Socioecology and Phylogeography of the Yellow-bellied Glider (*Petaurus australis*)

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Abstract

Marsupials have complex and interesting socioecology and life history strategies that differ quite markedly to much-studied eutherian mammals. However, the socioecology and life history strategies of a number of Australian marsupials are most often observed only within the context of a much larger study on their ecology. My aim was to study, using a combination of behavioural observations and molecular DNA techniques, aspects of the socioecology of a population of yellow-bellied gliders (*Petaurus australis*) in Rennick State Forest, south-western Victoria. Petaurid gliders feed on plant and insect exudates, pollen/nectar and arthropods. Yellow-bellied gliders are arboreal, rare, nocturnal and cryptic, have persistent pair bonds, are territorial and exist in low population densities. In particular, I sought to confirm that the Rennick population of yellow-bellied gliders maintained a predominantly monogamous mating system. I also sought to confirm that the timing of reproduction in this population of yellow-bellied gliders would be seasonal, and timed to coincide with peaks in the abundances of two indices of protein food resources (i.e. flowering and bark shed). In a more broadscale study, I sought to examine the geographic distribution of mitochondrial haplotyes and morphological variation of the yellow-bellied glider throughout its range.

Polymorphic microsatellite loci are the choice of genetic marker for fine-scale studies, such as relatedness and paternity. Microsatellite loci had previously only been characterised and optimised for *Petaurus norfolcensis* (squirrel gliders). However, close inspection of the GenBank sequences revealed the presence of replicates differing only by sequencing errors. A panel of seven polymorphic tetranucleotide loci in *Petaurus breviceps* (sugar gliders) and three polymorphic trinucleotide loci in *P. australis* were isolated and optimised. Five *P. breviceps* loci were polymorphic in *P. norfolcensis* and two were polymorphic in *P. australis*. Only one *P. australis* locus was variable in *P. breviceps* and *P. norfolcensis*. No locus showed a deficit in heterozygotes according to Hardy-Weinberg expectations, and the large number of alleles for some of the loci confirmed their usefulness for studies in relatedness and paternity.

A number of Australian arboreal marsupials have been reported to show monogamous and polygynous mating systems in different populations, but previous studies have not included genetic analyses to confirm the observations. My aim was to test the hypothesis that monogamy was the predominant mating system in a population of yellow-bellied gliders (*Petaurus australis*) in south-western Victoria. Home range overlap, cohesiveness of pairs, rates of den site co-occupancy and location of den trees within the home ranges of 13 gliders were determined via radio-tracking. A monogamous social system predominated, demonstrated by extensive home range overlap between cohabiting adult males and females (40-100%) and little home range overlap between adjacent territories (< 7%). Males spent approximately 55% of their active time within 25m of their female partners and 55-85% of their sleeping time in dens with their female partner. The paternity of all juveniles within the population was analysed using five microsatellite DNA markers. Of 37 individuals genotyped, 12 of 13 juveniles could be attributed to the resident adult male. My results suggest that social monogamy equates with genetic monogamy in this population of yellow-bellied gliders.

Mammalian taxa living in seasonal environments usually coincide energy-demanding reproductive activities with the seasonal availability of food resources. However, few studies on arboreal marsupial taxa in Australia have focussed upon the interplay of forest phenology and the timing of breeding. This study examined forest phenology in a temperate environment, and the timing of reproduction the yellow-bellied glider. I captured adult females once per month between August 2001 and August 2003 to determine reproductive condition, and monitored indicators for two key food resources over the same period. Flowering phenology (as an index of pollen availability) was assessed in 170 manna gum (*Eucalyptus viminalis*) and brown stringybark (*E. baxteri*) trees, while bark shed (as an index of arthropod availability) was assessed in 45 manna gum, the only eucalypt species at this site that sheds it bark. Aseasonal reproduction was indicated within this population of gliders, as distributions of births were not statistically different from random. However, yellow-bellied gliders did exhibit distinct birth peaks in spring, summer and winter, when data were combined for both years. The temporal distributions of flowering for both eucalypt species were statistically different from random, indicating seasonal availability of nectar and pollen. Peak flowering occurred in summer for brown

stringybark, and autumn for manna gum in both years, although for manna gum peak abundance of flowers was one month earlier in the second year. While the temporal distribution of bark shed on the trunks of trees did not differ from random, it did show seasonality on the main and outer branches, peaking in summer and declining thereafter. Thus, it appears that yellow-bellied gliders breed aseasonally in a predictable, seasonal environment. However, yellow-bellied gliders have a reliance on the complex temporal interplay of different seasonal food resources.

Subspecific status has often been used as a surrogate for conservation unit, but does not always reflect intra-specific lineages with different evolutionary histories. One contentious case of subspecific classification occurs in the yellow-bellied glider, a marsupial species showing considerable decline in population size and requiring conservation management. Our aim was to assess the current subspecific status of populations and define units of conservation using a combination of phylogeographical analyses of mitochondrial DNA and morphological analyses. Analyses of the mitochondrial ND4 gene provided evidence for significant phylogeographic structure within yellow-bellied gliders. Isolated populations in north Queensland (NQ) and Victoria/ South Australia were genetically distinct from populations in New South Wales and southern Queensland. Morphological analyses provided little evidence for discrimination of populations, although NQ specimens were generally smaller in size compared to southern forms. My analyses do not support the classification of subspecies, *P. a. reginae*, for the original type specimen from southern Queensland. Taking into account other behavioural and ecological data, and the disjunct distribution of NQ populations from southern populations, I propose that the NQ population represents a distinct Evolutionarily Significant Unit, a lineage showing highly restricted gene flow with the rest of the species.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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Meredeth Brown

26 June 2007

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This thesis is dedicated to my mum and dad

Chapter 1. General introduction

1.1 Introduction and definitions

In this introduction I provide background information to my research on yellow-bellied gliders and define certain terms. The thesis reflects two main themes: chapters 2, 3 and 4 comprise an investigation of the socioecology of the yellow-bellied glider. Chapter 2 describes characterisation and optimisation of a panel of microsatellite loci suitable for a paternity analysis to confirm the gliders' mating system, while chapters 3 and 4 detail an examination of the gliders' social and mating systems, and reproductive ecology. Chapter 5 examines the phylogeography of yellow-bellied gliders throughout their range and addresses some issues of conservation management. Thus, a fine-scale study of their behavioural ecology is complemented by a broad-scale study of the gliders' conservation.

1.2 Social and mating systems

1.2.1 Monogamous social and mating systems

In this thesis, social system is defined as the observed spatial distribution of adult males and females, with respect to one another and their conspecifics, and does not mean to imply the sexual mating system. Social polygamy may result whenever females are gregarious and live in home ranges that are defendable by a single adult male. Social monogamy is the close association of a single adult male and female to the exclusion of others, and may be for the length of the breeding season (e.g. migratory dabbling ducks (Sorenson 1992) or the life of the partner (e.g. Malagasy giant jumping rat, *Hypogeomys antimena*) (Sommer 2003). The use of the term mating system implies the sexual or genetic system amongst adult males and adult females that may, or may not, be evident from the observed social system.

Apart from birds, wherein approximately 90% of species have a monogamous social and/or mating system (Kleiman 1977), a diverse range of taxa are considered to be monogamous; socially and/or genetically, e.g. snapping shrimp (*Alpheus angulatus*)

(Mathews 2002a) and lizards (*Tiliqua rugosa* and *Egernia stokesii*) (Bull *et al.* 1998; Bull 2000; Gardner *et al.* 2002). Amongst the mammalian taxa, approximately 15% of all primate species are considered to be monogamous (van Schaik and Kappeler 2003). Examples of other mammalian monogamous taxa include some ruminants (*Oreotragus oreotragus* (klipspringer), *Capricornis crispus* (Japanese serow) and *Madoqua kirkii* (Kirk's dikdik)) (Kishimoto and Kawamichi 1996; Komers 1996; Brotherton *et al.* 1997; Roberts and Dunbar 2000; Kishimoto 2003), rodents (*Marmota marmota* (Alpine marmot), *Peromyscus californicus* (California mouse), *Hypogeomys antimena* (Malagasy giant rat) and *Castor* spp. (beavers)) (Ribble 2003; Sommer 2003; Sun 2003; Cohas *et al.* 2006), and prairie dogs (*Cymomys gunnisoni*) (Travis *et al.* 1996), although this list is not exhaustive.

Constructing a generalised theoretical framework within which to explain factors important in the evolution of monogamy has been difficult because there appears to have been no single evolutionary pathway that monogamy has taken in all species (Reichard 2003). For example, amongst some of the ruminants referred to above, mate guarding appears to be the most important factor contributing to the evolution of monogamy (Komers 1996; Roberts and Dunbar 2000; Brotherton and Komers 2003). Mate guarding also appears to be important for monogamy in some primate species e.g. fat-tailed dwarf lemurs (*Cheirogaleus medius*) and fork-marked lemurs (*Phaner furcifer*) (Fietz 1999; Schülke 2005). However, amongst prairie dogs, changes in food resource availability and demographics appear to be the most important factors (Travis *et al.* 1995; Travis *et al.* 1996), whereas biparental care may be important for the rodents, Malagasy giant rat and California mouse (Sommer 2000; Ribble 2003). Further, although eutherian taxa have been well described in the monogamy literature, monogamy in Australian marsupial taxa has been relatively unexplored, with many studies of social behaviour embedded within larger studies of behavioural ecology (although see Spencer *et al.* 1998; Runcie 2000; Martin 2005). Examining monogamy in marsupial taxa may provide supporting and independent evidence for existing hypotheses about the evolution of monogamy, or provide evidence of some new factors, and thus make a useful

contribution to understanding the evolution of this uncommon social behaviour in mammals.

1.2.2 Inter- and intraspecific variation in social and mating systems

Variation in social and mating systems may arise from the preparedness of females to be gregarious, which, in turn, may depend upon the distribution of resources or risk of predation (Emlen and Oring 1977). If females are not gregarious, but live in spatially dispersed, non-overlapping home ranges, and do not live with more than one adult male, monogamy ensues (Reichard 2003). However, the spatial dispersion of females is usually not sufficient to ensure the maintenance of monogamy, and behavioural strategies such as paternal care (Kleiman 1977; Clutton-Brock 1989), mate guarding (Brotherton and Komers 2003) and territoriality (Fricke 1986) may be important. Thus, quantifying behavioural traits and comparing them among populations with different mating systems may provide insights into the evolution and maintenance of monogamy. Although parental care has been studied in some monogamous mammalian taxa (see Terborgh and Goldizen 1985; Dunbar 1995; Sommer 2000; Ribble 2003), variations in the efficacy of mate guarding and territorial behaviour have not been well studied or quantified (see Reichard 2003). In part, this may be because of the difficulties involved in studying animals that may be dangerous, cryptic or nocturnal. However, use of microsatellite DNA technologies (see section 2.1.3 below) may provide an indirect measure of the efficacy of mate guarding by detecting the number of extrapair fertilisations (EPFs) (e.g. Bull *et al.* 1998).

In many instances, the social system may mask the mating system, in that observed social groupings may not accurately predict the relatedness of individuals within and amongst those groups. EPFs are also now known to be an important feature of many monogamous mating systems (Reichard 2003). These result from adult males and/or females seeking matings outside the pairbond or social group (Birkhead and Møller 1992; Birkhead and Møller 1995). The level of EPFs varies widely between species with monogamous mating systems (Birkhead and Møller 1995; Petrie and Kempenaers 1998) from species wherein EPFs have been not detected, e.g. the

Capricorn silvereye (Robertson *et al.* 2001), to species wherein up to 50% of offspring are from adult males not directly associated with the main social group, e.g. some species of lemur (Fietz *et al.* 2000; Schülke *et al.* 2004). Variation in the levels of EPFs also exists between different species of the same genus, and even different populations of the same species (see Petrie and Kempenaers 1998). Therefore, it is necessary to substantiate observations of the social system with paternity analyses, and confirmation of the mating system and detection of EPFs.

1.2.3 Use of DNA technologies in studies on behaviour

The best way to confirm the mating system or to detect the occurrence of EPFs is to conduct a paternity analysis with the use of microsatellite DNA technology. Microsatellites are short sections of DNA comprised of tandem repeats, which are usually repeats of two, three or four basepairs, e.g. AG, AAG or AAAG (Tautz and Schlotterer 1994). The advantage of microsatellites is that they are biparentally inherited, conforming to Mendelian inheritance, abundant throughout the genomes of eukaryotes, and are often highly polymorphic (Tautz and Schlotterer 1994). Microsatellite markers are commonly used in studies of fine-scale population structure, such as relatedness or kin structure (Bruford and Wayne 1993; Sunnucks 2000). Microsatellite loci are also useful for providing indirect information on behaviour in species that cannot be easily observed, such as cryptic or nocturnal species, or specific behaviours that cannot be easily observed, such as mating behaviour or dispersal. Panels of ≥ 5 polymorphic microsatellite loci are commonly used in paternity analyses, particularly in studies that include data on social behaviour, with much larger panels for studies that lack data on social behaviour; e.g. 16 loci were used in a study on the mating system of badgers (*Meles meles*) that did not have information on social behaviour (Carpenter *et al.* 2005).

1.3 Reproductive ecology

1.3.1 Life history strategies in a seasonal environment

Aspects of life history may profoundly influence the social or mating system. For example, synchronicity in breeding may determine whether adult males are able to monopolise more than one adult female (Westneat and Sherman 1997; Stutchbury

1998a; Stutchbury 1998b; Weatherhead and Yezerinac 1998; Isaac and Johnson 2003). Conversely, a relaxation of synchronicity in breeding may allow adult males to locate, court and mate with more than one sexually receptive female (see Stutchbury 1998b; Stutchbury 1998a; Weatherhead and Yezerinac 1998; Saino *et al.* 1999; Isaac and Johnson 2003). Thus, we may observe a monogamous social structure, but it may be characterised by high levels of EPFs because males are able to visit and mate with neighbouring adult females. Alternatively, if adult females breed highly synchronously, a monogamous social structure should be characterised by low levels of EPFs, because males are not able to monopolise more than one adult female during the breeding season. However, the timing of breeding in females depends largely upon the environmental conditions in which they live (Sadleir 1969). Living in seasonal environments can prompt seasonal breeding, because females usually time their reproductive activities with peaks in food resource abundance (Sadleir 1969). For example, many primates living in tropical regions breed seasonally (on a one year cycle) in accordance with the seasonal availability of high quality food resources, such as fruits (reviewed by Di Bitetti and Janson 2000). Although photoperiod was an important cue for the timing of reproduction in primates living at high latitudes, food availability explained much of the variation in timing of breeding for primates living nearer the equator (Di Bitetti and Janson 2000).

Food availability is important for females of different species at different times of their reproductive cycles. Females may breed at times when food availability is high thus ensuring enough energy throughout pregnancy, birth and lactation (capital breeders), or may breed outside peak food availability times relying on fat storage to ensure enough energy for pregnancy, birth and lactation (income breeders) (Jönssons, 1997). Marsupials are different from eutherian mammals in that their energy requirements for pregnancy and birth are low, with peak energy demands concentrating during lactation and in particular, late lactation. Increases in total solids, and protein and lipid content in milk during late lactation have been detected in a number of marsupials (Munks *et al.* 1991; Rose and Flowers 2005, Rose *et al.* 2003, also reviewed in Green and Merchant

1988), with similarities in changes in milk composition across different marsupial taxa (Green 1984; Green and Merchant 1988).

1.3.2 Seasonal conditions and forest phenology

In Australia, environmental conditions vary from temperate regions in the south, through to semi-arid and arid regions in central Australia and sub-tropical and tropical regions in the north. The milder, more temperate regions in the southern parts of Australia experience distinct seasonal changes with cool, wet winters and warm, dry summers. Associated with seasonal conditions in southern Australia are fluctuations in the relative abundance of flowering for a variety of plant species, as well as abundances in arthropods. Species from genera such as *Eucalyptus*, *Banksia*, *Acacia* and *Melaleuca* form important food resources for a number of glider species, including the sugar glider (*Petaurus breviceps*), squirrel glider (*Petaurus norfolcensis*) and yellow-bellied glider (*P. australis*) inhabiting eucalypt forests in southern Australia (Smith 1982; Henry and Craig 1984; Craig 1985; Goldingay 1986; Kavanagh 1987b; Kavanagh 1987a; Menkhorst and Collier 1987; Goldingay 1989b; Sharpe and Goldingay 1998; Carthew *et al.* 1999; Sharpe 2004). Accordingly, at least some of these glider species have been recorded breeding seasonally in these regions (Goldingay and Kavanagh 1990; Goldingay 1992; Quin 1995; van der Ree 2002). However, eucalypt forests vary throughout Australia with respect to their structural diversity. Some forests in NSW contain a high diversity of flowering species, including some winter-flowering species (Kavanagh 1987b; Kavanagh 1987a; Goldingay and Kavanagh 1991), while some forests in south-western Victoria contain a very low diversity of flowering species (Carthew *et al.* 1999). The low level of diversity in flowering species may mean that gliders inhabiting forests in areas such as south-western Victoria are not only likely to breed seasonally, but may be adapted to timing reproductive events to coincide with very narrow windows within which peak abundances in food resources are available.

1.4 Phylogeography and conservation units

1.4.1 Conservation units

There are few agreed upon criteria that objectively classify fauna, for the purposes of conservation management, below the level of species. Arguably, the most important and useful concept for classifying fauna below the level of species has been that of the Evolutionarily Significant Unit (*sensu* Ryder 1986; Moritz 1994a; Moritz 1994b). The concept of evolutionarily significant units (ESUs) was raised in an attempt to identify and prioritise conservation efforts towards populations that represented significant adaptive genetic variation (Ryder 1986). It was proposed that ESUs could be identified by concordance between independent datasets, such as taxonomic, ecological, geographic and molecular datasets (Ryder 1986). However, Moritz (1994b) proposed that molecular data alone be used to objectively allocate fauna to one of two conservation units that are hierarchically arranged. Specifically, Moritz (1994b) proposed that ESUs be distinguished by reciprocal monophyly of mitochondrial DNA and significant divergence of allele frequencies at nuclear loci between populations. Alternatively, management units (MUs) are defined by divergence of allele frequencies between populations, as evidenced by mitochondrial and/or nuclear DNA, but reciprocal monophyly is not a criteria (Moritz 1994b). For management purposes, ESUs may be considered as historically isolated and independently evolving units, whereas MUs represent geographically and/or demographically independent units (Moritz 1994b; Moritz 1999). Animals within ESUs would share mitochondrial haplotypes that may be unique within the species, and are considered more closely related to one another than animals from other populations (Avise 2000). It has been proposed that each ESU needs to be managed as a separate entity, and careful consideration should be given to decisions about supplementing such populations with animals from other populations, as it may lead to outbreeding depression (Moritz 1999). However, there has been some debate as to whether molecular data alone should be used as criteria for ESUs and MUs (*sensu* Moritz 1994b), as there is no universally agreed best method in all situations for the reconstruction of phylogeny (Waples 1995), and reciprocal monophyly may be not be evident in highly mobile animals, such as birds, that have high levels of gene flow (Crandall *et al.* 2000).

In this thesis, I have used the Fraser and Bernatchez (2001) definition of conservation units. They argue that the concept of ESUs should not be abandoned (as suggested by Crandall *et al.* 2000), but that criteria for conservation units should be flexible, and applied on a case-by-case basis (Fraser and Bernatchez 2001). This concept is known as 'adaptive evolutionary conservation' (AEC), and provides a framework within which criteria, other than strictly molecular, may be used to assign organisms to units for conservation management (Fraser and Bernatchez 2001). Thus, their definition of ESUs allows for the use of datasets other than strictly molecular as criteria for definition of conservation units, including ecological, morphological and biological data. The Fraser and Bernatchez (2001) definition of an ESU is 'a lineage demonstrating highly restricted gene flow from other such lineages within the higher organizational level (or lineage) of the species' (Fraser and Bernatchez 2001, p. 2747). It is consistent with those of other authors in that 'the accumulation of 'genetic differences' through reproductive isolating mechanisms are critical factors in defining evolutionary lineages for conservation' (pg. 2747). This definition is particularly appealing because it focuses upon the maintenance of evolutionary variants that are the result of historical processes (Fraser and Bernatchez 2001).

In this thesis, I used the mitochondrial ND4 gene (NADH dehydrogenase subunit 4, including tRNA-His, tRNA-Ser and tRNA-Leu) to examine genetic differences between populations of yellow-bellied gliders throughout their range. The use of mitochondrial DNA (mtDNA) markers for population genetic analyses has a number of advantages in that mtDNA evolves relatively rapidly, generally lacks recombination, and because it is maternally inherited, it has a lower effective population size compared to nuclear markers (Avise 2000). The latter is important, as mtDNA will more quickly go to reciprocal monophyly within populations showing highly restricted gene flow than nuclear DNA. In addition to the use of mtDNA markers, I examined differences in skull morphology in yellow-bellied gliders throughout their range, and finally compared ecological differences between populations of yellow-bellied gliders.

1.5 The study species: the yellow-bellied glider (*Petaurus australis*)

1.5.1 Description of the study species

The yellow-bellied glider belongs to the Family Petauridae, that contain a number of possum and glider species. Yellow-bellied gliders have a furred patagium extending from wrist to ankle that enables them to glide easily 25-30 m between trees. They have a pink rhinarium and large naked ears, grey or brown dorsal fur with a dark dorsal stripe. The ventral fur may range in colour from pale lemon or white to deep gold and may deepen in colour as the glider ages. Gliders have a range of calls, the most distinctive of which can be heard by humans up to 500 m away. Yellow-bellied gliders are exudivores that feed predominantly upon phloem sap from eucalypts and acacias, nectar and pollen, invertebrates and honeydew and manna. Phloem sap is obtained by gliders incising into the bark, usually in a v-shape, and licking the exudate that pools at the bottom of the v. This species lives in small family groups, usually consisting of an adult male and female plus offspring, although the size and composition of groups vary between populations. Yellow-bellied gliders sleep in tree hollows during the day and are active at night. They are totally arboreal and cryptic in behaviour. Adult males and females are sexually size dimorphic, with males weighing more than females. Adult males also have a scent gland on top of their heads that is lacking in adult females. Adult females have a pouch with one nipple in each of two compartments that is separated by a furred septum.

1.5.2 Distribution and conservation status

The range of the yellow-bellied glider extends from isolated populations in north Queensland, through south-eastern Queensland, eastern NSW, eastern and southern Victoria, with a single population remaining in south-eastern South Australia (see Fig. 5.1, Chapter 5). Yellow-bellied gliders are associated with mature eucalypt forests upon which they depend for foraging substrates and shelter, and occur patchily throughout their distribution (Goldingay and Possingham 1995; Carthew 2004). In particular, yellow-bellied gliders forage on plant and insect exudates, nectar/pollen and arthropods, and use tree hollows for shelter during the day (Smith and Russell 1982; Henry and Craig 1984; Craig 1985; Goldingay 1986; Goldingay 1989b; Goldingay 1990;

Goldingay and Kavanagh 1991; Quin *et al.* 1996a; Carthew *et al.* 1999). The species is of conservation concern because eucalypt forests have been subject to extensive habitat fragmentation and degradation, and many are targeted for hardwood timber logging (see Eyre and Smith 1997). Further, areas of suitable glider habitat are often surrounded by a matrix of inhospitable habitat, such as agriculture or softwood timber plantations. The isolated north Queensland (Wet Tropics) populations are listed as vulnerable under the *Environmental Protection and Biodiversity Conservation Act* (1999). The species is listed as vulnerable in NSW and endangered in South Australia. Although it is not listed as threatened in Victoria, populations throughout southern central and western Victoria are isolated and are likely to be subject to the problems that result from a lack of gene flow between populations and small population size, such as genetic drift and inbreeding depression (Frankham *et al.* 2002).

1.5.3 Social behaviour

Yellow-bellied gliders have been reported as being facultatively monogamous, due to the observed variation in their social systems throughout their distribution (Goldingay and Kavanagh 1991; Jackson 2003). They have a monogamous social structure in southern populations (Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990), but are mainly polygamous (Russell 1984) or exhibit a mix of monogamy and polygamy (Goldingay *et al.* 2001) in north Queensland. However, published studies to date have been solely observational, and thus, observations of polygamy in some populations of yellow-bellied glider may reflect failure to detect a monogamous mating system. Conversely, observations of a socially monogamous system may have missed extrapair copulations. Yellow-bellied gliders throughout their range live in small social groups of 2-6 individuals that exhibit territory exclusivity amongst social groups, and have large home ranges of approximately 20-65 ha in size (Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990; Goldingay and Kavanagh 1991; Goldingay 1992; Goldingay and Kavanagh 1993; Goldingay *et al.* 2001). Animals have been observed practising territorial behaviour, including regular loud calling (Kavanagh and Rohan-Jones 1982; Goldingay 1994), and aggressive repelling of intruders from their territories (Russell 1984; Goldingay *et al.* 2001). Loud

calling is a well-documented behaviour in this species (Kavanagh and Rohan-Jones 1982; Goldingay 1994), with calls being heard by humans from a distance of at least 400 m. However, due to the nocturnal, arboreal and cryptic nature of the yellow-bellied glider, territorial behaviour and mate guarding have been difficult to observe and quantify (although see Goldingay 1994).

1.5.4 Diet and reproductive behaviour

Yellow-bellied gliders rely on a diet comprised primarily of phloem sap, but supplemented by other plant and insect exudates, such as nectar, manna and honeydew, as well as protein resources, such as pollen and arthropods (Smith and Russell 1982; Henry and Craig 1984; Craig 1985; Goldingay 1986; Goldingay 1989b; Goldingay 1990; Goldingay and Kavanagh 1991; Quin *et al.* 1996a; Carthew *et al.* 1999). Environmental conditions vary throughout the yellow-bellied gliders' distribution, and the relative importance of food items in the diet, such as flowering or phloem sap varies markedly (Carthew *et al.* 1999). Nevertheless, although phloem sap in eucalypts is carbohydrate-rich, it is protein-poor (Ziegler 1975; also Pate *et al.* 1998 for composition of phloem sap in *Eucalyptus globulus*). Thus, the relative importance of protein food resources, such as flowering and arthropods, may influence the timing of reproductive activities in different populations of yellow-bellied gliders. Timing of breeding in yellow-bellied gliders has been documented from a number of populations throughout their range. Seasonality of breeding has been detected at two populations in southern NSW (Bombala and ~ 170 km to the north, Kioloa), although the timing of breeding was different in the two populations, with breeding taking place much earlier in the Kioloa population (Goldingay and Kavanagh 1990; Goldingay 1992). Births occurred in almost all months in a population in north Queensland (Goldingay *et al.* 2001). Differences in the timing of breeding throughout the range of the yellow-bellied glider may reflect adaptive differences to local fluctuations in food availability. Given the reliance of yellow-bellied gliders on flowering for nectar/pollen resources and arthropods, gliders in the southern parts of Australia are likely to coincide their reproductive activities with seasonal availability of these food resources.

1.5.5 Variation between populations of yellow-bellied gliders

The taxonomic status of the yellow-bellied glider has been historically controversial (Finlayson 1934; Tate 1952), with morphological characters of belly fur colour and smaller size being the basis for the designation of subspecies *P. australis reginae* in northern regions (Thomas 1923). Although yellow-bellied gliders from north Queensland were later reported as having paler belly fur colour than their more southern counterparts (Russell 1979; Winter *et al.* 1979; Russell 1983), this was questioned by other researchers who observed that belly fur colour varied according to the age of the glider (Goldingay *et al.* 2001). Despite contention over the taxonomic status of yellow-bellied gliders, no genetic study has been conducted, and only one unpublished study undertaken on skeletal characteristics of yellow-bellied gliders across populations (Chapman 1992).

1.5.6 Management considerations of isolated populations

There is some imperative to understanding the genetic relationship between populations of yellow-bellied gliders, as management concerns have been raised. The populations of yellow-bellied glider in north Queensland are under threat due to logging practices and the encroachment of rainforest upon wet sclerophyll eucalypt forest, reducing the amount of habitat available to yellow-bellied gliders. Further, the one remaining population in South Australia contains < 12 individuals and is at risk of inbreeding, genetic drift and extinction in the near future. In order to be able to make appropriate decisions about supplementing small populations of yellow-bellied gliders we must have some understanding of the evolutionary history of the different populations. Studies of the distribution of mitochondrial DNA haplotypes have usefully contributed to the management of small populations, e.g. the Thevenard Island mouse (Moro *et al.* 1998). Identifying where populations share mitochondrial haplotypes may assist in avoiding situations where animals used to supplement small populations lead to outbreeding depression and loss of local fitness (Frankham *et al.* 2002). In particular, plans are at an initial stage to connect isolated remnant patches in South Australia, using native corridors, to larger, more contiguous, forests in south-western Victoria (S.

Carthew, pers. comm.). It is, therefore, with some urgency that an investigation into the evolutionary history of isolated populations in this area be conducted.

1.6 Aims of the thesis

My first aim is to test the hypothesis that monogamy is the predominant mating system of a population of yellow-bellied gliders at Rennick in south-western Victoria. This is assessed using a combination of behavioural observations and microsatellite DNA techniques. I further seek to quantify behavioural traits, territoriality and the possibility of mate guarding, which may contribute to the maintenance of monogamy within this population.

My second aim is to assess seasonality of two indices of key protein food resources, flowering and bark shed, available to yellow-bellied gliders, and to test the hypothesis that seasonal breeding is associated with seasonal abundance of these food resources.

Finally, I seek to examine intraspecific morphological variation and the geographic distribution of mitochondrial DNA haplotypes throughout the range of the yellow-bellied glider, and test the hypothesis that yellow-bellied gliders in isolated populations of western Victoria/SA and north Queensland are genetically distinct and represent separate ESUs. I further seek to address issues of conservation management for these populations.

Chapters 3-5 have been written in journal format, incorporating an introduction, methods, results and discussion, and address the above specific aims. Chapter 2 has been written as a technical note and does not include a discussion.

