start pers. comm., N. Thurgate pers. comm.). This is particularly so when food resources are patchy due to fire or uneven rainfall (Letnic 2002).

6.2 Geographical sampling for molecular analysis

Good broad-scale coverage of the distributions of all species was achieved, largely through supply of museum tissues from southern and western Australia and field biopsies of the Queensland distribution of *P. delicatulus* (table 6.1 and fig. 6.2). New Guinean material from *P. delicatulus* was not available, and coverage of coastal and far northern Queensland was sparse. Many tissues samples were formalin-preserved, and only some of these have yielded good sequence. Those that have not are not listed in table 6.1, and an extensive collection awaits further analysis. The Pilliga was a location of special interest (section 6.4.3) and almost eighty tissue samples were provided by H. Tokushima, and will form the basis of future microsatellite studies. Samples were obtained from the Nullarbor where *P.*

hermannsburgensis is sympatric with *P. bolami* and from the northern Pilbara where *P. hermannsburgensis* is sympatric with *P. delicatulus*. One individual of *P. delicatulus* from of Weipa (site 50 in figure 6.1) was sequenced during the final stages of writing-up. Analysis of that individual's sequence was restricted to distance analysis to establish general relationships.

Table 6.1. Sampling locations and tissue sources for the delicate mice.
(ABTC)- tissue from Australian biological tissue collection with museum voucher; ABTC- no voucher; NTMU- Northern Territory Museum; QMJM- Queensland Museum; SAMAM- South Australian Museum. N= sample size. Sequence ID's are used to identify individuals from a particular site in results trees.

Site	Lat.	Long.	N	Type	Source/voucher	ID's
P. bolami						
1. Balcanoona	-30.6097	139.4436	2	Liver	(ABTC) SAMAM 19518, 19512	b16,17
2. Balladonia	-32.0728	124.0500	1	Liver	ABTC 8074, 8075	b8,b9
3. Black Flag	-30.5833	121.2667	2	Liver	ABTC 8058, 8059	b2,3
4. Morgan Vale	-33.2445	140.5722	2	Liver	(ABTC) SAMAM 12925, 12926	b10,11
5. Mt Gairdner	-32.2417	135.9583	2	Liver	(ABTC) SAMAM 12475, 12479	b12,13
6. Rawlinna	-29.7167	125.3167	1	Liver	ABTC 8070	b5
7. Vivian	-30.9000	135.7333	2	Liver	(ABTC) SAMAM 17966, 27292	b14,15
8. Whyalla	-33.0000	137.5750	1	Liver	(ABTC) SAMAM 18279	b1
P. delicatulus						
9. Bede	-23.3661	145.5660	2	Tail	Field biopsies (A. Kutt)	d5,6
10. Burra Range	-20.7237	145.1860	1	Tail	Field biopsy (Ford)	d1
11. Camp Milo	-25.9833	153.0667	1	Skin	QMJM 2620	d35
12. Clemant	-19.1140	146.4330	1	Liver	Field biopsy (A. Kutt)	d2
13. Fortuna	-22.6500	145.5333	1	Skin	QMJM 13835	d57
14. Great Sandy Desert	-19.6666	123.3500	1	Liver	ABTC 8068	d19
15. Litchfield NP	-13.2530	130.7335	2	Liver	(ABTC) NTMU 4191, 4192	d23,24
16. Melville Island	-11.5053	130.9032	2	Liver	ABTC 8035, NTMU 4418	d18,25
17. Mitchell Plateau	-14.8917	125.7597	1	Liver	(ABTC) WAMM 21624	d20
18. Monklands	-23.4847	146.4691	2	Tail	Field biopsies (N. Thurgate)	d3,15
19. Mount Inkerman	-19.7500	147.4830	1	Liver	(ABTC) QMJM 11350	d29
20. Nathan River	-15.2500	135.5167	1	Liver	(ABTC) NTMU 751	d22
21. Oakleigh	-23.5861	146.5425	1	Tail	Field biopsy (N. Thurgate)	d4
22. Patonga	-12.9600	132.5770	1	Liver	ABTC 8034	d17
23. Pellew Is.	-15.5500	136.8333	2	Liver	(ABTC) NTMU 1510, 1521	d30,31
24. Ramingining	-12.2047	134.9813	1	Liver	(ABTC) NTMU 4745	d28
25. Wickham R	-16.8252	130.1923	2	Liver	(ABTC) NTMU 4712, 4715	d26.27
26. Woodstock	-21.6069	119.0397	2	Liver	(ABTC) WAMM 29247, 34326	d33,34
P. hermannsburgensis						
27. Beetoota	-25.6940	140.7440	2	Liver	ABTC 8047, 8048	h10,11
28. Big Red	-25.8792	139.0531	1	Tail	Field biopsy (Ford)	h22
29. Curtin Springs	-25.5667	131.7972	2	Liver	(ABTC) SAMAM 12570, 12571	h8,9
30. Helen Springs	-18.4978	133.8738	1	Tail	Field biopsy (Ford)	h23
31. Kurundi	-20.6000	134.8333	2	Liver	ABTC 8052, NTMU 3429	h12,15
32. Laverton	-27.7622	123.3663	1	Liver	ABTC 41428	h16
33. MacDonnell Range	-23.7000	132.5000	1	Ear	Desert Wildlife Park	h3
34. Nifty Mine	-21.6666	121.5833	1	Liver	(ABTC) WAMM 43170	h20
35. Nullarbor	-29.5167	127.0500	2	Liver	ABTC 8076, 8077	h25,26
36. Pieracoola WH	-29.0117	146.0597	1	Liver	(ABTC) SAMAM 15017	h1
37. Purni Bore	-26.2833	136.1000	2	Liver	(ABTC) SAMAM 15773, 15777	h4,5
6. Rawlinna	-29.7167	125.3167	2	Liver	ABTC 8069, 8072	h14,24
38. Tallaringa	-28.2167	133.3667	2	Liver	(ABTC) SAMAM 17483, 17488	h6,7
26. Woodstock	-21.5917	119.0792	3	Liver	(ABTC) WAMM 29450, 29451, 29466	h17,18,19
P. novaehollandiae						
39. Anglesea	-38.4050	144.1890	2	Ear	Field biopsies (B. Wilson)	no1,2
40. Glenrock	-27.4900	152.1600	1	Liver	QMJM 11978	no10
41. Goobang	-32.6924	148.3441	2	Tail	Field biopsies (M. Ellis)	no3,4
42. Helidon	-27.9583	152.3281	1	Liver	QMJM 14454	no9
43. Loch Sport	-38.0450	147.5850	2	Ear	Field biopsies (B. Wilson)	no5,6
44. Port Stephens	-32.7000	152.1500	1	Seq.	Sequence SCU (M. Elphinstone)	no15
45. Toowoomba	-27.3650	152.1850	2	Tail	Field biopsies (P. Sparshott)	no12,13
46. Wilsons Promontory	-38.9190	146.3660	1	Ear	Field biopsy (B. Wilson)	no8
P. pilligaensis						
47. Binnaway NR	-31.4648	149.4550	2	Ear	Field biopsies (H. Tokushima)	pil2,11
48. Pilliga NR	-30.9872	149.3017	4	Liv/Ear	Field/Lab biopsies (H. Tokushima)	pil4,13,14,15
49. Pilliga SF	-30.6265	149.7210	6	Ear	Field biopsies (H. Tokushima)	pil1,3,5,6,7,8

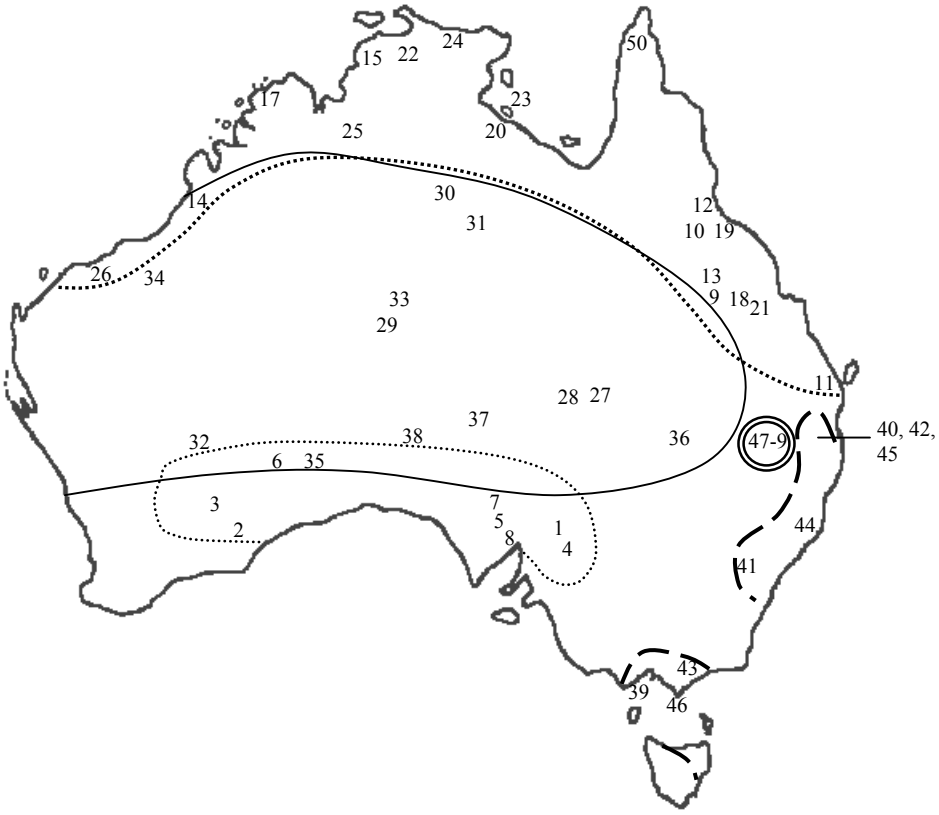


Figure 6.1. Distribution of delicate mice and sampling locations.
Distributions from Braithwaite and Covacevich (1995), Breed (1995a), Kemper (1995), Watts (1995a) and sampling locations. *Pseudomys bolami* ····· ; *P. delicatulus* ····· ; *P. hermannsburgensis*—— ; *P. novaehollandiae* – – ; *P. pilligaensis* ═══ . Site 50 (Weipa) is not included in table 6.1 (see text).

6.3 Molecular evolution

6.3.1 Mitochondrial control region

Four major clades are evident in distance and maximum-likelihood analysis of control region sequence (fig. 6.2; minimum evolution score: 0.50881: fig. 6.3; likelihood tree score: -ln 1591.52371; substitution model: TrN+I+G Base=(A 0.3259; C 0.0775; G 0.2222; T 0.3744) Nst=6 Rmat=(A-C 1.0000; A-G 19.9700, A-T 1.0000; C-G 1.0000; C-T 10.0920; G-T 1.0000) Rates=gamma Shape=0.4357). Two clades correspond to the currently described species *Pseudomys bolami* and *P. hermannsburgensis* although 3

individuals of *P. hermannsburgensis* were miss-identified as *P. bolami*. One contains five individuals from north-western Australia identified as *P. delicatulus* (*P*. cf. *delicatulus*). The largest clade, the "*P. delicatulus* complex", contains all individuals identified as *P. novaehollandiae*, *P. pilligaensis*, and the majority of individuals identified as *P. delicatulus*. Bootstrapping of trees strongly supports each of these major clades (fig. 6.2 and 6.3). Distance and likelihood analyses reveal three relatively deep clades within the *P. delicatulus* complex, but bootstrapping of maximum-likelihood trees provides marginal support (51%) for only one of these clades, "*P. novaehollandiae*", which includes all individuals identified as *P. novaehollandiae*, five individuals identified as *P. pilligaensis* and one Queenslnd museum voucher identified as *P. delicatulus* (QMJM 2620- Camp Milo, d35). The remaining two clades correspond to; *P. delicatulus* from the north Kimberley and Top End ("*P. delicatulus* (NT)"), and *P. delicatulus* from Queensland and seven individuals identified as *P. pilligaensis* ("*P. delicatulus* (QLD)"). The individual of *P. delicatulus* from Weipa not included in the trees (section 6.2) yielded sequence that was close to *P. delicatulus* from central Queensland, and more distantly related to haplotypes d2 and d29, thereby falling well within the *P. delicatulus* (QLD) clade. The likelihood tree is rooted on *P. hermannsburgensis* because that taxon retains ancestral characters when compared to the *P. delicatulus* complex, and is ancestral to the group in all control region phylogenies. The only difference between outgroup and midpoint rooted trees was the branching of *P.* cf. *delicatulus* from the base of the *P. hermannsburgensis* branch in midpoint, but not outgroup rooted trees.

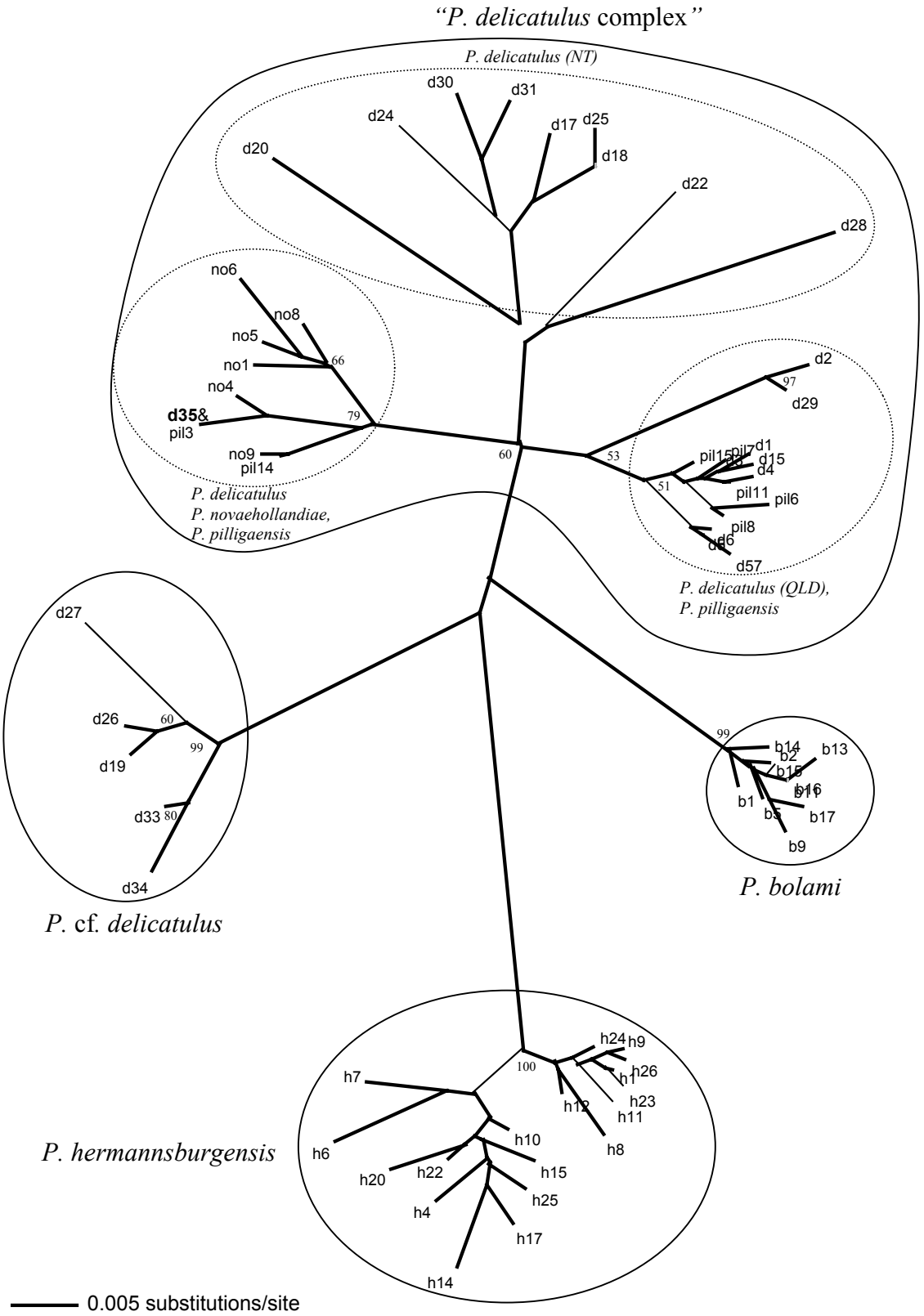


Figure 6.2. Genetic distance between 64 delicate mouse control region haplotypes.
Unrooted neighbour-joining tree of Kimura two-parameter distances between 64 haplotypes of 387bp isolated from 87 individuals. Labels are one individual that carried a haplotype, plus individuals (miss-)identified as another species (bold). Major clades shown as solid circles. Current species boundaries in "*P. delicatulus*" as labelled for broken circles. Node labels= bootstrap support (>50%) for major nodes based on 100 replicates.

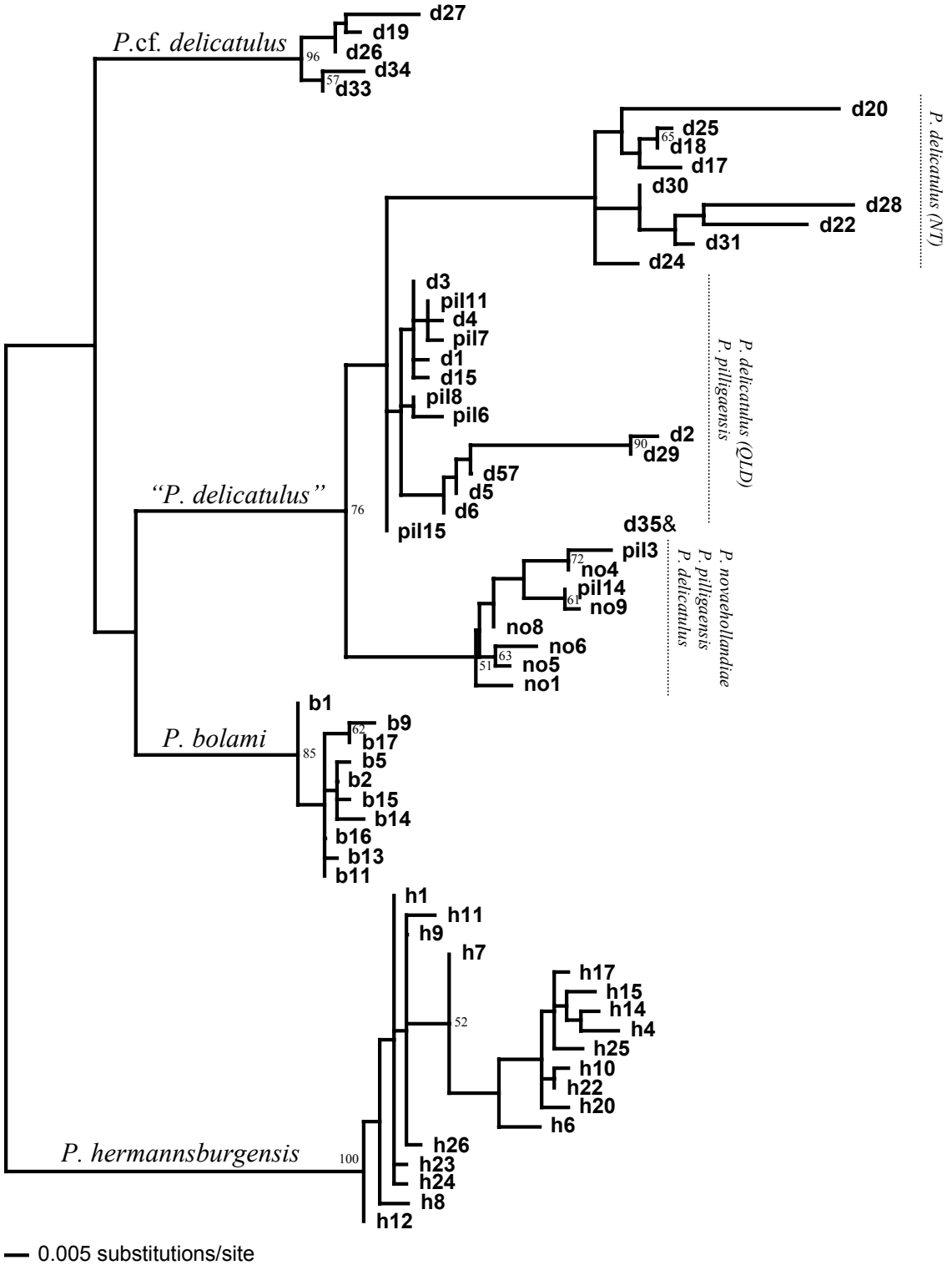


Figure 6.3. Maximum-likelihood tree of delicate mouse control region haplotypes.
Maximum likelihood tree for 64 haplotypes based on parameters derived from Modeltest for 387bp of sequence isolated from 87 individuals. Tree is rooted using *P. hermannsburgensis*. Taxon labels correspond to a single individual that carried a particular haplotype, plus (miss-)identified individuals. Node labels= bootstrap support (>50%) based on 100 replicates.

6.3.2 NUCLEAR INTRONS

Introns were of particular interest among the delicate mice due to the differences between established taxonomy of the group and results of control region analysis. Sequencing of five introns, ARAF, ACTC, CHAT, DHFR and MYH2, showed that they were relatively invariable, produced ambiguous products, or yielded sequence that aligned with exon sequences from Genbank. DHFR intron 1 was conserved among members of the group, but was useful in clearly separating *Pseudomys* cf. *delicatulus* from the *P. delicatulus* complex on the basis of two mutations and one 14bp deletion (fig. 6.4). A single fixed mutation characterised the *P. delicatulus* complex when compared to *P. bolami*, *P. hermannsburgensis* and *Pseudomys* cf. *delicatulus.* Phylogenetic use of the locus was not possible. Specimens of *"P. pilligaensis"* carrying *P. delicatulus* and *P. novaehollandiae* control region haplotypes were sequenced, and individuals of both types shared a unique DHFR intron 1 mutation, but were not significantly divergent from other populations of *P. novaehollandiae* or *P. delicatulus*. ARAF intron 1 sequences were highly conserved, but separated *Pseudomys* cf. *delicatulus* from the *P. delicatulus* complex (table. 6.2).

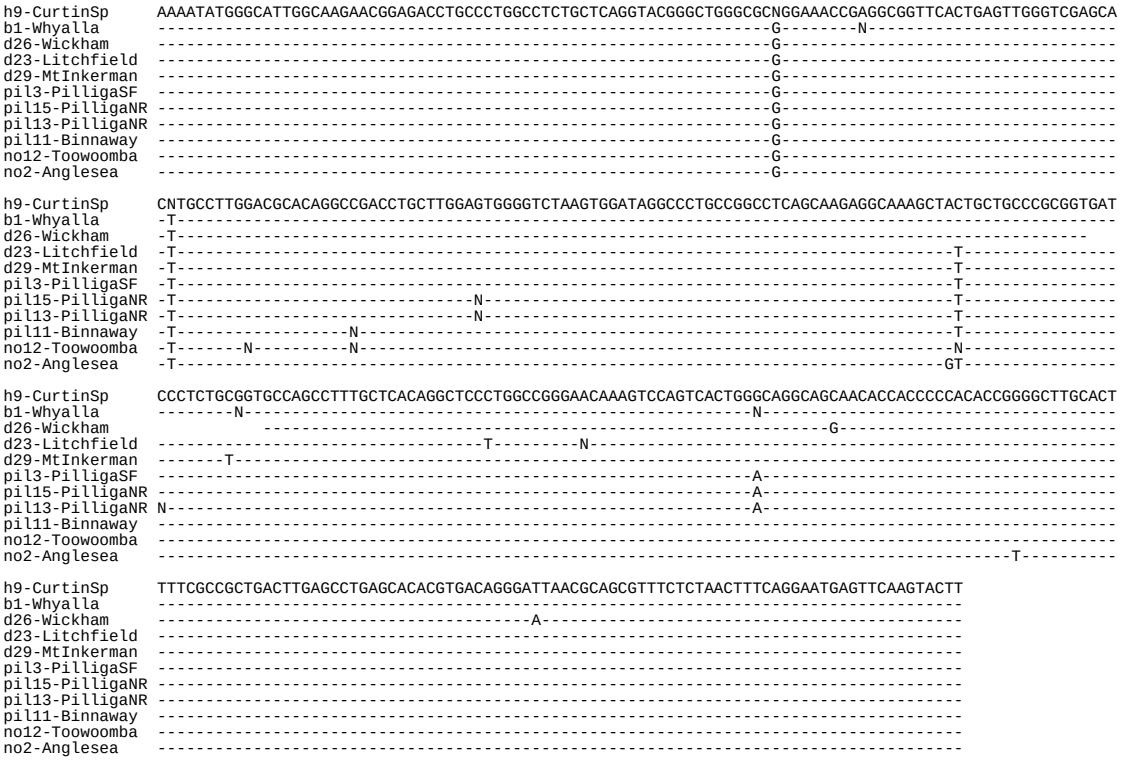


Figure 6.4. Alignment of DHFR intron 1 sequence from eleven delicate mice.
Codes represent a single individual and its site of origin. bx- *P. bolami*; dx- *P. delicatulus*; hx- *P. hermannsburgensis*; nx- *P. novaehollandiae*; px- *P. pilligaensis*. d26-Wickham is an individual identified as *P. delicatulus* but regarded here as *P.* cf. *delicatulus* on the basis of control region analysis. N is recorded for ambiguous sites in heterozygote individuals.

Table 6.2. Genetic distance at the ARAF intron 1 locus between *Pseudomys delicatulus*, *P. novaehollandiae*, *P. pilligaensis* and *Pseudomys* cf. *delicatulus*

	P.pill	*P.nov*	*P.del*(QLD)	*P.del*(NT)	*P.*cf.*del*
P.pill	-				
P.nov	0.0037	-			
P.del	0.0000	0.0075	-		
P.del	0.0000	0.0038	0.0037	-	
*P.*cf.	0.0156	0.0193	0.0194	0.0117	-

6.4 Evolution of the delicate mice

6.4.1 How many species of delicate mice are there?

There are six distinct molecular taxa within the group (table 6.3). Which of these constitute species is difficult to establish based on results presented here and in past studies. The (very) tentative conclusion of this thesis is that there are probably four, *P. bolami*, *P. delicatulus*, *P. hermannsburgensis* and *P.* cf. *delicatulus* but there needs to be critical evaluation of the species status of *P.* cf. *delicatulus* and *P. novaehollandiae* (included in "*P. delicatulus*") before this question can be resolved. *Pseudomys hermannsburgensis* and *P. bolami* are currently defined species that are strongly supported. Conversely, *Pseudomys delicatulus*, *P. novaehollandiae* and *P. pilligaensis* do not represent discrete molecular evolutionary units. *Pseudomys pilligaensis* is not a mitochondrial taxon, and individuals described as *P. pilligaensis* yield either *P. delicatulus* or *P. novaehollandiae* control region sequence. Three clades exist within *Pseudomys delicatulus* as currently recognised (figs. 6.2 and 6.3). One is a previously unrecognised species, referred to here as *P.* cf. *delicatulus*. This conclusion is supported by mtDNA and two nuclear introns. The remaining two clades within *P. delicatulus* are significantly less divergent from each other, but form lineages of roughly equal depth to their divergence from *P. novaehollandiae*. The nature of genetic structuring, biogeography, and probable evolutionary history of these two clades of *P. delicatulus* and *P. novaehollandiae* suggests that they have not yet fully speciated, and are a sub-specific complex within *P. delicatulus* (section 6.4.3).

Table 6.3. Inter-taxon control region divergence among delicate mice.
Distances based on average pair-wise Kimura two-parameter distances. Species shown at upper left, proposed subspecies at lower right. Percentage differences given here are not directly comparable to those given for pebble-mound mice in table 5.2 because the sequence length here (387bp) contains an additional 51bp. "*P. delicat.*" includes all subspecies proposed here, but this does not significantly alter distances in species comparisons.

	P. bolami	*P. delicat.*	*P. herman.*	*P. cf. delicat.*	*P. novae*	*P. del* (NT)	*P. del* (QLD)
P. bolami	-						
P. delicat.	6.09%	-					
P. herman.	7.98%	8.10%	-				
P. cf. delicat.	5.60%	7.05%	7.91%	-			
				P. novae	-		
				P. del (NT)	4.86%	-	
				P. del (QLD)	4.31%	4.34%	-

6.4.2 GEOGRAPHICAL DISTRIBUTION OF DELICATE MOUSE CLADES

Sequencing studies have revealed that the distribution of evolutionary clades within the delicate mouse group differs from the distribution of morphologically defined taxa. *Pseudomys hermannsburgensis* is Eyrean, and found throughout central Australia, while all other species of delicate mouse are distributed around the margins of the continent (fig. 6.5). *Pseudomys bolami* has a distribution along the southern coasts of the arid zone, and into the southern arid and semi-arid interior. Based on the distribution of mtDNA haplotypes and the known distribution of "*P. delicatulus*" in north-western Australia, *Pseudomys* cf. *delicatulus* is found in arid areas between the southern VRD and the Pilbara, including the southern Kimberley and Great Sandy Desert (section 6.4.3). The Torresian and Bassian subregions are home to three clades within the *P. delicatulus* complex. The first is found in the north Kimberley and the Top End. Although distribution maps in Braithwaite and Covacevich (1995) and Watts and Aslin (1981) show *P. delicatulus* to be distributed through the Carpentarian Barrier, this does not appear to be the case, and I could not find evidence of captures of the species from the Carpentarian plains or Mitchell Grass Downs in the Australian, Northern Territory, Queensland or Western Australian Museum collections, or from other field workers. De Vis (1884) does record a possible individual from the mouth of the Norman River, but was not confident if the identification. Based on control region haplotypes, this thesis is the first study to reveal the presence of *P. delicatulus* in New South Wales, and also reveals a broader distribution of *P. novaehollandiae* in Queensland than previously thought. These two clades meet in a region of secondary contact from the Pilliga in New South Wales to the Maryborough-Gladstone region of southeast Queensland (section 6.4.3), an area that roughly marks the Bassian-Torresian divide.