Chapter 2. Characterisation and optimisation of microsatellite loci in *Petaurus australis*, *P. breviceps* and *P. norfolcensis*

2.1 Preamble

This chapter was submitted to and published in Molecular Ecology Notes in 2004 (a copy of which appears at the end of this thesis) and presents work that was carried out in the laboratory by others and myself. Specifically, Mr Huw Cooksley, School of Molecular and Biomedical Science, The University of Adelaide, extracted DNA from some *Petaurus australis* and *Petaurus breviceps* specimens, isolated and characterised and optimised Petb1-9 microsatellite loci in *P. breviceps*. Ms Kathy Saint, Evolutionary Biology Unit, SA Museum isolated and characterised Peta10-20 microsatellite loci. Optimisation of microsatellite loci Petb1-9 and Peta13,16&18 in *P. breviceps* and *P. norfolcensis* was carried out by Ms Trish Kendal and, her supervisor, Dr Andrea Taylor, School of Biological Sciences, Monash University. The author of this thesis extracted DNA from some *P. australis* specimens and optimised all microsatellite loci from *P. breviceps* and *P. australis* in *P. australis*.

2.2 Introduction

Gliding marsupials of the Family Petauridae are associated with continuous tall, mature sclerophyll forests, which provide food resources and hollows for animals to shelter. However, agricultural, industrial practices, and urbanization have resulted in native habitat disappearing or becoming highly fragmented, leading to the decline of many populations of gliding marsupials (Goldingay and Possingham 1995). *Petaurus australis*, *Petaurus breviceps* and *Petaurus norfolcensis* are listed as either endangered, vulnerable or rare within some states of Australia, and each has been targeted for research and conservation efforts. However, their cryptic and nocturnal habits have made observations of their social behaviour difficult, and a low trap success rate for species such as *P. australis* (e.g. Henry and Craig 1984; Craig 1985; Goldingay 1992; Goldingay and Kavanagh 1993; Quin *et al.* 1996a; Carthew *et al.* 1999; Goldingay *et al.* 2001) have limited demographic investigations. Microsatellite loci will be invaluable in

investigations of social organization, mating systems and conservation genetics of these species. Although microsatellite markers have previously been reported for *P. norfolcensis* (Millis 2000), inspection of the associated GenBank sequences suggested the same loci were sequenced multiple times, with replicates differing only by sequencing errors. This hypothesis was supported by linkage disequilibrium data from *P. breviceps* and *P. norfolcensis* populations (Kendal and Taylor, unpublished data). The Millis (2000) primer set thus amplifies only two independent polymorphic loci (Pn3=Pn16 and Pn49) in each of Victorian *P. breviceps* and *P. norfolcensis*. In this chapter I describe the subsequent characterization of microsatellite loci from *P. breviceps* and *P. australis*.

2.3 Methods

Total DNA was extracted from liver tissue in *P. breviceps* and blood in *P. australis* using a salt extraction method (Miller *et al.* 1988) and DNAzol (Molecular Research Center, Inc.; Chomczynski *et al.* 1997) respectively. Microsatellite loci $(AAAG)_n$ from *P. breviceps* were isolated using the protocol of Gardner *et al.* 1999). A minor modification included screening for the presence of $(AAAG)_n$ repeats using a colony-based DNA hybridization technique (see below). Given the availability of only small amounts of DNA from *P. australis*, an alternative isolation technique was used based on the method of Schable *et al.* 2002). Minor modifications included size selection of DNA prior to enrichment and, ligation of enriched products in *pGEM* T vector (Promega). Positive clones in both species were detected using biotin-labelled olignucleotides for $(AAAG)_n$ in *P. breviceps* and $(AAG)_n$ or $(AAC)_n$ in *P. australis* and an alkaline phosphatase/streptavidin colorimetric detection system (Roche). Plasmid inserts were PCR-amplified using T7 and SP6 promoter primers (Promega) and were sequenced on both strands using a cycle sequencing approach (ABI PRISM BigDye Terminators, version 3.0). DNA sequences were determined using an ABI 3700 DNA Analyser. Primers were designed from microsatellite flanking sequences using Oligo version 4.0-s software.

DNA extractions from skin biopsies, stored in 100% ethanol, were carried out using either a salting-out method (*P. breviceps* and *P. norfolcensis*; Sunnucks and Hales 1996) or the Gentra Puregene Extraction Kit (*P. australis*). PCR-amplifications of microsatellite loci (Petb1-9) from *P. breviceps* and *P. norfolcensis* were carried out in 10 μl volumes with approximately 100 ng genomic DNA, 10 pmol of each primer, 0.1U Taq polymerase (MBI Fermentas), 2.5 mM $MgCl_2$, 0.02% Bovine Serum Albumin (Progen), 0.2 mM each of dTTP, dCTP and dGTP, 20 μmol of dATP and 0.02 μl of [α33P]-dATP at 1000 Ci/mmol (Geneworks), 1x PCR buffer (MBI Fermentas). PCR-amplifications of Peta (*P. australis*) loci were carried out as above, with the exception of using 0.25 mM dNTPs, 2 pmol of each primer (forward primers were synthesized with fluorescent tags FAM™, TET™ or HEX™ (Applied Biosystems) at the 5′ end) and Ampli*Taq* Gold (Perkin Elmer). Thermocycling was performed using a touch down program: 94° C 2 min, then cycles of 94° C, 15 s; annealing 30 s; 72° C, 45 s. Annealing temperatures ranged from 55° C to 47° C (Petb loci), 65-50° C (Peta13, 18) and 60-48° C (Peta16), decreasing by 2° C per cycle, with 30 or 40 cycles for the final annealing temperature. Microsatellite alleles were detected either by electrophoresis on 6% polyacrylamide sequencing gels and autoradiography (*P. breviceps* and *P. norfolcensis*) or using an ABI 3700 DNA analyser (*P. australis*).

2.4 Results and Conclusion

Variability of the nine loci from each of the target species is shown in Table 2.1. Five Petb loci (Petb1, 4, 6, 7, 9) were polymorphic in *P. norfolcensis*, (H_0 = 74%-93%; n = 251-256) and two Petb loci (Petb1, 6(a)) were polymorphic in *P. australis* (H_0 = 81%-86%; n = 36). Only one of the Peta loci was polymorphic in *P. breviceps* and *P. norfolcensis* (each 2 alleles, n = 5). None of the loci showed evidence for a deficit of heterozygotes based on Hardy-Weinberg expectations. The high heterozygosity and large number of alleles for many of the loci should, in combination, be useful for both mating system and population genetic analyses of these three species.

Table 2.1 Polymorphic microsatellite loci isolated from *Petaurus breviceps* (Petb1, 4, 6, 7, 8 and 9) and *P. australis* (Peta13, 16 and 18), including the primer sequence (F, forward; R, reverse), core repeat motif, size of alleles (bp), number of alleles at each locus, observed (H_o) and expected (H_e) heterozygosities* (information on allele size, no. of alleles and heterozygosity for Petb1, 4, 6, 7, 8 and 9 is for *P. breviceps*; Peta13, 16 and 18 is for *P. australis*) and GenBank Accession numbers. Petb6(a) primers were designed for specific amplification of the Petb6 locus in *P. australis*.

Locus	**Primer sequence (5'-3')**	**Repeat motifs**	**Allele size**	**No of alleles**	**H_o**	**H_e**	**Accession no.**
Petb1	F: CTTGAGTTCCTAGTATGAGC R: ATCACAGTGTAGAGGTAACC	*$(AAAG)_{24}$	208-478	45	0.902	0.940	AY633628
Petb4	F: CTTTCCAGTGCTATATGT R: GCTCCTAACAAGTTGCCA	*$(AAGG)_{14}(AAAG)_{14}$	214-362	32	0.833	0.906	AY633629
Petb6	F: AATGTCTTTGGGATATGGAC R: CCAGGACTTAGCAAACATC	*$(AAAG)_{16}(GAAG)_{14}(AAG)_{17}$	198-338	29	0.917	0.906	AY633630
Petb6(a)	F: CTTTGGGATATGGACTTATC R: ACATCTCCCTCTCCTCTATA	*$(AAAG)_{16}$	160-210	6	0.806	0.807	
Petb7	F: TCACCAGTACCCAAATAATG R: GGATAGGAAACTAGGTCACC	$(GAAG)_{14}$	202-274	17	0.864	0.895	AY633631
Petb8	F: AGAAAACTGAGGTAGAGAA R: ATTACCAGACATAGTGAGG	*$(AAG)_{23,18}$	356-480	25	0.917	0.903	AY633632
Petb9	F: TTGGAAAAATCAAAATACTG R: CCCTAGTCTTACTTCTTGAGTG	$(AAAG)_{19}$	218-366	40	0.909	0.916	AY633633
Peta13	F: CTTTTGAGACATTGGTTTGG R: GGCCCACTCACCTTTCATA	$(AAC)_{22}$	260-310	6	0.568	0.664	AY633634
Peta16	F: AAATGGGGGTTCAAAGAGTC R: GCCTTATGTGGTTTCTTCAA	$(AAC)_9$	290-340	3	0.459	0.523	AY633635
Peta18	F: TAATTCTACACCAAGTCCAG R: GGAGTCATTTTCATCAGG	$(AAC)_7$	230-270	4	0.541	0.578	AY633636

* compound locus, only repeat numbers > 14 given. H_o and H_e for Petb loci are from one population of *P. breviceps* (n=132) from Paddy Ranges, Central Victoria. H_o and H_e for Petb6(a) and Peta loci are from one population of *P. australis* (n=37) from Rennick State Forest, Victoria.

Chapter 3. Monogamy in the yellow-bellied glider

3.1 Preamble

The following chapter has been submitted to Australian Journal of Zoology and presents the results of an analysis of paternity and social behaviour in a population of yellow-bellied gliders at Rennick State Forest in south-western Victoria.

3.2 Introduction

Monogamous mating systems have been well studied in a broad range of vertebrate taxa, but it has been difficult to find a comprehensive theoretical framework within which to explain its evolution (Reichard 2003). Several hypotheses have been proposed to explain the maintenance of monogamous mating systems, e.g. the dispersion of resources and/or females (Emlen and Oring 1977) or the provision of paternal care (Kleiman 1977; Clutton-Brock 1989), but few provide a sufficient explanation for how monogamy has evolved (Brotherton and Komers 2003). One hypothesis that has gained increasing popularity in recent decades is that of resource defence, i.e. defence of territory (Fricke 1986) or mate guarding of females (Wittenberger and Tilson 1980; Brotherton and Komers 2003). Inter-specific variation in the level of extrapair fertilisations (EPFs) has also provided insights into the evolution of monogamy in a range of taxa, including lizards (Bull *et al.* 1998), crustaceans (Mathews 2002b; Mathews 2003), birds (Birkhead 1998) and mammals (Brotherton and Komers 2003). However, few studies have examined intra-specific variation in mating systems (monogamy/polygamy), in particular, between different populations. Such studies may provide unique insights into the ecological and evolutionary forces (e.g. role of mate guarding and/or territorial behaviour) that maintain monogamous mating systems.

In Australia, intra-specific variation among populations in social and mating systems (polygamy/monogamy) has been reported for a number of possum and glider species, including the yellow-bellied glider (*Petaurus australis*) (Goldingay and Kavanagh 1990; Goldingay 1992; Goldingay *et al.* 2001), sugar glider (*Petaurus*

breviceps) (Quin 1995), Leadbeater's possum (*Gymnobelideus leadbeateri*) (Lindenmayer and Meggs 1996; Harley 2005) and mountain brushtail possum (*Trichosurus caninus*) (Lindenmayer *et al.* 1997; Martin *et al.* 2004; Martin 2005). However, because possums and gliders are arboreal, cryptic, nocturnal and often exist at low population densities, they are difficult to study, and many of the above studies have been largely observational, of short duration and/or with small sample sizes. Furthermore, these studies of social behaviour did not include a genetic component to determine whether the observed social system equated with the genetic or sexual mating system (although see Martin 2005). Further research is therefore required to confirm the existence of intra-specific variation in the mating systems of these species.

The yellow-bellied glider is an arboreal and nocturnal marsupial, with a patchy distribution extending from north Queensland down the eastern seaboard to south-eastern South Australia. It usually lives in small family groups of 2-6 individuals. Populations in the northern part of its distribution have been described as being mainly polygynous (Russell 1984; Goldingay *et al.* 2001), while those in the more southerly parts of its distribution have been described as being mainly monogamous (Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990; but see Goldingay 1992). It has been suggested that differences in social organisation in different populations, and even within populations, may be associated with differences in food resource availability and abundance throughout their range (Goldingay 1992; Goldingay *et al.* 2001). In particular, the abundance of flowering eucalypts, from which yellow-bellied gliders obtain pollen and nectar food resources, may be important in driving the composition of social groups (Goldingay 1992; Goldingay *et al.* 2001). However, in forests, such as those in south-western Victoria, where eucalypts may not provide sufficient year-round nectar and pollen resources, the maintenance of monogamy may be associated with territorial and/or male mate guarding behaviour. There is some evidence for territorial behaviour, as gliders have been observed aggressively repelling intruders from their territories (Russell 1984; Goldingay *et al.* 2001). Further, adult male yellow-bellied gliders have a scent gland on top of their heads, lacking in adult females, which is used to mark members of a group and areas within their territory (Russell 1984). Loud calling

is a well-documented behaviour of this species, with calls being heard by humans from a distance of at least 400m (Kavanagh and Rohan-Jones 1982; Goldingay 1994). However, data on territorial behaviour has mostly been collected on an opportunistic basis (although see Goldingay 1994) and, therefore, further quantitative data are required.

A population of yellow-bellied gliders in south-western Victoria has been the subject of a long-term study of foraging behaviour and socioecology. Data from this population on home range size and exclusivity indicate that home ranges are stable over time, and similar in size for males and females (~30 ha), and that social groups generally consist of adult male and female pairs, and juveniles (Carthew and Goldingay, unpublished data). Therefore, I investigate the hypothesis that a monogamous mating system predominates in this population. I do this by using a combination of behavioural observations and molecular genetic analyses based on microsatellite DNA markers.

3.3 Methods

3.3.1 Study area and sampling times

The study population was located in Rennick State Forest (37°55'S 140°58'E) in south-western Victoria. Rennick State Forest is a little over 5000 ha in size, with the 400 ha study site located on the western edge. The sclerophyll forest there contains two dominant species of eucalypt; brown stringybark (*Eucalyptus baxteri*) and manna gum (*E. viminalis*). This study area has been the focus of a long-term study on the behavioural ecology of the yellow-bellied glider (Carthew *et al.* 1999; Carthew and Goldingay, unpublished data). It is at the extreme western end of the yellow-bellied gliders' distribution, where large blocks of sclerophyll forest are isolated from one another by agricultural land and pine (*Pinus radiata*) plantations. Sampling and field observations were conducted during 24 field trips, each of 7-10 days duration, between August 2001 and August 2003.

3.3.2 Trapping and processing techniques

The composition of social groups in this population had already been determined from the ongoing long-term study on their ecology and, therefore, the capture of adults

and juveniles was directed towards areas occupied by these groups. Yellow-bellied gliders were captured in cage traps placed 3-6 m above the ground on manna gum (*E. viminalis*) showing signs of recent use by gliders for sap feeding. Yellow-bellied gliders make v-shaped incisions on these trees in order to feed on phloem sap released by the tree after wounding (see Carthew *et al.* 1999 for details). Traps were baited with creamed honey placed on a cloth at the back of the trap. Trap trees were also sprayed liberally with a mixture of boiled water and honey, around the trap and higher, as an attractant. During wet weather, traps were covered with plastic to provide animals with shelter. Traps were checked near midnight and at first light, and any captured animals removed and placed into holding bags. Gliders were released the following evening at the point of capture.

All yellow-bellied gliders were weighed, identified as adults, subadults or juveniles (see below) and checked for reproductive status. Gliders were given an individually numbered metal ear tag (National Band & Tag Co., USA), with a piece of coloured, reflective tape to help identify the individual whilst spotlighting. A small piece of skin tissue from the bottom of the ear (~ 3 × 3 mm) was removed from all captured individuals and placed in a vial of 50 : 50 ethanol/saline and stored at room temperature for later extraction of DNA (see below). Fifteen adults (eight females and seven males), as indicated by tooth wear and colour of belly fur (see Goldingay 1989a; Goldingay 1992; Goldingay *et al.* 2001), were collared with brass loop collar radiotransmitters (Biotelemetry and Titley Electronics) for data collection on social behaviour. Collars had coloured, reflective tape glued to them to assist in observing and identifying individual gliders. Radiotransmitters did not weigh more than 5% of the body weight of the individual, and did not appear to detrimentally affect body condition, as assessed by variations in body weight (Brown and Carthew, unpublished data).

3.3.3 Collection and analysis of radio-tracking data

Yellow-bellied gliders were located on foot, between one and three times per night, using a TX3 (Biotelemetry) receiver and 3-element yagi hand-held antenna. Locations were spaced at least one hour apart because gliders can traverse their entire

home range easily in this time (Goldingay 1989b). Spotlighting was conducted using 55 W hand-held spotlights and 12V sealed lead acid batteries. Gliders were visually sighted in trees and an accurate location obtained using a Magellan or Garmin Global Positioning System (GPS). This usually did not obviously disturb their behaviour because they are strictly arboreal and inhabit tall eucalypts (around 25 m). Data from 13 individuals, representing six social groups, were included in home range analyses. Although 15 adult gliders were fitted with radiotransmitters during the study, some of the social groups in this population of gliders underwent changes in their composition and home range areas, leading to the disappearance (one presumed and one confirmed death) of two collared adults.

Home range analyses were carried out using Arcview 3.2a software with the Animal Movement Extension (Hooge and Eichenlaub 1997). Minimum Convex Polygons (MCPs) were used to construct home range areas. Although Kernel estimators are often used to evaluate the size of home ranges, these estimators make assumptions about the distribution of the animal's use of space within the home range when constructing the boundary (Worton 1987; Worton 1989; Seaman and Powell 1996). Areas less visited on the boundary may be removed by the Kernel smoothing parameters because animals do not tend to distribute themselves around the boundary, but towards a core area within the home range. This bias provides for a less conservative estimate of overlap between adjacent territories and because den trees were often on the boundary of the polygon, possible points of contact between neighbours may have been removed. Therefore, MCP 95% (adjusted to remove outliers using the Harmonic Mean Method) was used to calculate the sizes of home ranges, and MCP 100% was used to calculate the amount of home range overlap. The percentage of home range overlap was calculated for each individual home range. The number of observations per individual (n = 13 gliders) used in home range size analysis ranged from 13-82 (mean $\pm$ SE = 50.8 $\pm$ 5.4 observations) (Appendix 1). Bootstraps were carried out on 100% MCP data in Arcview with 20 replicates per interval and an interval size of 3. Asymptotes occurred between 35 and 46 observations. However, some gliders that had more than 46 observations still did

not show an asymptote. Home range cumulative curves for each glider are available in Appendix 2.

3.3.4 Observations of associations between paired adult males and females

Quasi-simultaneous observations of paired adult males and females radio-tracked whilst foraging at night proved to be the best method of determining close associations and possible mate guarding within this highly mobile and cryptic species. Close associations have been used to infer mate guarding activities previously in both birds (e.g. Wallander *et al.* 2001) and mammals (e.g. Schülke and Kappeler 2003). I defined individuals located within 50 m of a partner as a close association. The forest structure is reasonably open, and yellow-bellied gliders are likely to be capable of seeing their partner within 25 m, and certainly of reaching their partner using one or two glides at 50 m. Moreover an area of 50 m diameter represents a small proportion (≤ 2.6%) of a 30 ha home range area. Observations of up to 15 minutes apart were included. Interindividual distances were calculated using a simple formula based on the difference between x-y coordinates between individuals. During inactive daylight hours, gliders were tracked to their sleeping sites (i.e. den trees) to determine patterns of den sharing. Yellow-bellied gliders rely on tree hollows in mature eucalypts for shelter during the day. When gliders were in separate trees, den watches were conducted to find out whether other uncollared or untagged animals were present. Den watches involved remaining within view of the den tree until after dark and observing the gliders emerging from the hollow.

3.3.5 Spatial distribution of den trees

Indirect evidence for territoriality was obtained by assessing where den trees for a group were distributed in relation to their home range boundary. For this, 50 m buffers were calculated for the interior of MCP 100% polygons and a comparison made of the number of den trees within the buffer compared to the remainder of the home range. A distance of 50 m was selected as appropriate because it can be covered by yellow-bellied gliders in one or two glides (Goldingay 1989a; pers. obs.). Leaving scent, or loud calling within this 50 m buffer zone would advertise the presence of adult gliders to potential

intruders. Yellow-bellied gliders have often been observed loud calling after leaving their den trees (Goldingay 1994; pers. obs.) and depositing scent (Russell 1984).

3.3.6 Paternity analysis

A panel of two tetra- and three trinucleotide repeat microsatellite loci were used to genotype each individual within the study population. Two tetranucleotide microsatellite loci, Petb1 and Petb6, were isolated from sugar gliders (*P. breviceps*) and three trinucleotide microsatellite loci, Peta13, Peta16 and Peta18, from yellow-bellied gliders (Chapter 2; Brown *et al.* 2004). DNA was extracted using the Gentra Puregene kit according to the manufacturer's instructions. One primer of each pair was fluorescently labelled with either HEX, TET or FAM (Applied Biosystems), and PCR-amplifications performed using touchdown programs with annealing temperatures ranging between 47-65° C (see Brown *et al.* 2004) for annealing temperatures of each locus). Per-locus heterozygosities for yellow-bellied gliders ranged from 46-81% (no locus showed a deficit of heterozygotes) and the number of alleles per-locus ranged from 3-12 (Brown *et al.* 2004). Microsatellite alleles were detected by using an ABI3700 DNA analyser and scored using the software program GENOTYPER (Applied Biosystems).

Paternity analyses were conducted using the likelihood analysis software CERVUS 2.0 (Marshall *et al.* 1998). This program calculates the ratio of likelihoods that a candidate parent is the true parent versus not the true parent for each genetic locus, which is converted to a likelihood of difference (LOD) score by taking the log (to base e). LOD scores of zero, or less than zero, imply that the candidate parent is, respectively, equally, or less likely, to be the true parent as a randomly chosen individual. Positive LOD scores imply a better than random chance that the candidate parent is the true parent (Marshall *et al.* 1998). The genotypes of all parents with positive LOD scores were also compared with the genotypes of each offspring in the paternity analysis to assess the number of allelic mismatches. Putative parents were discounted as true parents if they had two or more mismatches with offspring. In addition, to assess the degree of confidence in the assigned parent, a Delta statistic was generated using CERVUS to 80%

and 95% confidence levels. Delta is defined as the difference in LOD scores between the most likely parent and the next most likely parent, and the significance of this statistic is determined by comparison with the distribution of Delta values obtained from simulated parentage tests (Marshall *et al.* 1998). Default parameters of 10,000 cycles with a rate of typing error of 0.01 were used for the simulation. Allele frequencies and pairwise relatedness of the 21 known adults in the population were determined using RELATEDNESS 5.08 (Queller and Goodnight 1989). Standard errors for average relatedness (R) were calculated by jackknifing over loci. All statistical analyses, other than paternity and relatedness analyses, were performed using SPSS V.13.0 and Microsoft EXCEL. Data were checked for normality and non-parametric equivalent tests performed if data were not normal.

Juveniles (males that had not yet developed a scent gland on top of their heads, and females whose pouches were pale pink in colour and tight) were usually sampled once they became independent. Early on in the study difficulties were experienced in removing tissue from pouch young, and for ethical reasons this practice was discontinued. Because of the potential ambiguity in maternity created by sampling juveniles that had already reached independence, results from the maternity analysis were determined using criteria from the 'no parent known' feature of CERVUS. This feature allowed all putative female alleles to be assessed as equally likely candidate mothers. However, yellow-bellied gliders within the Rennick population usually have one young at a time (Chapter 4), and juveniles have not been recorded as leaving the home range area of the family group until they are approximately 18-24 months. By this time, males have developed an active scent gland on top of their heads and females start showing signs of being reproductively active, i.e. pouches with loose skin and elongated teats. Radio-tracking data collected on adults within the study population, combined with capture data allowed a social mother and father to be determined for most juveniles. Therefore, parentage analyses were also conducted under the 'one parent known' option of CERVUS by assigning a likely social mother or father to each juvenile.

It was difficult to assess what proportion of males within the population were sampled as possible parents. There was potential yellow-bellied glider habitat to the north, east and south of the study population and in particular, a group of gliders living directly to the north of the study site were not captured. However, some of the habitat (particularly to the east of the study population) was dominated by stringybark with few patches of manna gum. Although transient gliders would be able to move with ease throughout these parts of the forest, the likelihood that these areas immediately surrounding the study population was inhabited by permanent groups of gliders was low.

3.4 Results

3.4.1 Home range overlap between individuals

Yellow-bellied glider group home ranges were (mean ± SE) 29 ± 7.2 ha (MCP 95%, range = 7-42 ha) and 22 ± 4.8 ha (KHR 90%, range = 13-67 ha), *n* = 7 glider groups. Adult female home ranges were 25 ± 4.0 ha (MCP 95%, range = 13-39 ha) and 19 ± 5.5 (KHR 90%, range = 6-40 ha), *n* = 7, whilst adult male home ranges were 22 ± 3.0 ha (MCP 95%, range = 12-28 ha) and 28 ± 6.9 (KHR 90%, range = 10-60 ha), *n* = 6. Eight gliders formed four monogamous pairs (i.e. shared a home range area with a glider of the opposite sex), one polygynous/polyterritorial group was observed (i.e. one adult male shared his home range area with those of two adult females, but the females did not share a home range) and one adult female did not share her home range with an adult male (i.e. remained single) (Fig. 3.1(a)(b)). Data collected on two other gliders prior to their disappearance in August 2002 indicated they may have formed another monogamous pair, although observations on the adult female were few (B group in Fig. 3.1(a)(b)). Monogamous glider pairs (not including B group) showed extensive overlap in their home ranges, with females and males having between 59-100% (females: 86.4 ± 7.4% overlap; males: 88.2 ± 9.7% overlap) of their home ranges overlapped by their cohabiting partner (Table 3.1). The male whose home range overlapped those of two females had nearly 95% of his home range overlapped by these females. The females, however, had only 42% and 65% of their home ranges overlapped by this male. Only female gliders in adjacent territories showed overlap with other gliders, but this

was quite low being between 0.56% and 26% (Table 3.1). Male and female gliders in adjacent territories had even less overlap, between 0.04% and 0.9%.

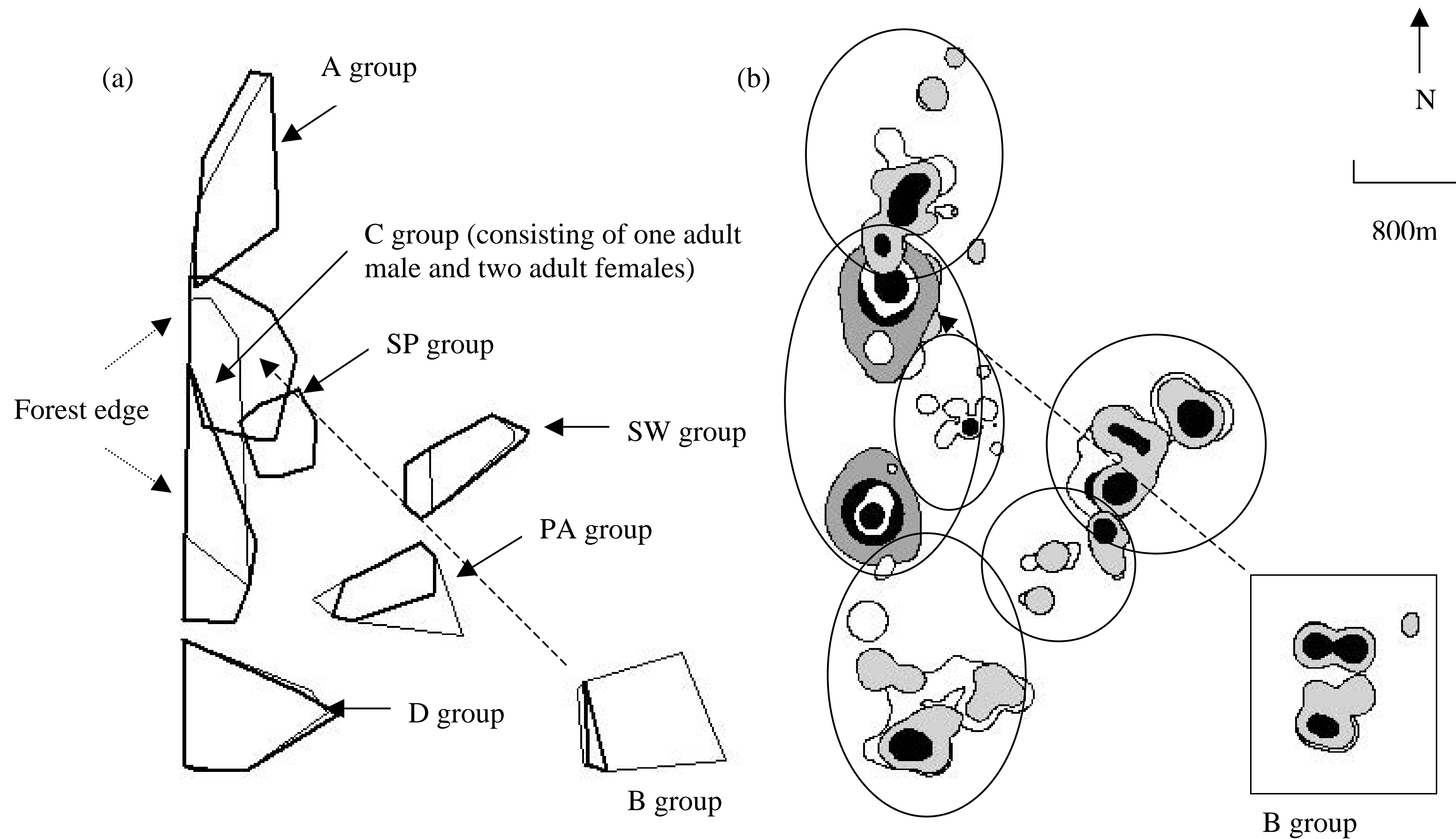


Fig. 3.1 (a) Yellow-bellied gliders' home ranges using MCP 100% (female home ranges are in bold outline, whilst male home ranges are in fine outline); and (b) home ranges using Kernel 50% (all core home ranges are in black) and 90%; female home ranges are in white, males are in grey (the darker grey showing the adult male in C group). Group names are indicated on MCP 100% home ranges but are also applicable to Kernel home ranges. Kernel home ranges are circled to make group identification easier. The location of a home range for the adult male and female in group B is indicated by the dashed arrow (prior to the disappearance of both the adult male and female after August 2002). The adult male and one adult female from C group resided in this area from August/September 2002 until the end of the study in August 2003. The forest is bordered by *Pinus radiata* plantations on the west, but continuous native habitat exists to the north, south and east.

Table 3.1 Amount of overlap (%) for cohabiting males and females, and between adjacent territories. Overlap is calculated from MCP 100% home ranges of each individual. Mean ± SE and range, in parentheses, is presented. *n* = number of overlaps observed. Monogamous pairs = one male cohabiting with one female. Polygynous/ polyterritorial group = one male overlapped his home range with those of two females.

	Amount that each home range is overlapped by the adjoining home range (%)	
	Females	Males
Monogamous pairs	86 ± 7.4	88 ± 9.7
	(67-100), $n = 4$	(59-100), $n = 4$
Polygynous/	54 ± 11.2	48 ± 7.5
polyterritorial group	(42, 65), $n = 2$	(40, 55), $n = 2$
Adjacent territories		
Female-female	6 ± 7.4	
	(0.6-14), $n = 3$	
Male-male		No overlap
Female-male	0.4 ± 0.3	0.5 ± 0.4
	(0.1, 0.8), $n = 2$	(0.04, 0.9), $n = 2$

[a]One adult male was overlapped by two females, therefore an average and SEM was not calculated and actual overlap values are shown.

3.4.2 Associations within adult male and female pairs

Quasi-simultaneous locational observations from the four pairs (monogamous pairs, *n* = 70 observations) and one group of three adults (polygynous/polyterritorial group, *n* = 27 observations) were obtained. Distances between individuals in pairs ranged from 0-1370 m (mean ± SE = 92 ± 24 m), whilst distances between the polygynous group members ranged from 0-1120 m (95 ± 60 m). However, the mean was affected by some extreme values in the dataset. Adult males were often observed within close proximity of their female partner; approximately 55% of the time within 25 m and 66% of the time within 50m of their partner (Fig. 2). Interactions with known individuals from adjacent territories were never observed.

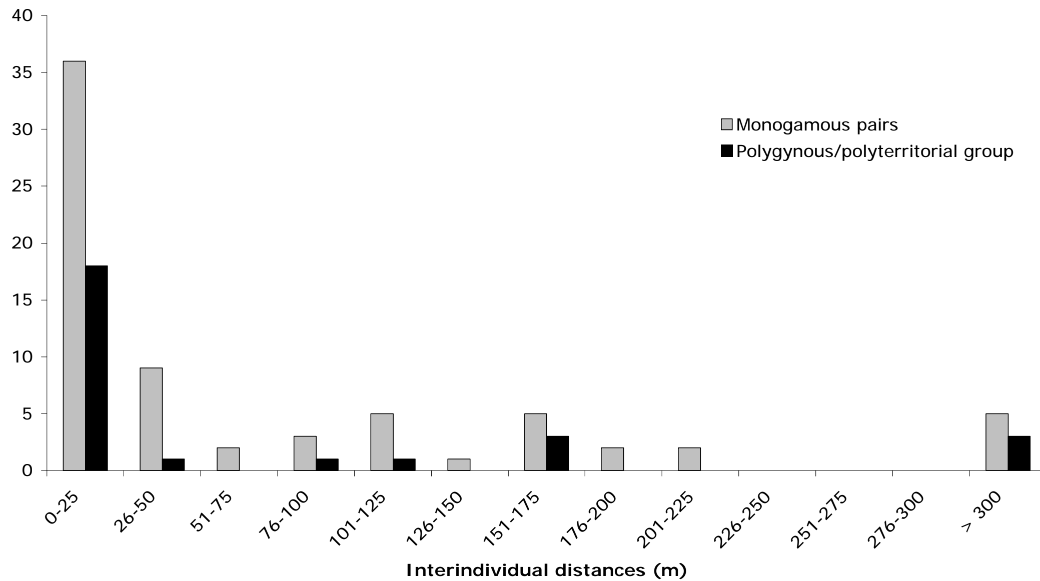


Fig. 3.2 Interindividual distances of monogamous pairs (cohabiting adult male and adult female) and a polygynous group (one adult male and two adult females). Monogamous pairs (*n* = 4 pairs) are compared with polygynous group (*n* = 1 group). Distance classes contain 25m ranges. Total number of observations = 117.

Den sharing data from six adult females (*n* = 221 observations) and five adult males (*n* = 195 observations) were included for analysis. Between 40% and 85% (mean ± SE = 67.2 ± 8.8%) of observations during the day were of adult males denning with his female partner. Adults were observed, during den watches (see section 3.3.4), to spend their denning time either alone or with presumed offspring when they were not denning with their partners. Individuals were never observed denning with known individuals from adjacent territories.

3.4.3 Distribution of den trees within the home range

Males and females used similar numbers of den trees over the two-year study; males (*n* = 6): mean ± SE = 7.8 ± 1.1, range 3-11 dens, and females (*n* = 7): 7.3 ± 1, range 5-12 dens (Mann-Whitney U = 17.0, P = 0.6, *N* = 415 observations). The number of den trees within the outer 50 m buffer zone was also similar for males (5.3 ± 0.7, range 2-6 dens) and females (4.9 ± 0.6, range 3-8 dens) (Mann-Whitney U = 14.5, P = 0.3). The

area covered by the 50 m buffer was between 29.1% and 54.5% (39.2 ± 2.2%, n = 13) of the total home range area. However, the percentage of den trees within this area was much higher; between 50% and 85.8% (68.5 ± 3.1%, n = 13). The hypothesis (H_0) that the percentage of den trees inside the 50 m buffer zone was not different to the percentage of area inside the buffer zone was rejected ($t = 9.3$, $P < 0.001$).

3.4.4 Genetic analyses of parentage

Thirty-seven yellow-bellied gliders were genotyped at all five loci. Of these, 16 were juveniles. The genotypes of all juveniles were analysed with all adult female genotypes to determine whether the social mother was the most likely genetic mother (as determined by the capture of juveniles within a female's home range). All but one putative mother had positive LOD scores with their presumed offspring (Table 3.3). The tissue sample from this offspring was taken whilst the young was still in the pouch, and the mismatch in genotypes occurred only at one locus (Petb1). At this locus, the genotype of the mother (4893f) was 380/420 (allelic sizes in bp), whilst the genotype of the pouch young, 5001j, was 384/400 (see Appendix 2 for the genotypes of all gliders). Both pouch young and the mother were genotyped twice to remove the possibility of typing error, or allelic size differences between typing runs. It was therefore most likely that the mismatch at this locus was due to a new mutation of one of the mother's alleles. The putative father's (4892m) alleles at this locus were 388/400, thus, the new mutation in the pouch young was 384. Another juvenile, 4435j, mismatched with her putative mother (5004f) at the Petb6 locus. Again, both the mother and juvenile were genotyped twice to remove the possibility of typing error. The mismatch may be due to a new mutation or, because the juvenile was first captured whilst independent, the social mother may not be the true mother. CERVUS resolved maternity of juvenile 4435j as being 5004f with 80% confidence, with the next most likely mother (5152f) having a negative LOD score and mismatching 4435j at the Petb1 locus. These results suggest the mismatch may have resulted from a new mutation at the Petb6 locus of 5004f.

Table 3.3 Likelihood analysis for putative mothers based on 10,000 simulations where no adults were assigned as known parents. LOD scores and Delta statistics for the female putative parent for each juvenile are provided. Putative mothers are in order of most likely candidate as defined by CERVUS. Delta statistics are between the most likely candidate mother and the next most likely candidate mother. ID numbers are the DNA in alcohol numbers assigned by the Evolutionary Biology Unit, SA Museum. #=juveniles/subadults where the social mother was not known, ^=true mother did not return a positive LOD score. *=95% and +=80% confidence interval, NS=not significant.

Juvenile ID	Social mother	Putative mothers	LOD scores	Delta statistic $\Delta_{obt} \geq \Delta_{crit}$ (neither parent known)
5384	4896	4896	1.86	1.56 ≥ 1.49 *
		4890	0.31	
5099	4896	4896	0.95	0.23 < 0.37 NS
		4890	0.71	
4898	4896	4896	1.96	1.58 ≥ 1.49 *
		5000	0.39	
5458	5459	5459	2.43	0.77 ≥ 0.37 +
		5004	1.66	
5855	5459	5459	1.82	0.05 < 0.37 NS
		5000	1.77	
5055	5004	5004	2.98	1.88 ≥ 1.49 *
		4890	1.10	
4435	5004	5004	1.37	0.44 ≥ 0.37 +
		5152	0.93	
5276	4893	4893	1.43	0.59 ≥ 0.37 +
		5152	0.83	
5001	4893^	5152	0.75	0.04 < 0.37 NS
		5000	0.71	
5421	4890	5003	2.01	0.33 < 0.37 NS
		4890	1.68	

Juvenile ID	Social mother	Putative mothers	LOD scores	Delta statistic $\Delta_{obt} \geq \Delta_{crit}$ (neither parent known)
5100	4890	4890	1.25	0.22 < 0.37 NS
		4893	1.03	
5422	5154	5154	1.72	0.96 ≥ 0.37 +
		5152	0.76	
5854	5000	5000	1.81	1.32 ≥ 0.37 +
		5003	0.49	
5278#	Not known	5459	1.42	0.93 ≥ 0.37 +
		5000	0.49	
5853#	Not known	5459	2.18	2.18 ≥ 1.49 *
5385#	Not known	5003	1.91	0.96 ≥ 0.37 +
		4893	0.95	

For paternity analyses, juveniles were assigned putative fathers under two conditions. The first was with 'no known' parent's alleles entered and the second was with the social mother's genotype entered as the known parent. In the few instances where data on the social mother were not available (see below) the candidate female with the highest LOD score (based on the 'no parent known' analysis) was chosen as the known parent. Analyses under both conditions provided the same fathers as the most likely parent, for all juveniles that were able to be assigned social groups. Difficulty was experienced in determining the paternity of juvenile 5854j. The social father, 5423m could not be excluded as a potential father, however, he was not calculated by CERVUS to be the most likely father. Both adult males, 5423m and 4895m, shared 50% of their alleles with juvenile 5854j, even with the maternal (5000f) alleles assigned. The pairwise relatedness value between adult males 5423m and 4895m was 0.4, indicating that they also may be related. Although a number of pairwise relatedness values suggested some adult males were related, overall relatedness values between adult males in this population were low (relatedness, R = 0.02, N_x = 11, N_y = 11, SE = 0.035, P = 0.9).

Any further difficulties experienced in assigning maternal alleles were usually resolved when the social father's alleles ('known parent') were assigned prior to the maternal alleles. In four of the five cases where the Delta statistic was not significant in assigning a putative mother, the statistic was resolved to 80% or 95% confidence once the social father's alleles were assigned as a known parent. The exception was with 5100j, whereby adult female, 4893f, (not the social mother) was assigned the true mother with the paternal (4891m) alleles assigned. Adult females 4893f and 4890f share more than 50% of their alleles with each other, and each share 50% of their alleles with the juvenile 5100j, with the paternal alleles assigned. A pairwise relatedness value of 0.17 was calculated for 4893f and 4890f, suggesting the possibility that these females may be related. However, consistent with the overall relatedness between adult males, the relatedness between adult females in this population was low (average relatedness, R = -0.005, N_x = 10, N_y = 10, SE = 0.032, P = 0.1). Maternity was resolved with 80 or 95% confidence for all the other juveniles once paternal alleles were assigned.

Of the 16 juveniles sampled, three juveniles were not captured within a known adult female's home range and thus were not assigned a social mother or father. Of the 13 juveniles that were assigned a social mother, only one, 5422j, could not be attributed to the adult male (5153m) whose home range area overlapped that of the mother. The male, 5153m, was homozygous at two loci, Peta13 and Peta18 (his alleles were 280/280 and 228/228, respectively). The juvenile 5422j possessed these alleles, but was heterozygous at locus Peta13 (274/280) and homozygous at locus Peta18 (234/234). Once the mother's (5154f) alleles (280/280 and 228/234, respectively) were assigned, adult male 5153m was not the most likely candidate for paternity. The most likely candidate for paternity of this juvenile was 5155m, an old adult male, who died prior to the first capture of the juvenile 5422j (Table 3.4). Only a very small radio-tracking dataset (*n* = 8 observations) was collected on adult male 5155m, and although it seems likely that his home range was situated adjacent to that of the juvenile 5422j, it is not known whether the home range of adult male 5155m overlapped that of the mother at the time of conception of 5422j.

Table 3.4 Likelihood analysis for putative fathers based on 10,000 simulations where no parent was assumed and where the social mother was assigned the known parent. LOD scores and Delta statistics of each putative father are provided. Putative fathers are in order of most likely candidate as defined by CERVUS. Delta statistics are between the most likely candidate father and the next most likely father. *=95% and +=80% confidence interval, NS=not significant. #=subadults that were assigned by Cervus the most likely female candidate.

			No parent known			One parent known		
Juvenile ID	Social mother (known parent)	Social father	Putative fathers	LOD scores	Delta statistic $\Delta_{obt} \geq \Delta_{crit}$	Putative fathers	LOD scores	Delta statistic $\Delta_{obt} \geq \Delta_{crit}$
5384	4896	4897	4966	3.24	0.66 ≥ 0.37 +	4966	4.13	0.67 ≥ 0.67 *
			4897	2.58		4897	3.46	
5099	4896	4967	4967	1.89	0.51 ≥ 0.37 +	4967	3.60	2.56 ≥ 0.67 *
			5423	1.38		5279	1.04	
4898	4896	4967	4967	0.73	0.65 ≥ 0.37 +	4967	2.53	1.17 ≥ 0.67 *
			4892	0.08		4892	1.36	
5458	5459	5279	5279	3.25	1.94 ≥ 1.49 *	5279	4.22	4.22 ≥ 0.67 *
			5153	1.31				
5855	5459	5279	5279	1.55	1.12 ≥ 0.37 +	5279	2.34	2.34 ≥ 0.67 *
			5277	0.43				
5055	5004	4895	4895	0.33	0.11 < 0.37 NS	4895	3.14	3.14 ≥ 0.67 *
			5423	0.22				

Juvenile ID	Social mother (known parent)	Social father	No parent known: Putative fathers	No parent known: LOD scores	No parent known: Delta statistic $\Delta_{obt} \geq \Delta_{crit}$	One parent known: Putative fathers	One parent known: LOD scores	One parent known: Delta statistic $\Delta_{obt} \geq \Delta_{crit}$
4435	5004	4895	Father not assigned	Father not assigned	Father not assigned	4895	1.73	$0.67 \geq 0.67$ *
						5155	1.07	
5276	4893	4892	4892	1.32	$0.09 < 0.37$ NS	4892	2.08	$0.93 \geq 0.67$ *
			4895	1.23		4967	1.15	
5001	4893	4892	5155	1.31	$0.11 < 0.37$ NS	5155	2.47	$0.47 \geq 0.00$ +
			4892	1.21		4892	1.99	
5421	4890	4891	4891	2.17	$2.17 \geq 1.49$ *	4891	3.32	$3.32 \geq 0.67$ *
5100	4890	4891	4891	2.04	$2.04 \geq 1.49$ *	4891	2.88	$2.88 \geq 0.67$ *
5422	5154	5153	5155	2.47	$1.41 \geq 0.37$ +	5155	2.02	$0.63 \geq 0.00$ +
			4892	1.06		4966	1.39	
5854	5000	5423	5153	0.77	$0.03 < 0.37$ NS	4895	1.84	$0.04 \geq 0.00$ +
			5279	0.64		5423	0.74	
5278#	5459	Not known	5277	2.50	$2.11 \geq 1.49$ *	5277	2.59	$1.84 \geq 0.67$ *
			4967	0.39		4967	0.75	
5853#	5459	Not known	5153	2.29	$1.02 \geq 0.37$ +	5153	2.41	$0.34 \geq 0.00$ +
			4897	1.27		4897	2.07	

Juvenile ID	Social mother (known parent)	Social father	No parent known Putative fathers	LOD scores	Delta statistic $\Delta_{obt} \geq \Delta_{crit}$	One parent known Putative fathers	LOD scores	Delta statistic $\Delta_{obt} \geq \Delta_{crit}$
5385#	5003	Not known	4895	1.62	$0.81 \geq 0.37$ +	5155	1.54	$0.55 \geq 0.00$ +
			5277	0.81		4895	0.99	

One instance of serial genetic monogamy was detected. The adult male, 4967m, the breeding male in C group until his disappearance at the beginning of the study period, fathered two offspring (5099j, 4898j) with the breeding adult female, 4896f. The adult male, 4897m, became the next breeding male in this group and fathered one offspring, 5384j, with the adult female, 4896f (see Appendix 2 for individual genotypes). Thus, instances in which extra-pair fertilisations (EPFs; offspring that were not related to the current resident adult male at the time of the study) were detected may have been instances of serial genetic monogamy, with females changing partners because of the disappearance or death of the previous partner.

3.5 Discussion

My results show that the mating system of the Rennick population of yellow-bellied gliders is predominantly socially and genetically monogamous, and is one of only a few studies to confirm genetic monogamy within a marsupial species (although see Spencer *et al.* 1998; Martin 2005). The social organisation is characterised by a great deal of home range overlap between single adult males and females (86-88%), but almost no overlap of adjacent territories. This result is consistent with previous studies on mahogany gliders (*Petaurus gracilis*) (average overlap between pairs of 85.9%), which are also thought to be socially monogamous (Jackson 2000). In addition, most yellow-bellied glider pairs were stable over the two-year study period, which was consistent with previous observations of this population (Carthew and Goldingay, unpublished data) and other populations within south-eastern Australia (Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990). My results are also generally consistent with those found for other populations of yellow-bellied gliders in that members of glider groups share common home ranges (Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990; Goldingay 1992; Goldingay *et al.* 2001). In the present study, one exception to monogamous pairs was found, with an adult male, 4897m, partly overlapping the home ranges of two adult females (4896f and 4898f, a juvenile at the time of sampling and discussed further below), which may represent an instance of social polygyny/polyterritoriality. Also, a single adult female (5003f, SP group in Fig. 3.1

above) was not observed sharing her home range with an adult male during the study period.

Genetic monogamy was confirmed, with parentage of 12 of 13 (92.3%) juveniles from known social groups, assigned to a socially monogamous pair. A further three juveniles were not able to be placed into social groups. Although only a small panel of five microsatellite markers was used, confidence levels of 80% or 95% were obtained for putative mothers where neither parent was known with eight of the 13 juveniles able to be assigned a social group. Further, paternity was assigned at 80% or 95% confidence for eight of the 13 juveniles using the 'no parent known' criteria, and paternity assigned for all juveniles at 80% (three juveniles) or 95% (10 juveniles) when maternal alleles were assigned. Despite these generally high levels of confidence in the Delta statistic, LOD scores for putative parents, although positive, were often relatively low (i.e. < 2; see Tables 3.3 and 3.4), suggesting that paternity analyses would benefit from the use of additional polymorphic microsatellite loci.

The one juvenile that could not be attributed to the social father represents a potential case of an EPF. However, it is also possible that the juvenile's father may have had a monogamous partnership with its mother, prior to the father's death/ disappearance, therefore, representing a case of serial monogamy. One case of serial monogamy is apparent from my study: the female 4896f was genetically monogamous with consecutive partners (adult males, 4967m and 4897m), partnering 4897m after the disappearance of 4967m. More long-term information on the yellow-bellied gliders' life history strategies and social behaviour, coupled with genetic analyses are needed to determine the likely incidence of EPFs. However, taken overall, the genetic analyses provide strong evidence that monogamy is the main mating strategy used by this population of yellow-bellied gliders.

Differences in environmental conditions (Emlen and Oring 1977) and demographic effects (e.g. Travis *et al.* 1995) may lead to variation in social and/or mating behaviour. In particular, variation in local food resource abundance and productivity have been proposed to account for variation in the social system among, and within, populations of yellow-bellied gliders (Goldingay 1992; Goldingay *et al.* 2001). Staggered flowering periods that allow nectar and pollen food resources to be

available through much of the year may allow larger group sizes, and the possibility of polygyny (Goldingay 1992; Goldingay *et al.* 2001). However, smaller group sizes, and monogamy, may result when flowering becomes scarce (Goldingay 1992; Goldingay *et al.* 2001). At the Rennick study site, there are only two species of eucalypt that only flower during the summer and autumn seasons, and phloem sap is the most prominent dietary item (Carthew *et al.* 1999; Chapter 4). That the mating system within the Rennick population of gliders is predominantly monogamous is consistent with the hypothesis that food resources (in particular, flowering) may be insufficient to allow adult males to defend an area large enough to contain more than one adult female (Goldingay 1992; Goldingay *et al.* 2001). Nevertheless, further investigation of food resource abundance at a fine spatial and temporal scale needs to be conducted in order to assess the importance of the hypothesis of variations in local food resource abundance in affecting the social system (*sensu* Goldingay 1992; Goldingay *et al.* 2001).

The size and exclusivity of female glider home ranges may prevent males from being able to establish and defend territories over more than one female's home range (see Rutberg 1983). However, one male, 4897m, in the Rennick population did expand his territory to overlap another available, unmated female with the females maintaining exclusive territories. Reasons for the polygynous/polyterritorial grouping adopted by one adult male within the Rennick population of yellow-bellied gliders are unclear. There may exist differential abilities of individuals to defend large enough areas, containing multiple adult females that may be teased out by examining body condition or weight of the gliders. The adult male, 4897m, was the second heaviest adult male glider in the study population (Brown and Carthew, unpublished data). The differential ability to defend a territory also may be influenced by the location of the territory within the forest. The adult male, 4897m and his two females, 4896f and 4898f, had territories that abutted the forest edge (see Group C in Fig. 3.1 above). They, therefore, only needed to defend three sides of their territories. However, whether such a position would allow for defence of a larger area by males is speculative, and further behavioural data from groups of gliders, particularly those living close to the forest edge, is needed in order to test this tentative hypothesis. Interestingly, the two females associated with this one male were closely related (i.e. mother, 4896f, and daughter, 4898f), and the male, 4897m, was unrelated to either

female (see Group C in Appendix 2). The adult male, 4897m, partnered the mother after the disappearance of the previous male partner, 4967m (discussed above with respect to serial genetic monogamy). When the daughter, 4898f, became sexually mature, within her mother's home range, both she and adult male, 4897m, moved into the area north of, and abutting, her mother's territory (Group C in Fig. 3.1 above). Adult male, 4897m, then moved between the home ranges of 4896f and 4898f, and often spent his time with one female or the other. It is likely there are a number of factors contributing to the maintenance of this polygynous/polyterritorial group.

Regardless of one adult male's behaviour, the home ranges of most males overlapped those of only one adult female. The maintenance of closely overlapping home ranges of male and female partners and the low incidence of EPFs, may result from mate guarding. Male yellow-bellied gliders spent up to 50% of their active time within 25m of their female partner. This time was greater than that found with the similarly-sized monogamous fork-marked lemurs (*Phaner furcifer*) (330g versus 400-600g for yellow-bellied gliders), which spent about 25% of active hours within 25m of their female partners (Schülke and Kappeler 2003). However, comparisons across studies of the cohesiveness of pairs needs to be treated with caution, as not only were methods in obtaining the data different, but home range sizes and habitat types were also different. Home ranges for fork-marked lemurs were around 5 ha (Schülke and Kappeler 2003), whereas home range sizes for yellow-bellied gliders were around 25 ha. Given the disparity in home range sizes, fork-marked lemurs had a much greater chance of meeting one another than did yellow-bellied gliders, but did so far less. Moreover, male yellow-bellied gliders also spent more sleeping time (on average 67%) with their female partners than fork-marked lemurs, which co-occupied sleeping sites every third day (Schülke and Kappeler 2003). Female fork-marked lemurs are larger than, and dominant over males, and actively repel them from feeding and sleeping sites (Schülke and Kappeler 2003). Thus, mate guarding activities by fork-marked lemur males may be ineffective in assuring paternity, as evidenced by the high level of EPFs within this species (Schülke *et al.* 2004). In contrast, male yellow-bellied gliders at Rennick are around 10% heavier than females (Brown *et al.* 2006) and thus male mate guarding activities may be more effective. Although mate guarding remains a plausible hypothesis to explain my behavioural

data on yellow-bellied gliders, I cannot entirely rule out the possibility that the close proximity of male and female partners during active and resting periods results from the dispersion of suitable feeding trees and tree hollows in which to shelter, rather than through mate guarding behaviour. Some evidence for the latter comes from observations that yellow-bellied gliders forage together on 39-46% of occasions when engaged specifically in sap feeding (Henry and Craig 1984; Goldingay 1989b), although for these data males and females were not separated.

Aggressive encounters between conspecifics were not observed within this population, although they have been observed in other populations, with encounters often ending with gliders injured, and even falling from trees to the ground (Russell 1984; Goldingay *et al.* 2001). A heavy investment in territorial behaviour may deter intruders, and be preferable to the possibility of being seriously injured. Territoriality is an important component of most socially monogamous taxa (Mathews 2002a), and it may be one of the factors constraining male and female attempts to solicit EPFs. Although joint territory defence has been perceived to be unimportant in the evolution of monogamy (see Brotherton and Komers 2003; van Schaik and Kappeler 2003), it is likely to be important in the maintenance of monogamy in yellow-bellied gliders. The costs of maintaining a territory may be lowered by strategic locations of den sites near the boundaries of home ranges. Territorial behaviour may complement a male mate guarding strategy, as it lowers the costs of defending a female (Brotherton *et al.* 1997). Yellow-bellied gliders often produce loud calls upon exiting their den trees (Goldingay 1994; pers. obs.) and may deposit scent in the area before moving to feeding areas (Russell 1984). Having a number of den sites in the narrow band around the border of their home range, relative to the number within the core area, may provide an effective advertisement that an adult male and female yellow-bellied glider occupy a particular area. The frequency of occurrence of yellow-bellied gliders' loud calls is greater in the boundary than core areas (Goldingay 1994), possibly letting conspecifics know that an adult male and an adult female inhabit that area. Such behaviour may limit interactions between males and females from adjacent territories.

There appear to be a number of factors affecting male and female mating strategies within this population of yellow-bellied gliders. Dispersion of food

resources, male mate guarding and territoriality may interact to limit males and females to mating within the pairbond. One single factor does not appear to be sufficient to explain the evolution of monogamy as a mating strategy for male and female yellow-bellied gliders at Rennick. Experimentally manipulating food and/or shelter resources and monitoring social behaviour may be practically very difficult, but would be likely to provide data on variables leading to monogamous or polygamous mating systems. Further research on populations in other parts of their range that have been suggested as being polygynous may help to identify the behavioural or ecological variables that lead to polygynous versus monogamous mating systems in this species.

Chapter 4. Forest phenology and the timing of reproduction in the yellow-bellied glider

4.1 Preamble

This chapter has been written in journal format, although it has not yet been submitted to an appropriate journal, and presents data and analyses on forest phenology and the timing of reproduction in yellow-bellied gliders at Rennick State Forest.

4.2 Introduction

Animals that inhabit temperate environments where the abundance of food resources fluctuates seasonally may coincide energy-demanding reproductive activities with peaks in food resource abundance (Sadleir 1969). For many vertebrate species, food resources high in protein are important for lactating females and young as they grow and develop (Herrera 1998; Herrera *et al.* 2001). Protein food resources are often in the form of pollen or arthropods, or both, for a number of small (< 1 kg) mammalian species, including many Australian possum and glider species, e.g. sugar and squirrel gliders (*Petaurus breviceps* and *P. norfolcensis*) (Smith 1982; Henry and Suckling 1984; Menkhorst and Collier 1987; Sharpe and Goldingay 1998; van der Ree 2002; Sharpe 2004; Dobson *et al.* 2005), and the much smaller eastern and western pygmy, and honey possums (*Cercartetus nanus*, *C. concinnus* and *Tarsipes rostratus*) (Turner 1984; Wooller *et al.* 1999; Wooller *et al.* 2000; Cadzow and Carthew 2004). Thus, mammals that are dependent on the seasonal supply of these food resources may time their reproductive activities to coincide seasonally with peaks in food abundances (Sadleir 1969; Tyndale-Biscoe 1973; Tyndale-Biscoe 2005). However, determining what selective advantages (i.e. ultimate causes) are associated with the timing of their seasonal reproductive activities has been difficult (see Brown and Shine 2006). The relationship between the timing of reproductive activities and food abundance is complex, because seasonal peaks in food abundance, and particularly, the availability of protein-rich foods, such as flowers and arthropods, may be associated with rainfall or latitude (Di Bitetti and Janson 2000). Further adding to the complexity is that long-term environmental cues, such as

photoperiod, may be important in underpinning the timing of breeding (Wikelski *et al.* 2000).

Many Australian marsupials are opportunistic or seasonal breeders (Tyndale-Biscoe 1977). Macropods, in particular, have evolved reproductive strategies well suited to unpredictable Australian arid and semi-arid conditions, in that the development of blastocysts may be activated only when food resources are abundant, or pouch young may be evicted if food resources become scarce (Low 1978; Tyndale-Biscoe 2001). Thus, larger herbivorous macropod species living in unpredictable semi-arid or arid environments often lack seasonality and breed opportunistically, e.g. euros and red kangaroos (*Macropus robustus* and *M. rufus*) (Newsome 1966; Newsome 1975; Tyndale-Biscoe 1989). An alternative strategy shown by some macropods, such as the western grey kangaroo (*Macropus fuliginosus*, Arnold 1991), living in cooler, temperate environments, is to breed seasonally such that young exit the pouch following autumn or winter rains (Tyndale-Biscoe 1977; Bolton *et al.* 1982; Arnold 1991).

Seasonal breeding in southern Australia is usually limited to the warmer months of the year (Tyndale-Biscoe 1973; Tyndale-Biscoe 2005), with many insectivorous marsupials following such a strategy, e.g. long-nosed bandicoot (*Perameles nasuta*), bush rat (*Rattus fuscipes*), New Holland mouse (*Pseudomys novaehollandiae*) and white-footed dunnart (*Sminthopsis leucopus*) (Kemper 1980; Wilson *et al.* 1986; Press 1987; Wilson 1991; White *et al.* 1996; Scott *et al.* 1999). Food resources in Australian eucalypt forests are usually more seasonally predictable than in arid and semi-arid regions, although less extreme in seasonality than those of the northern hemisphere (Majer *et al.* 2000). However, in a number of forests in southern Australia summer and winter-flowering eucalypts and banksias may povide year-round food resources for flower-dependent marsupials. For example, flower-dependent honey possums, living in temperate south-western Australia, are capable of breeding throughout the year (Renfree *et al.* 1984; Wooller *et al.* 1999; Wooller *et al.* 2000). However, interactions between the availability of food resources and the timing of reproduction in marsupials that are dependent upon arthropods and flowers has been little studied (although see Kavanagh 1987b; Millis and Bradley 2001).