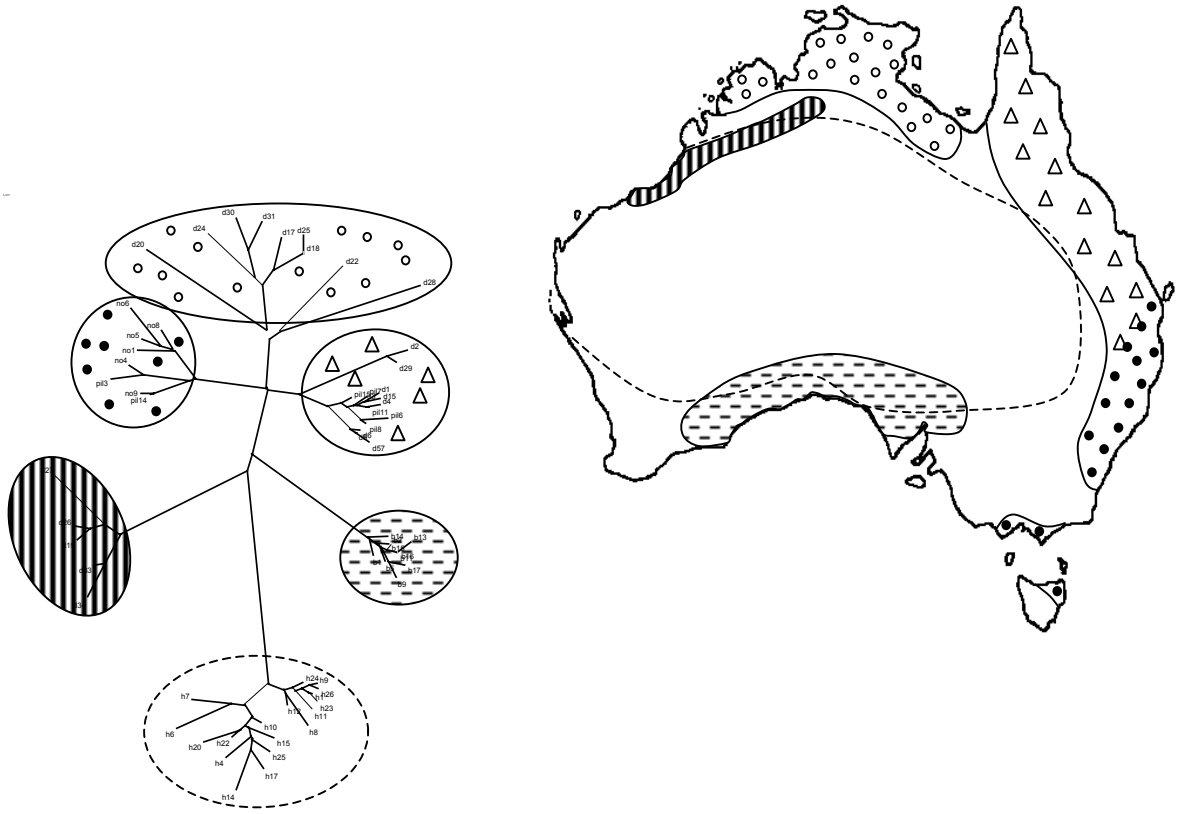


Figure 6.5. Geographical distribution of delicate mouse clades.
P. bolami ⊖ ; *P. delicatulus*- Kimberley-Top End ○ ; *P. delicatulus*- Queensland△ ;
P. (delicatulus) novaehollandiae ● ; *P. hermannsburgensis* ◌ ; *Pseudomys* cf. delicatulus ◍ .

6.4.3 EVOLUTIONARY HISTORY OF DELICATE MOUSE SPECIES

The distributions of delicate mouse species appear to be climatically determined, and all species overlap with closely related taxa in regions of climatic transition such as the Pilbara, southern Kimberley, the southern arid zone and central and south-eastern Queensland. The ability of these species to react to climatic change by expanding their range to occupy previously unsuitable habitat, and vice-versa, makes it difficult to successfully reconstruct the deeper evolutionary history of the delicate mice, as repeated range shifts may well obscure evolutionarily significant patterns. However, constructive comment can be made on several features of delicate mouse evolution important to general

evolution of Australian faunas, and detailed analysis of more recent evolutionary phenomena can be undertaken.

Pseudomys bolami

The re-description of *P. bolami* by Kitchener et al. (1984) identified differences in ear length, pes length, skull and dental features that separated *P. bolami* from *P. hermannsburgensis*. There were eight fixed differences at 15 variable isozyme loci. These results are matched by the genetic distinctness of the species in control region sequencing. *Pseudomys bolami* has an unusual distribution that includes the inhospitable Nullarbor Plain and southern margins of the arid zone. Few terrestrial mammals are restricted to the region inhabited by *P. bolami*. The southern hairy-nosed wombat (*Lasiorhinus latifrons*) had a largely concordant natural distribution (Wells 1995), and the southern Ningaui (*Ningaui yvonneae*) occurs in limited areas within the distribution of *P. bolami* (Kitchener 1995b). Among rodents *Notomys mitchelli* has a similar distribution but is also found in less arid areas to the southwest of the distribution of *P. bolami* (Watts 1995c). Some confusion over the respective distributions of *P. bolami* and *P. hermannsburgensis* may have arisen from past taxonomic synonymy, and three of 17 museum vouchers of *P. bolami* sequenced in this study were actually *P. hermannsburgensis* (these were considered to be individuals captured prior to the re-description of *P. bolami* and re-assigned to *P. hermannsburgensis* for the purposes of analysis). Sympatry of such ecologically and morphologically similar *Pseudomys* species is uncommon, particularly in southern Australia, but mirrors patterns between species of delicate mice elsewhere.

There is little genetic variation across the range of *P. bolami*, and no indication of geographical structuring. Although sample sizes are low, a maximum of one non-sequenced mutational step separates any of the nine recorded haplotypes from the closest related haplotype. The sites sampled include the geographical limits of the species range. Lack of contemporary genetic structuring is readily explained by a fairly continuous distribution with no geographical barriers. However, the overall lack of genetic diversity, and relative genetic isolation from related species, indicates that the recent evolution of *P. bolami* has

probably involved a population bottleneck, and that haplotype extinction has reduced the genetic diversity of this species well below that of any other delicate mouse species, or subspecies (as defined here). A population bottleneck due to geographical restriction seems a more likely scenario given the widespread evidence of range reductions during the Pleistocene and LGM (sections 2.3.2 and 5.4.3).

Pseudomys hermannsburgensis

Two shallow but distinct mitochondrial clades are apparent within *P. hermannsburgensis*. Each is distributed widely in the arid zone, and there is no spatial separation of the clades. Nested clade analysis did not reject the null hypothesis, no geographical association of alleles, for any clade. This is expected given the lack of correlation between geographical location and genetic structuring of haplotypes (fig. 6.6). No statistically significant evidence of fragmentation was detected between the two major clades. More intense geographical and population sampling may be able to resolve whether a bottleneck or allopatry was responsible for restriction of genetic diversity in the species, but this is unlikely because past geographical structuring will almost certainly be masked by current panmixia.

The ecology of *P. hermannsburgensis* is such that a lack of geographical structure in mtDNA studies is readily explicable. Individuals and populations move long distances to exploit patchy resources resulting from erratic rainfall patterns (Dickman et al. 1995, Letnic 2002). The species is notable for its ability to go without water and concentrate urine (MacMillen and Lee 1967, Kotler et al. 1998), and has a variable and low basal metabolic rate that Predavec (1997) suggested was an adaptation to variable environments of the arid zone. The relatively higher overall genetic diversity of *P. hermannsburgensis* when compared to *P. bolami* shows that it did not undergo a severe bottleneck as seems to have occurred in *P. bolami* (*P. hermannsburgensis*: avge. 1.77%, max. 3.52%; *P. bolami*: avge. 0.74%, max. 1.33%) during the LGM. Instead, better adaptation to arid conditions and high mobility would possibly have seen the species' range move towards the coastal margin, but not contract to the point where major loss of genetic diversity occurred. The fact that there

is no genetic structure within the Eyrean region is indicative of the lack of significant barriers to movement in the region, even for a small-bodied species.

Pseudomys *cf*. delicatulus

The recognition of cryptic species of *Pseudomys* has been a feature of recent Australian rodent taxonomy. However, most recently described species have been pebble-mound mice. *Pseudomys* cf. *delicatulus* is clearly a delicate mouse, and is similar to *P. bolami* in its genetic distinctness. Like *P. bolami*, its control region sequence is marginally more similar to that of the *P. delicatulus* complex than to *P. hermannsburgensis*, and like *P. bolami*, its recognition may be upheld on molecular grounds, and a revision of morphological characteristics. No morphological revision has compared animals from the Pilbara with Kimberley-Top End *P. delicatulus* (true *P. delicatulus*). The five individuals found to be members of this species were museum specimens previously referred to *P. delicatulus*. Norah Cooper of the Western Australian Museum briefly examined four individuals from the Pilbara and the northern Kimberley following the initial genetic findings, and noted that Kimberley animals were smaller overall, had a shorter rostrum, narrower braincase, smaller bullae, wider palate, and narrower pterygoids. A detailed review and species description are pending. Should the species be upheld, the proposed name will be *Pseudomys aphanomorphus* derived from the Greek for “hidden form”.

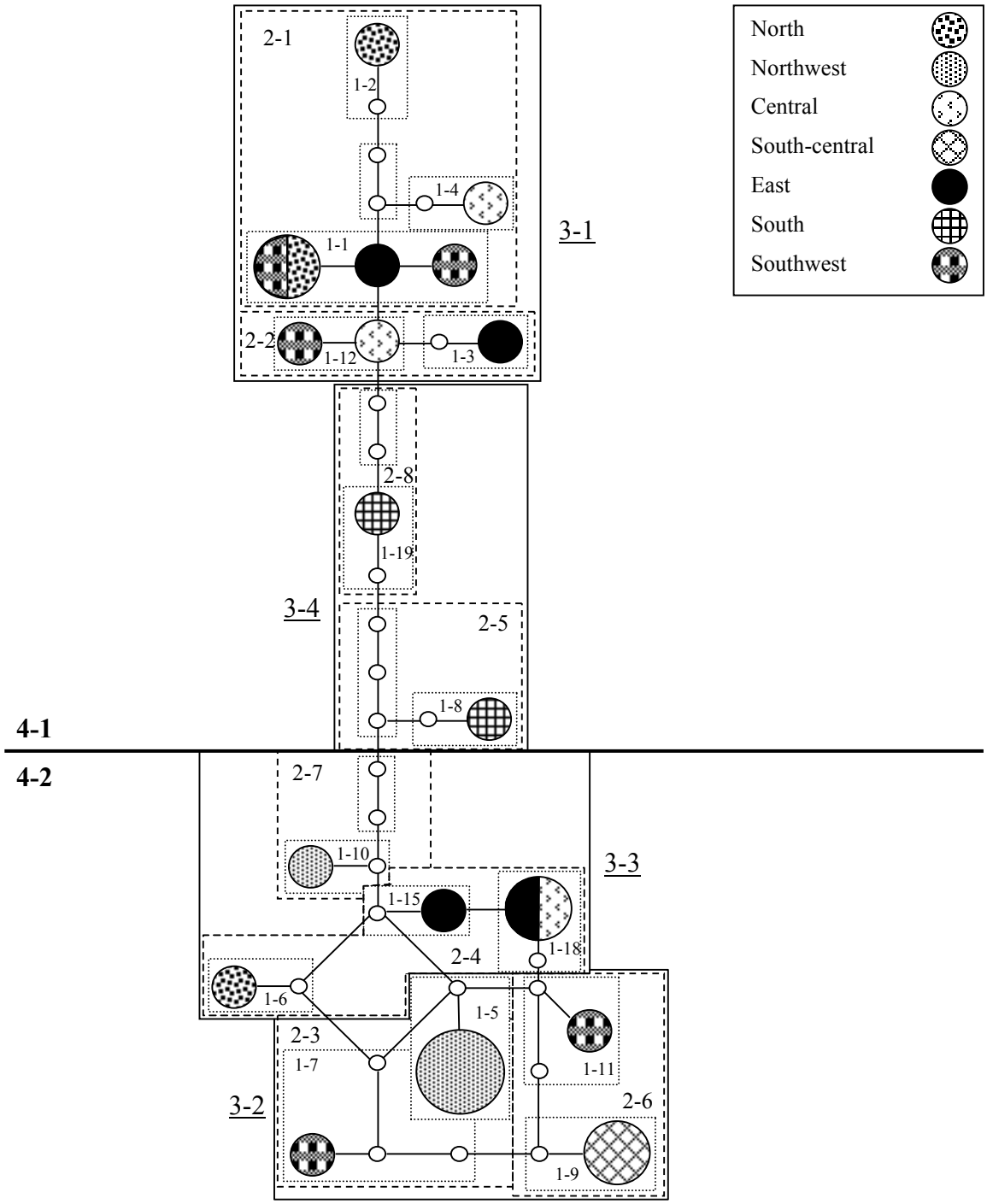


Figure 6.6. Nested haplotype network of *Pseudomys hermannsburgensis* control region haplotypes.

Large circles represent sequenced haplotypes (size= n). Small circles are unrecorded mutational steps. Shading as per key (represents multiple sites within a region of the arid zone). Significant clades: none.

A single individual of "*P. delicatulus*" studied by Baverstock et al. (1977a) and Baverstock et al. (1977b) was reported to come from a site 189km south of Broome. This site lies in the region where *Pseudomys* cf. *delicatulus* probably occurs. Baverstock et al. (1977a) found only three variable loci of seventeen they screened between *P. delicatulus* from Queensland, the Northern Territory and Western Australia. The individual from Western Australia differed from all Northern Territory and Queensland individuals at a single isozyme locus (Alb). This represents little significant genetic difference between forms. However, Baverstock et al. (1976) found no difference at the Alb locus between *P. hermannsburgensis*, *P. delicatulus* from QLD and *P. novaehollandiae*, and only detected a single variable locus among the seven they screened for these three species. A karyotype involving two peri-centric inversions and a medium-sized metacentric Y was shared between Queensland and Western Australia (possible *P.* cf. *delicatulus*), but not the Northern Territory.

It is likely that all "*P. delicatulus*" from the Pilbara, Great Sandy Desert and southern parts of the Kimberley and VRD are members of *Pseudomys* cf. *delicatulus*. The exact distribution of the species is unknown, but appears to have a strong ecological basis when compared to that of *P. delicatulus*. BIOCLIM modelling based on the five definite location records of *P.* cf. *delicatulus* and on populations from which individuals of *P. delicatulus* were sequenced in the northern Kimberley, the Top End and Queensland shows spatial separation of the two species, and predicts that *P.* cf. *delicatulus* may occur in a relatively narrow band of habitats between the savannahs of the Top End and central deserts to the south (fig. 6.7). The species is sympatric with *P. hermannsburgensis* at Woodstock Station in the Pilbara, and is probably sympatric with that species across the southern parts of its distribution, and with *P. delicatulus* in the north. Records of *P. delicatulus* from the Newcastle Waters region (Parker 1973) and Northern Tanami Desert (Gibson 1986) are likely to represent this species if the preliminary distribution modelling proves correct, and the fact that the species is found in significantly drier habitats than *P. delicatulus* is apparent without the need for any modelling. The five individuals sequenced in this study exhibited significantly greater genetic divergence than observed in *P. bolami*, but nothing further can be said of its genetic structure.

Despite the deep mtDNA and nuclear intron divisions between this putative species and *P. delicatulus*, I have some reservations of its specific status based on the similarity of chromosomes and isozymes between a possible individual of this species with *P. delicatulus* (above), and more particularly with the reported sperm morphology (Breed 2000) of an individual of "*P. delicatulus*" from the northern Tanami Desert. This individual, like the one from south of Broome examined by Baverstock et al. (1977a) and Baverstock et al. (1977b), came from an arid area I would expect *P.* cf. *delicatulus* to inhabit. Its sperm morphology was identical to that of other Northern Territory and northern Kimberley *P. delicatulus* examined by Breed (2000). The reported similarity of isozymes between these forms may be of little consequence. The studies involved loci that show minimal differences between *P. delicatulus* and *P. novaehollandiae*, and little more between *P. delicatulus* and *P. hermannsburgensis*. Loci used to separate *P. bolami* from *P. hermannsburgensis* may be more appropriate, as that study incorporated all loci that were variable within the group in other studies, and employed an additional seven loci, four of which had fixed differences, and another two of which were variable. Chromosomes and sperm morphology must be described for individuals with known control region identity, because the suggestion from past studies is that *P.* cf. *delicatulus* has a karyotype different to that of *P. delicatulus* in the Top End, but the same as Queensland *P. delicatulus*, but has sperm morphology identical to that of the Top End and northern Kimberley, but different to that of Queensland animals. Should *P.* cf. *delicatulus* prove to be a deep subspecies of *P. delicatulus*, the recognition of *P. novaehollandiae* as a species would render *P. delicatulus* paraphyletic. If *P.* cf. *delicatulus* is a species, then the currently recognised taxon *P. delicatulus* is polyphyletic. The working hypothesis in this thesis is that *P.* cf. *delicatulus* is probably a species, and that resolution of other characters states must be accompanied by control region sequencing.

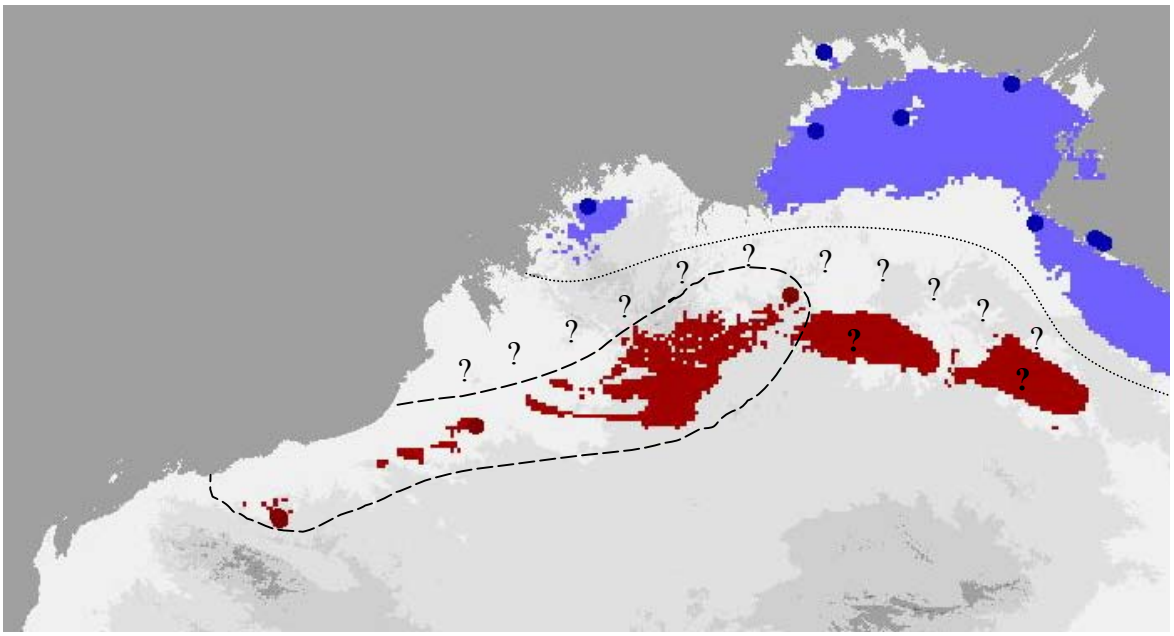


Figure 6.7. Bioclimatic prediction of the ranges of *Pseudomys delicatulus* and *Pseudomys* cf. *delicatulus*
BIOCLIM prediction based on known sites of occurrence. Lines indicate known boundaries of each species distribution: *Pseudomys delicatulus*- blue ····· ; *Pseudomys* cf. *delicatulus*- red – – - . Mice from areas indicated "?" are of unknown affinity. Dots indicate sampling localities. Shading indicates 250m asl contours.

Pseudomys delicatulus *complex* (*excluding* Pseudomys *cf.* delicatulus*, and including* P. novaehollandiae *and* "P. pilligaensis")

Pseudomys delicatulus and those taxa included in the "*P. delicatulus* complex" have a confused taxonomic history (section 6.1.1). However, although these taxa have erroneously been thought to be more divergent than sibspecies, this thesis is the first study to suggest that *P. delicatulus* and *P. novaehollandiae* are possibly subspecies. *Pseudomys pilligaensis* has been thought to be *P. novaehollandiae* (Fox 1995), but the suggestion that there is no taxon corresponding to the described *P. pilligaensis,* and that it is comprised of individuals belonging to both *P. delicatulus* and *P. novaehollandiae* is novel. Such radical changes in nomenclature, particularly of *P. delicatulus* and *P. novaehollandiae,* which were described over 150 years ago, require very strong evidence and should not be based on mitochondrial evidence alone, particularly as this study only involved a short segment of the mitochondrion. However, the results presented in this thesis do not contradict the results of

any previous research, rather, they provide a new and comprehensive context in which to interpret those results, and reveal previously unrecognised evolutionary phenomena.

Morphology vs molecules

Morphological variation within the *P. delicatulus* complex does not reflect evolutionary history. This is illustrated by the presence of the morphologically cryptic lineage *P.* cf. *delicatulus* in north-western Australia, the assignment of individuals carrying *P. novaehollandiae* mtDNA to *P. delicatulus* in southeast Queensland and the description of populations containing individuals carrying both *P. delicatulus* and *P. novaehollandiae* mtDNA as *P. pilligaensis*. Morphologically, *P. novaehollandiae* from the southern Queensland coast are "good" *P. delicatulus* based on museum voucher identification (S. Van Dyck, pers. comm.). Likewise, all individuals referred to *P. pilligaensis* are morphologically consistent with the species description provided by Fox and Briscoe (1980) and with each other. The strong geographical association of morphological forms is indicative of clinal variation in morphology. Current species definitions are based on what were previously considered allopatric segments of this cline. However, recent population discoveries (Van Dyck 1998, Ellis pers. comm., H. Tokushima pers. comm., S. Townley, pers. comm.) and genetic results presented here show that there is an effectively continuous distribution of the *P. delicatulus/novaeholladiae* group along the eastern seaboard. Basically, morphology of Queensland *P. delicatulus* and *P. novaehollandiae* is determined primarily by contemporary environmental conditions (natural selection), and is not necessarily indicative of evolutionary history. It is common for phylogenetic patterns to be masked by selection on morphological traits in many vertebrate species, including those with a known history of isolation (Chernoff 1982, Kidd and Friesen 1998, Driskell et al. 2002, Salzburger et al. 2002b). It is therefore misleading and difficult to use morphology alone to describe and identify taxa currently described as species, particularly in the Queensland-New South Wales border regions.

An interrupted cline and the deceptive "Gonzo syndrome"

Many characters differ between the type of *P. delicatulus* Gould 1842 from the Cobourg Peninsula, Northern Territory, and the type of *P. novaehollandiae* Waterhouse 1843, from the Hunter Valley, New South Wales. There is a pronounced accessory cusp on the M1 molar of *P. delicatulus* that is lacking in *P. novaehollandiae* (Thomas 1910, Troughton 1937). *Pseudomys novaehollandiae* is darker, exhibits less contrast between the upper and lower pelage, has a relatively shorter tail, and is heavier (Watts and Aslin 1981). It possesses dark shading behind the nose that Van Dyck (1998) described as the "Gonzo syndrome" (pg. 51) in reference to the fact that it creates a somewhat elongate appearance of the nose that is not seen in *P. delicatulus*. Like other Bassian *Pseudomys* species (*P. fumeus*, *P. higginsi, P. oralis* and *P. shortridgei*), *P. novaehollandiae* has a bi-coloured tail (Kemper 1995). *Pseudomys delicatulus* from the Cobourg Peninsula lack an apical hook on their sperm head that is present in *P. novaehollandiae*, and the tail of the sperm is attached to the head at different points in the two taxa (Breed 1997). *Pseudomys novaehollandiae* differs from *P. delicatulus* in having a shorter, telocentric, Y chromosome and lacks a second metacentric autosomal pair (Baverstock et al. 1977b). It shares the peri-centric inversion in pair 8 present in Queensland *P. delicatulus* but not Northern Territory *P. delicatulus* (Baverstock and Watts 1974, Baverstock et al. 1977b, Baverstock et al. 1983).

The type localities of *P. delicatulus* and *P. novaehollandiae* are, however, over 3000km apart, not allowing for the linear near-coastal distributions of these species. Intermediate geographical locations yield animals with intermediate forms of many characteristics that distinguish these species (fig. 6.8). Body weight and colouration are the best indicators of clinal variation, particularly along the eastern coast. Body weight varies from less than ten grams in *P. delicatulus* of the Top End and north Queensland (Braithwaite and Brady 1993) to over 25g in *P. novaehollandiae* in southern Victoria and Tasmania (Pye 1991). Through Queensland, body weights are intermediate (field data, A. Kutt pers. comm., N. Thurgate pers. comm.). Adult mice in the Pilliga carrying both *P. delicatulus* and *P. novaehollandiae* mtDNA haplotypes weigh 10-12g (Fox 1995a). New South Wales coastal *P.*

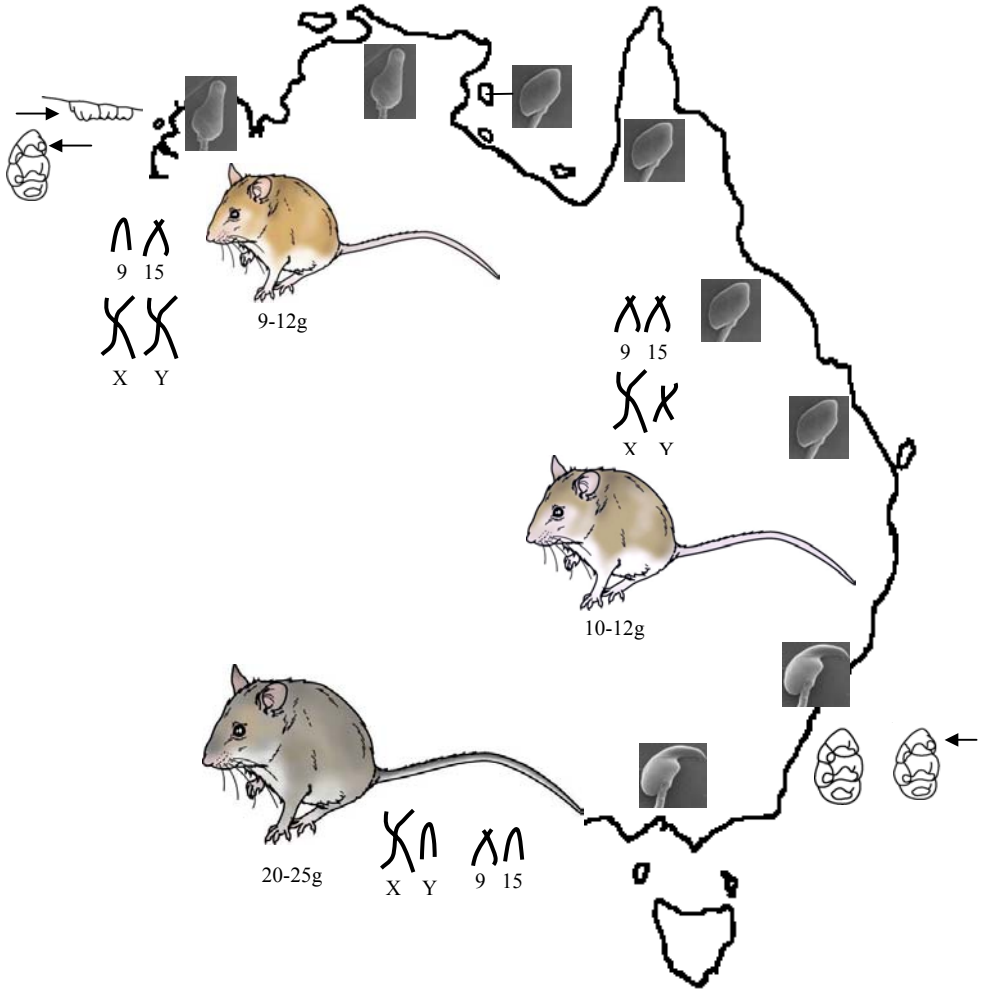
novaehollandiae weigh 16g on average (Kemper 1995, Haering and Fox 1997). Animals tend to have a darker or more muted pelage further south, with the description of *P. pilligaensis* intermediate between that of *P. delicatulus* and *P. novaehollandiae* (Fox and Briscoe 1980).