The yellow-bellied glider (*Petaurus australis*) (Shaw and Nodder 1791) inhabits sclerophyll forests that extend from north Queensland, down the eastern seaboard to south-eastern South Australia, but are patchily distributed throughout their range (Goldingay and Possingham 1995; Carthew 2004). Faecal analyses and observations of foraging animals indicate that they forage on pollen and arthropods as protein food resources, and also make use of other plant and insect exudates, including phloem sap from eucalypts (Smith and Russell 1982; Henry and Craig 1984; Goldingay 1986; Goldingay 1989b; Goldingay 1990; Quin *et al.* 1996a; Carthew *et al.* 1999; Goldingay and Jackson 2004). The frequency with which yellow-bellied gliders forage for different food resources varies throughout their distribution (see Carthew *et al.* 1999 for a review). For example, nectar/pollen and insect exudates (honeydew)/arthropods contributed around 20-30% to the gliders' diet in most populations, but up to 70% at one site in New South Wales (Smith and Russell 1982; Henry and Craig 1984; Goldingay 1986; Goldingay 1989b; Goldingay 1990; Quin *et al.* 1996a; Carthew *et al.* 1999). Given the presumed importance of protein to lactation and development of offspring (Herrera 1998), gliders, if giving birth seasonally, would be expected to time their reproduction with peak abundance of arthropods, and/or peak abundance of flowers. In southern Australia, the peak period for arthropod abundance is spring/summer (Recher *et al.* 1996). Eucalypts flower at different times of the year, depending on site and species. However, at some sites, winter-flowering species provide an important protein resource in months when arthropods are least active and not readily available for foraging gliders (Kavanagh 1987b; Goldingay and Kavanagh 1991).

Some forests such as in south-western Victoria do not contain winter-flowering species and in such areas, gliders are dependent upon exudates as foraging substrates. For example, one population of yellow-bellied gliders in south-western Victoria was observed as being heavily dependent upon phloem sap as a food resource; sap feeding accounted for 83% of foraging observations, and sap was the most used substrate for 10 months of the year (except December and March) (Carthew *et al.* 1999). However, although phloem sap is carbohydrate-rich, it is protein-poor (see Ziegler 1975; also Pate *et al.* 1998 for composition of phloem sap in *Eucalyptus globulus*), and whenever eucalypts were in flower, gliders utilised nectar/pollen as a food resource (Carthew *et al.* 1999). Given that protein is

important for reproductive activities, yellow-bellied gliders may thus be expected to show seasonal breeding, consistent with seasonal availability and abundance of arthropods and flowers.

In this study I investigate the proposition that yellow-bellied gliders time reproduction with the availability of high quality protein resources. I quantify the phenology of flowering (as an index of pollen availability) and bark shed (as an index of arthropod availability) in eucalypt trees, and the timing of reproduction (births and late lactation) in female gliders, and assess the seasonality of each.

4.3 Materials and Methods

4.3.1 The study area

The study was conducted at Rennick State Forest (Rennick) in south-western Victoria, Australia (37°55'S 140°58'E), a forest of more than 5000 ha in size, with the 400 ha study site located on its western side. The sclerophyll forest is dominated by two eucalypt species: brown stringybark (*Eucalyptus baxteri*) and manna gum (*E. viminalis* ssp. *cygnetensis*), with brown stringybark much more widespread through the forest. Other eucalypt species present in the forest, but either not within, or just on the edge of, the study area are swamp gum (*E. ovata*), shining peppermint (*E. willisi*) and *E. viminalis* ssp. *viminalis*. Blackwood (*Acacia melanoxylon*), black wattle (*A. mearnsii*) and coastal wattle (*A. longifolia*) are also present at the study site. Understorey vegetation consists of silver banksia (*Banksia marginata*), *Leptospermum* spp., *Astroloma* spp., common heath (*Epacris impressa*), grass tree (*Xanthorrea* spp.) and bracken (*Pteridium* spp.).

Rennick experiences a seasonal, temperate climate with cool, wet winters from July to August: 4º C (min. average daily temp.) and 13º C (max.), and warm, dry summers from December to February: average 12º C (min.) and 26º C (max.). The average annual rainfall at Rennick is 787 mm, with the highest rainfall in winter (average > 100 mm per month) and the lowest in summer (average < 40 mm per month; Fig. 4.1).

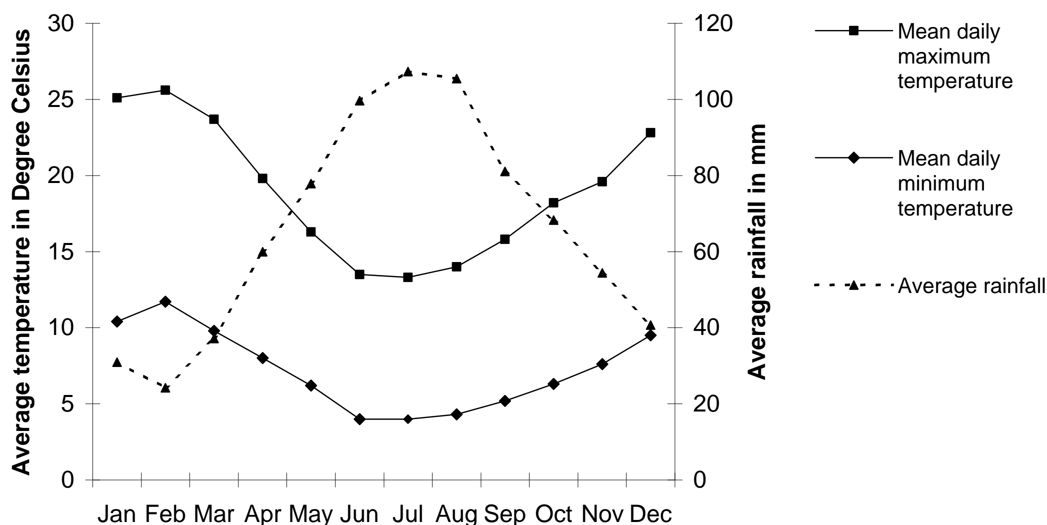


Fig. 4.1 Monthly mean daily maximum and minimum temperatures and average monthly rainfall for Rennick. Temperature data from 1948 to 2001 from the Australian Government Bureau of Meteorology, http://www.bom.gov.au/climate/averages/tables/cw_090092.shtml. Rainfall data from 1953 to 2003 from Hancock Victorian Timber Plantations.

4.3.2 Trapping and processing techniques

Yellow-bellied gliders in this population have been the subjects of a long-term study on their behavioural ecology, social organisation, mating system and habitat requirements (Carthew *et al.* 1999, Carthew and Goldingay, unpublished data, Chapter 3). In order to assess reproductive condition over time, seven social groups, each comprised of a single adult male and female (monogamous pairs, Brown *et al.*, in submission) and juveniles, were targeted for monthly trapping of adult females. The number of animals that could be captured each field trip was limited (see Appendix 3(a)(b) for trap success rates) because yellow-bellied gliders occur at very low population densities (Goldingay and Possingham 1995), are totally arboreal, and are particularly time- and labour-intensive to trap (e.g. Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990; Goldingay 1992; Goldingay *et al.* 2001). Nevertheless, trapping the same adult females over a period of 24 months provided a detailed assessment of the timing of breeding in a wild population. All gliders were individually marked with numbered metal ear tags (National Band & Tag Co., USA), sexed and aged according to the amount of tooth wear and colour of belly fur (*sensu* Goldingay 1989a; Goldingay and Kavanagh 1990; Goldingay 1992; Goldingay *et al.*

2001). Pouches of all females were investigated and reproductive condition noted (see below). Gliders generally behaved calmly whilst being handled, and were processed without the use of anaesthesia.

Yellow-bellied glider pouches have two deep compartments, each containing one teat, separated by a furred septum (Craig and Belcher 1980). Pouch condition was described using one of the following categories (*sensu* Goldingay 1989a):

a) Young females who had not yet bred (nulliparous) had pouches with a small, tight opening. The fur along the septum separating the two compartments was white, or very pale yellow. Teats were usually not visible and pouches were clean and pink.
b) Females who had bred, but were not reproductively active at the time of capture had yellow fur along the septum, a pouch opening that was loose (compared to females that had not bred), at least one teat was visible, and pouch was not vascular, but usually clean and pink.
c) For females with visible pouch young, the crown-rump length of pouch young was estimated by measuring with Verniers callipers from outside the pouch. It was also noted whether or not the pouch young was furred.
d) Females who were lactating, but did not have pouch young, had pouch openings that were loose and flabby. Lactation was accompanied by large, swollen and lumpy mammary tissue. As animals only had one young at a time (see results below), one teat was elongated, and milk could be expressed. Pouches usually contained dark spots (scale) and were deep.
e) Females in late lactation (females who had young in the process of being weaned or who had been weaned recently) had an elongated teat, and sometimes a swollen area around the mammary. Mammary tissue was not lumpy and liquid could not be expressed from the teat. Pouch openings were loose, but the pouch was shallower and lacked the scale present during lactation.

The birth of a glider was observed in this population during a previous study on the socioecology of the yellow-bellied glider (Gilbert 1993), with the young measured (crown-rump) as approximately 10 mm. We therefore used 10 mm as the size of pouch young at birth. In the current study 80 days was observed, for two

young, to be the maximum length that young were recorded as being in the pouch and, therefore, time of pouch exit was estimated by adding 80 days to the estimated date of birth. At the time of last measurement before pouch exit, the two young measured 73.5 mm and 80 mm (crown-rump length). Time to independence was estimated to be a further 60 days after pouch exit, as it has been estimated that juveniles spend approximately two months in the nest (Goldingay 1989a). Pouch exit (defined here as the time when young gliders leave the pouch permanently) and nestling stage (where young gliders are deposited in the nest) were considered an important energy-demanding period, because marsupials are known to make a much greater investment in late lactation, compared with early and mid lactation (Tyndale-Biscoe 2005). Unlike eutherian mammals, the composition of milk in marsupials changes throughout lactation (Green 1984; Green and Merchant 1988; Nicholas 1988). There is evidence that the composition of milk in a number of marsupial taxa increases in total solids, and protein and lipid content during late lactation (Munks *et al.* 1991; Rose and Flowers 2005, Rose *et al.* 2003, also reviewed in Green and Merchant 1988). There are similarities in the qualitative and quantitative changes in milk composition across different marsupial taxa (Green 1984; Green and Merchant 1988), and although milk composition has not yet been studied in petaurid gliders, they are likely to follow a similar pattern.

4.3.3 Forest phenology

Flowering and percentage of bark shed, as an index of arthropod availability (see below), were monitored monthly between August 2001 and August 2003 (except September 2001) on a sample of eucalypt trees distributed along transects throughout the forest. A total of 160 trees on 17 transects were monitored. A further 10 randomly chosen trees were monitored from January 2002 increasing the total number of monitored trees to 170. Because manna gum were not abundant in this forest, but were important for the assessment of bark shed (see below), transects were placed in areas that contained patches of manna gum. Most transects were located within the home range areas of known yellow-bellied glider groups, and thus represented what was available to the animals. Monitored trees comprised 125 brown stringybark, 44 manna gum and one shining peppermint. One brown stringybark died during the study and was removed from all analyses. Shining peppermint is a summer flowering species, of which a very small number occurred only on the south-eastern edge of the

study site, and has been excluded from analyses of flowering data. However, it is a smooth-barked species, and has been combined with manna gum for the percentage of bark shed. Only trees that were reproductively mature (i.e. had at least some buds or fruit) were included in the study.

During monitoring each month, the canopy of each tree was examined for flowers through binoculars (10 × 50 magnification). The canopy was first scanned for flowers, and if present, an estimate was made of the abundance of flowers. An exact count was made on trees with fewer than 50 flowers. Otherwise, the canopy was divided into smaller, manageable units of approximately 1 m^2. These were the terminal points of branches of the tree where flowers clustered as 3-5 per inflorescence. An average number of flowers were determined for the 1 m^2 units, and the number of units counted across the canopy. Whether trees flowered heavily or lightly was examined by sorting the data into three categories of light (≤ 1,000 flowers), medium (1,001-10,000 flowers) and heavy (≥ 10,001 flowers). These categories were subjectively chosen by looking at the raw data to see where natural breaks occurred. Analyses for both species were also conducted on the estimated abundance of flowers, the percentage of trees in flower and the number of months over which trees flowered.

Bark shed, as an index of the abundance of arthropods (see Kavanagh 1987b; Dickman 1991), was assessed monthly on 45 trees; 44 manna gum and one shining peppermint. Dickman (1991) measured seasonal changes in the abundance and activity of arthropods, and observed that in spring arthropods began migrating from leaf litter at the base of trees up the trunks of smooth-barked eucalypts, and that this was associated with an increase in the amount of bark shed. Smooth-barked eucalypts shed their bark periodically and yellow-bellied gliders forage underneath decorticating bark for sheltering arthropods (see Goldingay 1986; Kavanagh 1987a). Gliders are able to peel bark away from the trunk or branches, or forage through bark that has already peeled away from the tree and is hanging down in ribbons. Brown stringybark was excluded from the bark shed assessment, as it has persistent fibrous bark, and does not shed in a manner that enables gliders to forage underneath for arthropods. During monitoring, estimates were taken of the percentage of bark being

shed from the trunk, main and outer branches and were categorised as either bark being shed by the tree (bark shed) or as curled pieces, still attached to the tree, but hanging down in ribbons (curled bark). The percentage of curled bark was estimated as a percentage of bark being shed. Estimates of bark shed and curled bark were placed into one of the following categories: 0-20% = low, 21-60% = medium, 61-100% = high. Bark shed data was collected between August 2001 and August 2003, but curled bark estimates commenced in January 2002.

To determine whether breeding, flowering and bark shed occurred seasonally I used circular statistics (*sensu* Batschelet 1981) to test the hypothesis that the distributions would differ significantly from random. Circular or directional variables, such as any time period, may be represented by a rotation of 360°. Frequencies of births, flowering and bark shed were clustered into 12 groups of 30° each, with each group representing one month of the calendar year. Time instants (i.e., months) were converted into angles, measured in degrees, with the mean and deviations from the mean measured by sine and cosine (*sensu* Batschelet 1981). Distributions differing significantly from random were taken to mean that data were seasonal, whilst distributions not differing significantly from random were taken to mean data were aseasonal. Circular statistics were conducted using ORIANA V2.0 (Kovach Computing Services, UK); other statistical analyses were performed using MICROSOFT EXCEL or SPSS V.13.0. Data were tested for normality and non-parametric versions of statistical tests used if assumptions of normality were not met.

4.4 Results

4.4.1 Flowering phenology

Flowering in both manna gum and brown stringybark showed distinct seasonality. Circular statistics indicated that distributions of the percentage of trees in flower differed significantly from random (manna gum: Rayleigh test $Z = 198.6$, $P < 0.001$, brown stringybark: $Z = 122.7$, $P < 0.001$), and were seasonal over both years (Fig 4.2). Monitored manna gum flowered one month earlier in the second year, but brown stringybark flowered at the same time in both years (manna gum: year 1: mean month ± SE° = April ± 2.8°; year 2: March ± 5.1°, brown stringybark: year 1: February ± 2.7°; year 2: February ± 5.2°). The percentage of manna gum in flower was significantly greater in the first year (Wilcoxon Signed Ranks $Z = 1.97$, $P =$

0.05), although the abundance of flowers did not show the same trend (Z = 0.47, P = 0.64). The percentage of brown stringybark in flower and abundance of brown stringybark flowers showed no significant differences between years (percentage of trees in flower: Z = 1.91, P = 0.06; abundance of flowers: Z = 0.89, P = 0.37), although the percentage of trees in flower was almost significantly different.

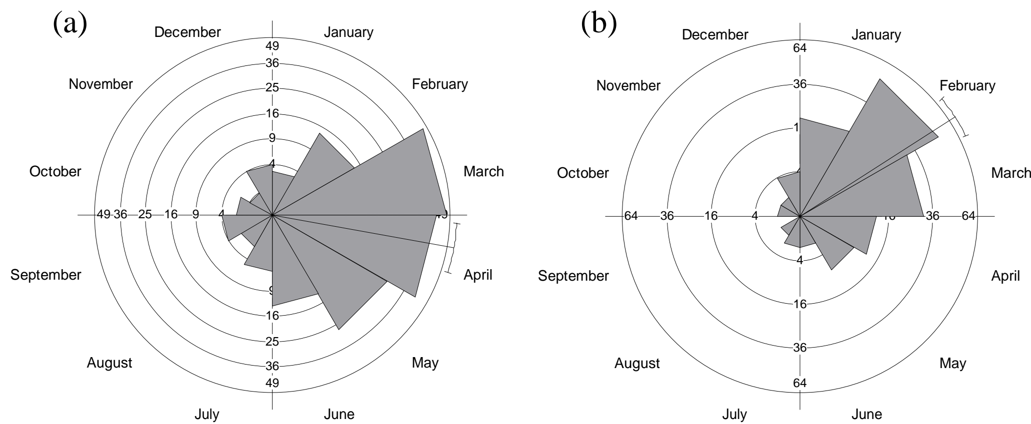


Fig. 4.2 Circular distributions of the number of (a) manna gum and (b) brown stringybark monitored trees in flower for both years combined. Number of trees in flower is indicated on the N-S, E-W axes. The length of each wedge is representative of the number of trees in flower for that month. The bold line emerging from the centre to the edge is the mean, whilst the arcs on the outside of the circle are the 95% confidence limits of the mean.

Most mann gum and brown stringybark trees flowered for one or two months (manna gum: year 1: 2.6 ± 0.2 months; year 2: 1.1 ± 0.2 months and brown stringybark: year 1: 2.0 ± 0.1 months; year 2: 0.7 ± 0.1 months). The number of months over which trees were in flower differed significantly between years, with flowering in both species progressing for longer in the first year (manna gum: Wilcoxon Signed Ranks Z = 4.80, P < 0.001; brown stringybark: Z = 8.14, P < 0.001).

In both years, increases in the abundance of flowering trees in the two eucalypt species were driven by increases in the number of trees flowering lightly (i.e. trees with fewer than 1,000 flowers) (Fig. 4.3). A small number of manna gum carried between 1,001-10,000 flowers (medium category), not only through the peak season (autumn), but also at various times throughout the year. Monitored manna gum trees that flowered heavily (> 10,001) tended to do so during the peak season,

but occasional trees were observed to flower heavily outside peak season. Except during November 2001, a small number of manna gum flowered lightly year round. The number of brown stringybark trees with between 1,001-10,000 (medium category) flowers tended to increase around peak season (summer) in both years, but were also observed in the months after peak seasons (autumn), particularly in the first year. Very few brown stringybark trees were observed to flower heavily in either year.

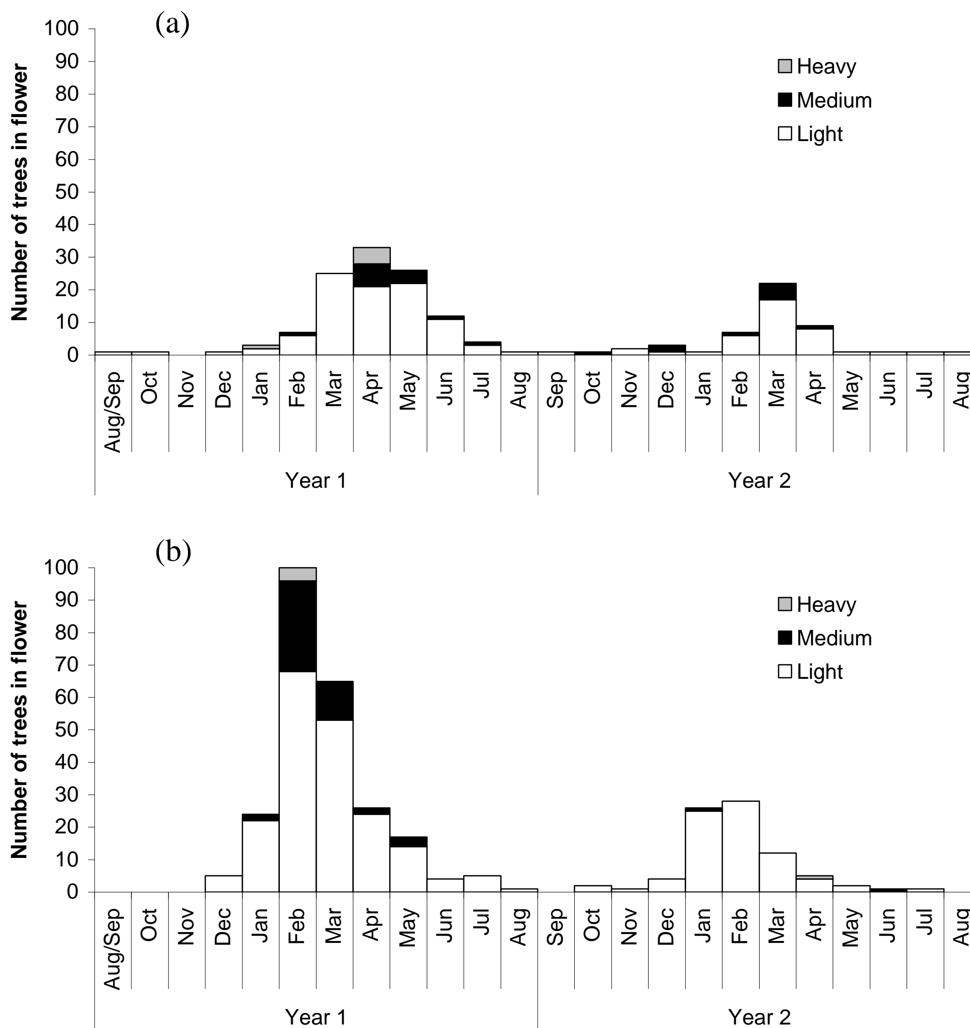


Fig. 4.3 Number of monitored (a) manna gum and (b) brown stringybark trees in various phases of flowering. Trees were categorised as having <1,000 flowers (light), between 1,001 and 10,000 (medium) flowers, and ≥ 10,001 (heavy) flowers. Data were collected between August 2001 and August 2003. n = 44 monitored manna gum trees, n = 124 monitored brown stringybark trees.

4.4.2 Bark shed phenology

High bark shed for the trunk and outer branches peaked over summer and declined thereafter, whilst on the main branches it also peaked in summer, but did not really decline until early winter. Bark shed showed some seasonality, but was neither consistent across all parts of the tree nor between years. For example, bark shed on the trunk for both years combined did not differ significantly from random for low (Rayleigh test $Z = 0.87$, $P = 0.42$), or medium amounts ($Z = 1.43$, $P = 0.24$), but did for high amounts ($Z = 4.00$, $P = 0.02$). This was because bark shed on the trunk in the high category was seasonal in the second year (year 1: $Z = 0.65$, $P = 0.52$; year 2: $Z = 4.10$, $P = 0.02$) (Fig. 4.4(a)). Distributions of bark shed on the main branches for both years combined differed significantly from random for the low and high categories (low: $Z = 22.66$, $P < 0.001$; high, $Z = 20.36$, $P < 0.001$), but not the medium category ($Z = 2.01$, $P = 0.13$) (Fig. 4.4(b)). Distributions for all categories on the outer branches for both years combined differed significantly from random (low: $Z = 33.06$, $P < 0.001$; medium: $Z = 40.60$, $P < 0.001$; high: $Z = 8.43$, $P < 0.001$). However, when data for each year were examined separately, the high category for the first year on the outer branches did not differ significantly from random ($Z = 2.17$, $P = 0.11$) (Fig. 4.4(c)).

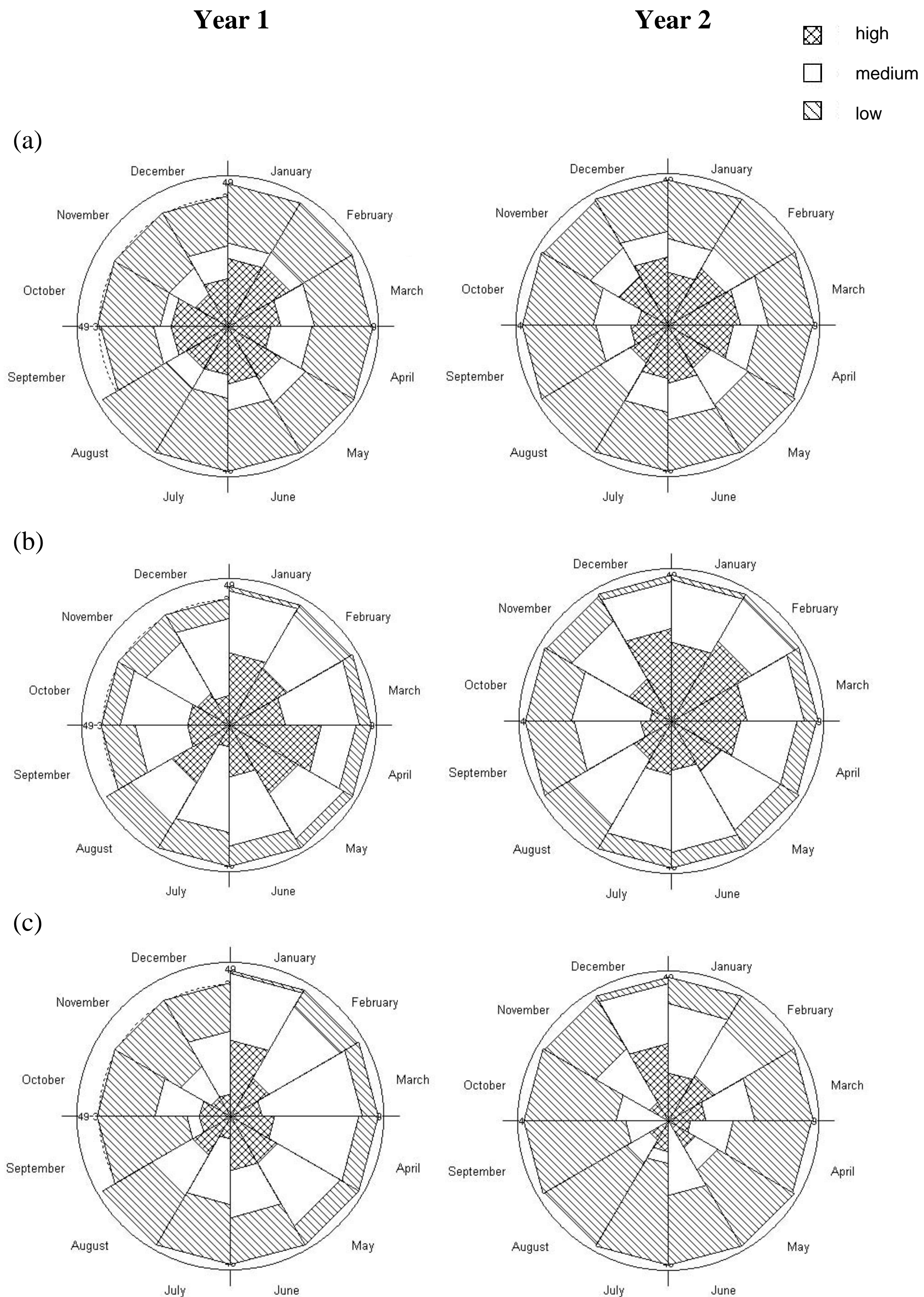


Fig. 4.4 Circular distributions of the number of trees in each of the high, medium and low categories of bark shed, where (a) = trunk, (b) = main branches, (c) = outer branches. High = > 60%, medium = 40-60%, low = < 40% bark shed. Number of monitored trees are on the N-S, E-W axes. Year 1 = August 2001-August 2002 (data not collected in September 2001), year 2 = September 2002-August 2003.

A peak in curled bark occurred in mid-late autumn, following the summer peak in bark shed. Distributions of curled bark in the high category differed from random on the main and outer branches (main: Rayleigh test $Z = 33.71$, $P < 0.001$; outer: $Z = 59.53$, $P < 0.001$) indicating seasonal availability of curled bark, the peak of which occurred from January through to August.

4.4.3 Timing of reproduction

Ten adult females were captured within the study area between August 2001 and August 2003. Nine females were captured five or more times during the study period (mean ± SE = 8.5 ± 1.3 captures/female, range = 2-15 captures/female). A total of 16 pouch young were recorded from the nine adult females, with all females having at least one pouch young during the study, and females never having more than one pouch young at a time. In this study, births occurred throughout most months of the year. Circular statistics indicated that the distributions of births did not differ from random, indicating a lack of seasonality (year 1: Rayleigh test $Z = 0.039$, $P = 0.96$, $n = 7$ births; year 2: $Z = 1.48$, $P = 0.23$, $n = 9$ births; both years: $Z = 1.09$, $P = 0.34$, $N = 16$ births). However, there were peaks of births in January (summer), June and July (winter) and September (spring) when data were combined for both years (Fig. 4.5a). There was a gap in births between March and May (autumn) in both years. The second year of births contained a more even spread of births throughout the year, however, there were two birth peaks in July (winter) and September (spring). Although sample sizes were small, birth peaks from this study over the two years, are consistent with what has been reported from the long-term study on the behaviour of this population of gliders (N = 38 births) (Carthew and Goldingay, unpublished data).