Isozyme studies showed fixed differences at only three loci (of twenty) between *P. delicatulus* and *P. novaehollandiae*, considerably less than between other *Pseudomys* species except *P. albocinereus* and *P. apodemoides* (4) (Baverstock et al. 1981). Baverstock et al.'s (1981) study did not include central Queensland individuals, which were shown previously to exhibit different patterns of variability to the north Queensland specimens included in their analysis (eg, at the PGM_2 locus: individual 14M (Baverstock et al. 1977a)). It would be expected that central Queensland individuals might be more similar to *P. novaehollandiae* on the basis of results presented in this thesis, although variability at isozyme loci used to examine these taxa is apparently low, and little difference may have been observed.

A characteristic that has lead to much taxonomic confusion in the delicate mice is the presence or absence of an accessory cusp on the M1 molar. Thomas (1910) Troughton (1937, 1941), Tate (1951) and Crabb (1977) attached a great deal of taxonomic significance to the expression of this cusp, but other authors have noted that there is considerable variation in expression of the cusp within *P. delicatulus* and *P. novaehollandiae,* and other rodents both within and outside the conilurine fauna (Ellermann 1941, Keith and Calaby 1968), and rightly regard it as a poor taxonomic character (Ride 1970, Mahoney and Marlow 1968). Mahoney and Marlow (1968) noted that within a single population of *P. novaehollandiae* the cusp was variably present or absent, and *P. novaehollandiae* was the type of a subgenus based around not possessing a cusp (Thomas 1910). No complete examination of the geographical distribution of this variation has been presented. However, it is worth noting that Troughton (1936) described the species *Gyomys pumilus* from the Rockhampton-Maryborough area in southeast Queensland due to the absence of an M1 cusp. Troughton noted that it was the smallest member of the genus, which also contained *P. novaehollandiae*, at that time known only from localities well to the south. *Gyomys*

pumilus was synonymised with *P. delicatulus*, yet results in this thesis show that at least one individual from the Maryborough area identified as *P. delicatulus* possesses a *P. novaehollandiae* mitochondrial haplotype, and recent discoveries of *P. novaehollandiae* in southern Queensland indicate that Troughton's *G. pumilus* was a small "*Gyomys*"; northern members of *P. novaehollandiae* that exhibit smaller body size due to clinal variation in that character (fig. 6.8).

Aside from clinal characters there are also a number of "fixed" differences between the three clades belonging to the *P. delicatulus* complex. Each clade possesses a small number of fixed control region mutations that are the basis of their separation in phylogenetic trees (fig. 6.8). Queensland *P. delicatulus* and *P. novaehollandiae* share a sub-acrocentric autosomal chromosome pair 9 that was not found by Baverstock et al. (1977b) in Top End animals, while Queensland and Northern Territory *P. delicatulus* share a sub-acrocentric autosomal pair 15 that was not found in *P. novaehollandiae*. The pair 9 form may be ancestral within the delicate mouse group, as it is present in some *P. hermannsburgensis* and possibly *P.* cf. *delicatulus*, so *P. delicatulus* in the Northern Territory and Queensland may share the derived chromosomal form (15). The attachment of the tail to the sperm head in Queensland *P. delicatulus* is more similar to *P. novaehollandiae* than to *P. delicatulus* from the Kimberley and the Top End (Breed 1997, 2000). However, the sperm head lacks the apical hook of *P. novaehollandiae*, instead having a reduced process that is presumably a rudimentary expression of the hook of *P. novaehollandiae* (fig. 6.8; Breed 2000). Such characteristic clade features indicate periods of isolation. *Pseudomys delicatulus* from Groote Eylandt, close to the Top End coast of the Gulf of Carpentaria possess a Queensland type of sperm morphology (Breed 2000). However, mtDNA sequence is not available to determine the significance of this fact. Sperm morphology has also not been determined for individuals with a known mtDNA haplotype from the Pilliga region, so it is not entirely clear whether sperm morphology is correlated with mitochondrial lineages.

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P. del NT     NAGNTNNTNNANNNNNNTNNGNANNNNNNNNNNAAG-NNNNGTNNAAANNNTANNATT-TANTT
P. del QLD    TGNTT-ANAGNNNNTANNNGGNAAANA-ANNA--AATANAGNTAAGATTANANAANT--NNTT
P. novae      TNNTN-ATANAANNTGNTNGTNNAGTA-GGAN--A-TNAANTTANANTTGTNNANTCNTAANN
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Figure 6.8. Clinal and "fixed" differences between members of the *P. delicatulus* complex. Bold bases indicate fixed control region base differences between taxa, "N" indicates intra-taxon variation at a site. Only variable bases shown. Rodent and tooth drawings Alice Wetherell. Sperm photos Bill Breed. Weights etc Braithwaite and Covacevich (1995), Fox (1995a) and Kemper (1995). Chromosomes Baverstock et al. (1977b), Baverstock et al. (1983).

Incipient, sub- or sib-species?

It appears there is contemporary isolation of Kimberley/Top End *P. delicatulus* from Queensland *P. delicatulus* (section 6.4.2), but the division apparent in genetic analysis almost certainly pre-dates this separation. These clades have successfully interbreed in captivity, and Baverstock et al. (1977a) found no reasons to recognise the clades as distinct species on the basis of isozyme divergence. The presence of *P. delicatulus* on islands in the Gulf of Carpentaria (Woinarski 2000) and in New Guinea (Flannery 1995) provides evidence that populations of delicate mice occupied habitats between Cape York, New Guinea and the Top End during the last glacial period. Reconstructions of habitats surrounding the pre-historic Lake Carpentaria also indicate suitable habitats for the species existed in areas that were inundated by the Gulf 10ka (section 2.3.2). The region now flooded by the Gulf has also been subject to periods of aridity on an ~2500 year cycle (De Dekker 2001), and aridity may have caused periodic geographical separation of Cape York and Top End *P. delicatulus* in the absence of a water barrier. Given the probable frequent contact between these areas, it is surprising that they are appear to be reciprocally monophyletic and to possess traits such as different sperm morphology that are constant among individuals within a region. Greater sampling of Cape York may well alter this apparent pattern, as movements of animals from the Gulf Plain and Arafura Plain presumably would occur equally to the Top End and Cape York as sea level rose. The presence of "Queensland" sperm type on Groote Eylandt off the Top End coast is potentially an indication of that fact.

The genetic distinctness of Queensland *P. delicatulus* from *P. novaehollandiae* is similar to the distinctness of *P. delicatulus* from the Top End and Queensland (average pair-wise distances for control region 4.3% in both cases; minimum divergence between QLD and NT= 3%, between QLD and *P. novaehollandiae*= 2.9%). This is harder to explain than divergence of Queensland and Northern Territory populations due to the continuous land connection between distributions of the two species throughout the Pleistocene. Nested clade analysis of Queensland *P. delicatulus*, "*P. pilligaensis*" and *P. novaehollandiae* suggested a possible explanation of factors leading to divergence of these clades. Three

major clades are identified in the nested cladogram (fig. 6.9). The majority of *P. delicatulus* haplotypes fall within clade 4-1, and all Victorian and most inland New South Wales *P. novaehollandiae* haplotypes fall within clade 4-3. The fact that there is slightly less genetic distance between than within the two "species" results in clade 4-2 containing both *P. delicatulus* haplotypes from central Queensland and *P. novaehollandiae* haplotypes from coastal New South Wales and southern Queensland. Haplotypes derived from animals referred to *P. pilligaensis* occur in all three major clades. Two individuals from the northeast Queensland coast form a distinct clade within Queensland (clade 3-1). The branches between "*P. delicatulus*" and "*P. novaehollandiae*" entailed more mutations than the 95% confidence of the maximum parsimony network created by TCS, but the effects of fragmentation are obvious (clade 4-2; χ^2 30; p= 0.030: total cladogram; χ^2 60.219; p= 0.003). Range expansion through central Queensland is suggested by the limited genetic diversity found over two thousand kilometres of latitude from Weipa to the Pilliga, although some evidence of IBD was found within clade 3-3 (χ^2 22.028; p=0.013). Nested clade analysis did not detect a significant effect of range expansion. This was probably due to the fact the Weipa haplotype was not included, and the clade diagram did not identify the major molecular clades as clades for nested analysis, a similar result to that obtained for *P. johnsoni,* which also had long inter-clade branches. Populations of *P. novaehollandiae* show relatively greater genetic divergence than Queensland *P. delicatulus* over similar distances, and no evidence of range expansion or fragmentation was found. Myroniuk (1998) has previously shown evidence of range fragmentation in a detailed study of *P. novaehollandiae.* He noted isolation of Tasmania and subsequent isolation of western Victoria from the remainder of mainland populations as important processes creating genetic structure within the species.

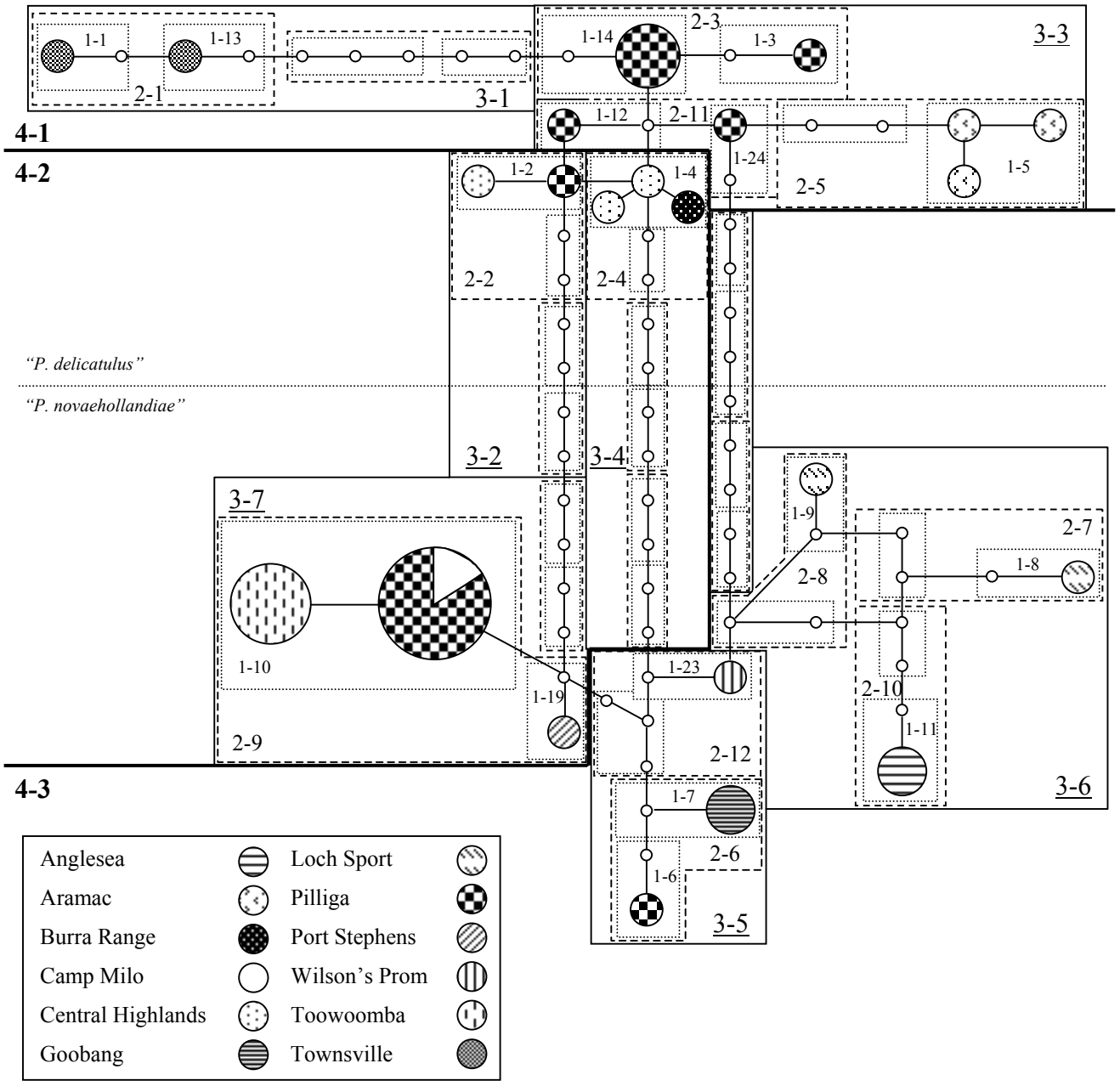
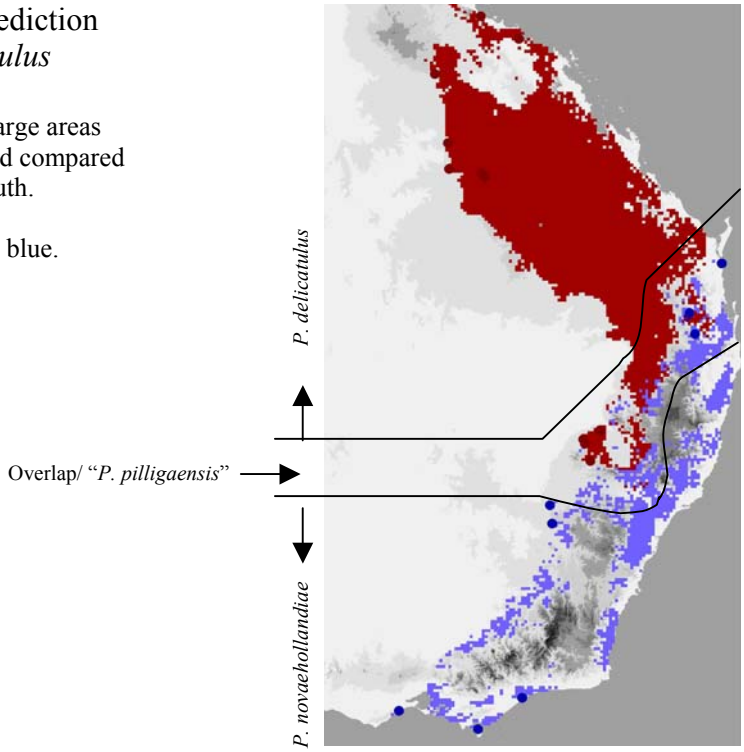


Figure 6.9. Nested haplotype network of *Pseudomys delicatulus* (QLD), *P. novaehollandiae* and "*P. pilligaensis*" control region haplotypes.
Large circles represent sequenced haplotypes (size= n). Small circles are unrecorded mutational steps. Shading as per key (may represent multiple sites within a region). Significant clades: 3-3 IBD, 4-4 PF, Total cladogram PF.

BIOCLIM distribution modelling of Queensland *P. delicatulus* and *P. novaehollandiae* haplotypes predicts a zone of contact between the taxa stretching from the vicinity of the Pilliga through south-eastern Queensland to the Maryborough region (fig. 6.10). Sampling along this boundary is poor away from the Pilliga, but the fact that both mitochondrial lineages are found in the Pilliga, and that at least one "*P. delicatulus*" from Maryborough carries *P. novaehollandiae* mtDNA strongly supports the notion of secondary contact along this zone. Contemporary convergence of suitable climatic factors and distributions implicates changes in those factors in promoting past fragmentation of the species distributions. The fact that much of central Queensland is predicted as climatically suitable for *P. delicatulus* on the basis of a few reference points suggests a homogeneity of conditions not present in the range of *P. novaehollandiae*. This also indicates a uniform loss of habitat is a likely outcome of changing climate, unlike the complicated interactions between microclimate and topography that may have prevented regional extinctions of *P. novaehollandiae* despite changing climate causing local extinctions or range movement.

Figure 6.10. Bioclimatic range prediction of Queensland *Pseudomys delicatulus* and *Pseudomys novaehollandiae*.
Dots indicate sampling localities. Note large areas of predicted habitat in central Queensland compared to the fragmented distribution further south. Shading indicates 250m asl contours
P. delicatulus- red ; *P. novaehollandiae*- blue.



Secondary contact in the Pilliga

The Pilliga mouse, *Pseudomys pilligaensis* Fox and Briscoe 1980 is a "species" of some note. It is a threatened taxon, and is the only described rodent endemic to New South Wales (Dickman et al. 2000). Following morphometric analysis and description of the Pilliga mouse (Fox and Briscoe 1980), the authors pointed out "no single character, apart from capture locality, can be cited to unambiguously distinguish *P. pilligaensis* from its nearest relatives" (Briscoe et al. 1981 pg. 89). They therefore relied on corroborative evidence from an isozyme study to justify recognition of a new species. They found that *P. pilligaensis* was as distinct from *P. novaehollandiae* and *P. delicatulus* as these species were from each other in isozyme studies, and considered this good evidence of specific distinction. Differences between *P. delicatulus*, *P. novaehollandiae* and *P. pilligaensis* amounted to; two alleles unique to *P. delicatulus*, two to *P. pilligaensis*, and three to *P. novaehollandiae*, at seven variable loci. The study used tissues from a captive colony of *P. delicatulus* held in Adelaide. Based on the results of Baverstock et al. (1977a) Briscoe et al. (1981) felt these tissues would be suitable because *P. delicatulus* was invariable across its distribution. They further concluded that differences between *P. delicatulus* and *P. pilligaensis* were greater than differences between Queensland and Western Australian *P. delicatulus*. However, only 11 of 25 enzyme systems screened in the two studies were common to both. One of these (G6PD) produced different results for Baverstock et al. (1976) when examining *P. delicatulus* from central Queensland than it did for Briscoe et al. (1981) using lab stock. Briscoe et al. (1981) were correct in suggesting on the basis of their results that mice in the Pilliga were as distinct as populations of *P. delicatulus* and *P. novaehollandiae* that they examined. However, the context of the genetic study was probably inadequate, and the differences they found between taxa were minor. Their study was unavoidably confounded by the unexpected circumstance of having both their critical comparative taxa present within the Pilliga population, a fact that has not been recognised until the results of this thesis.

Two important points arise from the studies of Fox and Briscoe (1980) and Briscoe et al. (1981); 1) mice in the Pilliga are intermediate, both morphologically and genetically,

between *P. delicatulus* and *P. novaehollandiae*, 2) There is very little genetic difference between *P. delicatulus* and *P. novaehollandiae*. The alternative to Fox and Briscoe's (1980) and Briscoe et al's (1981) interpretation of these facts is provided by mtDNA sequencing, and was outlined above; the Pilliga is a region of secondary contact between recently diverged sib-taxa. The important issue is now to determine what the status of those two taxa is. Haplotypes belonging to both mitochondrial lineages occur at every site in the Pilliga from which I have tissue (fig. 6.11), and the northern lineage, *P. delicatulus*, also occurs in Binnaway Nature reserve, 85km south of the Pilliga. The inconsistency between species assignment based on morphology and mtDNA also stretches over 650km to the northeast of the Pilliga to the Maryborough region. Overlap between mtDNA haplotypes of *P. delicatulus* and *P. novaehollandiae* therefore occurs over a very large area, considerably wider than typical murine hybrid zones (Sage et al. 1986, Bonhomme and Guenet 1989).

Pseudomys bolami and *P. hermannsburgensis*, *P. hermannsburgensis* and *P.* cf. *delicatulus,* and *P. hermannsburgensis* and *P. delicatulus* are all sympatric in parts of their ranges. It would therefore not be surprising if *P. delicatulus* and *P. novaehollandiae* were also sympatric where climatic conditions were favourable for both species. However, *P. delicatulus* and *P. novaehollandiae* are not so genetically distinct as other species. DHFR intron 1 provided circumstantial evidence of gene flow between mitochondrial haplotypes, but more work using rapidly evolving microsatellite loci is required to establish whether that is the case. *Pseudomys novaehollandiae* and individuals described as *P. pilligaensis* have successfully bred in captivity. However, Fox (1995a) states that laboratory breeding indicates that hybrid offspring of *P. novaehollandiae* and *P. pilligaensis* are infertile. This is not the case, as trials were never completed and the results were inconclusive (L. Lim pers. comm.). It seems highly unlikely that individuals from the Pilliga that differ by only one or two control region mutations from nearby populations of "good" *P. novaehollandiae* would be unable to inter-breed. Whether *P. delicatulus* from the Pilliga could do so is another question. Resolution of the species integrity of *P. novaehollandiae* is a priority, and in conjunction with microsatellite studies may rely on the further determination of the rate at which morphological change occurs through the Pilliga region from "*P. delicatulus*" to "*P. novaehollandiae*", and on the change in characters such as sperm morphology and

karyotype, which must be isolated from individuals with a known mtDNA profile. So far, only *P. delicatulus*-type sperm has been reported from Pilliga animals (Breed 2000). It seems that Van Dyck's (1998; pg. 50) concern that discovery of new populations of small *Pseudomys* in southern Queensland and northern New South Wales might cause a "taxonomic mega-toothache" has become a reality.

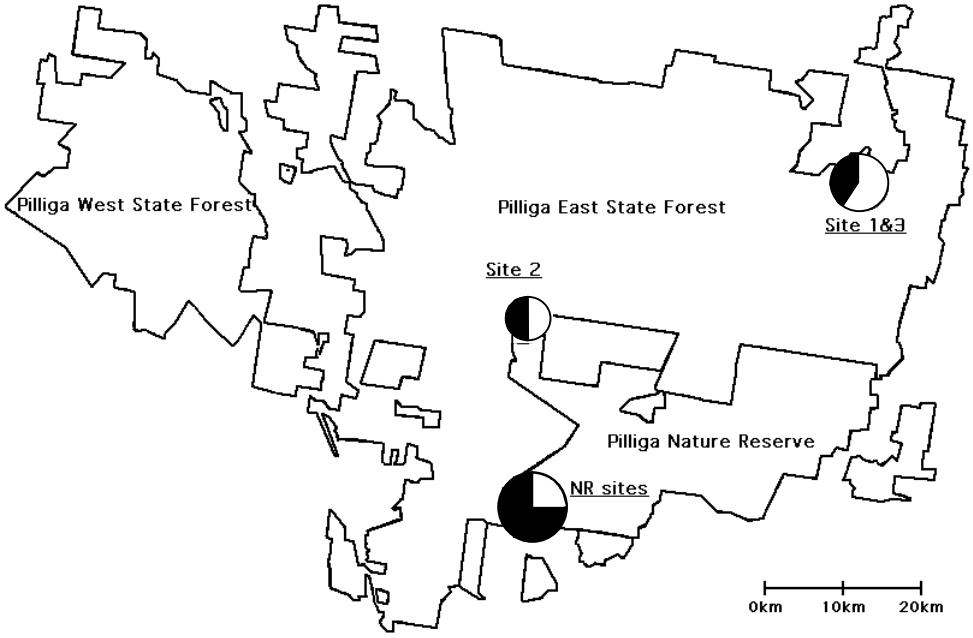


Figure 6.11. Location of sampling sites and control region clade frequency in the Pilliga. n= Site 1 and 3 (3), Site 2 (2), NR (4). "*Pseudomys delicatulus"*○ ; "*P. novaehollandiae"*● . Map and samples provided by Hideyuki Tokushima.

Complex evolution

Evolution within the *P. delicatulus* complex has apparently proceeded as shown in figure 6.12. As with any such interpretation, there are numerous alternative scenarios, but until better resolution of the situation in the Pilliga-Maryborough region is forthcoming, a simple account seems the most appropriate. An ancestral Pleistocene taxon was distributed

throughout northern and eastern (non-arid) Australia. It was probably clinal in features such as body size and colouration and had increasingly complex sperm morphology from northwest to southeast. The ancestral *P. delicatulus*' distributional limits would have fluctuated with climate, but the overall pressure of increasing aridity would have forced the species outwards (coast-wards) in the long term. At some stage, severe cyclical aridity began to fragment the near-coastal distribution via repeated flooding of the Gulf of Carpentaria, arid corridors through the southern Gulf (when exposed), and aridity in central and southern Queensland.

A potential arid barrier through central-southern Queensland may have been present where belt of dry habitats of the Brigalow Belt currently reach the coast near Gladstone and Rockhampton (Thackway and Creswell 1995). The Burdekin Gap is a similar coastal extension of the Brigalow Belt in north Queensland that had a significant effect in isolating populations of many species during the Pleistocene (Moritz et al. 2000). Populations in the Top End and south-eastern Australia would have experienced more mild climatic extremes than those in central Queensland, and there is high genetic diversity or IBD still evident in populations from those regions, rather than range expansion or bottlenecks, suggesting limited extinction when compared to populations in central Queensland. The genetic diversity of the Top End presumably corresponds to the contraction of populations from the exposed Arafura plain to the region as sea levels rose. I see no reason that the same should not be true of Cape York. The preservation of high diversity in the relatively small area of the Top End suggests that the area exploited by taxa such as *P. bolami* and intra-specific lineages within *P. johnsoni* during the LGM must have been very small indeed to produce the minimal genetic diversity of those taxa.

Queensland mice and *P. novaehollandiae* are now apparently in secondary contact. Although *P. delicatulus* and *P. novaehollandiae* have evolved some distinct features in isolation, the resumption of local selection regimes on previously clinal characters such as body size has resulted in identical morphology where the two mtDNA lineages are sympatric. This phenotype has been described as *P. pilligaensis*, but has a broader

distribution through southeast Queensland and northern New South Wales. Whether there is a smooth gradient in morphology across this region is unclear.

Mice from the Kimberley-Top End and Queensland do not exhibit the same degree of morphological distinctness as Queensland *P. delicatulus* do from *P. novaehollandiae* despite an equal degree of genetic distinctness. This fact has resulted in the description of Kimberley-Top End and Queensland animals as a single species, including the genetically very distinct *Pseudomys* cf. *delicatulus* from more arid parts of north-western Australia. This indicates a strong north-south cline in the phenotypes of the species involved, but less variation east-west. Therefore, the northwards contraction of Queensland animals did not create significant morphological differences between Queensland, the Kimberley and the Top End, when compared to the degree of differences compared to the southeast. Based on the loss of intermediate Queensland forms, distinctness at several characters, and evidence of clinal morphology, it could be argued that *P. delicatulus* and *P. novaehollandiae* do represent true species, and that their sympatry and identical morphology in the Pilliga region is the result of the southern end of a *P. delicatulus* cline coming into contact with the northern end of a *P. novaehollandiae* cline (but see above).

The Kimberley has emerged from this study, and that of the pebble-mound mice, as an area of dynamic evolutionary processes. Unfortunately, sampling of the mice from the Kimberley and the genetically diverse populations of the Top End was not sufficient to allow more detailed investigations of phylogeography or to determine the geographical distributions of *P. delicatulus* and *Pseudomys* cf. *delicatulus* in the region. However, as expected based on general area relationships, populations of mice in the northern Kimberley are genetically most similar to *P. delicatulus* from the Top End. In this respect *P. delicatulus* differs from the pebble-mound mice. *Pseudomys delicatulus* would have exploited exposed habitats of the North-west Shelf and Arafura Plain during periods of heightened aridity and lowered sea level, and this would have allowed re-colonisation of the Kimberley from that region even if habitats became unsuitable during glacial maxima, or the species may have been better able to exploit moist gorge refugia within the Kimberley than the pebble-mound mice. The opportunity for Top End populations to move

or expand their range during significant periods of the Pleistocene is probably responsible for the very high levels of genetic diversity still present in the region. The described subspecies *Pseudomys delicatulus mimulus* Thomas 1926 from Groote Eylandt and the Sir Edward Pellew group has no evolutionary basis. Although no animals from Groote Eylandt were sequenced, mtDNA sequence from the Pellew group differed by only a few mutations from nearby mainland populations.