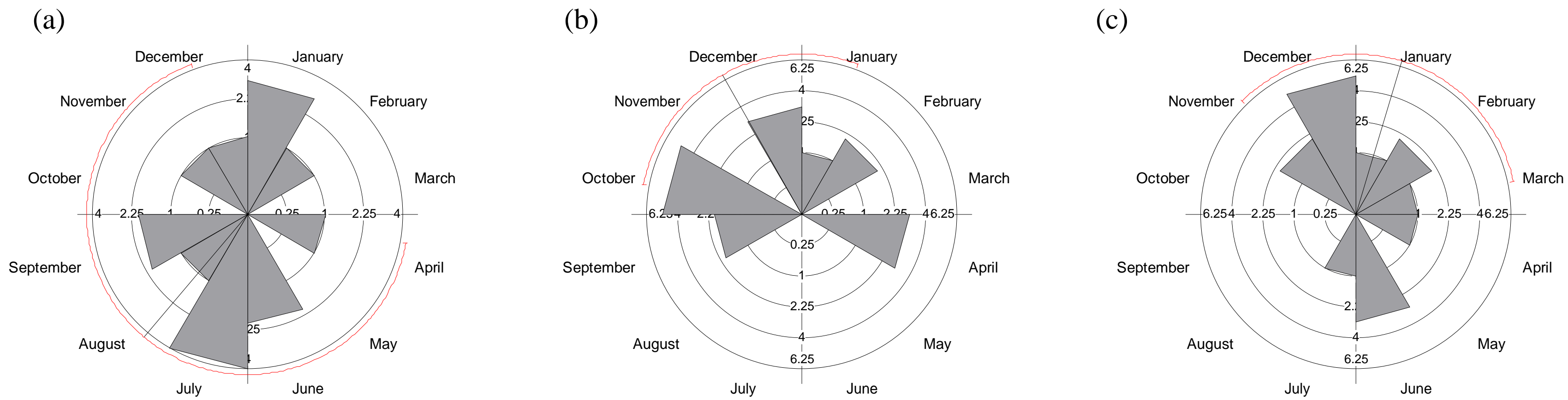


Fig. 4.5 Circular distributions of estimated dates of (a) births, (b) pouch exit and (c) independence. Number of births are on the N-S, E-W axes. The length of each wedge is representative of the number of births, pouch exit and independent young for that month. The line emerging from the centre to the edge is the mean, whilst the arcs on the outside of the circle are the 95% confidence limits of the mean.

Further, results from statistical tests on the distribution of births in the latter dataset were consistent with those here, in that the distribution of births did not differ from random (data not shown), and thus births were not seasonal. Observations taken here of the development of pouch young over time suggest that late lactation and weaning take place four-five months from the estimated date of birth, with approximately 70-80 days in the pouch and approximately two months in the nest (see Goldingay 1989a). Thus, pouch exit (coinciding with late lactation) during this study would have taken place in spring, early summer and autumn (Fig. 4.5b above). Young gliders would have been independent by summer and winter (Fig. 4.5c above).

Interbirth intervals (the interval from the estimated date of birth of one pouch young to the estimated date of birth of the next pouch young), were estimated for five adult females. Five such intervals were calculated for three females whose pouch young were presumed to have survived to weaning and were subsequently captured as juveniles. This gave a mean ± SE of 292 ± 27 days, with a range of 213-369 days. Interbirth intervals of two females whose pouch young were presumed not to have survived to weaning were much shorter (62 and 117 days). These data suggest that a post-partum oestrous may occur in females that have lost their young.

4.5 Discussion

The relationship between forest phenology and the timing of reproduction in the Rennick population of yellow-bellied gliders is not a straightforward one. Although birth peaks were evident when data were combined for both years, statistical tests on distributions of births in each year, and in both years combined, indicated that they did not differ from random and thus, were not seasonal. However, the distributions of flowering and bark shed on the main and outer branches did differ from random, indicating seasonality. Thus, yellow-bellied gliders appear to breed aseasonally in a seasonal environment.

Birth peaks in January and June/July (summer and winter, respectively), were followed by pouch exit/late lactation in April and October (autumn and spring, respectively). This means that while some females give birth in winter when protein resources are scarce, the more energetically-demanding reproductive activity of late

lactation (see Green 1984; Green and Merchant 1988) would commence in autumn and spring. Ample protein is available for lactating mothers and developing offspring in the population at this time, with an increase in the abundance of arthropods in spring and flowering of manna gum in autumn. Interestingly, a summer birth peak (with pouch exit in autumn) was only recorded in the first year, and coincided with the later flowering of manna gum that year. There may also be a peak in spring, but limited data preclude firm conclusions. Females that gave birth in spring sould have been in late lactation in summer, which would coincide with abundance of arthropods and flowering in brown stringybark.

Although yellow-bellied gliders at Rennick were not limited to breeding within distinct seasons, they have been described as breeding seasonally at two populations in NSW. For example, at Bombala in southern NSW, births in late winter-spring were followed by weaning in summer, a time when bark shed was at its peak, and arthropods readily available (Goldingay and Kavanagh 1990). However, at Kioloa in NSW (some 170 km north of Bombala), young yellow-bellied gliders were weaned earlier in the year, when spotted gum (*E. maculata*) was in flower (Goldingay 1992). The lack of birth seasonality in the Rennick population and differences in the timing of onset of breeding season in the two NSW populations make it unlikely that gliders were responding to seasonal changes in photoperiod (see Tyndale-Biscoe 2005). Births occurred in almost all months in a population of yellow-bellied gliders in north Queensland (Goldingay *et al.* 2001), possibly reflecting the more continuous availability of arthropods in the northern areas of Australia. This lack of seasonality in northern areas of Australia has also been found in a number of small insectivorous marsupial species (e.g. *Antechinus melanurus*, *A. naso* and *Planigale maculata sinualis*) (Lee *et al.* 1982; although see Watt 1997).

Similarly to yellow-bellied gliders, sugar gliders (*Petaurus breviceps*) have been recorded as seasonal breeders in the southern parts of their distribution (winter to early summer) (Suckling 1983; Henry and Suckling 1984; Quin 1995), but bred in almost all months in a population in north Queensland (Jackson 2000b). However, births recorded in a population of mahogany gliders (*Petaurus gracilis*) in north Queensland over two years were seasonal, and occurred from April to October (Jackson 2000b). Gliders in this population were observed feeding on insects/

arthropods during the study period, but a far greater percentage of the foraging time was spent gleaning flowers for nectar/pollen (see Jackson and Johnson 2002). Thus, mahogany gliders were likely to be more reliant upon seasonal availability of flowers from a variety of *Eucalyptus* and *Melaleuca* species, which is reflected in their seasonal breeding, than a continuous supply of arthropods.

Seasonality of reproduction was evident within a population of squirrel gliders (*Petaurus norfolcensis*) in south-east Queensland, with females anoestrous over the summer months (Millis and Bradley 2001). Seasonality was also detected in a population of squirrel gliders inhabiting remnant linear patches at Euroa in central Victoria (van der Ree 2002). However, births were recorded in most months in a population of squirrel gliders on the central-north coast of NSW (between the Queensland and Victorian populations), although there were distinct winter peaks either preceded by a spring peak, or followed by an autumn peak (Quin 1995). Thus, neither photoperiod nor variation in latitude provides a convincing explanation for variation in the timing of reproduction within petaurid species. Rather, these observations provide support for the contention that local environmental conditions, such as climate and rainfall, drive seasonal abundance in food resources, and thus timing of breeding in petaurids. Further, glider birth peaks have been recorded as varying from year to year (Quin 1995; Jackson 2000a; van der Ree 2002, this study), providing support for the contention that gliders follow seasonal differences in flowering or arthropod availability (*sensu* Kavanagh 1987b).

It has been assumed here and in other studies (e.g. Kavanagh 1987b) that seasonal availability of protein is primarily responsible for variation in the timing of reproduction in yellow-bellied gliders. However, in at least two populations of yellow-bellied glider, foraging on phloem sap accounts for significant amounts of foraging time (Quin *et al.* 1996a; Carthew *et al.* 1999). Previous work has also shown that phloem sap was used at most times of the year, and was the most heavily used substrate through winter in the Rennick population of gliders (Carthew *et al.* 1999). Although data from this study indicate that curled bark, which may provide shelter for over-wintering arthropods, is available in winter, there is little evidence that gliders feed on arthropods at that time of the year (Carthew *et al.* 1999). Rather, it would appear that gliders may rely on carbohydrate-rich food resources (i.e. phloem

sap and insect exudates) during winter, whilst they give birth, but protein-rich food resources (i.e. pollen and arthropods) during late lactation and weaning. This suggests that yellow-bellied gliders, as with other petaurids, may be described as income breeders (*sensu* Jönsson 1997), that give birth during resource-poor seasons and coincide the more energy-demanding reproductive activities (i.e. late lactation) with resource-rich seasons. However, further research is required to determine what exactly (e.g. rainfall) prompts the timing of births in yellow-bellied gliders 4-5 months before pouch exit. Also, there are a number of biological features of yellow-bellied gliders that are not well understood, such as length of oestrous cycle and gestation, and whether there is embryonic diapause. In particular, it is not known whether offspring that are born outside favourable times survive to weaning.

It would appear that describing petaurids as either seasonal breeders or not, is oversimplifying the complex interplay between these species and their environment. The variety of food resources available to petaurids means that there may be little need to time births to short periods during which only one protein food resource is abundantly available. Further, different populations may be reliant upon different food types and show local adaptation of the timing of breeding to that particular food resource (e.g. Goldingay and Kavanagh 1990; Goldingay 1992). This suggestion is in contrast to many other Australian marsupials in southerly latitudes that are annual seasonal breeders (Tyndale-Biscoe 1973; Tyndale-Biscoe 2005).

Chapter 5. Phylogeography of the yellow-bellied glider

5.1 Preamble

The following chapter was submitted to Australian Journal of Zoology and is now in press. It presents data and analysis on phylogeography and conservation units for yellow-bellied gliders throughout their range. Some of the work described in this chapter was carried out by others, and is indicated further in Appendix 4.

5.2 Introduction

The removal of mature eucalypt forests for agriculture and timber production has resulted in the decline in population sizes and distribution of a number of species of arboreal marsupial that were once widespread throughout eastern and southern Australia. In particular, the yellow-bellied glider, *Petaurus australis*, now has a patchy distribution throughout its range and is listed as endangered or vulnerable in three of the four states in which it occurs. The species has large home range requirements and its dependence upon mature forests, which provide foraging substrates and tree hollows, means it is sensitive to forest disturbance and habitat fragmentation (Goldingay and Possingham 1995). Yellow-bellied gliders have come close to extinction in south-eastern South Australia on the western edge of their range. Here more than 80% of the former forest cover has been removed for agriculture and softwood production, and only a single small population of these gliders survives in an isolated eucalypt forest patch (Carthew 2004). There is clearly a need for conservation management of yellow-bellied gliders, particularly in timber production forests. However, management programs are difficult to formulate, as despite considerable research on some aspects of the ecology of the species (see Goldingay and Kavanagh 1991), there is limited knowledge of gene flow between populations and uncertainty about the most appropriate units to be used for conservation management.

At the extreme ends of the range of yellow-bellied gliders, in northern Queensland (NQ) and south-western Victoria (Vic.)/South Australia (SA), populations have become isolated and disjunct from other parts of its range (Fig. 5.1). Long-term isolation of these populations may have resulted in significant

genetic divergence, raising the possibility that these populations may be genetically distinct at some level and should be given particular conservation significance. The isolated populations in NQ are recognised as a separate subspecies under the Environmental Protection and Biodiversity Conservation Act 1999 (EPBC Act) as *Petaurus australis unnamed subsp*, Fluffy Glider, Yellow-bellied Glider (Wet Tropics) and have been afforded the conservation status 'vulnerable'. However, subspecies status was originally conferred from a type specimen, named *P. australis reginae* (Thomas 1923), sourced from a population at Gin Gin in southern Queensland (Thomas 1923). This region is not part of the isolated north Queensland (Wet Tropics) populations, but is at the northern end of the distribution for southern populations (indicated in Fig. 5.1). Later publications (e.g. Russell 1983) have referred to only the north Queensland populations as being *P. a. reginae*, with southern Queensland (SQ) populations included within the southern subspecies *P. a. australis*.

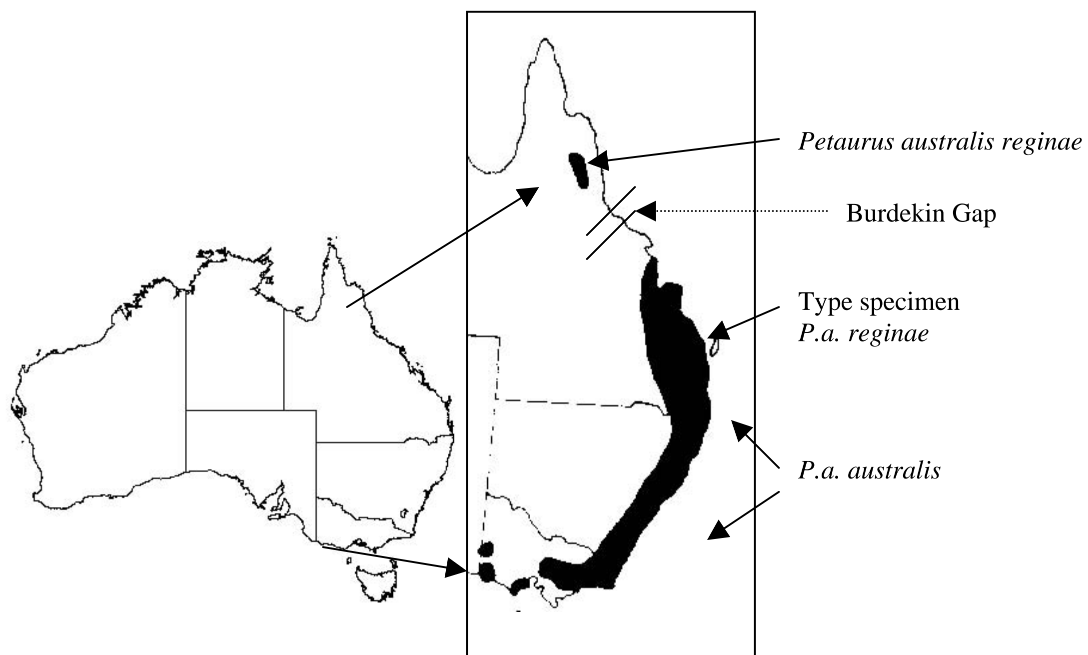


Fig. 5.1 Distribution map of the yellow-bellied glider (*P. australis*) showing both subspecies as they are recognised in the literature (e.g. Russell 1983). The location of the type specimen, *P. a. reginae* (Thomas 1923), is indicated.

The designation of *P. a. reginae* was based on differences in colour of the pelage, with the original type specimen described as having lighter ventral fur colour than yellow-bellied gliders from populations further south in the distribution

(Thomas 1923). Subsequent investigations of the subspecies in the northern isolated populations provided support for the taxonomic distinctiveness of this subspecies (Winter *et al.* 1979; Russell 1983). However, the distinctiveness of this subspecies with respect to its fur colour has been historically contentious (Finlayson 1934; Tate 1952). Further, a number of studies on yellow-bellied gliders in the southern and northern parts of its distribution suggest that the colour of belly fur varies according to the age of the individual (Goldingay and Kavanagh 1990; Goldingay 1992; Goldingay *et al.* 2001). Despite the contention over morphological characters, no comparisons of the genetics or skeletal morphology of the northern and southern populations have been published.

One critical issue for the conservation management of yellow-bellied gliders is the identification of appropriate conservation units, whose delineation and preservation will help conserve both evolutionary processes and the ecological viability of populations. Although the concept of a subspecies is widely used as a surrogate for a conservation unit, it does not always effectively describe intra-specific units with different evolutionary histories (Burbrink *et al.* 2000; Zink 2004). In particular, it is claimed that the subspecies concept obscures the amount of intra-specific biodiversity (Zink 2004), and thus should be abandoned (Wilson and Brown 1953; Barrowclough 1982). The concept of the evolutionarily significant unit (ESU) was proposed by Ryder 1986) as an alternative unit for conservation. The use of molecular tools, particularly based on phylogeographic analyses of mitochondrial sequence data, for objective criteria in assigning populations to ESUs (*sensu* Moritz 1994b) has become popular (e.g. Pope *et al.* 2000). However, the use of genetic criteria alone for the establishment of ESUs has been criticised as being restrictive, as it may not necessarily reflect ecological exchangeability and adaptive potential of populations (Paetkau 1999; Crandall *et al.* 2000). However, it is not always feasible to assess ecological exchangeability, particularly for species, such as yellow-bellied gliders, showing extensive, but patchy distributions. Thus, Fraser and Bernatchez (2001) advocate a more flexible approach under their concept of "adaptive evolutionary conservation". This concept defines an ESU as "a lineage demonstrating highly restricted gene flow from other such lineages within the higher organizational level (lineage) of the species" (Fraser and Bernatchez 2001).

This study uses a combination of mitochondrial DNA (mtDNA) sequencing and morphological analyses to investigate the distinctiveness of yellow-bellied glider populations across its range in Australia. In particular, this study focuses on populations at the limits of the distribution in NQ and Vic./ SA. The combination of approaches provides independent data sets that can be used to define ESUs under the criteria proposed by Fraser and Bernatchez (2001). Specifically, I aimed to (i) determine the phylogeographic structure of yellow-bellied gliders based on mtDNA sequences and assess whether isolated Vic./ SA and NQ populations represent distinct genetic lineages; (ii) determine if variation in morphological characters is consistent with the genetic analyses; and (iii) address issues of conservation management for this species.

5.3 Materials and Methods

5.3.1 Tissue samples

Thirty-two yellow-bellied glider samples were obtained from populations distributed across the range of the species in Australia (Appendix 4). Most samples were obtained by live trapping or were museum specimens. Overall, sample sizes were limited because of the difficulty of trapping gliders in tall eucalypt forests, which leads to very low trap success rates (see Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990; Goldingay 1992; Carthew *et al.* 1999; Goldingay *et al.* 2001). These difficulties stem from the arboreal and nocturnal nature of the species, low population densities (Goldingay and Possingham 1995) and exclusive home ranges of adults - home ranges of around 30ha (M. Brown; S. M. Carthew and R. L. Goldingay, unpublished data). Further, specimens suitable for DNA analysis are poorly represented in museum collections within Australia and prior to the commencement of our project only two samples, both museum skins, were available from populations in northern Queensland. Intensive trapping by the first author, M.B., in northern Queensland forests over a four-week period led to the collection of four further specimens. The endangered and vulnerable status of most populations of gliders meant that sampling techniques involving the removal of small skin biopsies were preferable to the collection of voucher specimens. Skin biopsies (~3×3mm) were removed from the bottom of the ear of each animal, with the exception of individuals from a captive colony at Taronga Zoo in Sydney, NSW, from which

blood samples were removed (see Brown *et al.* 2004). The zoo specimens were sourced from populations in NSW, but precise location details were recorded for only one of these samples (see Appendix 4). Skin biopsies were stored in vials of 50:50 ethanol/saline at room temperature. Samples used as outgroups for phylogenetic analyses included frozen liver tissue from two specimens of *Petaurus breviceps*, one from Australia and one from Papua New Guinea, one specimen of *Petaurus norfolcensis* from South Australia (Malekian *et al.* in press) and one specimen of *Petauroides volans*, sourced from the Australian Biological Tissue Collection at the South Australian Museum (see Appendix 4 for details).

5.3.2 mtDNA sequencing

DNA extractions for all samples were by salt extraction (Miller *et al.* 1988), DNAzol (Chomczynski *et al.* 1997) or the Gentra Puregene Extraction Kit (according to manufacturer's instructions). I initially used primers L15999M and H16498M (Fumagalli *et al.* 1997) to PCR-amplify and sequence an approximately 550 bp segment of the mtDNA control region. Preliminary sequencing results revealed large regions of repetitive DNA, tandem repeats of a 25 bp repeat unit, and multiple PCR-amplification products. We, therefore, abandoned the use of the control region and utilised an approximately 900 bp segment of the NADH dehydrogenase subunit 4 (*ND4*) gene, using truncated versions of the primers *ND4* and Leu (Arevalo *et al.* 1994): mt10812H: 5'-TGA CTA CCA AAA GCT CAT GTA GAA GC-3' and mt11769L: 5'-TTT TAC TTG GAT TTG CAC CA-3'. Petaurid-specific internal primers were designed for *ND4*: mt11242H: 5'-AAA CAG CCT TAC CCC TCA TA-3' to be used with mt11769L, and mt11334L: 5'-TTA ATT CGC CTA GTA GGT TGA TTG T-3' to be used with mt10812H to amplify smaller fragments of DNA of approximately 520 bp each. When the two internal primers were used together a fragment of approximately 93 bp was PCR-amplified, which was useful to confirm the degradation of DNA extracted from museum specimens.

PCR amplifications were carried out in 20 µl volumes with approximately 100 ng genomic DNA, 2.5 mM $MgCl_2$, 0.20 mM dNTPs, 1 x PCR buffer (Applied Biosystems), 5 pmol of each primer (Geneworks) and 0.5 units of Ampli*Taq* Gold (Applied Biosystems). PCR amplification was performed under the following

conditions: 94° C 9 min, then 34 cycles of 94° C 15 s; annealing 50° C 30 s; 72° C, 45 s; with a final elongation step at 72° C for 5 min. A variation on the annealing temperature was 54°C 30s for the use of the internal Petaurid-specific primers. PCR product was purified using Ultraclean PCR cleanup columns (MoBio Labs) and sequenced in both directions using the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems). Sequencing was carried out on an ABI 3700 DNA analyser and edited using SEQED v.1.0.3 (Applied Biosystems). Sequences were submitted to GenBank (accession numbers provided in Appendix 4).

5.3.3 mtDNA analyses

Phylogenetic analyses of the *ND4* sequence data were conducted using Maximum Parsimony (MP), as implemented in PAUP* v.4.0b10 (Swofford 2002), and a Bayesian approach using MRBAYES v.3.1.1 (Huelsenbeck and Ronquist 2001). Concordance of trees from the different methods and bootstrap and posterior probability estimates were used to examine the robustness of nodes. MP analyses were conducted using a heuristic search option and default options with the exception of using random stepwise addition repeated 100 times. Character state optimisation for MP trees used the DELTRAN option; there was bug in PAUP* v.4.0b10 in the default ACCTRAN option that leads to erroneous branch lengths in output trees.

MP bootstrap analyses (Felsenstein 1985) were carried out using 500 bootstrap pseudoreplicates, using a heuristic search option with random input of taxa. Bayesian phylogenetic analyses were carried out using the program MRBAYES (Huelsenbeck and Ronquist 2001). A General Time Reversible model (Rodríguez *et al.* 1990), with a proportion of invariant sites and unequal rates among sites (Yang 1996), modeled with a gamma distribution (GTR+I+G) in MODELTEST (Posada and Crandall 1998), was found to be the most appropriate model to use in the Bayesian analyses. The MRBAYES analysis was carried out, applying one model to the entire data set without partitioning, using default uninformative priors, running four chains simultaneously for 1.5 million generations in two independent runs, sampling trees every 100 generations. After this number of generations the standard deviation of split frequencies had reduced to less than 1%, confirming that a good sample of the posterior distribution had been obtained. The likelihood values converged to relatively stationary values after about 5,000 generations. A burnin of

100 trees (equivalent to 10,000 generations) was chosen with a > 50% posterior probability consensus tree constructed from the remaining 14,901 trees.

To further examine haplotype relationships within yellow-bellied gliders, a haplotype network was constructed using the statistical parsimony method implemented in TCS v.1.21 (Clement *et al.* 2000). However, data were insufficient to perform a nested clade phylogeographic analysis on the parsimony network (*sensu* Templeton 1998).

Intra-specific sequence divergence among haplotypes was estimated using the HKY85 model (Hasegawa *et al.* 1985), implemented in PAUP*. This model was shown to be optimal by MODELTEST analyses after exclusion of outgroup taxa (Posada and Crandall 1998). Divergence estimates based on the HKY85 model were found to give very similar values to the Kimura 2-parameter (Kimura 1980) model (data not shown), the latter being used by other researchers to estimate inter and intra-specific divergence in marsupials (e.g. Osborne and Christidis 2001). Nucleotide diversity was estimated using the Kimura 2-parameter model in ARLEQUIN v.3.01 (Excoffier *et al.* 2005). An AMOVA was performed in ARLEQUIN to test the hypothesis that genetic divergence between each population differed from zero (Excoffier *et al.* 1992). Four populations (NQ, NSW, SQ and Vic./SA) were tested on the *a priori* assumption that geographical isolation formed two natural groups, NQ and Vic./SA, whilst the state border between Queensland and NSW was arbitrarily chosen to separate this region into two further groups (SQ and NSW). It was later found that SQ and NSW were not significantly differentiated and results were repeated by assigning haplotypes into three regions (NQ, Vic./SA and NSW/SQ). F_{ST} estimates among pairs of populations was conducted using the distance method as implemented in ARLEQUIN (Excoffier *et al.* 1992).

5.3.4 Morphological measurements and analyses

Morphological measurements were taken from 34 yellow-bellied glider skulls in collections from the Australian, Queensland and South Australian Museums, the Museum of Victoria and Australian National Wildlife Collection. Catalogue numbers are available in Appendix 5. Only adults, as assessed by the amount of tooth wear on the lower and upper incisors, were included in the analyses. Where possible, all

measurements were taken with vernier callipers to the nearest 0.05 mm on the right side of the skull by the first author, M.B. Measurement data are available in Appendix 6. Only cranial characters could be used, as few complete skeletons were available. Fourteen morphological characters (Fig. 5.2) were chosen according to several criteria: (i) measurements were easily taken and repeatable, (ii) relatively independent and covering most of the skull, and (iii) characters had to be preserved in all skulls examined (as the methods of analysis could not process missing data). Bivariate and multivariate analyses were conducted to investigate both sexual dimorphism and geographic variability. All measurements were log-transformed and analyses conducted using MESQUITE (Maddison and Maddison 2005). The bivariate analyses used condylobasal length, an index of absolute skull size (see also Quin *et al.* 1996b), as the independent (X) variable, the multivariate analyses used Principle Components Analysis (PCA).

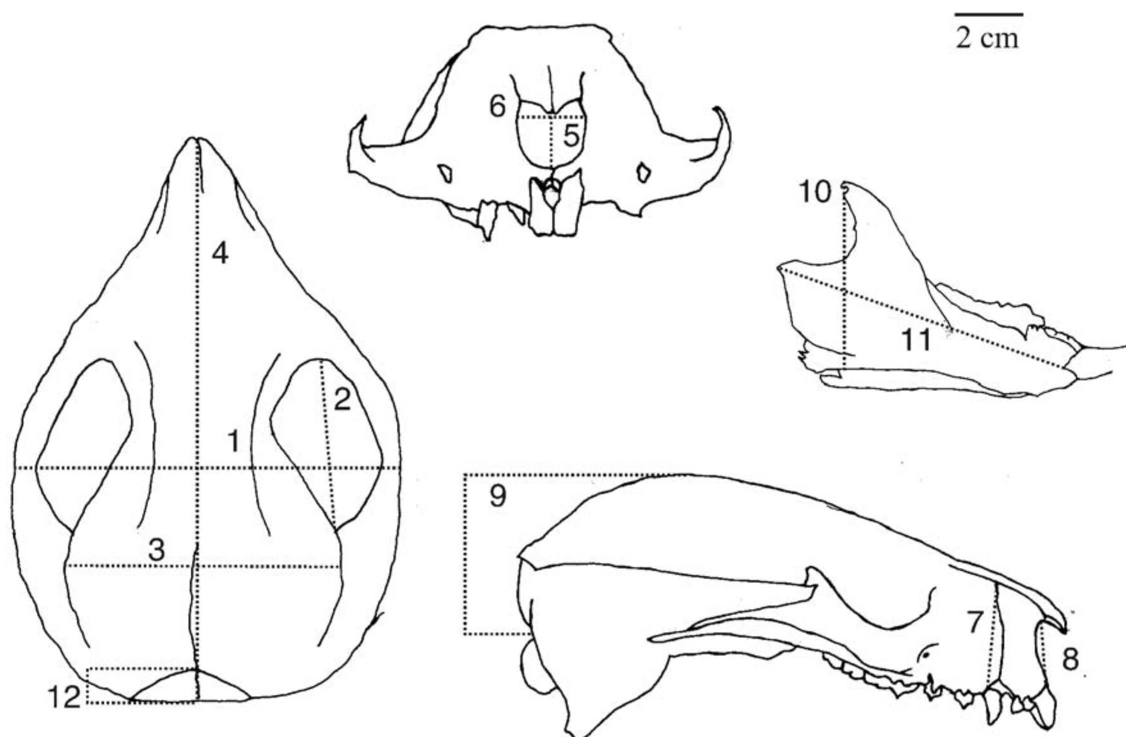


Fig. 5.2 Diagrams of cranial characters 1) zygomatic width, 2) zygomatic length, 3) brain width, 4) skull length, 5) nasal length, 6) nasal width, 7) lacrimal, 8) nasal angle, 9) brain height, 10) coronoid height, 11) mandible length, and 12) length of occipital. Measurements, not shown in the diagrams, were also taken of molar (M1) length and width. N = 34 skulls.

Consistent with genetic analyses, I examined four groups separated by isolation or state borders; NQ, SQ, NSW and Vic. No yellow-bellied glider skulls from South Australia are represented in collections within Australia.

5.4 Results

5.4.1 Variation and distribution of mtDNA haplotypes

Approximately 873 bp of sequence data from 32 yellow-bellied glider specimens were determined for phylogenetic analyses[1]. BLAST analyses confirmed that the sequence included 702 bp of the mtDNA *ND4* gene and a further 171 bp that included the tRNA-His, tRNA-Ser and part of the tRNA-Leu genes. The *ND4* sequence had an open reading frame in all sequences, suggesting it is a functional gene and unlikely to be a nuclear copy of mtDNA. This latter possibility, as with many phylogeographic studies using mtDNA genes, cannot be entirely ruled out, although I found no evidence for double PCR-amplification peaks and ambiguities in the sequence data to suggest the presence of nuclear copies in the data set. For the two museum specimens from NQ, only a short *ND4* fragment, approximately 90 bp in length, could be amplified from specimen QM JM8746. This amount of sequence data was uninformative for phylogenetic analyses and was, therefore excluded from further analyses. DNA samples from the second NQ specimen (QM JM6352) did not PCR-amplify, even with primers amplifying the short (90 bp) fragment. A total of 18 haplotypes was observed in yellow-bellied gliders, with 12 in NSW (n = 13), three in SQ (n = 3), two in NQ (n = 4) and only one in Vic./SA (n = 12) (Table 5.1). There were 45 variable sites among the 18 haplotypes of which 26 were parsimony informative. Overall nucleotide diversity was $\pi \pm SD = 0.010 \pm 0.005$, and nucleotide diversities within populations, ranged from 0 to 0.008 (Table 5.1). Pairwise distances between populations (Vic./SA, NSW, SQ and NQ), based on the HKY85 model, ranged from 0.81-2.33% and within populations they ranged from 0-2.25% (Table 5.2). In contrast to the low divergence among haplotypes within yellow-bellied gliders, divergence with the outgroup specimens *P. breviceps* was approximately 23-24%. Divergence between the two *P. breviceps* samples from Papua New Guinea and Queensland was 8.7%.