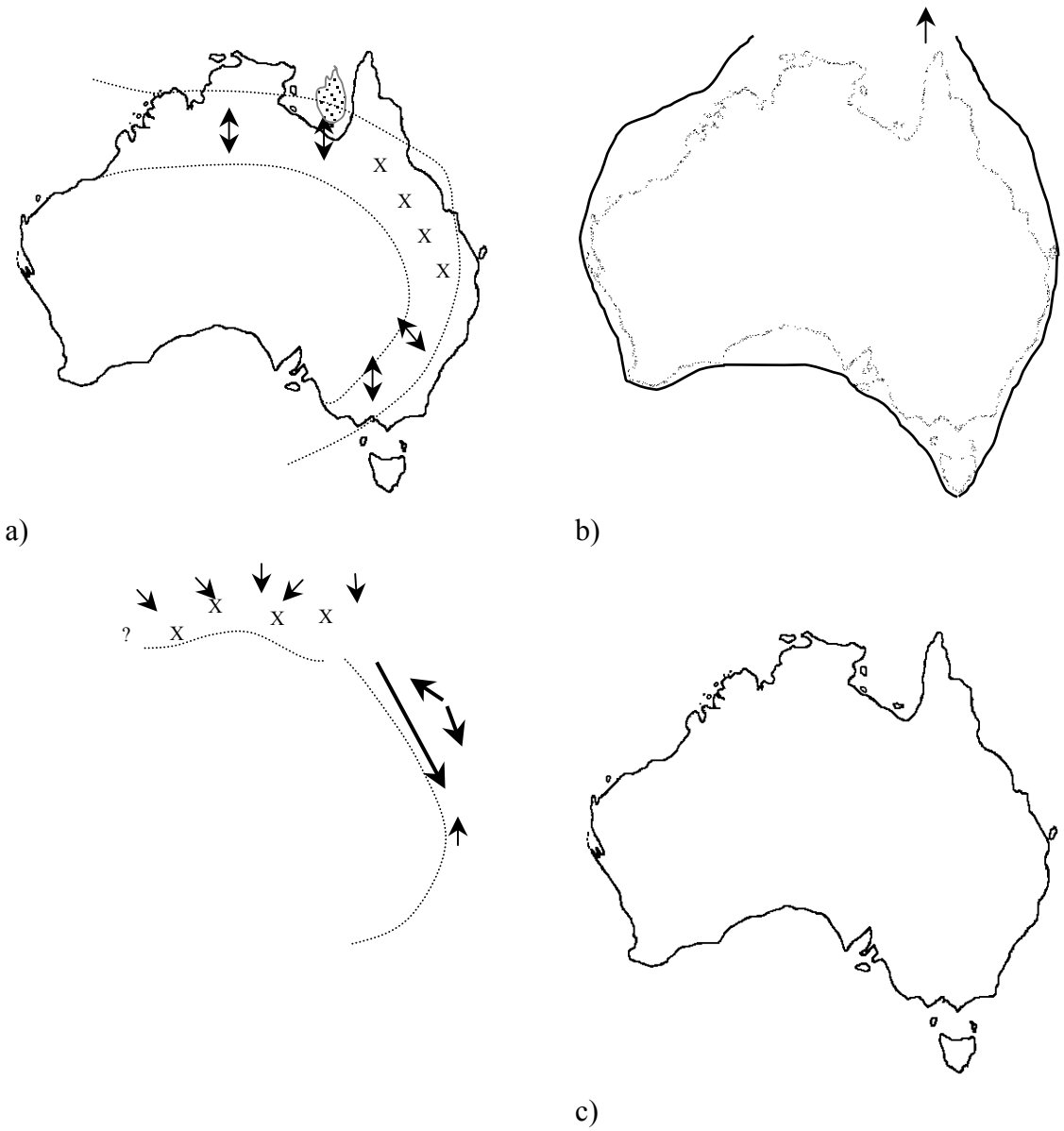


Figure 6.12. Evolution of the *P. delicatulus* complex (inc. *P. novaehollandiae* and "*P. pilligaensis*").

a) An ancestral, clinal, species is distributed widely throughout northern and eastern Australia. b) Increasing cyclical aridity during glacial phases eventually fragments the distribution and forces central Queensland populations to extinction. Morphological distinctions between the southern and northern end of a phenotypic cline are reinforced. Exposed sea beds to the north are exploited and New Guinea is colonised. These were not necessarily contemporaneous events and b) may cover several hundred thousand years until 20ka c) Southward colonisation by Queensland *P. delicatulus* post LGM results in secondary contact with *P. novaehollandiae*. Clinal phenotypic variation is partly renewed, but each clade retains some unique characters. The Kimberley is re-colonised from the Northwest Shelf?. Limit of arid zone ····· ; Coastline — ; Population extinction X; Range movement ▶ ; Lake Carpentaria .

6.4.4 SPECULATIONS ON DELICATE MOUSE EVOLUTION

The delicate mice were chosen as a study group because they are widespread generalists with distributions that largely reflect the major bioregions of Australia. Results presented in this chapter show that the evolution of delicate mice reflects general biotic patterns very well, in fact, better than current taxonomy indicates. There is a central Australian species (*P. hermannsburgensis*) that exhibits no genetic structuring. There is a suite of five taxa distributed around the continental periphery that are highly structured, that exhibit evidence of long-term occupation of some regions and periodic fragmentation and bottlenecking into refuge areas. The taxonomic status of these taxa needs critical examination through breeding trials and correlation between DNA sequence and other characters such as sperm morphology and karyotype.

Range movement and population extinction in response to arid conditions seem to be important factors in the recent evolution of the group. The *P. delicatulus* complex has largely been able to avoid arid conditions on the exposed sea bed of the Arafura Plain and in the Kimberley and the Top End, where the greatest intra-taxon genetic diversity of any species or taxon studied in this thesis is observed (5.8%). Northern and coastal Queensland and south-eastern Australian populations have also largely avoided severe arid conditions. However, genetic divergence of clades within the *P. delicatulus* complex is evident due to shorter periods of isolation and extinction of geographically intermediate populations during recent glacial maxima, and flooding of the Gulf of Carpentaria. The importance of preservation of intra-clade genetic diversity is apparent in the control region distance tree (fig. 6.2). Were populations of *P. delicatulus* from the Top End to undergo a series of extinctions that left a genetic diversity comparable to that seen in *P. bolami* a similar pattern of "species" would begin to emerge from the *P. delicatulus* complex. The evolution of *P.* cf. *delicatulus* has probably resulted from restriction to the Pilbara during a Pleistocene arid phase, but the (species?) has since had time to adapt to conditions currently found in the Great Sandy Desert and exhibits almost the same level of genetic diversity as *P. novaehollandiae* (based on only five sequences). This suggests that *P.* cf. *delicatulus* is now able to effectively track environmental change without losing genetic diversity. The

origin of *P. bolami* was possibly a similar bottleneck. However the recent history of this species has masked and hint of what may have been its origins. The current lack of genetic diversity seen in *P. bolami* is probably the result of a bottleneck along the southern coast. Re-invasion of the mainland from the flooding Great Australian Bight at the end of the LGM may have compounded the effects of the bottleneck.

The major lineages within the delicate mice seem to be (*P. hermannsburgensis*) vs (*P*. cf. *delicatulus*, *P. bolami* and the *P. delicatulus* complex). The origin of these lineages is obscure, but the central-periphery nature of their respective distributions suggests that aridity may have played a part in the split, as it has in subsequent divergence of species around the continental periphery. The dichotomy here lies in the fact that *P. hermannsburgensis* inhabits recent environments, and yet retains ancestral characters when compared to the derived karyotypes and sperm morphology of the *P. delicatulus* complex. The fact that *P. bolami* and *P*. cf. *delicatulus* have only recently been recognised means that their karyotype is not known, although that of a possible individual of *P*. cf. *delicatulus* was described by Baverstock et al. (1977b) (section 6.4.3). The sperm morphology of *P. bolami* is the ancestral form shared with *P. hermannsburgensis* and the majority of hydromyine rodents, assuming that specimens examined by Breed (1997) were correctly identified. That of *P*. cf. *delicatulus* is not known with any certainty, but may be the derived form of the *P. delicatulus* complex. Further intrigue is provided by the fact that although *P. hermannsburgensis* has an essentially ancestral karyotype, some individuals were polymorphic for the peri-centric inversion leading to sub-acrocentric chromosomes of autosomal pair nine. This re-arrangement is found among members of *P. delicatulus* (QLD), *P*. cf. *delicatulus*? and *P. novaehollandiae*.

Resolution of the states of these characters in *P. bolami* and *P*. cf. *delicatulus* will greatly aid in interpreting the evolution of this group. For instance, the origin of *P.* cf. *delicatulus* may have been a more complex event than the simple allopatric divergence from *P. delicatulus* suggested above. The current narrow distribution of *P.* cf. *delicatulus* is wedged between *P. hermannsburgensis* and *P. delicatulus*, and could represent an ancient hybrid zone, leading to a genetically and ecologically intermediate form.

6.5 Summary

- Current species taxonomy of the delicate mouse complex differs markedly from evolutionary lineages identifiable within the complex
- *Pseudomys pilligaensis* does not represent a distinct evolutionary taxon, and is not a species
- *Pseudomys delicatulus* as currently understood is comprised of at least two species, *P. delicatulus* and *Pseudomys* cf. *delicatulus*, which is not a sib-taxon to the remaining members of the currently recognised *P. delicatulus* (*P. novaehollandiae* is closer).
- *Pseudomys delicatulus* from the northern Kimberley and Top End, *P. delicatulus* from Queensland, and *P. novaehollandiae*, constitute evolutionary lineages of roughly equivalent standing and inter-relatedness.
- *Pseudomys delicatulus mimulus* is an insular phenotype and has no evolutionary basis
- There is evidence from captivity and DNA sequencing that *P. delicatulus* (QLD and NT) and *P. novaehollandiae* can interbreed. Members of all three lineages should be considered a sub-specific? complex pending microsatellite studies and/or inter-breeding experiments. According to taxonomic precedence the complex should be named *P. delicatulus delicatulus* (Kimberley and Northern Territory) *P. delicatulus novaehollandiae*, and a new taxon, *P. delicatulus stalkeri* ssp. nov. (Queensland). However *P. novaehollandiae* is entrenched in the literature and common usage, and is a readily identified taxon except in parts of northern New South Wales and southern Queensland. Therefore the nomenclatural status quo is a suitable compromise until species boundaries are fully resolved.
- Distributions of delicate mice are under climatic control, and sympatry is observed between all species in regions of climatic transition.
- Extinction and range movements have been central factors driving evolution of delicate mice subspecies and species boundaries through bottlenecking of regional populations. The effects of this are most pronounced in the current arid zone, where three species exist due to a longer history of aridity.

- Populations around the wetter northern and eastern periphery of the continent have been relatively untouched by aridity and extinction, except in central Queensland, which has been recently re-invaded from the north by *P. delicatulus*.
- Arid barriers and flooding of the Gulf of Carpentaria have caused periodic isolation of populations of the *P. delicatulus* complex in the late Pleistocene, but although these have reinforced some fixed differences between intra-specific clades (subspecies), speciation may not yet have occurred.

Chapter 7. Phylogeography of *Zyzomys argurus*

CHAPTER SUMMARY

Zyzomys argurus is distributed in three allopatric savannah regions across northern Australia. There is very little divergence of control region sequences between allopatric regions, and no historical signal of the LGM. Although IBD and geographical association of haplotypes are evident, only populations in the Pilbara form a shallow monophyletic clade, probably as a result of allopatric range fragmentation. Lack of genetic structure was an unexpected result, as the preferred habitat of *Z. argurus* is rocky landscapes similar to those preferred by the highly genetically structured pebble-mound mice. Ecological adaptability of the species observed in some contemporary populations is likely to explain the lack of genetic structure.

7.1 A medium-sized, specialised, taxon
Zyzomys argurus (Thomas 1889)

7.1.1 A (MOSTLY) ROCK RAT

There are four *Zyzomys* species described from northern Australia, however, only the common rock-rat (Djoorri; *Z. argurus*) is widely distributed, and is regarded as a rock-dwelling generalist by Trainor et al. (2000). *Zyzomys argurus* (<65g) is smaller than other *Zyzomys* species, but significantly larger than the pebble-mound mice and delicate mice. It appears to have a much wider tolerance to moisture regimes than other *Zyzomys*, and is found from the northern edge of the arid zone to rainforest margins. The three large *Zyzomys* species found in the Top End and the Kimberley are some of the few conilurine species to show a distinct preference for wetter habitat, being associated with monsoonal rainforest pockets near sandstone escarpments. Such a limited habitat preference has resulted in allopatric and restricted distributions (*Z. palatalis* has been found at only four

locations within 30km of each other; Churchill 1996). There is some evidence that *Z. argurus* is excluded from monsoonal forest habitats by larger *Zyzomys* species or has its number suppressed within those habitats (Kerle and Burgman 1984, Begg and Dunlop 1985). *Zyzomys argurus* has a varied diet that contains high levels of plant material (seed, leaves and stems) (Watts 1977, Begg and Dunlop 1985). It is usually associated with rock outcrops, and Watts and Aslin (1981) proposed a reliance on rocky refuges due to susceptibility to heat stress noted in captive animals that died when "accidentally left in the sun"(!) (pg. 139). However, individuals are occasionally captured some distance from major outcrops (Watts and Aslin 1981), and on the Wessel Islands of the Top End the species can be found in hummock grassland devoid of rock outcrop (Woinarski and Fischer 1996). Despite a tolerance of arid conditions and flexibility in specific habitat preference, *Z. argurus* is still apparently constrained by a similar general topographic preference to that which dictates the spatial distribution of pebble-mound mice. Thus, populations in the Pilbara are isolated from the Kimberley by the Great Sandy Desert and eastern Queensland populations lie to the east of the Mitchell Grass Downs and Gulf Plains. The known distribution of the species in eastern Queensland is enlarging rapidly with greater survey effort. Although restricted to allopatric regions, all populations across northern Australia are regarded as a single species. Lee et al. (1981) single out the species as having a large distribution exhibiting little genetic variation following the work of (Baverstock et al. 1977a) that found no fixed isozyme differences between allopatric populations. However, similar isozyme results were found for the highly genetically structured *P. delicatulus* (Baverstock et al. 1977a).

Studies of *Zyzomys argurus* aimed to establish the effects of allopatry, in particular whether any deep genetic structuring was evident within the species. *Zyzomys argurus* was chosen as a relatively specialised taxon of similar size to the chestnut mice. The chestnut mice, pebble-mound mice and delicate mice all exhibit species-level distinction of populations in at least one of the allopatric regions in which *Zyzomys argurus* is distributed. It seems unlikely that a taxon relatively restricted to rocky habitats ("specialised" for the purposes if this discussion) would be less structured than the widespread, generalist, chestnut mice, particularly given the deep division between specialised pebble-mound mouse clades in the

same regions. However, most other savannah rodents are considered single species across the regions in question (Watts and Aslin 1995, Strahan 1995), including *Leggadina lakedownensis*, a slightly smaller species with a very similar distribution to *Z. argurus* (Strahan 1995).

7.2 Geographical sampling for genetic studies

Sampling of *Z. argurus* populations provided a reasonable geographical coverage of its distribution, although only one or two individuals were sequenced from most populations (fig. 7.1; table 7.1). Control region sequence was obtained from all populations. DHFR intron 1 sequence was obtained from individuals from Inglis Gap, Litchfield National Park and Woodstock Station.

Table 7.1. Sampling locations and tissue sources for *Zyzomys argurus*.
(ABTC)- Australian biological tissue collection with museum voucher; ABTC- no voucher; NTMU- Northern Territory Museum; WAMM- Western Australian Museum. N= sample size. Sequence ID's are used to identify individuals from a particular site in results trees.

Site	Lat.	Long.	N	Type	Source/voucher	ID's
1. Bullita	-16.1167	130.4167	2	Liver	(ABTC) NTMU 263, 264	Za16,17
2. Depot Creek	-13.6467	131.5459	1	Tail	Field biopsy (J. Griffiths)	Za5
3. Enderby I	-20.6080	116.5210	2	Liver	ABTC 10594, 10597	Za14,15
4. Fortescue R	-21.2910	116.1420	1	Liver	ABTC 7897	Za8
5. Guluwuru Is	-11.5200	135.4167	2	Liver	(ABTC) NTMU 4101, 4102	Za19,20
6. Hidden Valley	-19.0085	146.0916	2	Tail	Field biopsies (Ford)	Za1,2
7. Inglis Gap	-17.1150	125.1760	1	Liver	ABTC 7898	Za9
8. Litchfield NP	-13.0685	130.7130	2	Liver	(ABTC) NTMU 4167, 4168	Za21,22
9. Mitchell Plateau	-14.8917	125.7500	3	Liver	(ABTC) WAMM 21522, 21527, 21908	Za10,11,27
10. Nathan River	-15.2500	135.5167	2	Liv/Tl	(ABTC) NTMU 752, Field Biop (Ford)	Za3,18
11. Nourlangie	-12.7800	132.7000	1	Liver	ABTC 8024	Za13
12. Pellew Is.	-15.6666	137.0167	2	Liver	(ABTC) NTMU 1423, 1426	Za23,24
13. Woodstock	-21.6597	119.0417	2	Liver	(ABTC) WAMM 28632, 32732	Za25,26
14. Wyndham	-15.4860	128.1200	1	Liver	ABTC 8023	Za12

Figure 7.1. Distribution of *Zyzomys argurus* and sampling locations.
Distribution of *Z. argurus* Western Australian Museums faunabase (http://www.museum.wa.gov.au/faunabase).
Numbers refer to population numbers given in table 7.1.

7.3 Phylogeography of *Zyzomys argurus*

7.3.1 Mitochondrial control region

Zyzomys argurus populations across northern Australia are striking in their lack of genetic differentiation and geographical structuring when compared to the pebble-mound mice and delicate mice (fig. 7.2). Twenty-two of 24 individuals carried unique control region haplotypes, but most differed from closely related haplotypes by only one or two mutations. In a sequence alignment of 380bp only 27 sites were variable (18 parsimony informative). Some shallow geographical structure is evident in distance analysis (fig. 7.2; minimum evolution score: 0.11141), with Pilbara individuals forming a monophyletic clade, but this

clade differs by only two mutations from Kimberley haplotypes (figs. 7.2 and 7.3). Queensland and Top End haplotypes do not form monophyletic clades. Bootstrapped maximum-likelihood trees and parsimony trees do not reveal any well-supported clades beyond uniting haplotypes from some island populations, and are not presented.

Nested clade analysis suggested few significant cases of restricted gene flow (fig. 7.3). Populations in the Pilbara that showed slight evidence of allopatric fragmentation from Kimberley populations due to the Great Sandy Desert yielded internally significant geographical associations of haplotypes within clade 3-1, but the exact contingency test was not significant (χ^2= 6; p= 0.235). Fragmentation or IBD is evident between populations distributed through the Top End, Gulf and VRD (clade 3-3; χ^2= 26.667; p= 0.005). Sampling was not detailed enough for the analysis to differentiate between these hypotheses. However mainland populations are probably only affected by IBD. Island populations may show evidence of fragmentation, but this is not the case for haplotypes from the Sir Edward Pellew group (clade 1-9), which fall between haplotypes from the adjacent mainland (clade 1-8; 1-11). There is a general geographical association of haplotypes evident in the nested haplotype network, and IBD is inferred for the total cladogram (χ^2= 43.429; p= 0.000). The high level of haplotype diversity per individual, general geographical association of haplotypes, and low number of missing mutational steps in the haplotype network, suggests that population extinction has not been a major factor forcing genetic structuring in this species. As a result, much more intensive sampling of individuals is required to examine inter-population relationships in this species.

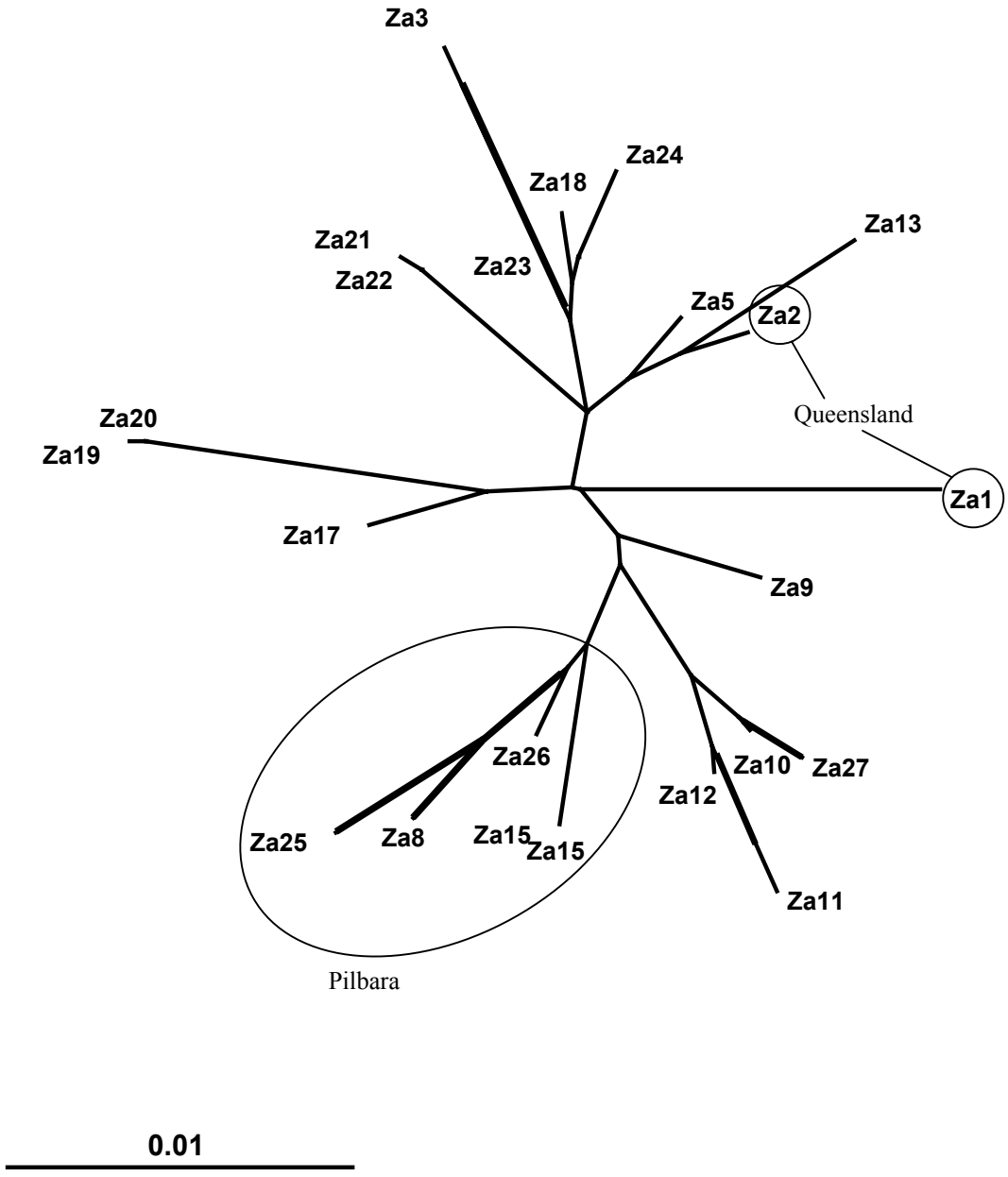


Figure 7.2. Genetic distance between *Zyzomys argurus* control region haplotypes.
Unrooted neighbour-joining tree of Kimura two-parameter distances between 22 haplotypes of 381bp from 24 individuals. Codes correspond to a single individual that carried a particular haplotype. Allopatric populations are highlighted (other populations= Kimberley and Top End).

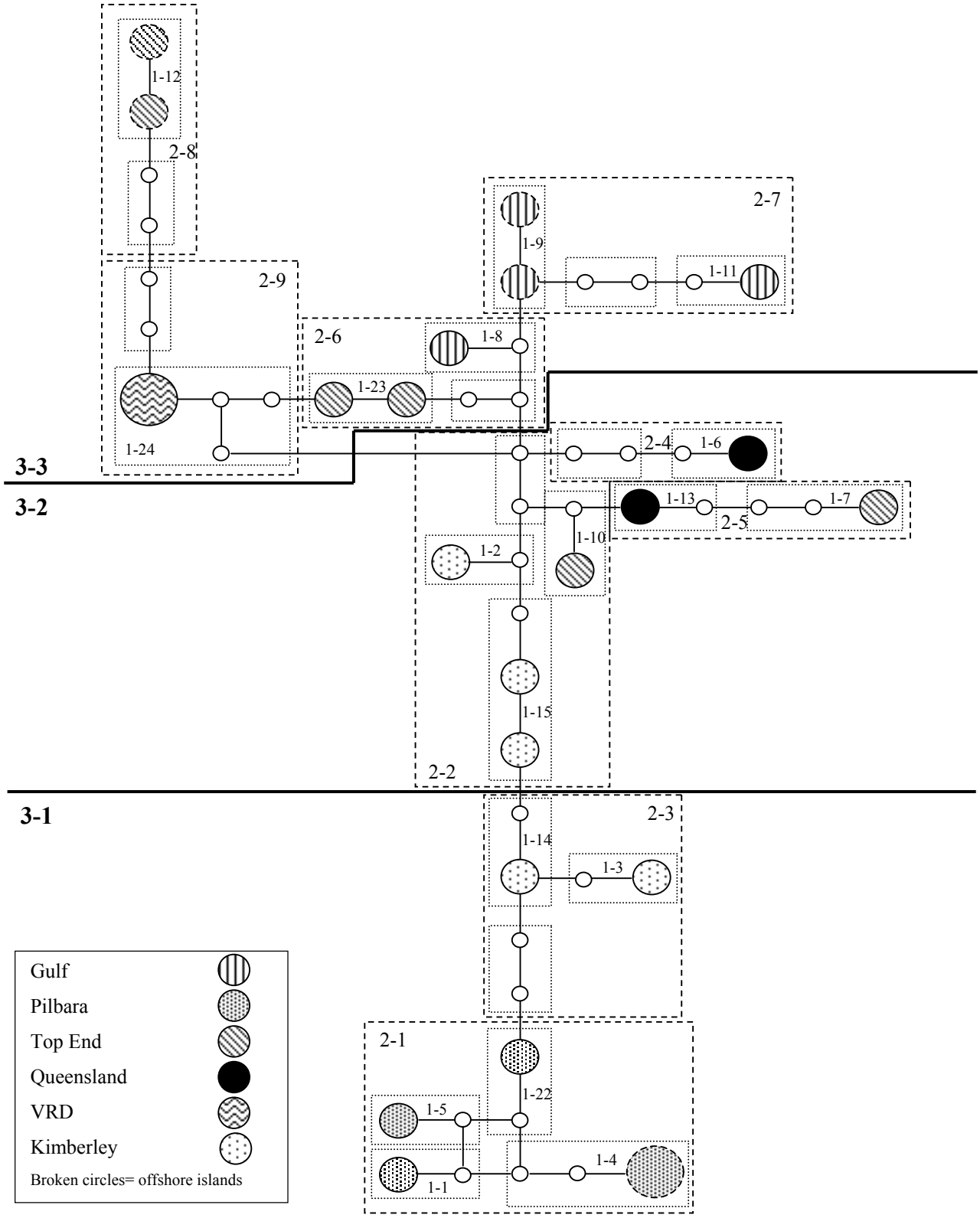


Figure 7.3. Nested haplotype network of *Zyzomys argurus* control region haplotypes.
Large circles represent sequenced haplotypes (size=n). Small circles are unrecorded mutational steps. Shading as per key (may represent multiple sites within a region). Clades exhibiting significant effects: 3-1 AF, 3-3 IBD or F, Total cladogram IBD.

7.3.2 DHFR INTRON 1

There is minimal genetic divergence at the DHFR intron 1 locus between populations of *Zyzomys argurus* from the Kimberley, the Top End and the Pilbara. Only three individuals were sequenced, specifically examining the level of divergence of the allopatric Pilbara populations compared to the Kimberley-Top End. The Kimberley and Top End individuals were identical, whereas the Pilbara individual showed ~0.25% divergence, which was significantly less than the ~2.5% divergence between all *Z. argurus* individuals and *Z. woodwardi* (table 7.2). This corroborates the findings of the control region, with Pilbara populations showing signs of allopatric divergence, but not nearly to the extent of speciation.

Table 7.2. DHFR distance matrix for three *Zyzomys argurus* populations.
Matrix based on Kimura-two parameter distances between 400bp sequences.

	Top End	Pilbara	Kimberley	*Z.wood.*
Top End	-			
Pilbara	0.0026	-		
Kimberley	0.0000	0.0025	-	
Z.wood.	0.0231	0.0256	0.0232	-

7.3.3 BIOCLIM MODELLING

Modelling of the distribution of *Zyzomys argurus* predicted larges areas of northern Australia as suitable habitat (fig. 7.4). These correlated well with the known distribution of the species, except in the Carpentarian Barrier and Great Sandy Desert, where topographically unsuitable habitats prevent occupation despite suitable climatic conditions. The climatic envelope of the species is actually significantly greater than shown, because large areas of the Queensland distribution of the species have only recently become known and animals from those areas were not sequenced. The climatic tolerances of this species are obviously central to its ability to “ride out” glacial periods.