[1] Sequencing of some gliders was carried out by Huw Cooksley, School of Molecular and Biomedical Science and Mansooreh Malekian, School of Earth & Environmental Sciences, The University of Adelaide. Details can be found in Appendix 3.

Table 5.1 Numbers of samples (n), haplotypes and diversity indices ± standard deviation (gene and nucleotide diversity estimated using ARLEQUIN v.3.01) in populations of yellow-bellied gliders.

	n	# haplotypes	Gene diversity	Nucleotide diversity
Vic./SA	12	1	0.00 ± 0.00	0.000 ± 0.000
NSW	13	10	0.95 ± 0.05	0.008 ± 0.004
SQ	3	3	1.00 ± 0.27	0.007 ± 0.006
NQ	4	2	0.50 ± 0.27	0.003 ± 0.002
Total	32	16	0.84 ± 0.06	0.010 ± 0.005

Table 5.2 Within and between regions pairwise distance (HKY85 model) comparisons. Mean ± standard deviation and range (in parentheses) are shown as percentages. Within regions comparisons are on the diagonal, between regions comparisons are above the diagonal.

	Vic./SA	NSW	SQ	NQ
Vic./SA	0	1.06 ± 0.17 (0.81-1.54%)	0.86 ± 0.06 (0.82-0.95%)	2.00 ± 0.07 (1.88-2.09%)
NSW		0.95 ± 0.55 (0-2.25%)	0.70 ± 0.38 (0.23-1.66%)	1.83 ± 0.19 (1.52-2.33%)
SQ			0.71 ± 0.52 (0.12-1.06%)	1.72 ± 0.31 (1.41-2.22%)
NQ				0.29 ± 0.32 (0-0.60%)

An AMOVA for population structure based on a single group containing all four regions showed strong genetic structuring. No haplotypes were shared between regions, and 69% of the variance was explained by among population variation (F_{ST} = 0.69, P<0.001). Pairwise F_{ST} estimates between each of the populations were also significantly different from zero, with the exception of the comparison between SQ and NSW, which was not significant. When a hierarchical AMOVA was conducted with three groups (SQ and NSW pooled) it was found that just 2% of the total variance was explained by differences between the SQ and NSW populations, and

69% of the total variance was explained by differences among the three groups, Vic./SA, SQ/NSW and NQ. Overall, the AMOVA analyses suggested considerable structure within yellow-bellied glider populations, with the presence of at least three distinct genetic lineages being supported.

5.4.2 Phylogeographic relationships of mtDNA haplotypes

Maximum Parsimony analyses resulted in 12 equally parsimonious trees of length 380, with all trees showing the existence of two reciprocally monophyletic groups of haplotypes, one containing haplotypes from NQ and a second group containing haplotypes from all the remaining populations in SQ, NSW, Vic. and SA (Fig. 5.3). This arrangement received moderate bootstrap support, with the NQ group supported by a bootstrap value of 79% and the second group receiving bootstrap support of 66%. A third monophyletic group containing all the Vic. and SA haplotypes was supported by a bootstrap value of 91%. The arrangement depicted in the MP tree was not supported by Bayesian analyses, which placed the root of the yellow-bellied glider tree in the branch connecting the Vic./SA clade and a group of haplotypes from NSW/SQ (tree not shown). Support for the monophyly of all Queensland and NSW populations was very low with a posterior probability of only 51%. However, monophyly of the NQ populations again received good support with a posterior probability of 89%. Overall, the outgroup phylogenetic analyses place some doubt on the position of the root of the yellow-bellied glider tree, possibly due to the long branch connecting the outgroup taxa, *Petauroides volans*, *Petaurus norfolcensis* and *Petaurus breviceps* to the ingroup.

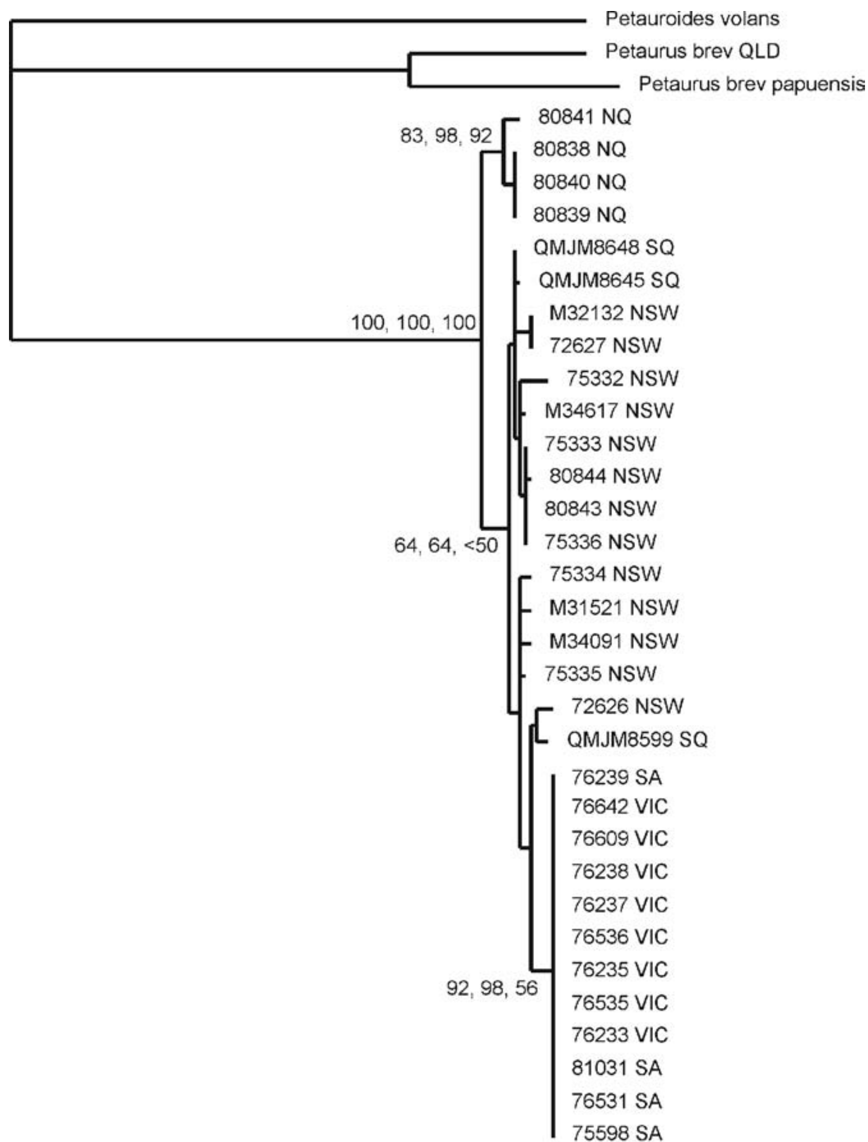


Fig. 5.3 Maximum Parsimony (MP) tree of length 380 showing evolutionary relationships among *ND4* haplotypes from yellow-bellied gliders. *Petauroides volans* and *Petaurus breviceps* were used as an outgroup for the analyses. Numbers adjacent to branches represent % bootstrap values for MP (left) and % MRBAYES posterior probabilities (right). Sample numbers refer to ABTC numbers (no prefix) or museum voucher numbers, given in Appendix 4.

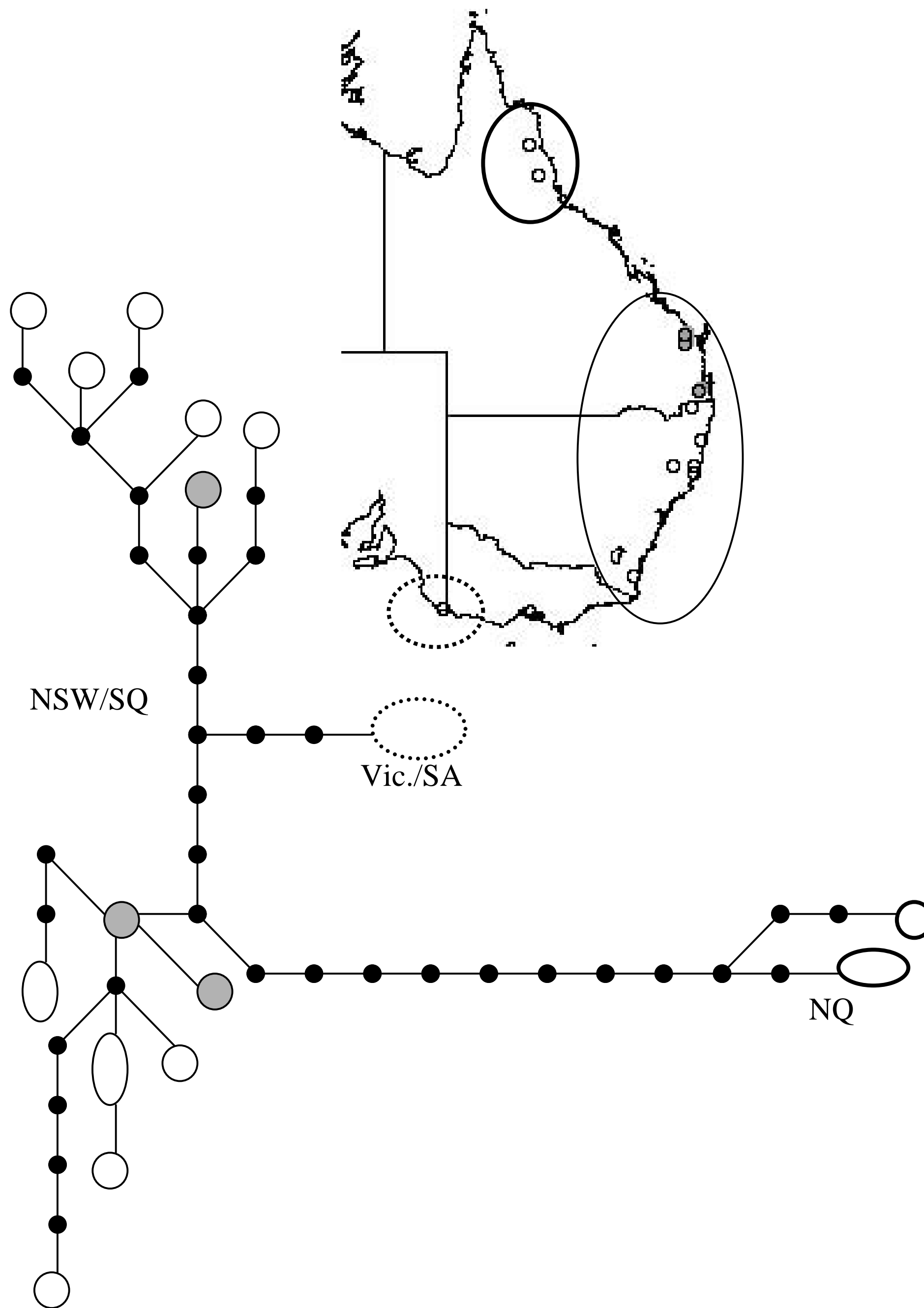


Fig. 5.4 Minimum spanning haplotype network, assuming statistical parsimony, constructed in TCS v.1.21 (Clement *et al.* 2000). A total of 50 steps were required to link all *P. australis* haplotypes. Size of the circles is proportional to sample size. Each black node represents a haplotype change (missing haplotype). The stippled circle on the haplotype network corresponds with the locations of specimens obtained from Vic./SA as indicated on the map. The heavily outlined circles on the haplotype network correspond with the locations of specimens obtained from NQ. All empty circles are from NSW. The grey filled-in circles are specimens from southern Queensland. Localities from which specimens from NSW/SQ were obtained are also indicated on the map.

The haplotype network provided further evidence for the separation of the NQ population from all other populations of yellow-bellied gliders, with 12 mutational steps (1.4% divergence) to the nearest related haplotype in SQ (Fig. 5.4 above). The network also illustrated the distinctiveness of the Vic./SA haplotype from all other yellow-bellied glider haplotypes with 7 mutational steps (0.8% divergence) to the nearest haplotype, also from SQ. The haplotype network further confirms the similarity of haplotypes from SQ and NSW, suggesting relatively recent connections between populations from these regions.

5.4.3 Morphological data: sexual dimorphism and geographic variation

The bivariate and multivariate plots revealed neither sexual dimorphism nor consistent geographic variability for any of the morphometric characters examined. In plots from both analyses the sexes were overlapping (results not shown) and were combined for geographic analyses. When examined according to geographic origin, specimens from each of three regions (Qld, NSW and Vic.) clustered together (Fig. 5.5). The percentage of variability expressed on each of the first two PCA vectors was also quite low (30%, 27%). Also, apart from three of the four NQ specimens being at the smaller end of the size variation for skull length (data not shown), the NQ gliders did not appear to differ in shape to southern forms. However, in this study only two age classes were identified from skulls: young and adult (and only adults were included for analyses). Small sample sizes in this study precluded further breakdown of data into more age classes (e.g. Quin *et al.* 1996b), which may have provided better resolution of variation between sexes and thus between regions.

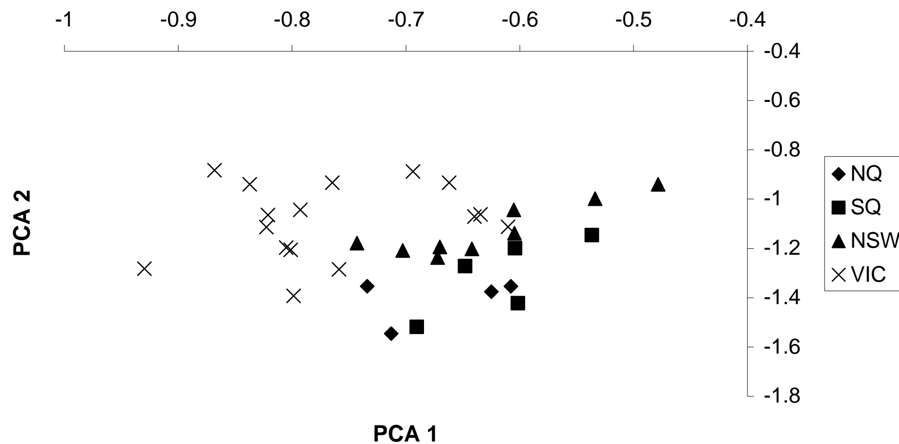


Fig. 5.5 Relationship between the first (PC1) and second (PC2) components of the Principle Components Analysis (PCA). Fourteen characters from 34 skulls of both sexes are included. NQ = north Queensland, SQ = south Queensland, NSW = New South Wales, VIC = Victoria.

5.5 Discussion

Evidence for significant phylogeographic structuring across the range of yellow-bellied gliders in Australia was provided from population genetic (AMOVA) and phylogenetic analyses of mtDNA sequence data. In particular, isolated NQ and Vic./SA populations each had monophyletic groups of haplotypes that were divergent in sequence from SQ and NSW haplotypes, suggesting the existence of three distinct phylogeographic lineages. In contrast, although there was some evidence for NQ gliders being slightly smaller in size than southern forms, morphological analyses, based on 14 cranial characters, revealed little distinction between gliders from these regions. Overall, the results have a number of important implications relevant to the current taxonomy and conservation management of the species.

5.5.1 Taxonomy

Population genetic and phylogenetic analyses provided no evidence for genetic differentiation of SQ and NSW populations, but gliders from both regions were significantly genetically differentiated from NQ populations. These findings, together with morphological data showing no clear distinction between populations from SQ and NSW for cranial characters, do not support the original (Thomas 1923) classification of SQ populations as a separate subspecies (evolutionary lineage) from

populations in NSW, Vic. and SA. Notably, the collection location of the type specimen of *P. a. reginae* in southern Queensland at Gin Gin is close to the location of one of my samples (QM JM8648), making it unlikely that the non-concordance of my data with the previous subspecific classification is due to a sampling artefact. My results are in accordance with a number of other studies that have questioned the subspecific status of the Queensland populations of yellow-bellied gliders, originally classified on the basis of colour of the pelage (Goldingay and Kavanagh 1990; Goldingay 1992; Goldingay *et al.* 2001). Consequently, I suggest that the name *P. a. reginae* should be dropped from future use for the taxonomic classification of *P. australis* populations. Although my results are consistent with the recognition, by the EPBC Act 1999, of the population in northern Queensland as a distinct subspecies, for reasons outlined in the introduction, I prefer to consider the ESU status of populations rather than use the subspecies concept, and further discuss the conservation status of the NQ populations below.

5.5.2 Conservation units and management

Evidence from MP phylogenetic analyses for reciprocal monophyly of mtDNA from NQ versus populations in SQ, NSW and Vic./SA, suggested that gene flow has been highly restricted between these two regions over a long time period. However, there was some uncertainty in the position of the root of the phylogeny with Bayesian phylogenetic analyses giving an alternative root showing the NQ populations to be paraphyletic with populations from NSW and SQ, and the Vic./SA population forming a separate reciprocally monophyletic group. These differences in the placement of the root are most likely due to the distant relationship of the outgroup taxa (*Petauroides volans, Petaurus norfolcensis* and *Petaurus breviceps*), a problem that cannot be resolved, given that the only other petaurid species available as an outgroup, *Petaurus gracilis*, is very closely related to *P. norfolcensis* (M. Malekian, S. J. B. Cooper and S. M. Carthew, unpublished data). However, the unrooted haplotype network, constructed using the statistical parsimony procedure, provided further evidence for the long-term isolation of the NQ and Vic./SA populations from populations in NSW/SQ, with multiple missing (intermediate) haplotypes connecting haplotypes from these populations and those in NSW/SQ. In addition, the NQ population is geographically isolated, by more than 500 km, from the nearest population in the south, with unsuitable habitat between these populations

coinciding with a major biogeographic break at the Burdekin Gap (see below). This level of isolation, particularly for a species with a small population size, would be expected to lead to significant genetic differentiation through processes such as drift and selection (Frankham *et al.* 2002).

Although genetic analyses provided evidence for phylogeographic structuring, morphological analyses, based on cranial characters, did not show a similar pattern of population structure. Skull proportions generally evolve very slowly and, although finding different skull morphologies would strongly indicate distinct evolutionary lineages, the lack of such differentiation cannot be taken to indicate gene flow among populations (e.g. Burbrink *et al.* 2000). My results are consistent with other studies on sugar and squirrel gliders that found size separation for skull length along a clinal gradient (Quin *et al.* 1996b). Specimens from three regions (Qld, NSW and Vic.) clustered together in the PCA analysis (see Fig. 5.5 above). However, in the PCA analysis, the NQ skulls did not separate from SQ skulls. Despite the lack of consistent skull shape differences, there are additional phenotypic differences between NQ and southern forms. The NQ gliders are smaller in size, as measured by weight (NQ males: mean 516g (s.d. = 7.7 g, n = 17); NQ females: 479.4g (s.d. = 7.8 g, n = 14) (Goldingay *et al.* 2001); Vic. males: 555.5g (s.d. = 13.5 g, n = 11); Vic. females, 508.1g (s.d. = 8.1 g, n = 9 (M. Brown and S. M. Carthew, unpublished data)), and are more likely to have darker dorsal fur (M. Brown, personal observations). The latter is likely to represent a genetically-based difference, but I cannot rule out phenotypic plasticity for the size variation.

There also appear to be sociobehavioural and life history differences between the NQ gliders and the southern forms. The NQ gliders have been reported to have a polygynous mating system (Russell 1984; although see Goldingay *et al.* 2001), whilst the southern populations are predominantly monogamous (Henry and Craig 1984; Craig 1985; Goldingay and Kavanagh 1990; Goldingay 1992; M. Brown, S. M. Carthew and S. J. B. Cooper. unpublished data). NQ gliders also appear to spend longer in the pouch (100 days vs. <80 days) (Russell 1983; M. Brown and S. M. Carthew, unpublished data). Whether reported ecological and behavioural differences are genetic or phenotypically plastic needs to be further investigated.

Taken overall, the weight of evidence from genetic, morphological, behavioural and ecological studies, together with the disjunct distribution of the NQ population, strongly suggest NQ should be considered as a separate ESU from all other populations of yellow-bellied gliders under the criteria of Fraser and Bernatchez (2001). There also is evidence that the Vic./SA populations show restricted gene flow from populations in NSW. However, I was unable to obtain samples of yellow-bellied gliders from the Otway region, west of Melbourne and from eastern Victoria, so it is possible that the pattern of population structure I have detected in the south has resulted from isolation by distance and inadequate sampling of intervening populations. Further sampling and analyses are required to resolve the conservation status of the isolated populations of yellow-bellied gliders in SA and Victoria.

The separate ESU status of the NQ populations has implications for its conservation management. First, the ESU concept was developed to provide objective criteria for prioritising populations that should be targeted for protection in order to preserve genetic diversity and the adaptive potential of a given species (Ryder 1986). I suggest conservation priority needs to be given to preserving the NQ population of yellow-bellied gliders, given its vulnerable status (EPBC Act 1999). This classification needs reviewing as wet sclerophyll forest in the Wet Tropics area is being increasingly encroached upon by rainforest (Harrington and Sanderson 1994), which would render habitat unsuitable for yellow-bellied gliders. Second, if populations in isolated forest patches in NQ severely decline in size there may be a future need to transfer animals to these populations to reduce the possibility of inbreeding depression, considered a significant factor in the possible extinction of populations (Spielman *et al.* 2004). The use of animals from southern populations (SQ or NSW) for this purpose may lead to outbreeding depression, further reducing the local fitness of populations (Frankham *et al.* 2002) and, therefore, should be avoided, if possible. Conversely, because of a lack of genetic distinctiveness within the south-western Victorian and SA region, yellow-bellied gliders from Rennick State Forest (the population sampled in this study) could potentially be used to supplement the single remaining population in SA. This may be best achieved by linking isolated native habitat through the planting of corridors of suitable habitat (see section 6.5 below for further discussion on corridors).

5.5.3 Levels of genetic variation

It is difficult to make direct comparisons of genetic divergence from my study with other marsupial species, as my analyses are based on the *ND4* gene, while other studies (e.g. Moritz *et al.* 1997; Firestone *et al.* 1999; Pope *et al.* 2000) were based on control region sequence data. I was unable to use the control region due to the presence of a repeat region that led to multiple PCR bands and sequencing problems in a number of my samples. Other studies of petaurid gliders and possums have been carried out using the ND2 gene (e.g. Osborne and Christidis 2001; Osborne and Christidis 2002b; Osborne and Christidis 2002a), which has a very similar level of divergence to *ND4* (M. Malekian, S. J. B. Cooper, S. M. Carthew, unpublished data on *P. breviceps* and *P. norfolcensis*). Divergence levels among different *ND4* haplotypes (0.24 to 2.33%) in yellow bellied gliders were similar to intra-specific haplotype divergence levels for *P. norfolcensis* and *P. breviceps* (1.7 to 2.5%; Osborne and Christidis 2001). Inter-specific divergence levels of *ND2*/ *ND4* among the three Petaurids were much higher, ranging from 11% between *P. breviceps* and *P. norfolcensis* (Osborne and Christidis 2001) to 24% between *P. breviceps* and *P. australis*.

One notable feature of the yellow-bellied glider data was the observation that mtDNA haplotypes had limited distributions, with most restricted to a single population. An identical haplotype was found in the Rennick population in south-western Victoria and Snowgum Native Forest Reserve population in SA, but these populations are only a few km away from each other. Restricted mtDNA distributions can result from female philopatry (Moritz 1999) and has been demonstrated for some marsupials (e.g. *Dasyurus maculatus;* Firestone *et al.* 1999). The lack of mtDNA variation in the Vic./SA populations may also have resulted from a small population size and/or inbreeding (Frankham *et al.* 2002). However, a recent study using microsatellite loci on the mating system of yellow-bellied gliders in the Rennick population, in south-western Victoria, indicated little evidence for relatedness within males or within females (M. Brown, S. J. B. Cooper and S. M. Carthew, unpublished data). Further, heterozygosity at each of the five microsatellite loci was high, ranging between 46-80% (Brown *et al.* 2004), with no evidence for fixation of alleles by genetic drift in a small population. My findings provide

tentative support for the 'female philomatry' hypothesis (*sensu* Firestone *et al.* 1999), with maternally-inherited alleles remaining within close proximity of each other due to limited dispersal of females, but additional data are required to determine whether there is evidence for sex-biased dispersal.

5.5.4 Phylogeography

The most likely break between the two populations in NQ and SQ coincides with a historical barrier known as the Burdekin Gap, which is dry woodland and unsuitable habitat for yellow-bellied gliders. A population break at the Burdekin Gap has been found in a number of vertebrate taxa distributed down the east coast of Australia. For example, yellow-throated and large-billed scrubwrens (Joseph *et al.* 1993; Joseph and Moritz 1994) and satin bowerbirds (Nicholls and Austin 2005), have distinct mtDNA lineages either side of the Burdekin Gap. Marsupials, such as *Isoodon* sps. (Pope *et al.* 2001), also show mtDNA differentiation across the Burdekin Gap. In contrast, mtDNA haplotypes from a disjunct north Queensland population of *Dasyurus maculatus* were found to be polyphyletic with southern haplotypes, indicating an absence of phylogeographic structure along the east coast of Australia (Firestone *et al.* 1999).

5.5.5 Conclusions

My analyses highlight the potential pitfalls in the use of subspecific classifications to prioritise conservation units for protection within species. A combination of molecular genetic, morphological and ecological analyses allows assessment of the adaptive genetic variance within species and provides a more rigorous framework for conservation management. Using this approach and flexible criteria for defining ESUs (Fraser and Bernatchez 2001) I suggest that populations of yellow-bellied gliders in north Queensland represent a distinct ESU and should be given special priority for conservation. Further analyses are required to assess the ESU status of isolated populations in SA and western Victoria.

Chapter 6. Concluding discussion

6.1 Summary of aims

The aims of my project were to:

1. examine the mating system of the yellow-bellied glider using behavioural observations and microsatellite DNA technologies, and test the hypothesis that a population at Rennick in south-western Victoria was monogamous.
2. quantify behavioural traits, such as mate guarding and territoriality that may have contributed to the evolution and maintenance of monogamy within this population.
3. test the hypothesis that seasonal breeding would be evident in yellow-bellied gliders at Rennick and would coincide with the time of seasonal abundance of two indices of protein food resources, i.e. flowering and bark shed.
4. investigate the conservation status of isolated populations of yellow-bellied glider in south-western Victoria/SA and northern Queensland by examining intraspecific morphological variation and the geographic distribution of mitochondrial DNA haplotypes.
5. address some issues of management for these isolated populations.

6.2 Mating system of yellow-bellied gliders

6.2.1 Evidence for a monogamous mating system

My results showed that the mating system of the Rennick population of yellow-bellied gliders was predominantly monogamous. The social organisation was characterised by a large amount of home range overlap between an adult male and adult female, but very little overlap of adjacent territories. Microsatellite analyses generally confirmed that social monogamy equated to sexual monogamy and that monogamy was maintained over more than one breeding season. Although a monogamous mating system predominated, varying group structures were evident within the Rennick population of yellow-bellied gliders. At least one male's territory overlapped those of two adult females, and one adult female remained single for much of the study (Chapter 3). Reasons that have previously been used to explain variation in the mating system of yellow-bellied gliders (e.g. Goldingay 1992; Goldingay *et al.* 2001) do not fully explain the appearance of one polygynous group

at Rennick. There was no evidence for variation in the availability of key food resources, such as flowering (Chapter 4), and no increase in female gregariousness, i.e. adult female yellow-bellied gliders did not share home ranges (Chapter 3). Other resources, such as tree hollows, were stable over time, with gliders changing den trees regularly, but spending most of their denning time in a small number of key trees (primary den trees) (see Appendix 7). Other factors, such as the density of animals (Travis *et al.* 1995), may affect female gregariousness, leading to changes in social structure between monogamy and polygamy. However, although demographics in the Rennick population of yellow-bellied gliders changed over the study period (see Appendix 8), the actual density of gliders remained mostly unchanged, with glider groups increasing and decreasing in size in response to offspring being born and subadults leaving the study area.

6.2.2 Factors contributing to monogamy in yellow-bellied gliders

It is clear that no single factor has led to the evolution of monogamy in all species (Reichard 2003). However, certain factors appear to be important in either the evolution or maintenance of monogamy in a number of taxa, including mate guarding, dispersion of females and resources, and paternal care (Emlen and Oring 1977; Kleiman 1977; Clutton-Brock 1989; Komers and Brotherton 1997; Brotherton and Komers 2003). Male yellow-bellied gliders do not appear to offer direct paternal care, e.g. feeding and carrying offspring (as seen in callitrichid primates, Dunbar 1995), in that offspring remain in the pouch until they are of an age where they are deposited in a tree hollow whilst the mother forages. However, males may offer indirect paternal care in the form of thermoregulation by denning with offspring, and possibly affording some protection from predation and incursions into the home range area from conspecifics. Although the dispersion of females has been observed as being an important predictor of monogamy (Komers and Brotherton 1997), it is unlikely to have been the only contributing factor to the maintenance of monogamy in yellow-bellied gliders. The lack of female gregariousness means that in order to have access to more than one sexually receptive female, males must overlap the home ranges of more than one female. In yellow-bellied gliders, this means a male's home range area must increase in size up to an average of 50 ha. Although this is double the average home range size at Rennick (Chapter 3), in other populations of yellow-bellied gliders, 50 ha is an average size for a glider home range, e.g. at

Nitchaga, north Queensland (Goldingay *et al.* 2001). Thus, it is certainly possible for male gliders to have home ranges that are 50 ha in size. The ability of adult males to overlap the home ranges of more than one female indicates there are likely to be other factors that have contributed to the evolution or maintenance of monogamy in yellow-bellied gliders.