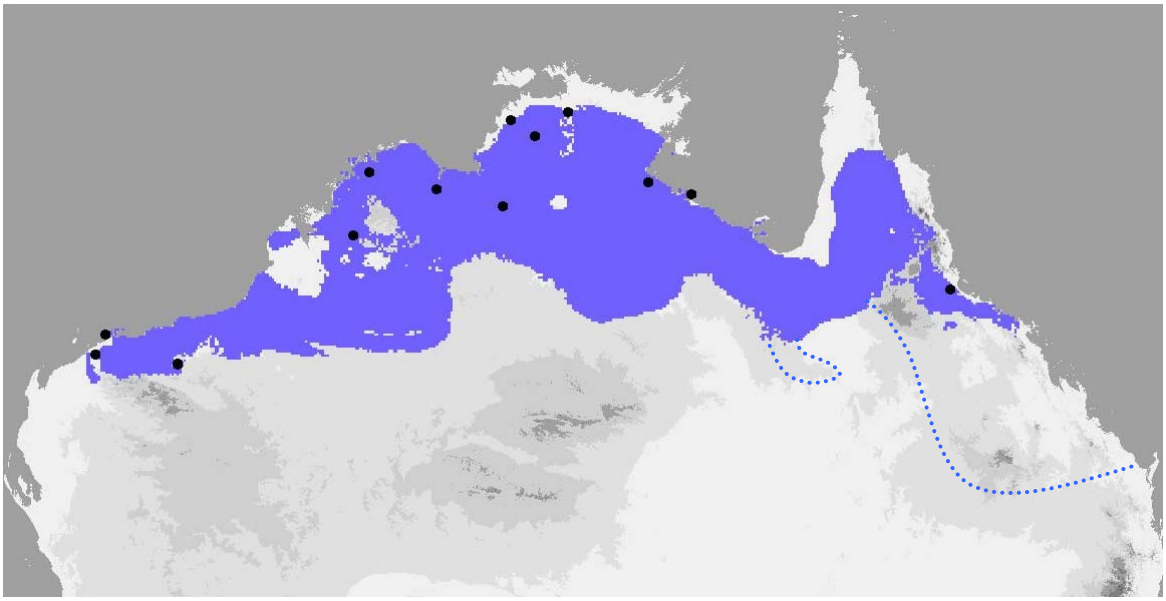


Figure 7.4. Bioclimatic modelling of the distribution of *Zyzomys argurus*.
Blue indicates modelled distribution, dots sampling locations. Shading is 250m asl contours. Dotted lines indicate areas in which *Z. argurus* occurs but from which no animals were sequenced.

7.3.4 EVOLUTION OF *ZYZOMYS ARGURUS*

Results presented here confirm that despite being found in allopatric regions across northern Australia, populations of *Zyzomys argurus* are a single species. Moreover, the species shows little sign of genetic structuring as a result of allopatry. Over 350km separates the closest known populations of *Z. argurus* in the Pilbara and Kimberley, and the distances between known Queensland populations are somewhat greater. Most northern Australian rodents are allopatric in savannah refuge areas inhabited by *Z. argurus*, and are also regarded as single species (Strahan 1995). However, for *Z. argurus* this result is difficult to explain. Other rodent species, such as *Leggadina lakedownensis*, *Mesembriomys gouldii* and *Pseudomys delicatulus* are probably able to exploit habitats that periodically unite the Top End and Cape York. Even so, *P. delicatulus* shows significant structuring across the divide. Long-distance dispersal or recent range expansion seem unlikely scenarios to explain the lack of genetic distance between populations of *Z. argurus*, because the bed of the Gulf of Carpentaria is a shallow sandy basin, and essentially a continuation of the flat landscape of the Mitchell Grass Downs and Gulf Plains. *Zyzomys argurus* does

show an ability to survive in areas free of rock outcrops, and the presence of populations on rock-free islands off the Top End suggests an ability to disperse over sandy plains. However populations restricted to rock-free islands are part of the rocky chain of the Wessel Islands (Woinarski and Fischer 1996), suggesting an historical association with nearby rocky landscapes. In general, landscapes inhabited by the species are the same as those inhabited by pebble-mound mice, but *Z. argurus* is obviously more adaptable to changing conditions, and does not have such a fundamental behavioural requirement for rocky areas as do the pebble-mound mice.

In western Queensland there are two populations of *Z. argurus* in the Kynuna-Winton hills to the west of the apparently substantial gap of the Mitchell Grass Downs (Fleming 1995, G. Mifsud pers. comm.). The presence of *Z. argurus* on these relictual, relatively isolated, hills is a function of either long-term population stability in small habitat patches, or an ability to disperse over moderately long distances between suitable habitat. However, although a single lateritic surface may have bridged the gap between rocky landscapes of eastern and western Queensland over time scales involved in the divergence of *P. patrius* and other pebble-mound mice (chapter 5), there is less chance of a connection recent enough to explain the lack of divergence of Queensland and Top End *Z. argurus* (R. Coventry pers. comm.). The plasticity of the species' habitat preference has apparently allowed migration through "unsuitable" environments that are not currently occupied. Cracking clay soils of the western Queensland plains may have facilitated movement, as these resemble rock crevices used as nesting sites by the species.

Suitable climates for the species exist throughout many regions of northern Australia. The fact that there is no historical signal evident from the LGM indicates an astonishing ecological resilience. The weak effects of IBD and geographical association of haplotypes, and the presence of a monophyletic clade in the Pilbara, indicate a fairly stable distribution since before the LGM. It seems very unlikely that the species' distribution would track habitat changes to the north, as that would entail wholesale changes in microhabitat preference over very short time scales. Whatever the case, *Z. argurus* does not exhibit the pattern of genetic divergence I expected of a "specialised" taxon distributed in three

allopatric regions separated by environments that seemingly lack key aspects of the species normal habitat. Population divergence is, however, consistent with the current taxonomy of the species and previous genetic findings (Baverstock et al. 1977a).

7.4 Summary

- Despite being comprised of allopatric populations that display a preference for the same general landscapes as pebble-mound mice, *Zyzomys argurus* shows only shallow genetic structure across its distribution.
- *Zyzomys argurus* populations in the Pilbara show signs of allopatric divergence due to the mid-late Pleistocene formation of the Great Sandy Desert.
- *Zyzomys argurus* populations in Queensland and the Northern Territory do not form monophyletic clades, and there is no evidence of range fragmentation in control region sequence data subjected to nested clade analysis.

Chapter 8. Genetic divergence among chestnut mice and *Pseudomys desertor* populations

CHAPTER SUMMARY

Results are presented from genetic studies of the chestnut mice (*P. gracilicaudatus* and *P. nanus*) and *P. desertor*. Assumed nuclear copies of the mitochondrial control region hampered study of the chestnut mice. Three clean sequences were obtained and they revealed moderate genetic divergence of the Kimberley and Top End, and relatively deep division of *P. nanus* (Northern Territory-Western Australia) and *P. gracilicaudatus* (Queensland-New South Wales). This agrees with current species descriptions, but until more comprehensive analysis can be performed, nothing can be said regarding the phylogeography of these species. *Pseudomys desertor* exhibits low levels of genetic divergence across the arid zone, similar to those found in *P. hermannsburgensis*. These species have very similar distributions, and have presumably shared a similar recent history. The range of *P. desertor* probably extended or moved to include central Queensland during the LGM, and previous arid phases, and the species is still found in semi-arid regions of central Queensland.

8.1 Generalist species

***Pseudomys desertor* Troughton 1932, *Pseudomys gracilicaudatus* (Gould 1845), *Pseudomys nanus* (Gould 1858)**

8.1.1 COMPARATIVE SPECIES

The species dealt with in this chapter were chosen for study because they have a similar habitat preference to the delicate mice, and their distributions are comparable to the distributions of *P. delicatulus* (including northern parts of the range of *P. novaehollandiae*) and *P. hermannsburgensis*. Like the delicate mice, areas of sympatry occur between the

desert mouse (Wildjin; *P. desertor*) and each of the chestnut mice (Karrooka; *P. gracilicaudatus* and Moolpoo; *P. nanus*) in areas of climatic interchange between wetter savannahs and the arid zone (Fox 1995b, Robinson 1995, Kutt et al. in press). The chestnut mice are allopatric sib-taxa distributed on either side of the Carpentarian Barrier. Despite the ubiquity among rodents of a discontinuous distribution across the Carpentarian Barrier, they are the only such sib-species pair in the savannah rodent fauna (excluding pebble-mound mice). Both species have (or had) natural distributions that included large areas of non-savannah habitats, including near-coastal areas along the eastern and western seaboard (fig. 8.1; Kendric and Porter 1973, Mahoney and Posamentier 1975). In northern Australia *P. nanus* is found as far inland as the northern Tanami Desert (Robinson 1995), and I have found the species to be common on rocky ridges in the vicinity of Renner Springs at the eastern margin of the Tanami. The northern distribution of *P. gracilicaudatus* is poorly known, but new captures are expanding the range and number of known inland occurrences of the species (S. Burnett pers comm., P. Williams pers. comm.)

There is no significant difference between skulls of the two chestnut mouse species (Watts and Aslin 1981), and Ride (1970) felt they may constitute a single species. However, chromosomal and electrophoretic studies revealed significant differences between the two taxa (Baverstock et al. 1977b). Chestnut mice are the Australian equivalent of voles, being large mice that consume substantial amounts of vegetation in their diet, but seeds are also important, and moderate quantities of insect and fungus are eaten (Luo et al. 1994, Fox 1995b, Robinson 1995, Luo and Fox 1996). This allows co-existence with smaller, more granivorous *Pseudomys* species such as *P. novaehollandiae* and *P. patrius*, and both *P. nanus* and *P. gracilicaudatus* are members of rich rodent communities in parts of their distribution (Luo and Fox 1995, Haering and Fox 1997, Woinarski 2000, personal observation). The aims of chestnut mouse studies were to examine the pattern of divergence across the Carpentarian Barrier, attempt to reconcile the widespread, generalist nature of the species with speciation across this barrier, and to provide a comparative data set for *Pseudomys delicatulus*, which is distributed in the same regions.

Pseudomys desertor is the ecological equivalent of the chestnut mice in central Australia, and has been historically confused with the superficially similar *P. nanus* (Gould 1863, Finlayson 1941), although the species are quite distinct (Troughton 1932), and would be included in different genera under the taxonomic scheme proposed in chapter 4. *Pseudomys desertor* is sympatric with *P. nanus* across the northern limit of its distribution, including the Tanami Desert and southern Kimberley, where I have collected both from the same trap line. Although Kerle (1995b) and Watts and Aslin (1981) show the distribution of *P. desertor* to be restricted to central parts of the Northern Territory, South Australia and Western Australia, the species is relatively common in central Queensland (Kutt et al. in press), where it is replaced to the east by *P. gracilicaudatus* (fig. 8.1). *Pseudomys desertor* can be found in a variety of habitats, including sand and clay plains and rocky ridges in central Australia (Watts and Aslin 1981, Kerle 1995b, Read et al. 1999), and *Eucalyptus* woodlands and grasslands in Queensland (Kutt et al. in press). I have recorded the species in low acacia woodland on rocky ridges, open rocky spinifex, sandy flats and grasslands on clay plains in central Queensland. It is unusual among *Pseudomys* species in exhibiting partly diurnal tendencies (Read et al. 1999, Kutt et al. in press), and unlike most desert conilurines is apparently solitary (Happold 1976). It has the ability to breed rapidly when conditions are favourable (Happold 1976, Read et al. 1999). Watts and Aslin (1981) described the range of *P. desertor* as "enormous" (pg. 182), yet most locations from which *P. desertor* was sampled for this thesis fall outside the distribution they recognised (over 1500km to the east in some cases). Sampling of *P. desertor* therefore had two main purposes; to confirm the identity of animals captured in Queensland, and to provide a set of genetic distances for comparison with the similarly distributed *P. hermannsburgensis*.

8.2 Geographical sampling for molecular studies

Sampling of chestnut mice was representative of the distribution of the two species (fig. 8.1; table 8.1). However, few individuals per site were sampled and results were limited due to the presence of nuclear pseudogenes (section 8.3.1). Sampling locations are concentrated in the east of the distribution of *P. desertor*, but provide suitable coverage of

the arid zone for comparison to *P. hermannsburgensis* sampled from the same sites (fig. 8.1; table 8.1).

Table 8.1. Sampling locations and tissue sources for chestnut mice and *P. desertor*.
(ABTC) Australian biological tissue collection and museum voucher; ABTC- no voucher; NTMU- Northern Territory Museum; QMJM- Queensland Museum; WAMM- Western Australian Museum. N= sample size. Sequence ID's are used to identify individuals from a particular site in results trees.

Site	Lat.	Long.	N	Type	Source/voucher	ID's
P. gracilicaudatus						
1. Hidden Valley	-19.0028	146.0705	4	Liv/Tl	Field biopsies (Ford)	g1,3,4,6
2. Blackbraes NP	-19.2290	144.0430	1	Liver	Biopsy (Ford/P. Williams)	g8
3. Mt. Pollux	-22.4764	147.8705	1	Tail	Field biopsy (Ford)	g2
4. Ravensbourne	-27.3650	152.1850	1	Liver	QMJM15094	g7
5. Yurragir NP	-29.6406	153.3099	1	Seq.	Sequence (M. Elphinstone)	g9
P. nanus						
6. Bradshaw	-15.3490	130.2740	1	Liver	ABTC 67932	n11
7. Gregory NP	-16.5976	130.4539	1	Liver	ABTC 27906	n10
8. Helen Springs	-18.4978	133.8738	2	Tail	Field biopsies (Ford)	n6,7
9. Jim Jim	-12.9667	132.5667	1	Seq.	Sequence (M. Elphinstone)	n12
10. Kakadu	-12.9767	132.5220	1	Tail	Field biopsy (Watson)	n3
11. Mitchell	-14.8861	125.7986	1	Liver	(ABTC) WAMM 21625	n8
12. Purnululu	-17.3996	128.2717	1	Tail	Field biopsy (Ford)	n4,5
13. Pellew Is.	-15.7000	136.6333	1	Liver	(ABTC) NTMU 1448	n9
P. desertor						
14. Albion Vale	-22.3032	145.5509	2	Tail	Field biopsies (A. Kutt)	des5,6
15. Alice Springs	-23.6990	133.8810	1	Liver	ABTC 8143	des18
16. Burra Range	-20.6701	145.1934	1	Tail	Field biopsy (Ford)	des1
17. Laverton	-28.3751	122.6756	1	Liver	ABTC 41464	des17
18. MacDonnell	-23.7000	132.5000	1	Liver	ABTC 24044	des19
19. Moorrinya	-21.4200	144.9600	1	Tail	Field biopsies (C. Johnson)	des4
20. Oakleigh	-23.5861	146.5425	2	Tail	Field biopsies (A. Kutt)	des10,11
12. Purnululu	-17.3996	128.2717	1	Tail	Field biopsy (Ford)	des16

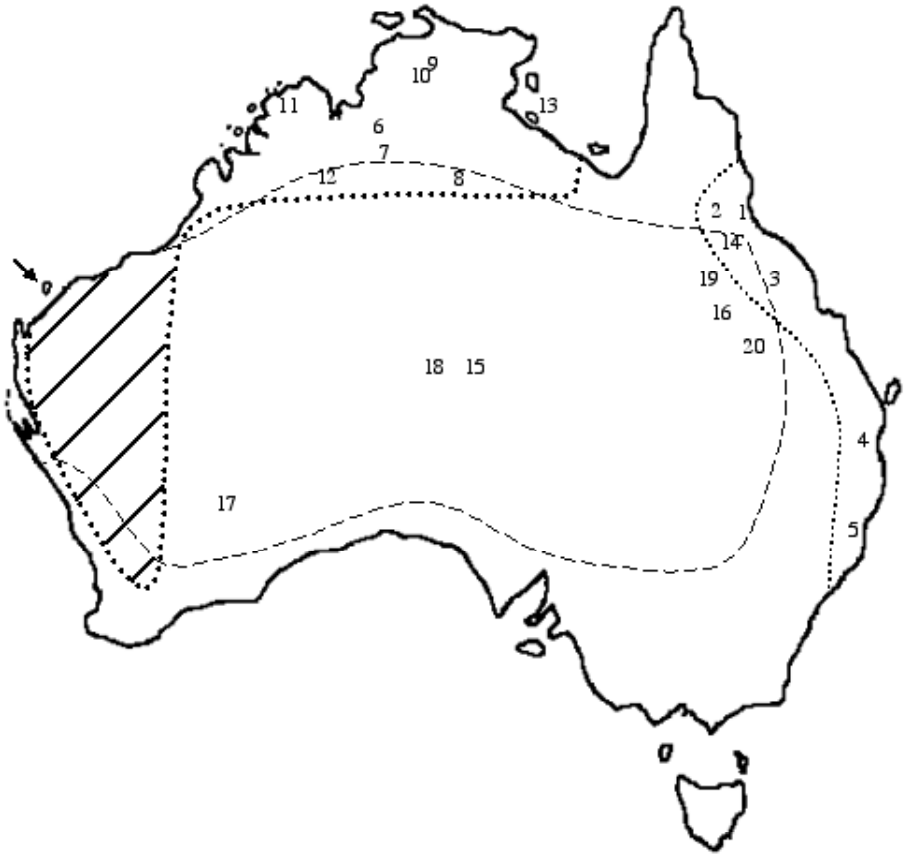


Figure 8.1. Distribution of the chestnut mice, *P. desertor*, and sampling locations.
Distributions based on Watts and Aslin (1981), Fox (1995b), Kerle (1995b), Robinson (1995) Kutt et al. (in press) and collection locations. *Pseudomys nanus* is extinct in the shaded area of its distribution, with the exception of Barrow Island (arrow). Numbers refer to population numbers given in table 8.1.
Pseudomys desertor – – – ; *P. gracilicaudatus* ····· ; *P. nanus* ▪▪▪▪▪ .

8.3 Genetic differentiation of chestnut mouse and *P. desertor* populations

8.3.1 MITOCHONDRIAL CONTROL REGION

Nuclear pseudogenes and population divergence among chestnut mice

Like the pebble-mound mice, the chestnut mice share a unique nuclear copy of their control region. The presence of the nuclear copy was highlighted by very noisy sequence obtained from tail tips when compared to liver samples and extremely low levels of genetic variation across the entire distribution of both *P. nanus* and *P. gracilicaudatus* in preliminary

sequencing. Unlike the pseudogene isolated from the pebble-mound mice the nuclear copy of the chestnut mice is quite similar to their actual control region sequence, differing at very few bases. In phylogenetic trees of the entire conilurine fauna the nuclear sequences still group with chestnut mouse sequences, although they differ from all conilurines at several highly conserved control region sites. The similarity of the pseudogene sequence to true control region sequence makes it difficult to construct primers that preclude its amplification during PCR within the region of the mitochondrion examined in this thesis. If all chestnut mouse sequences are examined in a Kimura two-parameter distance tree the pseudogene sequences are readily apparent (fig. 8.2). In "clean" sequence from liver samples it was usually possible to read two distinct peaks at ambiguous sites, and in nearly all cases the background noise was identical to the pseudogene sequence for that site. Likewise, in sequence from tail tips it was often possible to read two distinct peaks, and by inference, the base present in liver sequence or not present in the pseudogene was probably the correct one. Sequences inferred in this fashion were not acceptable for anaylsis, but indicate potential for solving the problem. I currently consider only the sequences from individuals g8, n8 and n12 to be true mitochondrial sequence. Distances between these three sequences (table 8.2) are still useful as they are from the Kimberley, Top End and Queensland, and therefore sample three of the main regions of northern Australia inhabited by chestnut mice. These show 3.9% divergence between *P. nanus* in the Top End and Kimberley, and 6.4-7.4% divergence between *P. gracilicaudatus* (QLD) and *P. nanus*. The sequences align easily, with only one indel creating a one bp gap in *P. nanus* sequence when compared to *P. gracilicaudatus*.

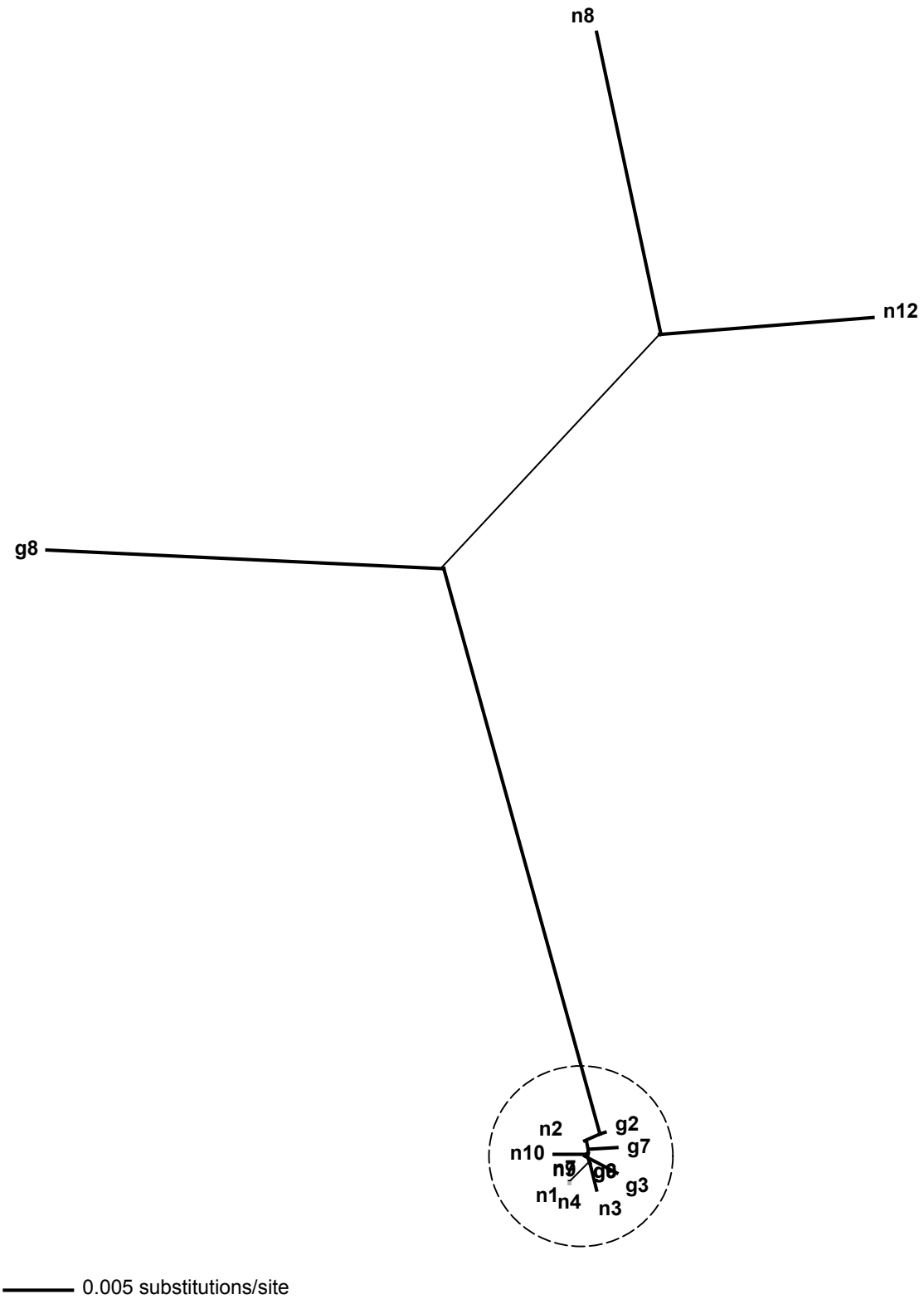


Figure 8.2. Genetic distance between sequences obtained from chestnut mice.
Unrooted neighbour-joining tree of Kimura two-parameter distances between 10 haplotypes of 379bp isolated from 15 individuals. Circle highlights the 12 sequences believed to be nuclear copies of mitochondrial sequence. Taxon labels refer to individuals carrying a particular haplotype.

Table 8.2. Control region distance matrix for three chestnut mice.
Matrix based on Kimura-two parameter distance between 379bp sequences. Only the three "clean" sequences from liver are shown here. Codes are individuals and region: gx- *P. gracilicaudatus*; nx- *P. nanus*.

	g8-QLD	n8-Kimberley	n12-Top End
g8-QLD	-		
n8-Kimberley	0.06429045	-	
n12-Top End	0.07366365	0.03850857	-

Population divergence in P. desertor

There is very little genetic differentiation of *P. desertor* populations across a very large distribution. Individuals from Queensland were clearly *P. desertor* (fig. 8.3 and table 8.3). A maximum divergence of 3% between some Queensland sites and Purnululu is indicative of a similar situation to that observed in *P. hermannsburgensis*, although in the few sequences analysed here there appears to be a weak association of haplotypes with geographical proximity. More detailed sampling is required to establish whether that is the case.

Table 8.3. Control region divergence among *Pseudomys desertor* populations.
Matrix based on Kimura-two parameter distance between 379bp sequences. Maximum distance between sites is shown.

Albionvale	0.0026							
Alice Springs	0.0265	-						
Burra Range	0.0079	0.0185	-					
Laverton	0.0211	0.0159	0.0185	-				
MacDonnell	0.0291	0.0132	0.0265	0.0132	-			
Moorrinya	0.0106	0.0212	0.0026	0.0212	0.0291	-		
Oakleigh	0.0026	0.0238	0.0079	0.0185	0.0265	0.0079	0.0026	
Purnululu	0.0317	0.0212	0.0291	0.0212	0.0238	0.0317	0.0317	-
	Albionvale	Alice Springs	Burra Range	Laverton	MacDonnell	Moorrinya	Oakleigh	Purnululu

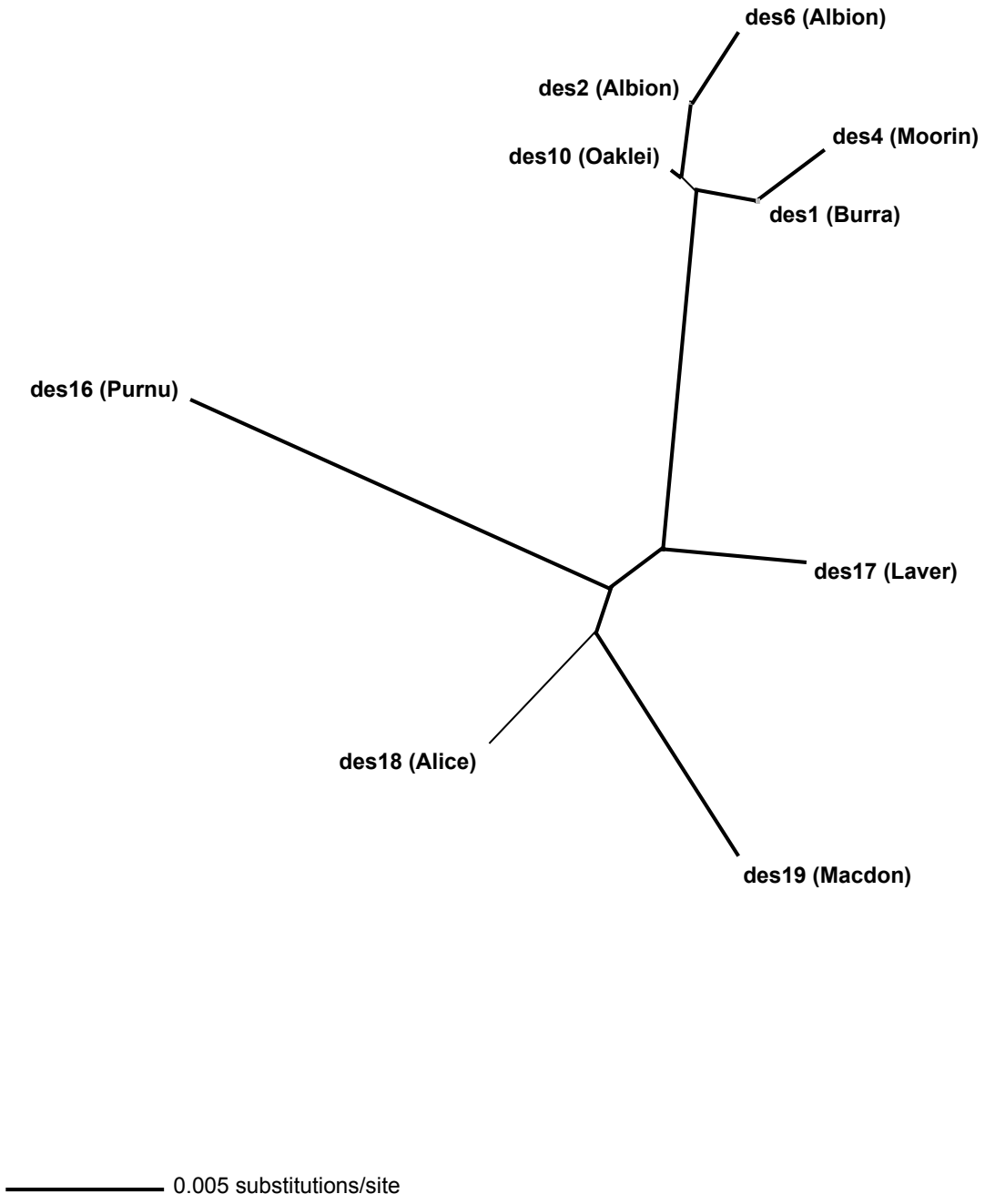


Figure 8.3. Genetic distance between nine *Pseudomys desertor* control region haplotypes.
Unrooted neighbour-joining tree of Kimura two-parameter distances between nine haplotypes of 379bp isolated from ten individuals. Taxon labels refer to a single individual that carried a particular haplotype, and to the source population (see also table 8.3).

8.4 Summary

- The chestnut mice possess a unique nuclear pseudogene that has thus far confounded detailed analysis of control region sequence.
- Based on three sequences *Pseudomys nanus* from the Kimberley and the Top End are significantly more closely related to each other than either is to *P. gracilicaudatus* in north Queensland.
- Recently recognised populations of mice identified as *P. desertor* in central Queensland are clearly *P. desertor* on the basis of control region sequence.
- *Pseudomys desertor* populations are genetically similar (<3% divergent) from locations up to 2400km apart in the north-eastern, north-western, south-western and central parts of the species distribution.

Chapter 9. Discussion

EXPLAINING THE CONILURINE RADIATION

In this discussion I focus on extending the results of chapters 4-8 in two ways. I briefly review patterns of molecular evolution within the conilurine rodents and attempt to describe the ways in which species ecology has created specific evolutionary consequences for different species and groups. The varied effects of Pleistocene arid barriers on northern savannah conilurines are reviewed. I suggest that the biogeographical practice of using areas of endemism in reconstructing evolutionary history may not be an appropriate method for describing the evolution of some Australian faunas because the history of current areas of endemism differs markedly from that of classic case studies in the Neotropics and elsewhere. I then describe some patterns that seem to be of evolutionary significance within the conilurine fauna. Having established ecological and environmental models that account for specific cases of evolution among conilurines I then briefly consider whether local-scale ecological processes are important in shaping macroecological and macroevolutionary patterns. Discussion builds on the observations that the phylogeny of conilurine rodents is imbalanced and that the distribution of range sizes among conilurine rodents is not log-normally distributed, while that of marsupials is. Recent neutral models of macroecology, biodiversity and biogeography provide a conceptual framework for discussion.

9.1 Describing diversity

9.1.1 Patterns of conilurine diversity

The trophic diversity of conilurine rodents is reflected primarily in the adaptations and morphology of different genera. I have proposed that this generic diversity is the result of an early savannah radiation. Aridity is the overwhelming influence on conilurine evolution following the ancestral savannah radiation, and is a predominantly Pleistocene phenomenon. Post-radiation diversification of conilurines has primarily involved the evolution of two arid-adapted genera, *Leporillus* and *Notomys,* and the radiation of the *Pseudomys* group, which includes a number of arid-adapted *Pseudomys* species and *Notomys*. Species diversity is only high in the *Pseudomys* group, which creates phylogenetic imbalance in the conilurine tree. The effects of aridity are obvious in causing population extinction and partitioning in model species groups (chapters 5-8), and there are obvious influences of species ecology in modifying the effects of aridity among these groups (section 9.2.3). Such climatic stimuli have affected all Australian species, but there are differences between patterns of species diversity in marsupials, birds and rodents that are not readily explained by structural differences in habitat, in particular the diversity of conilurines in seasonally arid, semi-arid and arid environments (section 2.3.3). In this discussion I therefore present the synthesis of the results chapters, and then explore the possibility that species ecology might not be the best way to investigate macro-evolutionary patterns in conilurine rodents.

9.1.2 Species

I suggest a number of taxonomic changes may be required in the wake of my studies. In particular, some species in the delicate mouse group that have long-standing recognition as good species may require reconsideration as a sub-specific complex. I have highlighted cases where there is doubt surrounding the significance of results, and I have explicitly stated that this thesis does not constitute a published work under article 9 of the Zoological

Code of Nomenclature. Those results will be tested further and published if they prove correct.

It should be noted that my taxonomic framework is based on the notion of biological species. I will not attempt to review the debate over the nature and definition of species, which is perhaps dealt with most evenly in Wheeler and Meier (2000). However, as they point out on the cover of their book, "No question in theoretical biology has been more controversial or perplexing than "What is a species?". Like many others (Paterson 1985, Coyne 1992, Gould 1994, Mayr 2000, Lee 2003), I believe that species are a real unit of evolution for sexual organisms, and in particular mammals and birds. Species represent perhaps the only meaningful rank in the taxonomic hierarchy; the point where the future and past evolutionary history of members are shared, but above which only common ancestry is important in demarcating taxonomic units. Species are therefore unique (Gould 1994). They are not an arbitrary point along an evolutionary continuum of divergence, although that continuum of divergence certainly exists, running from populations to genera, families, and so on. Should the concept of species representing unique evolutionary entities be abandoned, and species become viewed only as units of convenience, what then are we describing when we discuss speciation and diversification? Speciation literature, as opposed to literature on species definitions, is very clear on this subject; speciation is the formation of reproductive isolation (Mayr 1963, Templeton 1981, Barton and Charlesworth 1984, Butlin 1987a, Coyne and Orr 1998, Bridle and Jiggins 2000, Servedio 2000). Therefore I appeal to the notion that, for example, despite the fact *P. novaehollandiae* is a distinct taxon (barring the mismatch between molecular and morphological identification of individuals), it is not a distinct species if it can be shown that individuals of *P. novaehollandiae* in the Pilliga are freely inter-breeding with its very closely related, morphologically identical sib, *P. delicatulus*.

Taxa with no apparent basis

Two described taxa were shown to have no evolutionary basis (table 9.1). *Pseudomys delicatulus mimulus* Thomas 1926 is a relic of the past description of morphological island

variants as different forms. *Pseudomys d. mimulus* is much more closely related to nearby mainland populations than are most other mainland populations of *P. delicatulus*. *Pseudomys pilligaensis* Fox and Briscoe 1980 fails a simple test under nearly all species concepts (e.g. Templeton's (2001) null hypothesis 1) in that it does not consist of individuals belonging to a single monophyletic lineage. The Pilliga situation is similar to that of the Japanese endemic *Mus musculus molissinus*, which in reality was a mixed population of *M. m. castaneus* and *M. m. musculus* (Yonekawa et al. 1988).

Good taxa: good species?

The remaining problems in conilurine taxonomy (table 9.1) illustrate the problems associated with determining what constitutes a species, and also why an appeal must be made to a non-arbitrary definition of species. All involve assignment of real taxa to their correct place in the taxonomic hierarchy, rather than identifying cases where taxa do not exist. I have proposed that the taxonomic boundaries of *Pseudomys johnsoni* Kitchener 1985 should be enlarged to include *P. laborifex* Kitchener and Humphries 1986 because the level of mtDNA distance between these taxa is significantly lower than between other conilurine species, and these lineages apparently have a recent origin related to a straightforward process of isolation during the LGM. Only breeding trials can determine any greater significance arising from the isolation of these taxa. Problems in the *P. delicatulus* complex are outlined elsewhere (above, sections 6.4.4), but arise from relatively shallow genetic distance among taxa, inconsistent naming of taxa of equivalent depth, lack of congruence between the genetic and morphological assignment of individuals to "species", and incomplete knowledge of the outcomes of secondary contact in the Pilliga (i.e. is introgression or assortative mating occurring?).

Table 9.1. Proposed changes to *Pseudomys* species and subspecies taxonomy

Species (populations)	Proposed change to status	Grounds
P. delicatulus (Pilbara, sth Kimberley- sth VRD)	New species (proposal will be made for name *P. aphonomorphous*)	Very distinct mitochondrial lineage that is more isolated from nearby *P. delicatulus* populations than are *P. novaehollandiae* or *P. bolami*. Very distinct nuclear intron sequence at two loci. Problem: one possible individual resembles *P. delicatulus* (NT) in unique derived sperm morphology.
P. delicatulus mimulus	Not recognised	Minimal genetic divergence from nearby mainland populations. A very recent (~10ky) insular form not described on evolutionary grounds.
P. delicatulus (Queensland, nth New South Wales)*	Subspecies of *P. delicatulus* (proposal will be made for name *P. delicatulus stalkeri*)	Distinct mtDNA lineage within *P. delicatulus*, characterised by several morphological traits including unique sperm morphology and karyotype.
P. laborifex (Kimberley) and *P. laborifex* (VRD)	Subspecies of *P. johnsoni* (proposal will be made for name *P. johnsoni laborifex* for Kimberley and *P. j. kitcheneri* for VRD)	Very recent range fragmentation probable cause of clade formation. Shallow genetic divergence between clades. Geographical isolation of two *"P. laborifex"* clades not current, *P. johnsoni* probably not isolated from others. Inconsistent species assignment of genetically equivalent clades.
P. novaehollandiae	Subspecies of *P. delicatulus*	Previously unrecognised continuous, overlapping, distribution of the two taxa. Some evidence of ability to inter-breed in captivity and gene flow in the wild. Lack of spatial separation and identical morphology of both lineages when found in sympatry. Descriptions of this species and *P. delicatulus* were made from two sites over 3000km apart in a clinal distribution.
P. pilligaensis	Junior synonym of *P. delicatulus* and *P. novaehollandiae*	No mitochondrial lineage is unique to the species. Those mtDNA haplotypes that are present belong to either *P. delicatulus* or *P. novaehollandiae*, which are continuously distributed through the region (above). Description was an artefact of poor survey knowledge of *P. delicatulus* and *P. novaehollandiae*, and incomplete context for genetic description.

**Pseudomys delicatulus* from the mainland Northern Territory and north Kimberley retain the current name *P. delicatulus delicatulus*.

9.1.3 GENERA: A MATTER OF OPINION

I have defined new genera within the *Pseudomys* group because; 1) the currently defined genus encompassed a significantly greater genetic diversity than any other conilurine genus, 2) it contained several very distinct species assemblages, including the biologically unique, and relatively old, pebble-mound mice, 3) the genera *Notomys* and *Mastacomys* were not clearly separable from the currently defined boundaries of *Pseudomys*, and they are biologically distinct enough to warrant their previous generic status. Genera are essentially arbitrary units with no formal definition or conventions guiding their description (Lee 2003). It is therefore potentially of little importance how *Pseudomys* is taxonomically defined, as long as distinct evolutionary units are recognised in some form. Molecular phylogenies combined with a synthetic assessment of previous studies led to the description of genera in chapter 4. However, future work is required to resolve the doubts I have highlighted surrounding the assignment of some species to genera/subgenera. The removal of *Thetomys* and "*Phorolithomys*" is well supported.

9.2 Speciation and population divergence among conilurines

9.2.1 MODES OF SPECIATION

Just as there is a long-running debate over the definition of species, there is a protracted debate over how new species are formed (Mayr 1991). Again, this debate is reviewed at length elsewhere (Otte and Endler 1989, Coyne and Orr 1998), and I shall only touch on the subject by reviewing modes of evolution observed in the conilurine fauna. Predictably, there is evidence presented in chapter 5 of allopatric divergence of pebble-mound mouse species. Allopatric speciation (Mayr 1942, 1963) is accepted as the most common, and the "easiest", process of species formation (Otte and Endler 1989, Foster et al. 1998, Salomon 2001). Populations become geographically isolated, gene flow is reduced or ceases, and genetic mutations arise that eventually cause reproductive isolation between species should they come into secondary contact. Besides pebble-mound mice, *Pseudomys albocinereus*

and *P. apodemoides*, large *Zyzomys* species and probably many other conilurines are the result of allopatric speciation.

Bottlenecks and founder-flushes are particular cases of allopatric speciation that entail more rapid genetic change than may occur in large allopatric populations (Barton and Charlesworth 1984). The contraction of peripheral populations or migration by few individuals to form small founder stocks has been postulated to allow emergence of new co-adapted gene complexes that permit the bottlenecked stock to exploit novel evolutionary pathways (Meffert 1999). There are several modifications of this theory, but all entail small population size (Mayr 1963, Carson 1975, Templeton 1980, Barton and Charlesworth 1984). There is experimental support for the evolution of assortative mating and varied evolutionary trajectories due to founder-flushes (Templeton 1996, Regan et al. 2003). Baverstock (1982) has previously suggested bottlenecks may have been important in promoting conilurine speciation, and proposed they would have taken place in the semi-arid zone. *Pseudomys bolami* and *Pseudomys johnsoni* clades appear to have recently experienced severe bottlenecks, although whether the initial evolution of the species involved such a step is not clear. Nevertheless, climatic processes that led to the recent bottlenecks in these species were recurrent throughout the Pleistocene (section 2.3.1) suggesting that species currently residing in the arid zone may have had their origin as a result of founder-flushes that allowed previously maladapted species to exploit novel environmental conditions. I see no reason why bottlenecks could not have taken place throughout Pleistocene central Australia with populations taking advantage of relictual pockets of wetter habitat, although the number of refugia present in the region would have diminished with each successive arid cycle. The lack of genetic structure across the very large arid zone distribution of *P. hermannsburgensis,* and probably *P. desertor,* highlights the lack of geographical barriers to rodents in central Australia. Bottlenecks may therefore have been a particularly important factor leading to diversity of arid zone faunas.

Other modes of speciation are more difficult to observe, and to theoretically demonstrate. Modern authors largely dismiss parapatric speciation and cline theory, although the process had some influential supporters in the past (Huxley 1942, Bush 1975, Endler 1977). The

cline observable in the *P. delicatulus* complex between *P. delicatulus* and *P. novaehollandiae* almost certainly entailed past allopatric fragmentation (section 6.4.3), and resembles a "broken cline" proposed by Salomon (2002). Salomon's (2002) interpretation of a situation such as observed in the Pilliga is the same as the one I proposed in chapter 6. Where two taxa with clinal characters share identical forms of the clinal characters in secondary contact they are probably subspecies, and reproductive isolation has not evolved during a past period of allopatry.

Sympatric speciation suffers from the same theoretical problem as parapatric speciation. It is difficult to theoretically demonstrate a mechanism whereby assortative mating and reproductive isolation can occur within a single population because of the homogenising effects of gene flow (Lewontin 1974). Recent theoretical and empirical studies have revived acceptance of sympatric speciation as a biological reality (Butlin 1987a, Coyne 1992, Kaneko and Yomo 2000, Shaw et al. 2000, Danley and Kocher 2001). Sympatric speciation is often facilitated by some mechanism promoting reinforcement of incipient assortative mating (Butlin 1987b, Servedio 2000) and pebble mounds were potentially such a mechanism as they have been hypothetically linked to mating preference (Cermak 1999). However, pebble mounds proved not to be involved in mating preference, and no evidence of sympatric speciation was provided by studies presented here. Some suggestion that sympatric speciation may have occurred within conilurine rodents comes from the disparity in body size between sympatric species of *Mesembriomys* and *Leporillus* that could indicate niche-partitioning as a driving force of divergence, and ecological specialisation and rapid speciation among *Notomys* species that are sympatric with sibs. Ecological specialisation and rapid divergence have been linked to sympatric mechanisms of speciation (Butlin 1987a, Danley and Kocher 2001). However, the initial divergence of these taxa could easily have occurred in allopatry, and the extensive evidence of range movements among taxa studied here indicates that interpretation of speciation processes among now sympatric species is generally not possible.

9.2.2 ECOLOGICAL CORRELATES OF EVOLUTIONARY PATTERN

Flow on effects: a behavioural cause of macro-evolutionary pattern

The building of pebble mounds has dictated a very different evolutionary pathway for pebble-mound mice than for the morphologically similar delicate mice. As outlined in chapter 5, pebble-mounds promote a level of site fidelity remarkable among conilurine rodents. This applies to breeding females, and hence populations. Males of *P. patrius* are highly mobile, and individuals of *P. chapmani* have been caught some distance from rocky ridges (Start et al. 2000), but the centre of breeding and population survival is pebbly habitat. This has been the major factor promoting differences in macroevolution between pebble-mound mice and delicate mice. In addition pebble-mound mice do not seem to boom in response to very favourable conditions, probably because mounds are a critical limiting resource. Disturbances, even fire, do not seem to unduly affect their population dynamics. On the other hand delicate mice have mobile populations that undergo very large fluctuations in size, and site fidelity is low (section 6.1.2). Some indication of the relative global population size of pebble-mound mice and delicate mice comes from the number of haplotypes isolated from 87 individuals of each group; 64 in the delicate mice, and 37 in the pebble-mound mice. This is potentially due to uneven effects of extinction acting on the two groups, but even taking the most diverse site for *P. patrius*, Marodian, ten samples yielded three haplotypes. Seven "*P. delicatulus*" samples from the Pilliga yielded five haplotypes. Smaller population size of pebble-mound mice may therefore have accelerated the observed effects of allopatry enforced by geological change.

The major evolutionary consequence of these differing ecologies is secondary contact between delicate mice. Distributions of delicate mice are apparently under climatic control and their distributions inter-grade where prevailing climatic conditions inter-grade. More complete modelling using BIOCLIM has the potential to reveal which climatic factors are limiting the ranges of delicate mouse taxa, but can only be performed once the distributional limits of morphologically cryptic taxa such as *P. delicatulus* and *P. novaehollandiae* in southern Queensland and *P.* cf. *delicatulus* are established by mtDNA

sequencing. Speciation has probably been a straightforward process among allopatric pebble-mound mice. However, even when delicate mice have diverged in allopatry, cyclical climate and sea level change periodically re-unites them. In such a situation the population size while allopatric may be important in dictating the degree of divergence noted between taxa when in secondary contact. This could result in the ambiguous status of *P. delicatulus* clades and *P. novaehollandiae*, which have probably been repeatedly isolated as moderately large populations during the late Pleistocene, whereas severely bottlenecked taxa such as *P. bolami* may become reproductively isolated following a single allopatric interlude.

Effects of allopatry on the savannah fauna

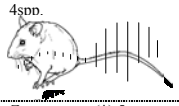
A major effect of Pleistocene cycles was the formation of habitat refugia in many parts of the world (Patterson 1999, Knowles 2001). At high latitudes ice replaced forests (Willis and Whittaker 2000), while in South America, Africa, India and Australia arid conditions had a greater effect, with deserts a major feature in Africa, Australia and India (Livingstone 1982, Linder 2001). Holocene climatic change in Africa has now reversed dominant local conditions to the extent that some ancient desert dunes are colonised by rainforest (Nichol 1999). However, in Australia arid corridors remain between mesic refuge areas (sections 2.3.1 and 2.3.3). Pleistocene arid barriers in northern Australia apparently had little affect on speciation in the savannah fauna. A review of the 17 conilurine species distributed across northern Australia reveals no consistent patterns of distribution or speciation in response to the formation of Pleistocene barriers (table 9.2). *Zyzomys argurus* and *Leggadina lakedownensis* are distributed in all refuge areas and have very different habitat preferences that see *L. lakedownensis* also distributed through the Great Sandy Desert, yet neither exhibit significant mtDNA structuring or divergence between regions (Moro et al. 1998, this thesis). Pebble-mound mice are unique in having a different species distributed in each of the major refuge areas, and in the subdivisions of the Kimberley and Top End. All savannah species are found in the Kimberley-Top End with the exception of the pebble-mound mice *P. chapmani* and *P. patrius*. Most species are also found in either the Pilbara or Cape York. Large *Zyzomys* species and *Conilurus penicillatus* are the exceptions. The

absence of *Conilurus penicillatus* from Cape York is difficult to explain, particularly given its presence in New Guinea (Flannery 1995) and on several islands in the Gulf of Carpentaria (Dickman and Van Dyck 2000, Woinarski 2000). The restrictive habitat preferences of large *Zyzomys* species dictate small contemporary distributions of all three, but there are fossils of large *Zyzomys* from Chillagoe (Godthelp 1997), at the base of Cape York, so recent (Holocene?) extinction of both *Conilurus* and large *Zyzomys* from Cape York is probable.

There are two cases in which Pleistocene barriers cannot be discounted as a cause of speciation, but where they are not directly implicated in any process beyond dictating the current distribution of species. These are *P. delicatulus/P. cf. delicatulus* and *P. gracilicaudatus/P. nanus*. Aridity and restriction to the Pilbara are probable causal agents of the initial divergence of *P. cf. delicatulus*. However, it was not conclusively established in chapter 6 whether *P.* cf. *delicatulus* is even a sib-taxon to *P. delicatulus*, and any number of alternative scenarios are possible, including divergence from *P. hermannsburgensis*, or an origin as a hybrid form (chapter 6). The separation of *P. nanus* and *P. gracilicaudatus* by the Carpentarian Barrier may be responsible for the chromosomal and genetic differentiation of the species (Baverstock et al. 1977a), but given the lack of such processes in species with similar habitat preferences and distribution (e.g. *Leggadina lakedownensis*) it is equally plausible that there is a co-incidental correlation between current distributions and species boundaries of the two. Speciation in the pebble-mound mice is related to allopatric isolation across the Pleistocene barriers, but divergence is related to loss of rocky habitats that may significantly pre-date the current climatic and vegetation barriers.

Table 9.2. Species distributions and areas of endemism in northern Australia.

Area of endemism: *barrier*. Each row represents a species or species group. Different species within a group are shaded differently and face in different directions. For example, there are four species of pebble-mound mice, each restricted to a single area of endemism, but five *Zyzomys*, three of which occur in the Top End, two in the Kimberley, and one that occurs in all areas of endemism (*Z. argurus*). The natural distribution of *Pseudomys nanus* (non-shaded member of chestnut mice) in the Kimberley-GSD-Pilbara region is not known and it may have been distributed through the GSD. * = study group in this thesis ((*) only *Z. argurus*).

Pilbara	*Gt Sndy Des*	Kimberley -	Top End	*Carpentarian*	Cape York
Conilurus penicillatus 1spp.					
Mesembriomys 2spp. 					
Leggadina 2spp. 					
Chestnut mice* 2spp. 					
?spp. 					
Pebble-mound mice * 4spp. 					
Zyzomys spp.(*) 5spp. 			 		

Areas of endemism as biogeographic units

A standard method in biogeography is the use of phylogenetic histories of taxa within areas of endemism to reconstruct a series of vicariance events describing area relationships (Anderson 1994, Brooks and McLennan 2001, Hausdorf 2002). The variable and unpredictable relationships between conilurine species inhabiting areas of endemism in the northern savannahs suggest that this approach may not be appropriate for much of the Australian continent. Many authors have highlighted the patterns of endemism within the Australian vertebrate fauna (Keast 1961, 1981, Archer and Fox 1984, Heatwole 1987), and Cracraft's (1982) oft-cited summary of evolutionary events for Australian birds was based on phylogenetic relationships between areas of endemism, including the northern savannahs. However, current areas of endemism in northern Australia have a very different history to the classically cited cases in Europe, Neotropical and African rainforests or the Wet Tropics rainforests of north-eastern Australia (Haffer 1969, Livingstone 1982, Moritz et al. 2000, Willis and Whittaker 2000). Savannah refuges have not been continuously inhabited areas to which species retreat at times of glacial activity and heightened aridity.

Making the reasonable assumption that rodents act as an indicator of the fauna as a whole, some species have probably been more continuously distributed at times during the Pleistocene because savannah habitats have moved northwards and united in a continuous band across exposed seas beds. Vrba (1992) has proposed that such migrations of habitats and their constituent species probably allowed most species to avoid detrimental effects of Pleistocene glaciations, and also reduced the potential for allopatric speciation during the epoch. Others, like the resilient *Zyzomys argurus*, have remained fixed in their distribution and unaffected by the climatic turmoil around them. Problems associated with the use of areas of endemism to reconstruct evolutionary history are probably amplified in central Australia. There has almost certainly not been a series of neat vicariance events such as that envisaged by Cracraft (1982). Even had there been, the lack of genetic structure across the distribution of *P. hermannsburgensis* suggests that reliable evidence for such events would be lacking in the modern fauna. Moreover, the unsolvable lack of knowledge of the natural (pre-European colonisation) distributions of terrestrial vertebrates in central Australia

probably compounds problems associated with the use of such methods. These issues all largely stem from one of the premises outlined in chapters 1 and 2; evolution of the Australian fauna is the result of climatic change that potentially affects each species in different fashion, and not the result of geological events that affect all species equally.

9.2.3 Some patterns of evolutionary interest

Correlates of (secondary) contact between conilurine sibs

Sperm morphology has often been referred to in this thesis and differs between *P. delicatulus* and *P. novaehollandiae* (Breed 1997). It also differs between Queensland and Northern Territory populations of *P. delicatulus* (Breed 2000). The significance of that variation is not clear because laboratory breeding has shown fertilisation can occur between Queensland and Northern Territory animals (Baverstock et al. 1977a), and between Pilliga animals (of unknown mtDNA haplotype) and *P. novaehollandiae* (L. Lim pers. comm.). However, variation in sperm morphology would appear to be in some way related to the evolution of reproductive isolation among conilurines, even if the process is not complete in the case of the *P. delicatulus* complex. Sperm morphology is conserved across all hydromyines, and many other Australasian rodent species (Breed 1997). In every case where divergent sperm morphology is observed, the species involved is sympatric or parapatric with its sib (fig. 9.1). In these cases there are also significant departures from highly conserved norms in sperm motility and abundance, and in testes mass relative to body size (Breed and Taylor 2000). These features have been linked to the unusual reproductive tract and possible breeding system of *N. alexis* (Suttle et al. 1988, Breed 1989a, Breed and Taylor 2000), but given their occurrence under particular circumstances, and in *Pseudomys* species that differ from *N. alexis* in reproductive physiology and breeding systems (Happold 1976, Breed 1989) they may have a more general implication for speciation processes. In the case of the delicate mice, there have possibly been two phases of sperm evolution, one involved in the divergence of the *P. delicatulus* complex as a whole (from *P. hermannsburgensis*?), and a second now evident with continued fragmentation of the *P. delicatulus* complex into sib-taxa.

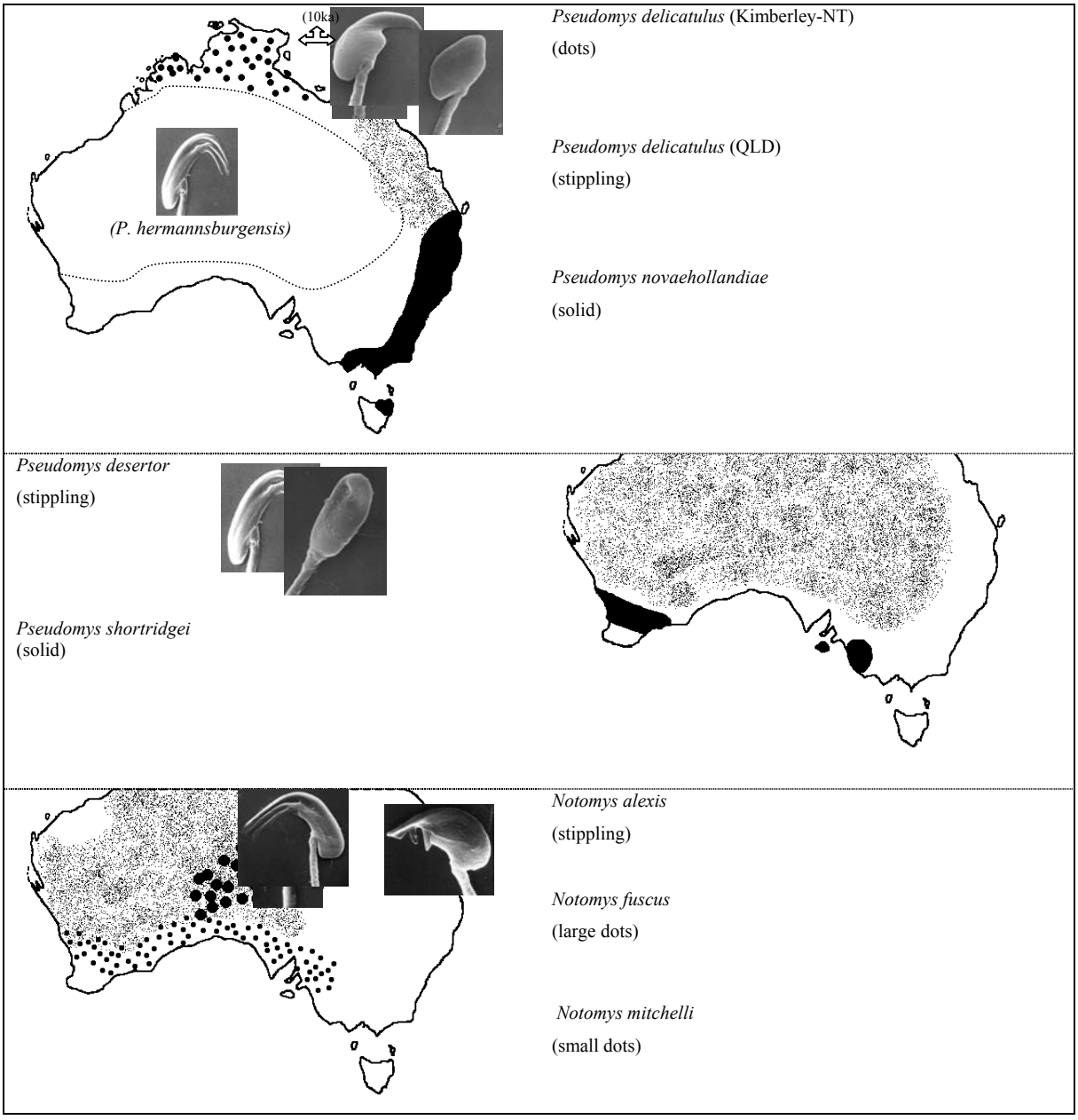


Figure 9.1. Biogeography of derived sperm morphology among conilurine rodents.
Sperm photos Bill Breed. Distributions Watts and Aslin (1981), Braithwaite and Covacevich (1995), Breed (1995b), Cockburn (1995a), Kemper (1995), Kerle (1995b), Watts (1995b) (modified as specified in chapter 6).