6.2.3 Mate guarding or group sap feeding in yellow-bellied gliders?

One factor that has recently become recognised as being important in the evolution and maintenance of monogamy is that of mate guarding (Brotherton and Komers 2003). Mate guarding behaviours can range from obvious behaviours, such as over-marking of females' scent deposits in Kirk's dikdik (Komers 1996) to a more general close association between an adult male and female, as in fork-marked lemurs (Schülke and Kappeler 2003). In animals such as petaurids, that are highly mobile, arboreal, cryptic and nocturnal, quantifying observations of a close association between animals was deemed the most feasible option, rather than trying to quantify behaviours such as aggressive repelling of intruders, that may have occurred infrequently and briefly. Quantifying close associations revealed that approximately 50% of the time, yellow-bellied gliders were observed within 25 m of their partner (Chapter 3). It is difficult to state for certain that this is evidence of mate guarding behaviour, because it has been observed in other populations that yellow-bellied gliders tended to be close to other group members whenever they were engaged in sap feeding (see Craig 1985; Goldingay 1989b). Thus, the hypothesis that adult males and females remained within close proximity because some specific trees are more productive than other trees needs to be tested. If other trees within the group's home range area are as productive, then it may be considered more likely that gliders are remaining in close association because they are mate guarding.

Alternatively, it is possible that increased sap feeding may lead to enhanced mate guarding, leading to a greater likelihood of monogamy. If so, we might expect populations of yellow-bellied gliders with monogamous mating systems to have been observed with phloem sap comprising a larger component of their diet compared to populations with polygamous mating systems. However, this is clearly not the case with the north Queensland population at Nitchaga, which, like the gliders at Rennick, was heavily dependent upon phloem sap, with ~ 80% of the dietary component

comprising tree exudates in both populations (Quin *et al.* 1996a; Carthew *et al.* 1999). This north Queensland population was observed to have a variable social structure comprised of monogamous, polygamous and polyandrous groups, although genetic methods have not been utilised to test these observations (Goldingay *et al.* 2001). The variation between sap feeding and mating system highlights not only the importance for exploring and quantifying behavioural associations between glider group members, but also confirming the mating system for the north Queensland populations using microsatellite DNA technologies.

6.3 Reproductive ecology of yellow-bellied gliders

6.3.1 Seasonality of food resources and aseasonality of births

Flowering was strictly seasonal, with the abundance of manna gum flowers increasing in autumn and brown stringybark flowers in summer (Chapter 4). Bark shed was less seasonally evident, but peaked in summer and declined thereafter (Chapter 4). Bark shed coincided with an increase in activity of most insects during the spring/summer period in southern Australia (Recher *et al.* 1996). In particular, Dickman (1991), at a study site in NSW, noted that arthropods began migrating up the trunks of manna gums at the commencement of spring in order to seek shelter under newly peeling bark. The timing of births in the population of yellow-bellied gliders studied here was statistically aseasonal, but with distinct birth peaks in summer and winter, and possibly spring (Chapter 4). Pouch exit, the most energetically-demanding time for both mothers and offspring, would commence in autumn and spring, respectively. Although data were limited, it was likely that yellow-bellied gliders at Rennick timed late lactation/pouch exit with peaks in indices of pollen and arthropod availability, i.e. flowering (particularly of manna gum in autumn) and bark shed. The apparent lack of seasonality in the timing of reproduction in yellow-bellied gliders in a temperate, seasonal environment at high latitude was surprising. Yellow-bellied gliders at two other populations at lower latitudes in southern NSW have been observed as breeding seasonally (Goldingay and Kavanagh 1990; Goldingay 1992).

6.3.2 Cues that may be important for initiating breeding in yellow-bellied gliders

Many mammals that breed seasonally rely upon cues to initiate breeding, such as photoperiod (e.g. many species of neotropical primate, Di Bitetti and Janson

2000), or the rate of change of photoperiod (*sensu* McAllan *et al.* 2006), ambient temperature (Tinney *et al.* 2001), food availability (Dennis and Marsh 1997; Di Bitetti and Janson 2000; Tinney *et al.* 2001) or rainfall (Bolton *et al.* 1982). It is difficult to determine what initiates the timing of reproductive events in yellow-bellied gliders at Rennick, partly because of a lack of seasonality in breeding, but also because the data here were collected on such a short-term basis. Nevertheless, the amount of rainfall, which may be associated with the abundance of flowering, may be a possible cue. Although not seasonal, birth peaks were evident in summer and winter. In particular, there was a pouch exit peak in April in the first year that coincided with the higher amount of, and slightly later, flowering of manna gum in that year. The higher abundance of flowering in summer 2002/2003 may have been associated with a higher amount of rainfall between January and December 2001 of 795 mm (although higher amounts of rainfall have been recorded in previous years). In 2002, approximately 175 mm less rain (609 mm) fell between January and December, and this may have been associated with the lower amount of flowering in both species of eucalypt the following summer 2002/2003 (Chapter 4; data for rainfall are presented in Appendix 9). However, although data are available for rainfall since 1953, data for flowering are not. Also, it is not known to what extent rainfall is associated with the abundance of flowering in manna gum and brown stringybark at Rennick, although an increase in rainfall has been associated with an increase in flower abundance in some eucalypt species (Law *et al.* 2000).

One way in which gliders may assess the amount of rainfall is through the rate of flow of phloem sap produced by eucalypts. Phloem sap is the most prominent dietary item in yellow-bellied gliders at Rennick (Carthew *et al.* 1999), and yellow-bellied gliders have been seen making test incisions on eucalypts, presumably to test the level of sap flow in trees, at Rennick (S. Carthew, pers. comm.; pers. obs.) and in other populations (Goldingay 1986; Goldingay 1987; Goldingay 1990). Rising phloem sap levels may be an indirect indicator of heavy rainfall, and that flowers (pollen/nectar) will be in abundance during the peak flowering season. However, this suggestion is largely speculative, and further research needs to be conducted to provide support for this tentative hypothesis and the potential role of rainfall in triggering breeding.

6.3.3 Opportunism in the timing of breeding?

An alternative explanation for the aseasonal pattern of breeding is that gliders breed on an opportunistic basis. The hypothesis that some marsupials breed regardless of conditions, but suffer high mortality of young when conditions are unfavourable (Tyndale-Biscoe 1973; Tyndale-Biscoe 2001) has been observed in some macropods, e.g. agile wallabies (*Macropus agilis*) and red kangaroos (*M. rufus*) (Bolton *et al.* 1982; Munn and Dawson 2003), but has not been observed in other smaller (i.e. < 1 kg) marsupials (Lee and Cockburn 1985). Although in this study pouch young were not marked, the parentage analysis (Chapter 3) provided some information on survival of offspring. Together, with the data collected on reproduction, these data indicate that 13 of 16 pouch young throughout the period of this study were raised to independence. However, small sample sizes and the short-term nature of this study make it difficult to be certain about whether this is indicative of the general population's survival of offspring. Further, it is not known whether survival of pouch young is correlated with environmental conditions, such as amount of rainfall and food availability. This would appear to be the case with survival of squirrel glider (*P. norfolcensis*) offspring being adversely affected by low rainfall and failure to flower of key eucalypt species (Sharpe, 2004). Data on the reproductive biology of yellow-bellied gliders are lacking, such as how long gestation periods are and whether yellow-bellied gliders have embryonic diapause. Such data may provide valuable clues as to whether an opportunistic reproductive strategy may be successful for yellow-bellied gliders.

6.3.4 Aseasonality of births and a monogamous mating system

Importantly, examination of some of the yellow-bellied glider life history traits has provided an insight into one of the benefits for males within a monogamous mating system. Given the lack of gregariousness amongst female yellow-bellied gliders, males may opt to remain and mate with only one female, or remain and mate with one female, but visit and mate with other sexually receptive females. The lack of synchronicity between females should allow adult males to locate, court and mate with other females. However, the adoption of such a strategy would be risky, given that he would be required to enter into unfamiliar territories, where he would not know the location of food and shelter resources (see also Brotherton and Komers 2003). Further, familiarity with his female partner's state of oestrous might be best

obtained if he remains in close contact with her (Sillén-Tullberg and Møller 1993; Brotherton and Komers 2003). This asymmetry of knowledge may provide the partnered males with surety of paternity (Brotherton and Komers 2003). This situation appears to be borne out with very few extrapair paternities evident within the Rennick population, as well as pair partners remaining within close contact throughout active and non-active hours (Chapter 3).

6.4 Conservation units in yellow-bellied gliders

6.4.1 ESU status of yellow-bellied gliders

My results showed that there were two distinct mtDNA lineages of yellow-bellied gliders; one comprised of gliders from the isolated north Queensland (Wet Tropics) (NQ) region and the other comprised of gliders from other populations throughout their remaining distribution (see Fig. 5.1, Chapter 5). Thus, I argued that the molecular data, combined with ecological data on differences in social structure and weight, suggested that the NQ populations comprised an ESU under the criteria of Fraser and Bernatchez (2001). Although the morphometric data showed no differentiation between north and southern Queensland forms, this cannot be taken to mean there has been genetic exchange between the populations. All other populations throughout the yellow-bellied gliders' range form a separate ESU (*sensu* Fraser and Bernatchez 2001). The Vic./SA populations may form a separate management unit (*sensu* Moritz 1994b) to NSW and southern Queensland populations, but further investigation and sampling of populations, particularly in central and eastern Victoria, needs to be carried out before the status of separate management unit may be confirmed. Further, data from nuclear markers may provide an independent assessment of whether the genetic differences detected in this study was a result of differing evolutionary lineages or subadult females remaining close to their natal home ranges.

6.5 Limitations to the study

Limitations to this chapter (and the entire study) included small sample sizes. In this chapter, morphological distinction of the north Queensland gliders may have been achieved with much larger sample sizes, in particular more skulls from the Wet Tropics and south-eastern Queensland areas. Small sample sizes plagued much of this thesis and was the unfortunate product of working with an animal that was

incredibly labour and time intensive to capture. Given more time, more specimens are likely to have been obtained from north Queensland, which would have provided more information about phylogeographic structure across the Burdekin Gap. Other techniques in obtaining specimens suitable for DNA analysis may have included the collection of fur via hair tubes or swabbing of incision sites on feed trees. Satellite (GPS or Argos system) tracking devices may have provided simultaneous (or close to simultaneous) data collection on gliders. However, a limitation of satellite tracking devices is that of not being able to obtain fixes whilst the glider is in a tree hollow. Nevertheless, some satellite tracking devices are also able to be fitted with VHS transmitters enabling hand-tracking to take place. A further limitation to the study involved the inability to sample pouch young without causing some distress to female gliders. Possibly the use of a very light anaesthesia to enable relaxation of the muscles around the pouch would enable the collection of skin tissue from pouch young.

6.6 Overall conclusion

Highlighting socioecological differences between populations of yellow-bellied glider will contribute to the growing literature on monogamy and mating systems in general. Much research into monogamy is carried out on primates (e.g. Reichard, 2003) with relatively little research being carried out on ecologically-similar marsupials (see Goldingay, 1989), such as possums and gliders (although see Martin, 2005). Research on the socioecology of marsupials in comparison with primates may provide insight into the evolution of monogamy without the confounding factor of phylogeny.

Research should also continue on the life history strategies of other populations of yellow-bellied glider, particularly with respect to the timing of reproduction. Further studies on the effect of rainfall on flowering and level of phloem sap in different species of eucalypt throughout the gliders' distribution would also contribute usefully to providing information about how gliders may be cued for breeding in this and other populations. Conservation management decisions will be best made with as much detailed information as possible on the yellow-bellied glider's life history strategies. The removal of trees that represent important resources, such as flowering and shelter for arthropods may be detrimental to

reproductive activities in yellow-bellied gliders and may lead to a loss of local fitness.

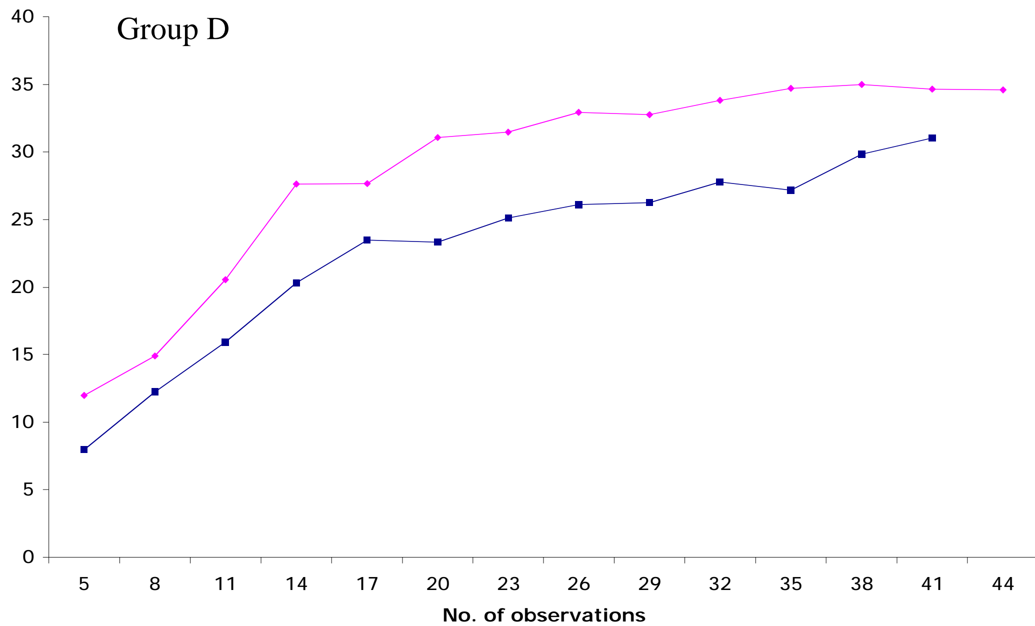
As the size of populations of yellow-bellied gliders become smaller due to loss of native habitat, the need to supplement small populations becomes more urgent. The patchy nature and low population density of the yellow-bellied glider makes them a prime candidate for such management strategies. In particular, there is a need to link up isolated populations of gliders with larger populations via the introduction of native habitat corridors. In south-eastern South Australia yellow-bellied gliders have been radiotracked into pine forest (S. Carthew, pers. comm.) and thus appear to utilise sub-optimal habitat (pine forests provide neither food nor shelter resources for yellow-bellied gliders) at least on occasion, although the reasons for using pine forest are unknown. Further, small (i.e. <20 ha patches of native habitat) are sometimes devoid of and at other times occupied by yellow-bellied gliders that may have moved between patches of suitable habitat through pine forest (S. Carthew, pers. comm.). Therefore, it is likely that yellow-bellied gliders would utilise native habitat corridors, particularly corridors that provide food and shelter resources (i.e. smooth-barked species, some of which are old enough to have formed hollows of a suitable size for gliders). Corridors have been seen as a suitable solution to linking isolated remnants in South Australia and Victoria, and many populations within central and eastern Victoria, as well as throughout NSW and Queensland, may also benefit from the establishment of habitat corridors between populations.

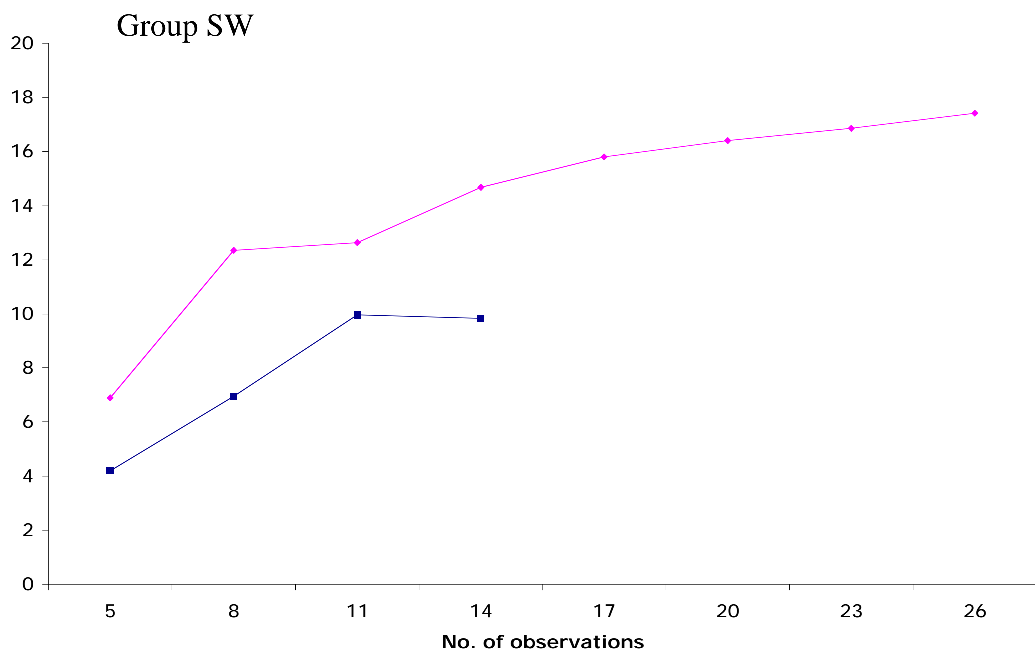
Appendix 1 Number of observations for each glider. Total number of observations for home range size analysis was comprised of trap, spotlight and den locations. Trap locations were included because traps were only placed on manna gum that were already being used by gliders. Den locations were included once per field trip or if gliders moved to a new tree during the field trip. F = adult female, M = adult male.

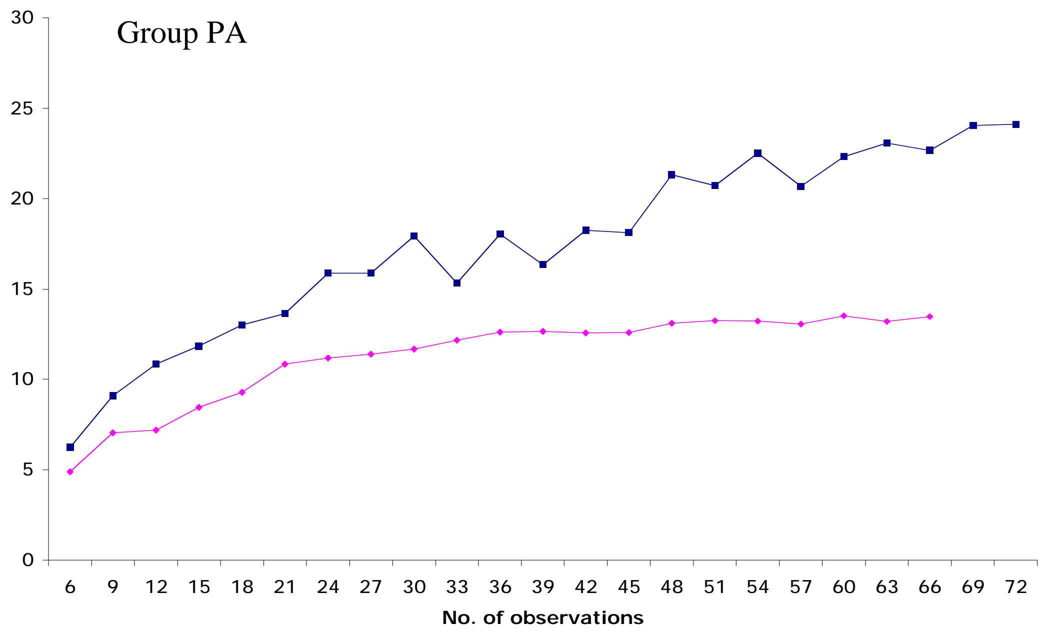
Glider	Group	No. of trap locations	No. of spotlight locations	No. of den tree locations	Total no. of observations
F	D	7	28	11	46
M	D	6	26	10	42
M	SW	3	6	4	13
F	SW	3	18	5	26
F	PA	11	42	14	67
M	PA	10	44	18	72
F*	B	6	4	0	10
M	B	6	26	12	44
F	C	3	24	9	36
F	SP	11	33	17	61
M	C	5	32	9	46
F	C	12	51	19	82
M	A	7	36	13	56
F	A	13	39	18	70
M*	G	1	3	4	8

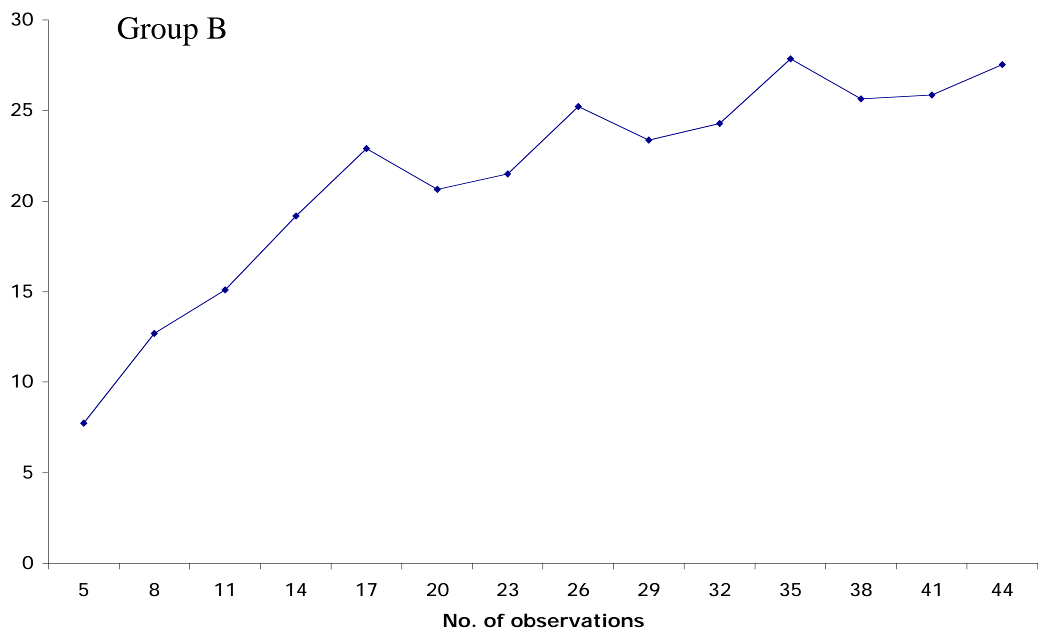
* These gliders were not included in analysis of home range size

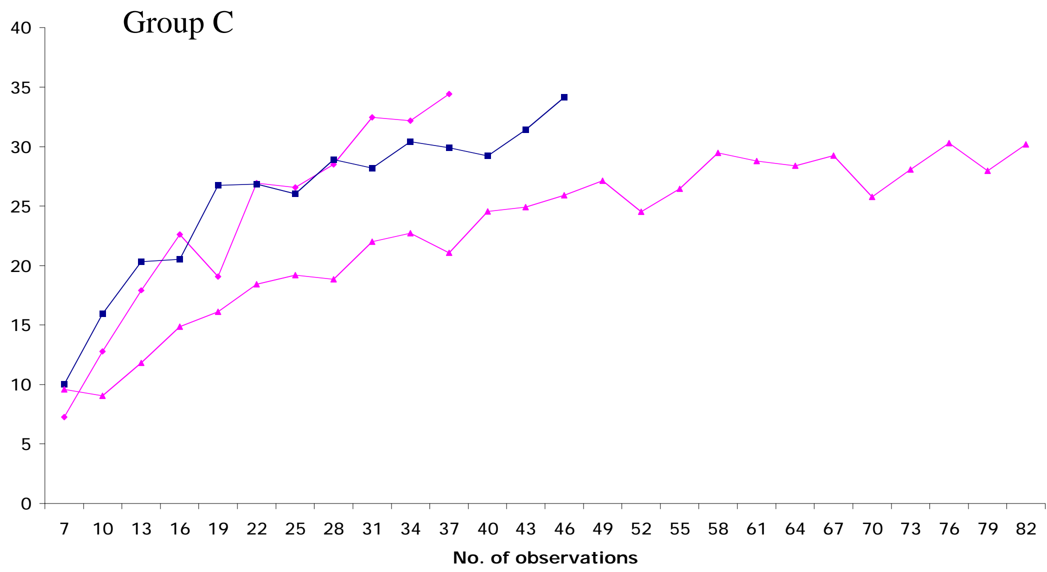
Appendix 2 MCP 100% sample size bootstraps. Bootstraps were carried out in Arcview with 20 replicates per interval and an interval size of 3. Adult females are in pink, adult males in blue. Each graph represents the adults for one group. Only curves for gliders included in the home range size analysis are shown ($N = 13$ gliders). Asymptotes were difficult to achieve for gliders, but where they did occur tended to be between 35 and 46 observations. However, some gliders that had more than 46 observations still did not show an asymptote.

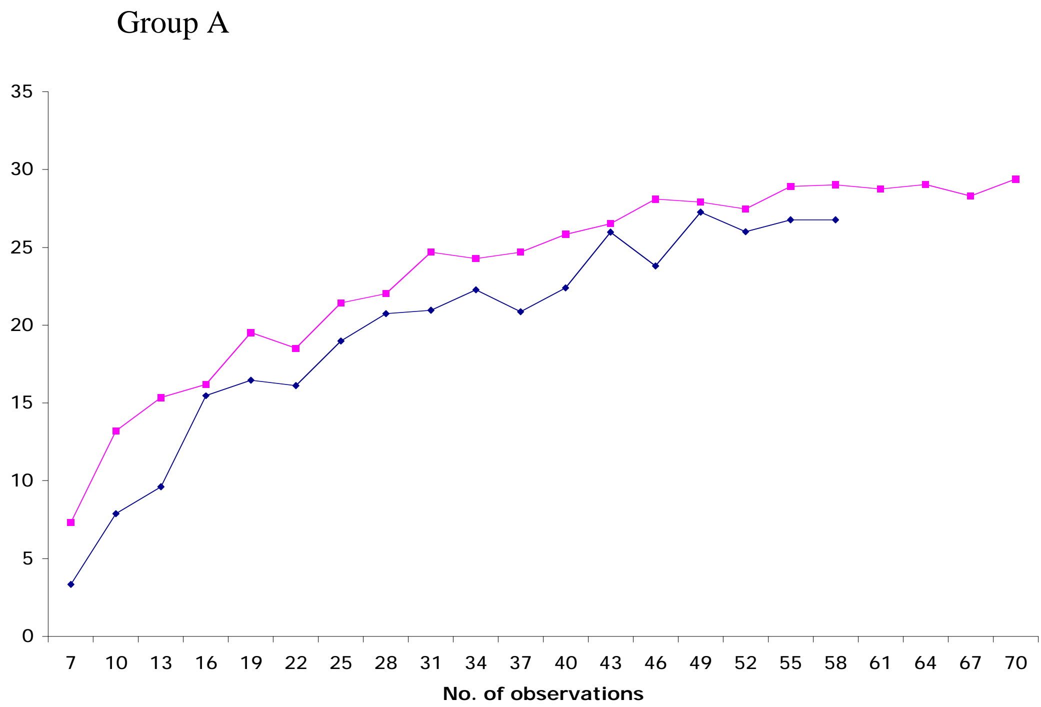


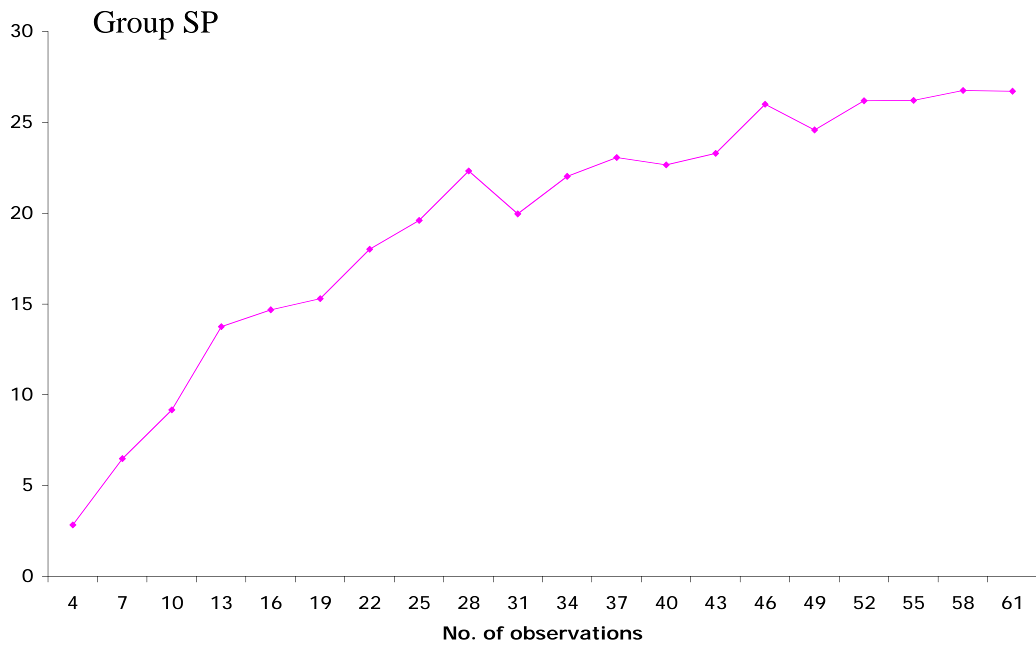



Group PA
30
25
20
15
10
5
0
6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72
No. of observations


Group B
30
25
20
15
10
5
0
5 8 11 14 17 20 23 26 29 32 35 38 41 44
No. of observations


Group C
0 5 10 15 20 25 30 35 40
7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82
No. of observations


Group A
0 5 10 15 20 25 30 35
7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70
No. of observations


Group SP
30
25
20
15
10
5
0
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
61
No. of observations

Appendix 3 Genotypes for all individuals at five microsatellite loci. Genotypes are provided for the social, or most likely true, mother, and the social, or most likely true, father. † = male disappeared or was known to have died during the course of the study, *=both the social* father and genetic** father's genotypes are shown.