Origins and affinities of the arid zone fauna

Baverstock (1982) proposed the semi-arid zone as the centre of diversification of conilurines, with subsequent invasion of the arid zone. I suggested in section 2.3.4 and in section 9.2.1 that the actual geographical location was likely to be within the confines of the current arid zone, because the area was previously savannah or semi-arid habitats and contained refuge areas through much of the Pleistocene. Relying only on well-resolved species groups from chapter 4, and on speciation studies in chapters 5-8, some general patterns of the origin of arid zone species can be explored. Within the *Pseudomys* group, each genus or species group exhibits a different set of habitat preferences, but there is a tendency to include at least one peripheral and one arid zone species. *Leggadina* too, contains one arid and one peripheral species. This tends to support Baverstock's (1982) proposal of an arid-periphery relationship among conilurine species, with little speciation having occurred within the arid zone. The *Pseudomys* group and *Leggadina* therefore present a series of independent "invasions" of the arid zone. However, in the case of the pebble-mound mice, and I would suggest most arid zone species, adaptation to the arid zone would have occurred *in situ*, and would not have involved a re-invasion of abandoned habitat (although the recent history of the group indicates some range movement may have occurred). The same can be said of *Zyzomys pedunculatus*. Both pebble-mound mice and rock rats were probably forced to adapt rather than move their ranges, and both are now restricted to relatively small ranges within the arid zone. *Notomys* has apparently undergone extensive speciation within the arid zone. *Leporillus* is the only exclusively arid zone conilurine genus, and speciation presumably occurred within the arid zone.

An early arid bottleneck?

Notomys species are well adapted to aridity. They can utilise hyperthermia when temperatures are high (MacMillen and Lee 1970), have an exceptional ability to exist free of exogenous water, and *N. alexis* can concentrate urine more efficiently than any other mammal for which data are available (MacMillen and Lee 1967). DNA sequencing and other molecular studies have demonstrated that *Notomys* forms a lineage within the

Pseudomys group phylogenetic bush, and is not an isolated lineage as once thought on the basis of its unique morphology (chapter 4). A brief consideration of *Notomys* morphology also suggests that this is the case. The large ears, hopping gait and elongated feet are all features shared by desert rodents of other continents such as jerboas (Dipodidae), Springhares (Pedetidae) and kangaroo rats (Heteromyidae). Such convergent morphology is therefore a direct result of intense selective pressure in arid environments. Australian arid environments are recent, and true deserts are probably less than 1Myo (section 2.3.1). So, even if a distinct *Notomys* lineage pre-dates the Pleistocene, it is unlikely that the morphology and arid-adaptations of the group existed long before that time. How could such a morphologically distinct group emerge from the *Pseudomys* group over such a short time period?

The early onset of aridity probably had a similar affect as it does now, the creation of small founder populations. There is potential evidence of a small founder population in the history of the *Notomys alexis* group. The bizarre reproductive morphology of the group is so distinct from all other mammals (Breed 1985, 1986) that it is difficult to envisage its evolution in any circumstance other than isolation of a small population. The initial evolution of *Notomys* from a *Pseudomys*-like ancestor may therefore have involved a bottleneck in the late Pliocene or early-mid Pleistocene. The *Notomys alexis* group was not the direct result of this initial bottleneck, however, as *N. cervinus* retains a suite of ancestral characters similar to those of *Pseudomys* (section 4.4.3). Alternatives to a bottleneck are also possible, and Breed (1989) favours a mechanistic origin of the reproductive tract of the *N. alexis* group.

Is the arid zone a sink?

The general view in Australian biogeography is that the arid zone has been a sink into which diversity has filtered from the non-arid or semi-arid periphery (section 2.3.4). *Notomys* species are probably a product of speciation within the arid zone, but are not restricted to the arid zone. This indicates range expansion with expanding arid conditions, and the formation of relict populations once arid conditions retracted to more central

locations. Similar processes may have lead to the presence of *P. desertor* in central Queensland. *Pseudomys fumeus* appears to be related to *P. albocinereus* and *P. apodemoides* that occur along the semi-arid southern margins of the arid zone. Both *P. apodemoides* and *P. albocinereus* nest communally (Cockburn 1981a, Morris 1995), a condition often related to habitation of arid conditions, and that occurs in a number of Australian desert rodents (Happold 1976, Watts and Aslin 1981). The mesic *Pseudomys fumeus* also breeds communally, a behaviour that is not observed in other low-density mesic rodents (Woods and Ford 2000). This could indicate an arid heritage for *P. fumeus*, suggesting expansion out of the arid or semi-arid zone. There is a suggestion then, that the arid zone may not have functioned simply as a sink for conilurine species diversity. The genera *Notomys* and *Leporillus* have speciated within the arid zone, and *Notomys* species have colonised non-arid areas due to past expansion of the arid zone. Some non-arid *Pseudomys* species may also have their recent origins within the arid zone fauna, not vice-versa.

9.3. Is the importance of ecology scale-dependent?

9.3.1 Explaining biodiversity

Throughout this thesis I have maintained that species ecology explains patterns of evolution in the conilurine fauna. I have outlined characteristics that determine species distributions, either climatic or geological, and have suggested that changes in climate or landform are responsible for promoting evolutionary divergence by altering population size and connectivity. However, despite describing patterns and causes of evolution within components of the conilurine fauna, I have not attempted to explain a number of large-scale evolutionary patterns. Patterns of interest include the phylogenetic imbalance among conilurine rodents, whereby the *Pseudomys* group contains 36 of 49 species, a fact borne out by a recent study that identified both *Notomys* and *Pseudomys* as containing significantly more species than expected in a comparison of most Australian mammal genera (Cardillo et al. 2003). Another pattern that seems potentially important in conilurine

biogeography is that few conilurines have, or had, small or restricted natural ranges when compared to marsupials.

In closing this thesis I would like to briefly explore these two evolutionary patterns and draw on the conceptual framework provided by neutral models of macroecology (Bell 2001) and biogeography (Hubble 2001), and on macroecology literature that deals with patterns of distribution, abundance and phylogenetics that these models address (Gaston et al. 1997, Webb and Pitman 2002, Jablonski and Roy 2003). The attraction of neutral models lies in their ability to predict large-scale pattern using simple population level parameters such as birth, death and migration (Bell 2001, Hubble 2001). The integration of these models with empirical studies linking large-scale biogeographical pattern to evolutionary pattern may offer the solution to Gould's (1985) "paradox of the first tier" which suggests that there is dissociation between ecological processes classically held responsible for evolutionary pattern (the "first tier") and large-scale, infrequent, processes that over-ride the effects of the first tier and result in macroevolutionary patterns (Gould 1985).

Neutral models

Neutral models consider the abundance of species in a local community to be the product of random extinction processes between ecologically identical species. This is Hubble's (2001) "ecological drift", and derives from the concept of genetic drift in the neutral theory of molecular evolution (Ohta 1992, Nei and Kumar 2000). The equivalence of species in a neutral model is hard for ecologists to accept (de Manzacourt 2001, McGill 2003), and the consideration of only one trophic level is a problem that needs addressing with further development of models (Ford 2003). The abundance of a species across a landscape in neutral models is contingent on its likelihood of migration between sites, which is determined by the migration rate and its abundance in local communities. That means that once a species becomes abundant in a local community through chance, it is more likely to emigrate and more likely to become abundant across a landscape. This leads to a result that is empirically demonstrable in natural communities; some species are very abundant while

most others are not (Marzluff and Dial 1991). In many cases species abundance in local communities approximates a log-normal distribution, although Hubble (2001) demonstrated that his zero-sum multinomial described species abundance under varying rates of immigration, and also accounted for the geometric distribution of species abundances observed in some communities. This seems at first to have little to do with diversification rates and range sizes among conilurine rodents. However, Bell (2001) demonstrated that large range size was a result of being abundant in the global community, essentially because as abundance increases, the likelihood of occurring in any given patch also increases, even in models that specifically include spatial correlation of patch occupancy as a proxy for local adaptation. A positive relationship between abundance in local communities and range size is a pattern commonly cited in macroecology literature (Brown 1984, Gaston et al. 1997). Large range size has in turn been linked to an increased likelihood of speciation (Rosenzweig 1995), as has abundance (Webb and Pitman 2002). Cardillo et al. (2003) reported a positive correlation between larger average range size and diversification rates among Australian mammals in the study that identified *Notomys* and *Pseudomys* as particularly speciose genera.

Phylogenetic imbalance

Cardillo et al's (2003) treatment of rodents was flawed, as they compared *Zyzomys* to *Uromys* and the tree-rat/*Leporillus* clade to *Melomys* to control for age of lineages. This is clearly not a suitable comparison in an Australian context as the conilurine lineages have probably been present for millions of years longer than have *Melomys* or *Uromys*. However, had their comparison taken a more appropriate comparison of diversification rates the result may have been more striking. Any of the tree-rat/*Leporillus* clade (6 spp.), *Leggadina* (2 spp.) or *Zyzomys* (5 spp.) would make a suitable comparison of equal age to the entire *Pseudomys* group (36spp.), and by comparing the entire *Pseudomys* group, issues relating to the actual taxonomic composition of "*Pseudomys*" are avoided. Cardillo et al. (2003) suggested that larger range size in some Australian mammal lineages had promoted faster rates of diversification. It has previously been suggested that large range size promotes speciation because there is a greater chance of range fragmentation and peripheral

isolates forming (Rosenzweig 1995, Wagner and Erwin 1995). However, the alternative, that large range size retards speciation because a species with a large range is adapted to a greater variety of conditions and is buffered against formation of small barriers and climate change, has also been supported (Mayr 1963, Gaston 1998, Jablonski and Roy 2003). Empirical data and "weight of opinion" suggest that small-medium to medium sized ranges are most speciation prone (Gaston 1998, pg. 227).

Range sizes, however, are not "heritable" (Webb and Gaston 2003), a fact evident in the very different range sizes of the conilurine sibs *Pseudomys desertor*/*P. shortridgei*, *P. higginsi/P. fieldi*, and *Notomys alexis/N. fuscus/N. mitchelli*, so the effect of larger range is probably not linked to speciation rates within a lineage over time. Range sizes between major conilurine lineages are also not significantly different (based on data below). Range size is therefore not a satisfactory explanation for phylogenetic imbalance in conilurines. Another aspect linked to both species abundance and phylogenetic imbalance is a fast life-history (Marzluff and Dial 1991). Cardillo et al. (2003) noted that speciose Australian mammal lineages have larger litter sizes than non-speciose lineages. Again, this cannot explain the disparity in species diversity between the *Pseudomys* group and *Leggadina*, and for rodents in general, life history does not seem to be correlated to speciation rates (Marzluff and Dial 1991). The relatively low abundance of *Leggadina* (Read 1984, Reid and Morton 1995, personal observation) may be related to lower species diversity compared to the *Pseudomys* group, as abundance has been correlated with membership of speciose lineages (Schwarz and Simberloff 2001). However, differential species diversity between related higher-level taxa is common (Purvis and Agapow 2002), appears to be heritable, and results from features such as differential rates of cladogenesis, extinction and key innovations for which there is no clear evidence within the conilurine fauna (Chan and Moore 2002, Savolainen et al. 2002).

Neutral models may be of particular value when considering this type of question. Bell (2001) did not consider the evolutionary implications of his neutral theory, but Hubble (2001) included speciation as a central parameter in his Unified Theory. He indulged in a wide-ranging demonstration of the Unified Theory's applicability to both biogeography and

evolution, including presenting results that suggest there is no need for ecological differences between taxa when explaining macroevolutionary patterns such as the lack of species in *Leggadina*. They can arise by chance from processes that have their root in birth, death and migration among ecologically equivalent species and individuals. However, neither Hubble (2001) nor Bell (2001) denied the importance of species ecology in dictating specific evolutionary patterns, and although neutral models might produce results describing the distribution and species diversity of pebble-mound mice and delicate mice, differences in the evolution of these two groups are still best dealt with in an explicit ecological framework as I have presented in earlier chapters.

Range size

A basic mathematical observation that begins to unite range size and species abundance is that, in general, they conform to a log-normal, or near log-normal distribution (Bell 2001, Webb and Pitman 2002). That is, most species have small ranges, significantly fewer have medium-sized ranges, and very few very large ranges (Gaston 1998). To formalise the observation that conilurines tend not to have small ranges I analysed data on range sizes of Australian mammals that had been previously calculated from maps in Strahan (1995) by Chris Johnson (unpublished data). I re-calculated the area for species where I knew of major departures from the distribution shown in Strahan (1995). Two important changes were the range of *P. desertor*, which is almost twice the area shown in Strahan (1995), and *P. patrius*, which is not listed at all. In general, ranges in Strahan are under-estimates of the actual range size, but this is also true of marsupials, so I did not alter data for the majority of species. The distribution of marsupial range sizes is log-normal (Shapiro-Wilk test: $p<0.0004$), while the distribution of range sizes for all Australian rodents is not ($p<0.4187$). The distribution for conilurine rodents alone is an even greater departure from a log-normal ($p<0.6367$). The average size of conilurine ranges (1,067,702km^2) was not significantly different from that of marsupials (1,056,326km^2; $p<0.9634$). Marsupials, however, constitute a group of significantly greater taxonomic rank than do conilurines, so I also compared only dasyurid marsupials, because they are roughly the same body size as conilurines, are as speciose, and have also been successful in the arid zone. Dasyurid range

sizes were log-normally distributed ($p<0.0368$), and were not significantly different in size to those of conilurines (1,103,425km^2; $p<0.8979$). Differences in range size distribution between these groups are due to the comparative lack of conilurines with very small ranges, and a comparatively high number with small-medium sized ranges (fig. 9.2). Unlike most size-class distributions, including that of marsupials, the smallest size-class is not the modal class. The three smallest range size-classes are approximately the same size, whereas, in a comparison where both marsupial and conilurine ranges are presented in classes of 200,000km^2, the smallest class is distinctly modal for marsupials (fig. 9.2 b and c). Many range size distributions may be slightly left-skewed due to species with very small ranges (Gaston 1998), however, following the results of chapter 6, I recognise only one conilurine that has a very small range, *Zyzomys palatalis*. One of the reasons *Pseudomys pilligaensis* seemed an unlikely taxon before any DNA sequencing was conducted was its very small range. This was made more unusual by the fact that all its close relatives have medium-large or large distributions conforming roughly to biogeographic subregions.

Bell (2001) showed that range size and abundance could be considered essentially products of the same process in a neutral model (above), and successfully predicted the general empirical correlation between large range size and abundance of a species in local communities (Brown 1984, Gaston and Blackburn 1996). Larger body size has also been linked to larger range size (Brown and Maurer 1987), but there is little support for this relationship from reliable data sets sampled over appropriate scales (Gaston and Blackburn 1996). Ecologists accept that range size is determined by a set of characteristics that include the fundamental niche of a species and historical contingencies (Begon et al. 1996), and I have endeavoured in earlier chapters to describe the factors that might determine the distribution of some species; pebble-mound mice need pebbles, rock rats need rocks, *P. delicatulus* in Queensland require different climatic conditions to *P. novaehollandiae*. It is in considering the origin and limiting factors determining a species' range that ecologists are most pressed to accept the usefulness of neutral models. I do not think that neutral models provide a suitable evaluation of patterns of range size distribution among conilurine rodents (below). However, the use of such models is not necessarily in identifying the cause of pattern, but in identifying significant deviations from the predicted pattern (Bell

2001). I have not attempted to test whether conilurine range size distribution is a significant departure from predicted values under a neutral model, but the following discussion of the probable cause of this pattern would be significantly strengthened by an accepted method for producing a null model against which to test the significance of the effects I discuss, and neutral theory can provide that.

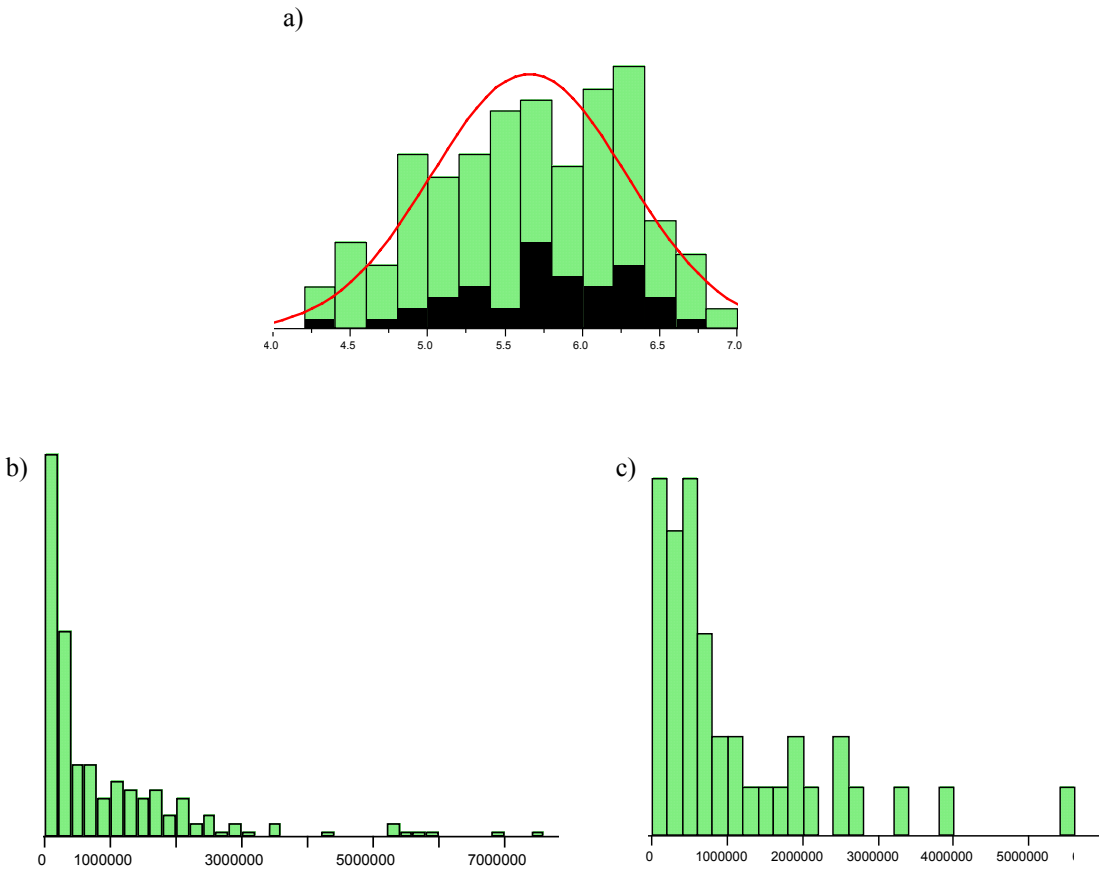


Figure 9.2. Range size distributions for Australian mammals.
Log range size: a) marsupials (n=145) and conilurine rodents (black) (n=40). Normal curve shown. Range size in km^2: b) marsupials, c) conilurine rodents (both in 200,000km^2 categories, but y-axes not proportional)

In agreement with results presented in this thesis, Jansson (2003) proposed Pleistocene climatic changes had been a central factor in determining range size. He proposed that in a region subjected to less severe climatic fluctuations, more endemic species would survive, and new endemic species would be more likely to establish and survive. Jansson (2003)

followed Stevens (1989) in suggesting that species with larger ranges were more adapted to variability, and proposed that species with larger ranges would occupy areas subject to greater disturbance through the Pleistocene. He offered this as an explanation and modification of Rapaport's rule, whereby species at higher latitudes tend to have larger distributions because these areas were subject to greater Pleistocene climatic fluctuation. Stephens and Wiens (2003) evoked the idea of time-for-speciation to explain higher species diversity and smaller range size of emydid turtles in the south-eastern United States when compared to the northeast. Turtles inhabited the southeast constantly throughout the Pleistocene, whereas they periodically retreated and re-invaded the northeast due to glaciation of the region (Stephens and Wiens 2003). Speciation has therefore not occurred in the northeast, while range fragmentation in the southeast has resulted in speciation and smaller range size, without increasing the local community diversity at any given site (Stephens and Wiens 2003).

Variability seems to be the key. Gaston (1998) also observed that in highly variable environments departures of range size distributions from the log-normal might occur because the effects of speciation are swamped. So why the contrast between marsupials and conilurines when both inhabit highly variable environments? Section 2.3.2 outlined the difference between marsupials, which are "forest animals", and conilurines that are "non-forest animals". Marsupials have a long history in Australia, and the legacy of their Gondwanan origin is a preference for less variable habitats. Compared to conilurines there are more endemic, restricted range species in eastern forests. This is also apparent in the Australian birds, which exhibit a pronounced left skew in the distribution of range sizes (Gaston 1998), because a proportionally large number have very small or small distributions in rainforests and forests. In contrast, arid zone mammals tend to be widespread. Of the 33 species with ranges greater than the 85th percentile in Australian mammal range sizes (excluding monotremes), 25 are restricted to the arid zone, and a further three have distributions centred on, or largely comprised of, arid regions. The arid zone makes up such a large part of Australia that this result could simply be related to the fact that to have a large range, it must encompass large parts of the arid zone. However, arid zone mammals, both conilurine and marsupial, have a significantly larger average

range size than forest mammals (e.g. for conilurines: t-test, t= 3.723 p<0.0006 df= 38). Disturbance in Australia was in the form of heightened aridity not glaciers, so unlike the situation with American turtles, central Australia was still potentially inhabitable during periods of "disturbance". This may explain why arid-adapted *Notomys* species show signs of specialisation, smaller range size, and rapid speciation within the arid zone in defiance of Greenslade's (1982) theoretical prediction that species adapted to arid conditions were unlikely to speciate. The very widespread *Pseudomys* species of the region have apparently not diversified within the highly disturbed habitats.

Does species ecology explain macroevolutionary patterns within conilurines?

I can only provide a classic "yes and no" answer: phylogenetic imbalance within conilurines could be partly related to body size, but no ecological attribute suitably accounts for the lack of species diversity within *Leggadina* compared to the *Pseudomys* group. Neutral models, however, produce such results without any need for ecological differences between clades. I favour ecologically-based explanations for the non log-normal distribution of range sizes within the group, and of differences in evolution evident between pebble-mound mice and delicate mice. However, the parameters and patterns involved in ecological explanations for conilurine macroevolution are entwined within the conceptual and predictive framework of neutral models, and once the ecological community has accept them, neutral models will provide a pivotal null model for assessing the real importance of ecological processes. The unification of these factors and the development of neutral models accounting for realistic levels of community structure may be in its infancy, but neutral models should be embraced and explored by the ecological community in an attempt to marry apparently small-scale realities such as habitat choice and competition with large-scale ecological and phylogenetic patterns.

9.3.2 FINALÉ

I believe I have shown here that species ecology is a fundamental influence guiding evolutionary processes in conilurine rodents, and that those effects have been translated

into macro-evolutionary patterns. A less empirical goal of this thesis was to promote the conilurine group as an interesting evolutionary model. I hope that I have illustrated the unique place conilurine rodents hold in the evolution of the Australian biota. They arrived late in development of modern Australia, and their evolution has taken place largely outside the environments that have been the centre of the evolutionary history for most Australian vertebrates. This means that with further characterisation of evolutionary processes and pattern, conilurines may provide a valuable contrast to studies of marsupial evolution. Many directions for future research are suggested by results presented here. In particular, studies of the *P. delicatulus* complex could prove of great theoretical interest regarding speciation processes, and the northern savannah fauna still provides excellent opportunities to investigate the importance of Pleistocene climatic cycles and species ecology in speciation, although such opportunities are unfortunately lacking in many other areas due to an horrific extinction record.

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Appendix i. Aligned control region and DHFR intron 1 sequences

Control region (3 pages):

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Xeromys   TAGCTACCCC CAAGTTTTAT GGGCCCGGAG CGAGAAGAGG GGCAT-G-GG TGGGCGGGTT GTTGGTTTCA CGGAGGATGG
Melcer    .......... .......C.. .......... .......... A....TA-A. .......... ....A..... ..........
Melbur    .......... .......... .......... .......... .....TA-A. .......... .A........ ..........
Urocau    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Urohad    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Lepcon    .......... ......G... .......... .......... .....-.-.. .......... ....A..... ..........
Mesgou    .......... ...A...A.. .......... .......... ..T..A-.. .......... ....A..... ..........
Conpen    .......... .......... .......... .......... ......AT.. .......... .A........ ..........
Mesmac    .......... ...A...A.. .......... .......... ......A-.. .......... .......... ..........
Leglak    .......... .......A.. .......... .......... .....TA-A. .......... .A..A..... ..........
Legfor    .......... .......... .......... .......... .....TA-.. .......... .A........ ..........
Zyzmain   .......... .......... .......... .......... .....TA-.. .......... .A..A..... ..........
Zyzarg    .......... .......C.. .......... .......... .....TA-.. .......... .A..A..... ..........
Zyzped    .......... .......... .......... .......... .....TA-A. .......... .A..A..... ..........
Zyzwood   .......... .......... .......... .......... .....TA-.. .......... .A..A..... ..........
Notmit    .......... .......... .......... .......... ......-.-.. .......... ....A..... ..........
Notale    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Notfus    .......... .......C.. .......... .......... ......A-.. .......... .......... ..........
Notcer    .......... .......A.. .......... .......... ......A-.. .......... ....A..... ..........
Pseocc    .......... ......GA.. .......... .......... ......-.-.. .......... ....A..... ..........
Pseaus    .......... .......... .......... .......... ......A-.. .......... .......... ..........
Psealb    .......... .......... .......... .......... ......A-.. .......... .......... ..........
Pseapo    .......... .......... .......... .......... ......A-.. .......... .......... ..........
Masfus    .......... .......... .......... .......... ......-.-.. .......... .......... ..........
Psehig    .......... .......... .......... .......... ......A-.. .......... .A.......T ..........
Psefie    .......... .......... .......... .......... ......A-.. .......... .........T ..........
Psesho    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Psedes    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Pseora    .......... .......A.. .......... .......... ......-.-.. .......... ....A..... ..........
Psejoh    .......... .......... .......... .......... ......T-.. .......... .......... ..........
Pselab    .......... .......... .......... .......... ......T-.. .......... .......... ..........
Psecal    .......... .......... .......... .......... ......T-.. .......... .......... ..........
Psepat    .......... .......... .......... .......... ......T-.. .......... ....A..... ..........
Psecha    .......... .......... .......... .......... ......T-.. .......... .......... ..........
Psedel    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Psepil    .......... .......... .......... .......... ......A-.. .......... .......... ..........
Psenov    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Pseher    ...TC..... .......... .......... .......... ......A-.. .......... .......... ..........
Psebol    .......... .......... .......... .......... ......A-.. .......... ....A..... ..........
Psenan    .......... .......A.. .......... .......... ......A-.. .......... .......... ..........
Psegrac   .......... .......... .......... .......... ......A-.. .......... .......... ..........
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Xeromys   TAGAAGTACC GACCACAT-G TGGAAGGGGA TAGTCATATG GAAGAGAAGA GTTTATGACT TAATGGTGTA TGTCTGATAA
Melcer    .....C..TA ..T-TA..-. .A.C...... .......G.. .........G .......... .......... .....A....
Melbur    .....C..TA ..T-TA..-. .A.C...... .......G.. .........G A......... .......... .....A....
Urocau    .....A..TA ..T-TG..-. .......... .......... .......... .......... .......... .....A....
Urohad    ....GA..TA ..T-TG..-. ...G...... .......... ........AG .......... .......... .....A....
Lepcon    ....GT..TA AGT-GT..-. .......... .....G.... ...A....AG A......... .......... .....A....
Mesgou    .....C..GA ..TT.A..-. .......... .....G.... ...A.....G A......... .......... .....A....
Conpen    .....C..GA .....A..-. G......... .......... ...A...... .......... .......... .....A....
Mesmac    .....C..GA .....A..-. .......... .......... ........AG A......... .......... .....A....
Leglak    .....A.TTA ..T-TA..-. G......... .......... .....T..AG .......... .......... .....A....
Legfor    ....TA.TTA ..T-CT..-. G......... .......... .....T..AG .......... .......... .....A....
Zyzmain   ....TA..TA ..TTTT..-. .......... .......... ..T..T..AG .......... .......... .....A....
Zyzarg    ....TA.TTA .TG-TT..-. G......... .......... .....T..AG .......... .......... .....A....
Zyzped    ....TA.TTA .TT-GT..-. G......... .......... .....T..AG .......... .......... .....A....
Zyzwood   ....TA.TTA .TT-TT..-. G..T...... .......... .....T..AG .......... .......... .....A....
Notmit    .....C-TTA ....CA..-. .......... .......... .....T.... .......... .......... .....A....
Notale    .....C-TTA ....CA..-. .......... .......... .....T.... .......... .......... .....A....
Notfus    .....C-TTA ....CA..-. .......... .....G.... ...A.T.... .......... .......... .....A....
Notcer    ....GA.TTA ATT-TA..-. .......... .......... .....T.... .......... .......... .....A....
Pseocc    ....GA.TTA ..T-TA..-. .A.C...... .......... .....T.... .......... .......... .....A....
Pseaus    .....A.TTA ..T-TT..-. .......... .....G..G. ..C..T.... .......... .......... .....A....
Psealb    .....A.TTA AGT-TA..-. G......... .....G.... .....T.... .......... .......... .....A....
Pseapo    ....GA.TTA A.T-CA..T. G......... .....G.... ..T..T.... .......... .......... .....A....
Masfus    .....A.TTA ..T-TA..-. .......... .....G.... ..TA.T..AG A......... .......... .....A....
Psehig    ....TA.TTA ..T-.....- .......... .......... ..GA.T.... .......... .......T.T A....A....
Psefie    ....TAATTA A.T-.T..-. .......... .......... .....T.... A......... .......T.T A....A....
Psesho    ....TA.TTA ..T-TA..-. .......... .....G.... ..CA.T.... .......... .......... .....C....
Psedes    ....TA.TTA ..T-TA..-. .......... .......... ..T..T.... .......... .......... .....C....
Pseora    .....A.TTA ..T-TA..-. .......... .....G.... ..T..T.... .......... .......... .....A....
Psejoh    ....GA.TTA ..T-CA..-. .......... .......... ...A.T.... .......... .......... .....A....
Pselab    ....GA.TTA ..T-TA..-. .......... .......... ...A.T.... .......... .......... .....A....
Psecal    .....A..TA A.T-TA..-. .A.C...... .......... .....T.... .......... .......... .....A....
Psepat    ....GA.TTA ..T-TA..-. .......... .......... .....T.... .......... .......... .....A....
Psecha    ....TAGTTA ..T-CA..-. .A.C...... .......G.. .....T...G .......... .......... .....A....
Psedel    .....A..TA ..T-TG..-. .......... .......... .....T..AG .......... .......... .....A....
Psepil    .....A..TA A.T-TA..-. .......... .......... .....T..AG .......... .......... .....A....
Psenov    .....A..TA ..T-TA..-. .......... .......... .....T..AG .......... .......... .....A....
Pseher    .....A.TTA .TT-TT..-. .......... .....G.... ..GA.T..AG .......... .......... .....A....
Psebol    .....A..TA ....TA..-. .......... .......... .....T..AG A......... .......... .....A....
Psenan    .....A.TTA A.T-TA..-. .......... .......... ........AG .......... .......... .....A....
Psegrac   ....TA.TTA N.T-TA..-. .......... .......... .....T..AG .......... G......... .....A....

Xeromys   CACAGATATG TCTTA-AAAG CATTAATTAA AATG--TGTT TCTT-AATAT TCATGAGTTT GT--GAATTT -TAAGTTGAA
Melcer    .....T.... ...A.-...A .......... ....--T.. .A.G-..... .....T.... ..--A..... -.........
Melbur    .......... ...AT-...A .......... ....--T.. CT.G-G.... .....T...A ..--A..... -......A..
Urocau    .......... ..C.T-...A .......... ....--T.A GG..-..... .......... A.--A..... -......A..
Urohad    .......... ...GT-...A .......... ....--T.G GT..-..... .....T.... A.--A..... -.....CA..
Lepcon    .....G.... ....T-.... .......... ....--CC. GT..-..... .....T.G.. A.--.GT... -C........
Mesgou    .......... .....-T... .......... ....--A.. GT..-..... .....T.G.. A.--..T... -.T......G
Conpen    .......... ...GT-G..A .......... ....--T.. GT..-G.... .....TTA.. A.--.GT... -.GG....G.
Mesmac    .......... .....-.... .......... ....--TC. AT..-..... .....T.G.. A.--..T... -.........
Leglak    .......... ...AT-...A .......... ....--T.. GT.C-..... .....T.... A.--A...A. -......A..
Legfor    .......... ...AT-...A .......... ....--T.. GT.C-..... .....T.... A.--..... -......A.T
Zyzmain   .......... ....T-.G.T .......... T...--A.. AG..-..... .....T...A A.--A.T.A. -..T...A.G
Zyzarg    .......... ...AT-.... .......... T...--A.. GG..-..... .....T...A ..--..T... -......AG.
Zyzped    .......... ...GT-...A .......... T...--A.. CG..-..... .....T...A ..--A..... -......AG.
Zyzwood   .......... .....-.G.A .......... T...--A.. CA..-..... .....T...A A.--A.T..G -.........
Notmit    .......... ....T-.G.A .......... T...--A.G .G..G..... .....T...A ..--A....A -.........
Notale    .......... ..C.T-...A .......... T...--ACG .G..A..... .....T...A ..--A....G -.........
Notfus    .......... ..C.T-.T.. .......... T...--AC. ....-..... .....T...A A.--A..A.G -.GGA....T
Notcer    .......... ...AT-.G.T .......... ....--AC. ....-..... .....T.... A.--..... -.T.....GG
Pseocc    .......... ..A.T-.... .......... ....--AA. .A..T..... .....T.... A.--A.T... -.....-.G.
Pseaus    .......... ....T-.... .......... ....--A.G ....-..... .....T.... A.--AGT.A. -.........T
Psealb    .......... ...AT-.G.. .......... T...--AC. ....-..... .....TT.G. C.--TGTA.. -.........T
Pseapo    .......... ...AT-.... .......... T...--AC. ....-..... .....T..A. A.--A.TA.. -AGG.....T
Masfus    .....G.... .T.A.-.... ........G. -...--AC. ....-..... .....T.... A.--.GTA.. -.......ACT
Psehig    .......... ...ATA.G.A .......... ....--AC. ....-..... .....T.... A.--..TA.A -......A.T
Psefie    .....T.... ...ATA.G.T .......... ....--..C. .T..TG.... .....T.... A.--..T... -.........
Psesho    .....G.... ...AT-.G.. .......... T...--A.. AA.G-..... .....TA.G. A.--.G.... -.........
Psedes    .......... ....T-...A .......... T...--AC. AA..-..... .....T..A. ..--A.T... -..G......
Pseora    .......... ...A.-G..A .......... ....--GAC. AA..-..... .....-.... A.--AG.... T........T
Psejoh    .......... .T..T-GT.A ........G. G...--AC. AG..-..... ..G..GA..A C.-AT....G T.T......G
Pselab    .......... .T..T-GT.A ........G. G...--AC. AG..-..... ..G..GA..A C.-AT....G T.C....AGG
Psecal    .......... .TC.T-...A .......... ....--A.. AA.G-....G .....TA... ..TTA....G T........G
Psepat    .....G.... .T..T-...A .......... ....--A.. GG.A-..... .....TT... A.--..T..G -.........
Psecha    .......... .T..T-.... ....G..... ....--A.. .G..-..... .....T.... A.--.GT..G -.GG.....G
Psedel    .......... ...AT-.G.A .......... ....--A.G GG..-..... .....G.G.A A---AGTA.. -.........
Psepil    .......... ...AT-.G.A .......... ....--A.. AA..-..... .......G.A A---A.TA.. -..G....G.
Psenov    .......... ...AT-...A .......... ....--A.. GA..-..... .....G...G A---AGTA.. -..GG.....G
Pseher    .......... ....T-TG.A .......... ....TG.A.G GG..-..... .....TA..C .A---.GTA.. -.........T
Psebol    .......... ...AT-.... .......... ....--A.. GA..-..... .....T.... AG---.TA.. -.........
Psenan    .....G.... .GGAT-TG.. .......... ....--A.G GT.G-..... C....TT.A. A.--.G-... --G.....G.
Psegrac   .......... .G.ATTT... .......... ....--A.N GT..-..... .....TT.G. T.--A..... --.......G.
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Xeromys   --ATATCATT TATGTC-TTA ATTCATTAAT GTTATG-TTC TTGCTTATAT GCTAGGGGTA AATAATTTAA TGTACGATAT
Melcer    --..T.G... A.....-... .......... T..T.A-.A. A.......... .......... .......... ..........
Melbur    --..T.G... A.....-... .......G.. T.AT.A-.A. A.......... .......... .......... ..........
Urocau    --T.T.A... A.....-... .......... T.AT.A-... G.......... .......... .......... ..........
Urohad    --..T.G... A.....-... G......... T.AT.A-... G.......... .......... .......... ..........
Lepcon    -T..TCT... ......-C.. .......... A.ATAA-.A. G.......... .....T.... .......... ..........
Mesgou    --T.T.A... A..A..-... .......... AGAT..-.A. .......... .......... .......... ..........
Conpen    --G.T.T..G A...A.-... .......G.. AAAT.A-.A. .......... ........G. .......... ..........
Mesmac    --..T.A... A.....-C.. .......G.. AAAT.A-.A. .......... .....C..A. .......... ..........
Leglak    --....T... A.....-... .......G.. A.AT.A-.A. .......... .....T.... .......... .......-..
Legfor    --....A... A.....-... .......G.. T.AT.A-.A. .......... .....T.... .......... ..........
Zyzmain   --G..AATG. A.....-C.. .......G.. A.AT..-.A. .......... .....T.... .......... .....A....
Zyzarg    --T..GG.G. G.....-... ...A...... ..AT..-.C. .......... .....T.... .......... ..........
Zyzped    --T..GG.G. A.....-... ...A...... ..AT.A-.C. .......... .....T.... .......... ..........
Zyzwood   --T.GAGG.. A.....-... .......... T.AT.A-... .......... .....T.... .......... ..........
Notmit    --T...T... A.....-..G ..C....G.. T-.T.AG.A. .......... ........C. .......... .....A....
Notale    --....T... A.....-..G .......G.. TA.T.AA.A. .......... .......... .......... ..........
Notfus    --........ A..A..-C.. G......G.. T..T.AG.A. .......... .......... .......... ..........
Notcer    -TTG.-AT.A A.....-... G......G.. T.ATAA-.A. .......... .......... .......... ..........
Pseocc    -TTA..AG.. A.....-... ...A...... T..T.A-.A. A.......... .......... .......... ..........
Pseaus    --....GG.. A.....-C.. .......G.. T.AT..-.A. A.......... ........A. G.G....... ..........
Psealb    --T....... A.....-C.. .......... T.AT.TT.A. A.......... ........A. .......... ..........
Pseapo    --........ A.....-... .......G.. T.AT.T-.A. A.......... ........A. .......... ..........
Masfus    --....AT.. A.....-... G......... AGAT.A-.A. A.......... ........A. .......... ..........
Psehig    --TA..A... A.....-... ..C....G.. TAAT..-.A. A.......... ........A. .......... ..........
Psefie    --TA..A..G A.....-... T......... T.AT.A-.A. G.......... ........A. .......... ..........
Psesho    --....A... A.....-... .......G.. T.AT.A-.A. A.......... ........A. .......... ..........
Psedes    --T...A... A.....C... G......G.. TAAT.A-.A. A.......... .......... .......... ..........
Pseora    --G....... A.....-... .......G.. T.AT..-.A. A.......... ........A. .......... ..........
Psejoh    -TTGTATG.. AGGT.T-..G .A........ .A.TAA-.A. .......... .....T..AG .......... .....T....
Pselab    -TTATAG... A.AT..-... .A.....G.. AA.TAA-.A. .......... .....T..AG .......... .....T....
Psecal    -TTGTAATA. G..T..-..G .A.....G.. AG.TA.-.A. .......... ........A. .......... .....T....
Psepat    -TTATAGT.. A..T..-... .A........ T..TAA-.A. .......... ........A. .......... .....T....
Psecha    -ATATAGT.. ...T..-C.. .......... .A.TAA-.A. .......... ........A. .......... .....T....
Psedel    -AT.G.AG.. A.....-.G. .........A TA.T.A-.A. A.......... .....T.... ...G...... ..........
Psepil    -TTA..A... A.....-... .......G.. TA.T.A-.A. G.......... .....T.... .......... ..........
Psenov    --T...A... A..A..-... .......... TG.T.A-.A. G.......... .....T.... .......... ..........
Pseher    --TA..A... A.....-C.. .......G.. T.GT..-.A. A.......... .....T.... .......... ..........
Psebol    --T...G... A.....-... ..A....G.. TAAT..-.A. A.......... .....T..A. .......... ..........
Psenan    TT....ATA. G.....-..C G..A...... A......-.A. A.......... ........A. T.......... ..........
Psegrac   TT....AT.. A.....-..T G..A...... AG.T.A-.A. A.......... ........A. .......... ..........

Xeromys   ACATT-AATG TTCTATAGTA CTATATAATT TT-ATGTACT
Melcer    .....-.... ......T... .......... ..-.......
Melbur    .....-.... ......T... ....G..... ..-.......
Urocau    .....-.... ......T... ....G..... ..-.......
Urohad    .....-.... .C....T... .......... ..-.......
Lepcon    ....A-G... ..A...T... .......... ..-.......
Mesgou    .....-.... ......T... .......... ..-.......
Conpen    ....A-.... .ATA..T... .......G.. ..-.......
Mesmac    .....-.... ......T... .......... ..-.......
Leglak    .....-.... ..AGT.T... ........A. ..-.......
Legfor    .....-.... ..ACT.T... ....G...A. ..-.......
Zyzmain   .....-.... .-....T... .......... ..-.......
Zyzarg    .....-.... .-T.G.T... .......... ..-.......
Zyzped    .....-.... .-........ .......... ..-.......
Zyzwood   .....-.... .-T.G.T... .......... ..-.......
Notmit    .....-.... .G...AT..G T......G.. ..-.......
Notale    .....-.... .A...AT..G .......G.. ..-.......
Notfus    .....-.... .AT..AT... .......G.. ..-.......
Notcer    .....-.... ..T..AT... .......... ..-.......
Pseocc    .....-.... .....AT... .......... ..T.......
Pseaus    .....-.... ..T..AT... ...A...T.. ..-.......
Psealb    .....-.... ..T...T... .......... ..-.......
Pseapo    .....-.... ..T...T... .......... ..-.......
Masfus    .....-.... .CT...T... ....G..... ..-......G
Psehig    .....-.... ..T..-T... ....G..... .--.......
Psefie    .....-.... .-T...T... ....G..... ..-.......
Psesho    .....-.... ......T... .......... ..-.......
Psedes    .....-.... ..T...T... .......... ..-.......
Pseora    .....-.... ..T...T... .......... ..-.......
Psejoh    .....T.... ..T..AT... .......... ..-......A
Pselab    .....TG... ..T..AT... .......... ..-.......
Psecal    .....-.... ..T..AT..G T......... ..-.......
Psepat    .....-.... ..T..AT... ....G..... ..-.......
Psecha    .....-.... ..T..AT... ....G..... ..-.......
Psedel    .....-.... ..T....... .......... ..-.......
Psepil    .....-.... ..-....... .......... ..-.......
Psenov    .....-.... .......... .......... ..-.......
Pseher    .....-.... ..T....... .......... ..-.......
Psebol    .....-.... ..T....... ..T....... ..-.......
Psenan    .....-.... .AA..AT... .......... ..-.......
Psegrac   .....-.... .AA..GT... .......... ..-.......
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DHFR intron 1(3 pages):

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Psealb    AAAATATGGG CATTGGCAAG AACGGAGACC TGCCCTGGCC TCTGCTCAGG TACGGGCTGG GCTGGGCGCG GGAAACCGAG
Pseapo    .......... .......... .......... .......... .......... .......... .......... ..........
Psefum    .......... .......... .......... .......... ..C....... .......... .......... .........C.
Pseher    .......... .......... .......... .......... .......... ...-----.. .........T ..........
Psepil    .......... .......... .......... .......... .......... ...-----.. .......... ..........
Psenov    .......... .......... .......... .......... .......... ...-----.. .......... ..........
Psedel    .......... .......... .......... .......... .......... ...-----.. .......... ..........
Psenan    .......... .......... .......... .......... ..C....... .......... .......... .....-....
Psegra    .......... .......... .......... .......... .......... .......... .......... ..........
Psehig    .......... .......... .......... .......... .......... .......... .......... ..........
Psefie    .......... .......... .......... .......... .......... .......... .......... ..........
Notfus    .......... .......... .......... .......... .......... ...C...... .......... ..........
Notale    .......... .......... .......... .......... .......... ...C...... .......... ..........
Pseocc    .......... .......... .......... .......... .......... ...C...... .......... ..........
Notmit    .......... .......... .......... .......... .......... ...C...... .......... ..........
Masfus    .......... .......... .......... .......... .......... ...-----.. .......... ..........
Psesho    .......... .......... .......... .......... .......... .......... .......... ..........
Pseora    .......... .......... .......... .......... .......... .......... .......... ..........
Lepcon    .......... .......... .......... .......... .......... .......... .......... ..........
Legfor    .......... .......... .......... .......... .......... ...C...... .......... ..........
Psepat    .......... .......... .......... .......... .......... .......... ........G. .....T....
Psecha    .......... .......... .......... .......... .......... .......... ........G. .....TT...
Psecal    .......... .......... .......... .......... .......... .......... ........G. .....T....
Pselab    .......... .......... .......... .......... .......... ....---... ........G. .....TT...
Urocau    .......... .......... .......... .......... .......... .......... .......... ..........
Zyzarg    .......... .......... .......... .......... .......... .......... .......... ..........
Zyzwoo    .......... .......... .......... .......... .......... .......... .......... ....G.....
Mesmac    .......... .......... .......... .......... .......... .......... .......... ..........
Mesgou    .......... .......... .........  .......... .......... ..T....... .......... ..........
Rattun    .......... .......... .........T .......... .......... ...TAA.C.. .......T.. .......C..
Melcer    .......... .......... .......... .......... .......... .......... .......... ..........
Psedes    .......... .......... .......... .......... .......... .......... .......... ..........
Psebol    .......... .......... .......... .......... .......... ...-----.. .......... ..........
Psejoh    .......... .......... .......... .......... .......... ....---... ........G. .....TT...
Conpen    .......... .......... .......... .......... .......... .......... .......... ..........
Xermyo    .......... .......... .......... .......... .......... .......... .......... ..........

Psealb    GCGGTTCACC GAGTTGGGTC GAGCACTTGC CTTGGACGCA CAGGCTGACC TGCTTGGAGT CGGGTCTAAG TGGATAGGCC
Pseapo    .........T .......... .......... .......... .......... .......... .......... ..........
Psefum    .........T .......... .......... .......... .....C.... .......... .......... ..........
Pseher    .........T .......... .......... .......... .....C.... .......... .G........ ..........
Psepil    .........T .......... .......... .......... .....C.... .......... .G........ ..........
Psenov    .........T .......... .......... .......... .....C.... .......... .G........ ..........
Psedel    .........T .......... .......... .......... .....C.... .......... .G........ ..........
Psenan    .........T ....A..N.. .......... .......... .....C.... .......... .......... ..........
Psegra    .........T ....A..T.. .......... .......... ..T..C.... .......... .......... ..........
Psehig    .........T .......... .......... .......... .....C.... .......... .......... ..........
Psefie    .........T .......... .......... .......... .T...C.... ........A. .......... ..........
Notfus    .........T .......... .......... .......... .....C.... .......... .......... ..........
Notale    .........T .......... .......... .......... .....C.... .......... .......... ..........
Pseocc    .........T .......... .......... .......... .....C.... .......... .......... ..........
Notmit    .........T .......... .......... .......... .....C.... .......... .......... ..........
Masfus    .........T ....A..... .......... .....----- ----C.... .......... .......... ..........
Psesho    .........T ....A..... .......... ......T... .....C.... .......... .......... ..........
Pseora    .........T ....A..... .......... ......T... .....C.... .......... .......... .......A..
Lepcon    .........T ....A..... .......... ....A..... .....C.... .......... ...A...... ..........
Legfor    .........T ....A..... .......... .......... .....C.... ........C. .......... .....G....
Psepat    .........T ....A..... ..T..T.... .......... .....C.... .......... .......... ..........
Psecha    .........T ....A..... ..T..T.... .......... .....C.... .......... .........C ..........
Psecal    .........T ....A..... ..T..T.... .......... .....C.... .......... .........C ..........
Pselab    .........T ....A..... ..T..T.... .......... .....CA... .......... .......... ..........
Urocau    .........T .--------- ---------- ---------- .....G.... .......... .........A C........
Zyzarg    .........T ....A..... .......... .......... .....C.... .......... .......... ..........
Zyzwoo    .........T ..A.A....T .......... .......... .....C.... .......... .......... ..........
Mesmac    .........T ....A..... .......... .......... .....C.... .......... .........C ..........
Mesgou    .........T ....A..... .......... .......... .....C.... .......... .........C ..........
Rattun    .......C.T ....A...G. .G..G....G .........G .....C.... ....C....A ....CG.G.. C---C.....
Melcer    .........T ....A..... .......... .......... .....G.... .......... .........A C........
Psedes    .........T ....A..... .......... ......T... .....C.... .......... .......... ..........
Psebol    .........T .......... .......... .......... .....C.... .......... .G........ ..........
Psejoh    .........T ....A..... ..T..T.... .......... .....CA... .......... .......... ..........
Conpen    .........T ..A.A..... .......... .......... .....C.... .......... .........C ..........
Xermyo    .........T ....A...-- ---------. .......... .....C.... .......... .......C.. C.....A...
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Psealb    CTGCTGGCTT CAGCAAGAGG CAAAGCTACT ----GCTGCC CGCGGTGATC CCCCTGCCGT GCCAGCCTTT GCTCACAGGC
Pseapo    .......... .....T.... .......... ----...... .......... .....A.... .......... ..........
Psefum    ....C..... .......... .......... ----...... .......... .......... .....T.... ..........
Pseher    ....C...C. .......... .......... ----...... .......... ..T...G.. .......... ..........
Psepil    ....C...C. .......... ........T. ----...... .......... .......G.. .......... ..........
Psenov    ....C...C. .......... .......GT. ----...... .......... .......G.. .......... ..........
Psedel    ....C...C. .......... ........T. ----...... .......... .......G.. .......... ..........
Psenan    ....C..... .......... .......... ----...... .........G .......... .......... ..........
Psegra    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Psehig    ....C...C. .......... .......... ----...... .......... .......... .......... ..........
Psefie    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Notfus    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Notale    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Pseocc    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Notmit    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Masfus    ....C..... ..........T.......... ----...... .......... .......... .......... ..........
Psesho    ....C..... ..........G.......... ----...... .......... .......... .......... ..........
Pseora    ....C..... .......... .........A ----...... .......... .......... .......... ..........
Lepcon    ....C..... ....G..... .......... ----...... ..G....... .......... .......... ..........
Legfor    ....C..... ....G..... .......... ----...... .N........ .......... .......... ..........
Psepat    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Psecha    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Psecal    ....C..... .......... .......... ----...... .......... .......... .......... ..........
Pselab    ....C..... .......... .......... ----...... .......... ........C. .......... ..........
Urocau    ....C..... ....G..... .......... ----...... .A........ .......... .......... .T........
Zyzarg    ....C..... .....G.... .......... ----...... .......... .......... .......... ..C.......
Zyzwoo    ....C..... .....G.... .......... ----...... .......... T......... .......... ..C.......
Mesmac    ....C..... ....G..... .......... ----...... .......... .......... .......... ..........
Mesgou    ....C..... ....G..... .......... ----...... .......... .......... .......... ..........
Rattun    ....C...C. ....G..... AC.....GTC ----.G.... G......... .......... .......... ..........
Melcer    ....C..... ....G..... .......... ----...... .......... .......... .......... ..........
Psedes    ...TC..... .......... .......... ----...... .......... .T........ .......... ..........
Psebol    ....C...C. .......... .......... ----...... .......... .......... .......... ..........
Psejoh    ....C..... .......... .......... ----...... .......... ........C. .......... ..........
Conpen    ....C..... ....G..... .......... TACT...... .......... .A......A. .......... ..........
Xermyo    ....C..... ....G..... .........C ----.G.... .......... ...-...... .......... ..........

Psealb    TCCCTGGCCG GGAACAAAGT CCACTCACTG GGCAGGCAGC AACACCACCA CC-CCCACAC CGGGGCTTGC ACCTTTCGCC
Pseapo    .......T.. .......... .......... .......... .......... ..-....... .......... ..........
Psefum    .......... .......... .......... .....----. .......... ..-....... .......... ..........
Pseher    .......... .......... ...G...... .......... .........- ---....... .......... ..T.......
Psepil    .......... .......... ...G...... .......... .........- ---....... .......... ..T.......
Psenov    .......... .......... ...G...... .......... .........- ---....... ..T....... ..T.......
Psedel    ...T...... .......... ...G...... .......... .........- ---....... .......... ..T.......
Psenan    .......... .......... ...G...... .......... ....A..... ..-....... ...--..... ..........
Psegra    .......... .......... ...G...... .......... ....A..... ..A.A..... ...--..... ..........
Psehig    .......... .......... ...G...... .......... .......... ..-....... .C........ ..T.......
Psefie    .......... .......... ...G...... ...G...... .........C ..C....... .C........ C.T.......
Notfus    .......... .......... ...G...... .......... .......... ..-....... .......G.. ..........
Notale    .......... .......... ...G...... .......... .......... ..-....... .......G.. ..........
Pseocc    ........T. .......... ...G...... .......... .......... ..-.T..... .A........ ..........
Notmit    .......... .......... ...G...... .......... .......... ..-....... .......G.. ..........
Masfus    .......... .......... ...G...... .......... ....A..... ..-....... .......... ..T.......
Psesho    .......... .......... ...G...... .......... .......... ..-....... .......... ..........
Pseora    .......... .......... ...G...... .......... .......... ..-....... .......... T.........
Lepcon    .......... .......... .......... .........T .......... ..-....... ....A..... ........T.
Legfor    .......... .......... ...G...... .......... .......... G.-....... ...AA..... .......CT.
Psepat    .......... .......... ...G...... .......... .......... ..-....... ....A..... ..........
Psecha    .......... .......... ...G...... .......... .......... ..-....... .......... ..........
Psecal    .......... .......... ...G...... .......... .........C ..-....... T......... ..........
Pselab    .......... .......... ...G...... .......... .......... ..-....... .......... ..........
Urocau    .......... .......... ...G...... .......... .......... ..-....... .......... ........T.
Zyzarg    .......... .......... ...G...... .......... .......... ..-....... ..A....C.. ........T.
Zyzwoo    .......... .......... ...G...... .......... .......... ..-....A.. .......C.. ..G.....T.
Mesmac    .......... .......... ...G...... .......... .......... ..-....... ....A..... ........T.
Mesgou    .......... .......... ...G...... .......... .......... ..-....... ....A..... ........T.
Rattun    ........T. A..G...... ..GGC..... AA..A..... .G........- ..-G.-.... .......C.. ........T.
Melcer    .......... .......... ...G...... .......... .......... ..-....... .......... ........T.
Psedes    .......... .......... ...G...... .......... .......... ..-....... .......... .........G
Psebol    .......... .......... ...G...... .A........ .........- ---....... .......... ..T.......
Psejoh    .......... .......... ...G...... .......... .......... ..-....... .......... ..........
Conpen    .......... .......... ...G...... .......... .......... ..-....... ....A..... ........T.
Xermyo    .......... .......... ...G...... .......... ..T....... ..-....... .......... ......A.GA
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Psealb    GCTGACTTGA GCCTGAGCAC ACGTGACAGG GATTAACGCA GTGTTTCTC- TAACTTTCAG GAATGAGTTC AAGTACTT
Pseapo    .......... .......... .......... .......... .........- .......... .......... ........
Psefum    .......... .......... .......... .......... .........- .......... .......... ........
Pseher    .......... .......... .......... .......... .C.......- .......... .......... ........
Psepil    .......... .......... .......... .......... .C.......- .......... .......... ........
Psenov    .......... .......... .......... .......... .C.......- .......... .......... ........
Psedel    .......... .......... .......... .......... .C.......- .......... .......... ........
Psenan    .......... .......... .......... .......... .........- .......... .......... ........
Psegra    .......... .......... .......... .......... .........- .......... .......... ........
Psehig    .......... .......... .......... ....G..... .C.......- .......... .......... ........
Psefie    ..........T......... .......... .......... .C.......- .......... .......... ........
Notfus    .......... .......... .......... .......... .C.......- .......... .......... ........
Notale    .......... .......... .......... .......... .C.......- .......... .......... ........
Pseocc    .......... .......... .......... .......... .........- .......... .......... ........
Notmit    .......... .......... .......... .......... .C.......- .......... .......... ........
Masfus    .......... .......... .......... .......... .C.......- .......... .......... ........
Psesho    .....T.... ........T. .......... A......... .C.......- .......... .......... ........
Pseora    .......... .......... .......... .......... .C.......- .......... .......... ........
Lepcon    .......G.. .......... .......... AG........ N.......- .......... .......... ....N...
Legfor    .......G.T .......... .......... .G........ .........- .......... .......... ........
Psepat    .......... .......... .......... .......... .C.......C .......... .......... ........
Psecha    .......... .......... .......... .......... .C.......T .......... .......... ........
Psecal    .......... .......... .......... .......... .C.......T .......... .......... ........
Pselab    .......... .......... .......... .......... .........- .......... .......... ........
Urocau    .......G.. .......... .......... AG........ .........- .......... .......... ........
Zyzarg    .......G.. .......... .......... AG....T... .........- .......... .......... ........
Zyzwoo    .......G.. .......... ...A...... -C-....... .........- .......... .......... ........
Mesmac    .......G.. .......... .......... AG........ .........- .......... .......... ........
Mesgou    .......G.. .......... .......... AG........ .........- .......... .......... ........
Rattun    CT.....G.. .......... ..ACATG.CT .G.....A.. .........- ----...... .......... ........
Melcer    .......G.. .......... .......... .G........ .........- .......... .......... ........
Psedes    .....T.... ........T. .......... .......... .C.......- .......... .......... ........
Psebol    .......... .......... .......... .......... .C.......- .......... .......... ........
Psejoh    .......... .......... .......... .......... .........- .......... .......... ........
Conpen    .......G.. .......... .......TA. AG........ .....-...- .......... .......... ........
Xermyo    .......G.. .......... .......... NG........ .........- .......... .......... ........
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