	Juvenile ID	Adult female	Adult male	Petb1	Petb6	Peta13	Peta16	Peta18
Group A		4893		380/420	159/183	268/280	289/289	228/234
			4892	388/400	179/183	274/277	289/289	228/234
	5276			388/420	179/183	268/280	289/289	228/234
	5001			384/400	159/179	274/280	289/289	234/234
Group B		5004		408/412	163/171	265/265	304/304	228/228
			4895	380/408	179/183	268/280	289/289	234/234
	5055			408/412	163/183	265/268	289/304	228/234
	4435			408/412	159/179	265/280	289/304	228/234
Group C		4896		380/404	159/183	280/283	289/304	234/234
			4967†	400/400	163/179	280/280	289/289	228/234
	5099			380/400	163/183	280/283	289/289	228/234
	4898			400/404	159/179	280/283	289/304	234/234
			4897	388/424	183/183	274/280	304/304	228/234
	5384			404/424	183/183	274/283	289/304	234/234

	Juvenile ID	Adult female	Adult male	Petb1	Petb6	Peta13	Peta16	Peta18
Group D		5000		404/420	159/159	280/280	289/289	228/234
			5423	384/408	163/183	280/283	289/304	234/234
	5854			404/408	159/183	280/280	289/289	228/234
Group SW		5459		404/404	171/171	280/280	289/304	228/234
			5279	392/404	159/163	280/280	289/304	228/228
	5458			392/404	163/171	280/280	304/304	228/228
	5855			392/404	159/171	280/280	289/289	228/234
Group PP		4890		380/412	167/183	268/283	289/304	228/234
			4891	404/420	159/171	268/280	292/304	228/228
	5421			380/420	159/183	268/283	304/304	228/228
	5100			380/404	171/183	268/268	289/304	228/228
Group PA		5154		392/392	179/183	280/280	289/289	228/234
			5155†**	392/408	179/183	274/280	289/289	228/234
			5153*	408/424	171/183	280/280	289/304	228/228
	5422			392/424	179/183	274/280	289/289	234/234

	Juvenile ID	Adult female	Adult male	Petb1	Petb6	Peta13	Peta16	Peta18
Social group of juvenile not known								
The genotypes of the most likely genetic parents (as assigned by CERVUS) is provided and the social group of the putative mother indicated								
Group SW		5459		404/404	171/171	280/280	289/304	228/234
			5277	380/420	171/179	280/280	289/289	234/234
	5278			404/420	171/179	280/280	289/289	231/234
			5153	408/424	171/183	280/280	289/304	228/228
	5853			404/424	171/183	280/280	289/304	228/234
Group SP		5003		380/404	159/159	268/280	289/304	228/234
			5155	392/408	179/183	274/280	289/289	228/234
	5385			380/392	159/179	268/280	289/304	234/234
Other adults within the population								
		5002		404/404	163/167	265/280	289/289	228/264
		5152		390/394	179/179	265/277	289/289	228/234
			4966	388/424	183/183	274/280	304/304	234/234

Appendix 4(a) Percentage trap success rate for all captures (total) and for yellow-bellied gliders (gliders). Including number of yellow-bellied gliders trapped more than once per field trip and number of animals other than yellow-bellied gliders trapped.

Month	No. of trap nights	Total no. captures of gliders	No. of animals caught more than once	Other animals	% trap success rate (total)	% trap success rate (gliders)
August 2001	N/A	5	0	0	N/A	N/A
October 2001	21	3	0	2	23.8	14.3
November 2001	19	5	0	0	26.3	26.3
December 2001	48	4	0	3	14.6	8.3
January 2002	88	10	1	5	17.0	11.4
February 2002	49	7	1	3	20.4	14.3
March 2002	49	7	0	0	14.3	14.3
April 2002	31	7	0	1	25.8	22.6
May 2002	20	10	2	1	55.0	50.0
June 2002	42	7	0	0	16.7	16.7
July 2002	49	10	0	0	20.4	20.4
August 2002	47	14	1	1	31.9	29.8
September 2002	26	9	2	1	38.5	34.6

Month	No. of trap nights	Total no. captures of gliders	No. of animals caught more than once	Other animals	% trap success rate (total)	% trap success rate (gliders)
October 2002	34	14	1	2	47.1	41.2
November 2002	51	13	3	1	27.5	25.5
December 2002	39	14	2	1	38.5	35.9
January 2003	32	6	0	0	18.8	18.8
February 2003	25	9	3	0	36.0	36.0
March 2003	26	6	0	1	26.9	23.1
April 2003	37	8	2	3	29.7	21.6
May 2003	25	13	1	0	52.0	52.0
June 2003	29	20	4	1	72.4	69.0
July 2003	15	8	0	0	53.3	53.3
August 2003	29	8	1	2	34.5	27.6
Total	**831**	**217**		**28**	**23.8**	**14.3**

Appendix 4(b) Percentage trap success rate for male and female captures of yellow-bellied gliders.

Month	No. of males caught	% trap success rate	No. of females caught	% trap success rate
August 2001	2	N/A	3	N/A
October 2001	2	9.5	1	4.8
November 2001	2	10.5	3	15.8
December 2001	2	4.2	2	4.2
January 2002	2	2.3	7	8.0
February 2002	2	4.1	3	6.1
March 2002	3	6.1	4	8.2
April 2002	3	9.7	4	12.9
May 2002	4	20.0	4	20.0
June 2002	4	9.5	3	7.1
July 2002	4	8.2	6	12.2
August 2002	7	14.9	6	12.8
September 2002	3	11.5	3	11.5
October 2002	6	17.6	7	20.6
November 2002	6	11.8	4	7.8
December 2002	6	15.4	6	15.4
January 2003	4	12.5	2	6.3
February 2003	1	4.0	4	16.0
March 2003	1	3.8	5	19.2
April 2003	2	5.4	3	8.1
May 2003	4	16.0	13	52.0
June 2003	7	24.1	8	27.6
July 2003	4	26.7	4	26.7
August 2003	4	13.8	2	6.9
Total	**85**		**107**	

Appendix 5 Yellow-bellied glider specimen ABTC numbers, voucher numbers, tissue type and location (latitude, longitude) from which specimens originated (if known) and GenBank accession numbers for haplotypes. AMS = Australian Museum, QM = Queensland Museum. ABTC specimens were provided from the South Australian Museum collection. Blood from specimens ABTC 75332-75336 was collected from a captive colony of yellow-bellied gliders at Taronga Zoo, NSW. ABTC numbers are provided for the outgroup specimens. The type specimen, *Petaurus australis reginae*, from southern Queensland (Thomas, 1923) was not available within Australian museum collections for analysis.

ABTC no. or voucher no.	Tissue	Lat.	Long.	GenBank #
New South Wales (NSW)				
ABTC 72627*	Liver			DQ889434[B]
ABTC 72626*	Liver			DQ889438
AMS M32132*	Liver	31°12'	152°49'	DQ889434
AMS M34091*	Liver	36°22'	150°04'	DQ889435
AMS M34617*	Liver	31°33'	152°48'	DQ889436
AMS M31521*	Heart	31°13'	151°53'	DQ889437
ABTC 80843, 80844	Skin	28°12'	152°43'	DQ889431[B],45
ABTC 75335*	Blood	30°01'	153°11'	DQ889433
ABTC 75332-4*, 75336*	Blood			DQ889430-2,31[B]
Southern Queensland (SQ)				
QM JM8648	Skin	25°06'	152°22'	DQ889440
QM JM8645	Skin	27°42'	153°03'	DQ889441
QM JM8599	Skin	26°11'	152°39'	DQ889442
North Queensland (NQ)				
QM JM8746[A]	Skin	17°49'	145°33'	
QM JM6352[A]	Skin	16°26'	145°12'	
ABTC 80838-40	Skin	16°13'	145°02'	DQ889443[B]
ABTC 80841	Skin	17°33'	145°27'	DQ889444
*Victoria (Vic.)**				
ABTC 76233, 76535-6, 76235, 76237-8, 76609, 76642	Skin	37°55'	140°58'	DQ889439[B]

ABTC no. or voucher no.	Tissue	Lat.	Long.	GenBank #
South Australia (SA)				
ABTC 76239*, 75598*, 76531*, 81031	Skin	37º56'	183º3'	DQ889439[B]
Petauroides volans				
ABTC 13802**	Liver			DQ889448
Petaurus norfolcensis (SA)				
ABTC 27085**	Liver	36º32'	140º45'	DQ889449
Petuarus breviceps (QLD)				
ABTC 7688**	Liver	17º06'	145º47'	DQ889446
P. b. papuensis				
ABTC 7606**	Liver			DQ889447

[A]DNA was degraded for specimens QM JM8746 and QM JM6352, and was not able to be PCR-amplified with primers for the ND4 gene for phylogenetic analyses. [B]Identical sequence under listed GenBank number.

*Sequencing of these specimens was carried out by Mr Huw Cooksley, School of Molecular and Biomedical Science, The University of Adelaide.
**Sequencing of these specimens was carried out by Ms Mansooreh Malekian, School of Earth & Environmental Sciences, The University of Adelaide.

Appendix 6 Catalogue numbers of yellow-bellied glider skulls, institution where skulls were kept, gender of the specimen and location from which the skull was obtained.

Catalogue no.	Specimen obtained from	Gender	Latitude	Longitude
Victoria				
DTC9	Vic museum	F	37º51'	148º4'
C26674	Vic museum	F	38º0'	145º16'
C3729	Vic museum	M	37º26'	149º32'
C29787	Vic museum	M	37º34'	149º9'
C3731	Vic museum	F	37º26'	149º32'
C5777	Vic museum	M	38º24'	143º6'
C2397	Vic museum	M	38º35'	143º30'
DTC10	Vic museum	M	37º51'	148º4'
C18873	Vic museum	M	37º44'	149º30'
C22660	Vic museum	F	37º34'	149º45'
C3728	Vic museum	F	37º15'	149º25'
C2396	Vic museum	F	38º35'	14º30'
C3884	Vic museum	Unknown	37º42'	145º50'
C3727	Vic museum	M	37º15'	149º25'
C3730	Vic museum	F	37º26'	149º32'
C8736	Vic museum	F	37º20'	149º25'
NSW				
M6820	Aust museum	M	34º11'	150º37'
M32132	Aust museum	F	32º13'	152º50'
CM10071	ANWC	F	37º04'	149º55'
CM00932	ANWC	F	35º16'	148º36'
CM03868	ANWC	F	34º53'	150º30'
CM00141	ANWC	F	30º13'	152º44'
CM15565	ANWC	F	36º59'	149º22'
CM15585	ANWC	F	36º59'	149º22'
CM15583	ANWC	F	36º59'	149º22'

Catalogue no.	Specimen obtained from	Gender	Latitude	Longitude
South-east Queensland				
JM15152	Qld museum	Unknown	25°57'	152°12'
M2747	SA museum	F	23°30'	150°40'
JM8598	Qld museum	F	27°43'	152°58'
JM8646	Qld museum	M	25°09'	152°23'
JM8599	Qld museum	M	25°57'	152°33'
North Queensland				
M2237	SA museum	Unknown	17°23'	145°23'
JM8747	Qld museum	M	17°49'	145°33'
JM8746	Qld museum	M	17°49'	145°33'
JM8503	Qld museum	F	17°50'	145°33'

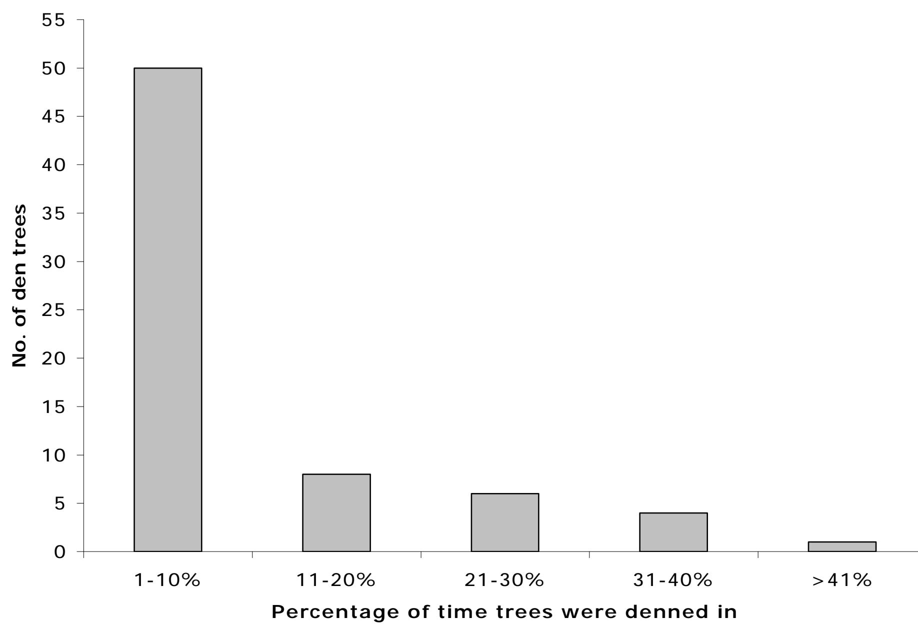
Appendix 7 Measurement data of 34 yellow-bellied glider skulls in mm. Shaded individuals are repeat measurements for a small subset of individuals taken on a different day, shaded data are where the data are different from the measurement presented in the results section. Data were within 1 mm accuracy for all 14 characters. ZygW = zygomatic width, ZygL = zygomatic length, BrainW = brain width, M1W = width of molar 1, M1L = length of molar 1, NasalL = nasal length, NasalW = nasal width, Lacr = lacrimal, Nangle = nasal angle, BrainH = brain height, Coron = coronoid height, ManL = mandible length, Occip = length of occipital.

	ZygW	ZygL	BrainW	SkullL	M1W	M1L	NasalL	NasalW	Lacr	Nangle	BrainH	Coron	ManL	Occip
JM8503_N_Qld	3.7	1.6	2.3	5.3	0.3	0.3	0.4	0.6	0.8	0.6	1.9	1.85	3.1	0.4
JM8503_N_Qld	3.7	1.6	2.4	5.3	0.35	0.3	0.4	0.6	0.85	0.6	1.9	1.9	3.1	0.4
JM8747_N_Qld	3.95	1.6	2.5	5.3	0.3	0.3	0.45	0.6	0.9	0.7	1.9	1.8	3.1	0.45
JM8746_N_Qld	3.8	1.65	2.4	5.3	0.4	0.3	0.4	0.6	0.9	0.7	1.9	1.9	3.1	0.4
JM8746_N_Qld	3.8	1.65	2.4	5.3	0.3	0.3	0.4	0.6	0.8	-	1.9	1.85	3.1	-
M2237_N_Qld	3.7	1.6	2.5	5.6	0.3	0.3	0.4	0.4	0.9	0.6	1.85	1.9	3.1	0.6
JM8598_S_Qld	3.5	1.55	2.25	5.4	0.35	0.4	0.5	0.55	0.8	0.6	1.8	1.8	3.1	0.45
M2747_S_Qld	3.7	1.7	2.5	5.4	0.3	0.25	0.35	0.5	0.9	0.6	1.9	1.85	3.1	0.5
JM8646_S_Qld	3.7	1.7	2.4	5.7	0.3	0.3	0.45	0.5	0.9	0.7	1.9	1.8	3.2	0.45
JM8646_S_Qld	3.75	1.7	2.5	5.7	0.3	0.3	0.5	0.5	0.9	0.6	1.95	1.85	3.3	0.5
JM8599_S_Qld	3.6	1.7	2.2	5.55	0.4	0.3	0.5	0.6	1	0.7	1.85	1.95	3.2	0.5
JM8599_S_Qld	3.6	1.7	2.2	5.55	0.3	0.3	0.5	0.6	1	0.7	1.85	1.8	3.2	0.6

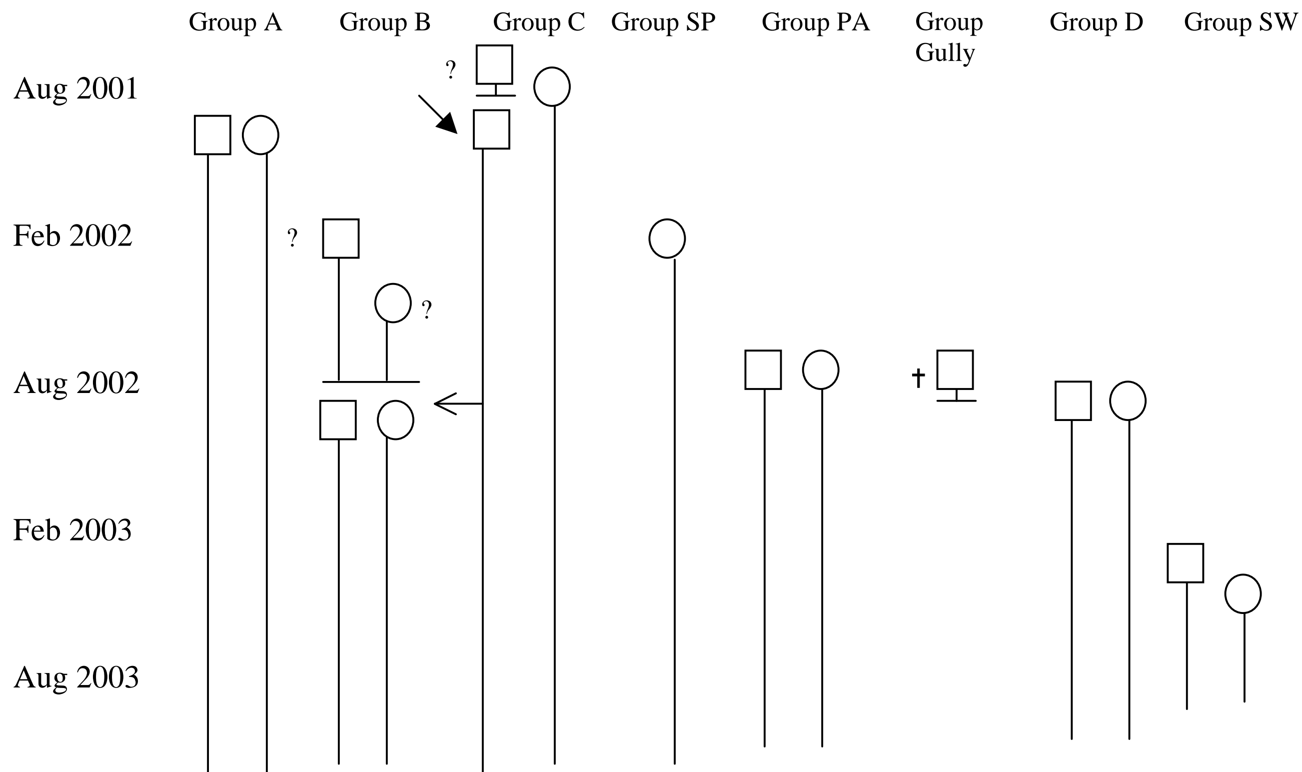
	ZygW	ZygL	BrainW	SkullL	M1W	M1L	NasalL	NasalW	Lacr	Nangle	BrainH	Coron	ManL	Occip
JM15152_S_Qld	3.8	1.55	2.3	5.4	0.35	0.35	0.5	0.6	0.9	0.6	1.9	1.8	3.1	0.5
M32132_NSW	3.8	1.7	2.5	5.5	0.35	0.35	0.5	0.6	0.8	0.8	1.9	1.85	3.1	0.5
CM15565_NSW	3.85	1.15	2.5	5.6	0.35	0.3	0.35	0.5	0.9	0.6	2	1.95	3.25	0.65
CM15585_NSW	3.6	1.6	2.4	5.5	0.4	0.3	0.35	0.5	0.85	0.7	2	1.9	3.15	0.6
CM15583_NSW	3.8	1.7	2.5	5.6	0.35	0.3	0.4	0.5	0.9	0.65	1.9	2	3.3	0.6
CM10071_NSW	3.9	1.65	2.45	5.8	0.35	0.3	0.4	0.5	0.85	0.7	1.95	2.05	3.3	0.55
CM932_NSW	3.85	1.6	2.55	5.4	0.4	0.3	0.5	0.55	0.9	0.7	2	1.95	3.1	0.7
CM3868_NSW	4.05	1.7	2.7	5.7	0.35	0.3	0.5	0.6	0.95	0.7	2	2	3.2	0.65
CM141_NSW	3.9	1.7	2.5	5.5	0.35	0.3	0.45	0.6	1	0.7	1.95	2	3.3	0.5
M6820_NSW	3.75	1.65	2.5	5.6	0.4	0.35	0.5	0.6	0.8	0.7	2	1.8	3.2	0.55
DTC9_VIC	3.85	1.7	2.5	5.5	0.35	0.35	0.4	0.6	0.9	0.7	2	1.9	3.3	0.5
C26674_VIC	3.9	1.5	2.55	5.35	0.4	0.4	0.5	0.6	0.95	0.6	2	2.05	3.1	0.6
C3731_VIC	3.8	1.8	2.55	5.6	0.35	0.35	0.5	0.55	0.85	0.7	2	1.75	3.2	0.55
C22660_VIC	4	1.8	2.6	5.6	0.4	0.35	0.4	0.6	0.9	0.7	2	1.85	3.4	0.55
C3728_VIC	3.8	1.7	2.5	5.6	0.4	0.35	0.4	0.6	0.9	0.65	1.9	1.8	3.4	0.6
C2396_VIC	4.1	1.7	2.55	5.6	0.4	0.35	0.4	0.6	1	0.7	1.9	1.95	3.25	0.5
C3730_VIC	3.9	1.85	2.5	5.8	0.35	0.4	0.4	0.6	0.95	0.7	1.95	1.8	3.35	0.4
C8736_VIC	3.9	1.65	2.6	5.7	0.4	0.35	0.5	0.6	0.9	0.7	2	1.9	3.3	0.5

	ZygW	ZygL	BrainW	SkullL	M1W	M1L	NasalL	NasalW	Lacr	Nangle	BrainH	Coron	ManL	Occip
C3884_VIC	3.75	1.6	2.6	5.5	0.3	0.35	0.4	0.6	0.8	0.6	2	1.8	3.15	0.45
C3729_VIC	4.1	1.7	2.6	5.7	0.4	0.4	0.4	0.6	1	0.7	2	2	3.4	0.65
C29787_VIC	4	1.7	2.6	5.7	0.35	0.35	0.4	0.6	0.9	0.8	2.05	2	3.3	0.7
C5777_VIC	4	1.7	2.55	5.6	0.4	0.4	0.5	0.65	0.9	0.7	2	1.9	3.1	0.6
C2397_VIC	3.9	1.7	2.55	5.7	0.35	0.4	0.4	0.6	0.95	0.65	1.9	1.9	3.2	0.7
DTC10_VIC	3.95	1.7	2.6	5.55	0.3	0.35	0.4	0.5	0.8	0.5	1.9	1.8	3.25	0.6
C18873_VIC	4	1.8	2.5	5.65	0.35	0.35	0.4	0.55	0.9	0.7	1.95	2	3.25	0.5
C3727_VIC	4	1.65	2.5	5.6	0.4	0.35	0.5	0.6	0.9	0.7	2	2.1	3.3	0.5

Appendix 8 The percentage of time gliders spent per den tree. Primary den trees were those that were denned in > 10% of the time, whereas secondary den trees were those that gliders denned in < 10% of the time. Thus, gliders maintained a few primary den trees per group in which they spent much of their denning time, but several secondary den trees within their range that were denned in only a few times. Data have been combined for all groups because raw data were converted to percentages, and thus were comparable, however, dens that contained more than one glider were included only once per day.



Appendix 9 Demography of the Rennick population of yellow-bellied gliders shortly before, and until the end of, the study period, between August 2001 and August 2003. Each group is shown with adult males as squares and adult females as circles. Immigration (black filled-in arrow) and movements between groups is indicated by arrows. A horizontal line represents the time at which the individual disappeared, question marks represent the fate of the individual is unknown, whilst a cross represents death of that individual. Subadults and juveniles are not included in this figure.



Appendix 10 Rainfall each month (mm) recorded at Rennick. Total = total rainfall for that year. Rainfall data provided by Hancock Victorian Timber Plantations.

Year	January	February	March	April	May	June	July	August	September	October	November	December	Total
1953	23.0	9.0	2.2	41.0	51.2	122.7	110.2	95.5	81.7	49.2	111.5	60.5	757.7
1954	17.7	11.2	30.5	92.0	63.2	102.0	53.0	53.7	75.0	41.0	49.7	55.0	644.0
1955	15.0	40.5	7.5	91.0	169.7	130.0	100.0	200.2	56.5	59.5	52.0	31.2	953.1
1956	23.0	0.2	23.5	134.2	47.7	168.2	109.0	108.0	76.0	67.0	41.7	52.2	850.7
1957	3.5	22.0	47.0	62.2	36.7	66.7	39.7	55.2	116.2	38.7	64.5	35.7	588.1
1958	3.5	18.5	22.5	26.7	147.2	39.2	148.0	154.2	58.0	103.2	46.2	2.0	769.2
1959	4.2	31.2	55.7	23.0	14.7	53.2	62.7	99.7	56.2	26.7	22.5	72.5	522.3
1960	45.7	108.5	41.5	90.7	148.7	63.5	129.5	81.7	119.7	52.5	43.5	19.5	945.0
1961	18.0	9.2	10.0	80.7	49.0	101.2	98.0	47.5	53.2	53.5	38.2	41.0	599.5
1962	27.0	46.7	25.5	25.2	122.2	138.5	60.0	105.5	47.2	107.2	31.0	38.7	774.7
1963	123.7	7.2	17.5	8.5	58.7	58.5	109.0	86.2	92.2	24.7	20.2	5.7	612.1
1964	38.0	32.7	46.7	76.2	169.2	237.2	114.0	89.0	88.2	68.0	72.5	20.0	1051.7
1965	13.5	5.5	43.2	82.5	129.7	78.7	107.5	99.0	62.7	29.2	75.2	33.5	760.2
1966	18.5	11.2	44.7	43.2	51.5	70.7	203.7	88.2	88.0	66.0	60.7	64.0	810.4
1967	18.5	35.2	16.5	10.0	33.5	20.5	112.2	83.7	49.5	16.0	24.2	28.7	448.5
1968	16.7	19.5	34.7	135.5	169.0	111.7	121.7	131.2	59.0	126.5	105.2	40.7	1071.4
1969	20.0	91.0	30.7	54.0	69.2	26.0	104.5	64.0	104.0	14.7	48.2	51.5	677.8
1970	50.7	10.0	60.5	67.7	68.7	102.5	146.5	202.2	74.7	33.7	62.0	87.5	966.7

Year	January	February	March	April	May	June	July	August	September	October	November	December	Total
1971	21.0	18.0	38.2	134.7	96.7	118.5	53.5	133.7	108.7	107.5	99.0	59.7	989.2
1972	49.2	44.7	4.2	76.9	30.5	66.7	113.2	92.0	38.0	33.5	35.7	6.2	590.8
1973	25.7	48.2	68.5	77.5	100.7	86.0	63.0	114.2	119.2	141.5	31.2	55.7	931.4
1974	13.7	71.7	17.2	105.4	37.5	76.4	191.3	104.9	104.9	86.2	62.2	49.5	920.9
1975	32.1	11.8	83.5	27.6	97.5	87.7	149.7	111.8	84.2	200.6	81.7	34.8	1003.0
1976	16.0	34.4	13.9	64.1	54.9	113.2	74.9	103.3	108.7	138.4	63.5	69.3	854.6
1977	47.0	32.1	47.1	38.6	125.9	123.3	99.7	57.0	40.5	47.2	116.4	28.1	802.9
1978	22.1	20.5	29.7	67.1	67.9	72.1	146.7	109.7	76.7	54.6	62.7	49.3	779.1
1979	25.1	42.5	17.9	52.7	70.9	92.3	63.6	138.7	115.8	90.3	57.3	33.4	800.5
1980	28.5	5.1	9.9	89.8	62.9	70.8	92.3	81.3	100.1	65.3	43.4	29.0	678.4
1981	39.2	7.6	46.3	27.4	73.0	160.3	166.9	211.3	42.4	64.8	52.4	12.4	904.0
1982	34.8	8.1	57.1	72.3	59.7	98.6	60.0	21.6	50.1	38.8	25.1	12.5	538.7
1983	37.2	1.5	184.8	68.9	110.2	105.2	100.6	97.9	112.6	27.5	65.6	19.6	931.6
1984	20.2	9.6	95.0	31.8	45.2	65.4	166.4	138.6	121.6	50.2	76.2	36.6	856.8
1985	23.6	10.0	41.2	54.4	74.6	103.6	74.0	103.7	50.8	78.8	76.8	89.0	780.5
1986	13.0	7.4	8.0	93.6	89.4	78.0	158.8	103.3	79.0	107.4	20.8	123.6	882.3
1987	15.6	19.2	27.8	30.4	171.6	83.4	87.6	58.2	32.2	89.8	32.4	27.8	676.0
1988	47.6	39.4	21.8	21.0	89.2	111.8	110.8	102.0	83.2	62.8	53.0	40.1	782.7
1989	51.0	6.8	32.4	70.2	66.2	135.0	126.2	139.8	67.4	112.8	17.6	21.2	846.6
1990	22.6	25.0	15.8	36.6	21.0	119.0	112.6	201.4	104.8	66.6	39.0	30.6	795.0
1991	95.4	0.2	40.2	46.4	14.0	158.2	71.4	156.6	94.6	26.4	50.6	37.6	791.6
1992	19.6	9.2	34.0	109.4	96.6	102.0	119.0	127.2	98.8	125.4	107.8	44.0	993.0

Year	January	February	March	April	May	June	July	August	September	October	November	December	Total
1993	61.4	43.0	19.8	8.0	57.6	82.2	95.6	98.2	90.0	95.0	14.8	77.2	742.8
1994	50.2	15.8	1.6	46.4	84.6	94.2	75.7	72.8	65.1	59.3	69.4	48.6	683.7
1995	90.2	20.4	47.2	109.6	30.5	92.0	150.4	76.2	62.8	32.8	26.4	40.8	779.3
1996	74.6	22.6	24.5	57.8	15.0	150.6	164.4	173.6	139.4	52.2	13.0	26.2	913.9
1997	33.4	10.0	48.8	17.6	121.9	53.4	59.0	85.8	95.2	28.2	97.8	10.8	661.9
1998	28.2	31.4	21.6	75.0	58.6	116.0	124.4	64.8	76.6	64.4	30.0	24.6	715.6
1999	4.8	33.0	49.0	19.2	75.8	119.8	58.8	49.0	59.0	53.0	71.2	66.2	658.8
2000	14.6	11.8	24.0	91.8	139.8	96.8	162.0	76.4	124.0	103.6	70.8	19.4	935.0
2001	1.2	11.9	88.2	31.2	68.4	87.8	59.6	171.8	79.8	92.4	58.2	44.8	795.3
2002	16.6	15.2	19.2	24.8	45.1	122.4	97.5	53.6	79.0	54.1	46.5	35.1	609.1
2003	22.1	36.6	58.2	33.5	16.8	147.6	91.7	102.1	72.6	52.6	35.8	34.0	703.6
2004	22.1	18.8	47.5	37.0	38.6								

NB Mar 2002 to Aug 2002 rainfall based on Heywood figures as the rain gauge at Rennick malfunctioned

March 2003 figures have some Mount Gambier figures, because rainfall on 29/3/03 failed to record.

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