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# Population structure of brush-tailed rock-wallaby (*Petrogale penicillata*) colonies inferred from analysis of faecal DNA

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## Abstract

**Genetic data obtained using faecal DNA were used to elucidate the population structure of four brush-tailed rock-wallaby (*Petrogale penicillata*) colonies located in Wollemi National Park, New South Wales. The results suggested that the four sampled colonies are genetically differentiated and do not form a panmictic unit. Based on assignment tests, approximately 5% of sampled individuals were inferred to be dispersers and both male and female migrants were detected. Multilocus spatial autocorrelation analyses provided evidence for increased philopatry among females compared to males within the largest colony in the valley. Females in close spatial proximity were more genetically similar than expected under a random distribution of females, and females separated by more than 400 m were less genetically similar than expected. In contrast, there was no evidence of a significant clustering of related males. This suggests that within-colony dispersal is male biased. We also investigated the best strategies for conserving genetic diversity in this population. All of the four sampled colonies were found to contain distinct components of the genetic diversity of the Wolgan Valley *P. penicillata* population and loss of any colony is likely to result in the loss of unique alleles. Conservation and management plans should take into account that these colonies represent genetically differentiated discrete subpopulations. This approach is also the best strategy for maintaining the genetic diversity of the populations in this valley.**

*Keywords*: dispersal, faecal DNA, noninvasive genetic sampling, population structure, rock-wallaby

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## Introduction

An accurate knowledge of local population structure and boundaries can provide vital insights into species biology, including social structure and dispersal patterns (e.g. Paetkau *et al*. 1995; Sugg *et al*. 1996; Chesser 1998; Dobson *et al*. 1998). Interpreting population structure is also critical for assessing the conservation status of populations and establishing the appropriate scale for conservation management strategies (Taylor & Dizon 1999). Moritz (1994) proposed a criterion for identifying discrete management units (demographically independent populations) based on significant divergence of nuclear or mitochondrial allele frequencies. However, the criteria for identifying units for specific conservation management activities should be flexible, and the amount of interpopulation exchange that defines management units will be influenced by the population biology of the species in question, the management aims and case-specific conservation issues (Taylor & Dizon 1999).

Recent advances in molecular genetic techniques, combined with existing and new statistical approaches, offer opportunities to interpret population structure across different geographical scales (e.g. Piertney *et al*. 1998; Smouse & Peakall 1999; Roach *et al*. 2001; Stow *et al*. 2001). Manel *et al*. (2003) provide a recent review of genetic approaches to delineating spatial population boundaries. Piertney *et al*. (1998) identified a cleared river valley as a barrier to gene flow in red grouse (*Lagopus lagopus scoticus*) populations in Scotland by spatial interpolation of principal component scores derived from microsatellite allele frequencies at each sampling location. Roach *et al*. (2001)

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recommended that the appropriate scale for management of black-tailed prairie dogs was a regional, rather than a colony-by-colony scale, since prairie dogs live in metapopulations, where their existence depends on a balance of local extinction and recolonization.

The identification of genetically differentiated groups also contributes to the design of conservation strategies to preserve the genetic diversity of the species. This is important for the preservation of evolutionary divergence among lineages within a species and, on a smaller scale, for preserving the genetic diversity among local populations (Moritz 1994). Assessment of the capacity of alternative management strategies to preserve the genetic diversity of a species requires estimates of the degree of diversity within, and differentiation between, local populations and regions. Petit *et al*. (1998) presented a framework for assessing the contribution of individual populations to the overall levels of genetic diversity within a species based on the overall loss of genetic diversity if that population were to be lost. The importance of a particular population to the conservation of the genetic diversity of a species is therefore assessed in terms of what it uniquely contributes, which permits the assignment of priority to particular populations for the conservation of the species' genetic diversity (Ji & Leberg 2002).

This study examines the population structure of colonies of the endangered brush-tailed rock-wallaby (*Petrogale penicillata*). The species was once widespread and abundant throughout southeastern Australia and found in a variety of habitats on rocky outcrops (Eldridge & Close 1995). Since European settlement, *P. penicillata* populations have declined markedly and the species is now rare or absent over most of its former range (Lunney *et al*. 1997). Currently, the main impacts on *P. penicillata* colonies are habitat disturbance and degradation, disease, competition from introduced herbivores (e.g. goats and rabbits), and predation by introduced carnivores such as the red fox (Short & Milkovitis 1990; Eldridge & Close 1995; Dovey *et al*. 1997; Ruming & Moss 2000).

There are four known *P. penicillata* colonies located in the Wolgan Valley, Wollemi National Park, New South Wales. Wollemi National Park has been classified as a World Heritage area and the colonies are located in a relatively remote and rugged part of the park. The largest colony (Main) was the first to be discovered and management and conservation strategies were initially designed to protect and conserve this colony only. Recently, three smaller colonies have been discovered. Very little is known about the population biology and intercolony dispersal patterns of *P. penicillata*, and it was initially unknown whether the colonies represented a single population in a geographically patchy habitat, or a series of demographically discrete populations. Conservation strategies have focused primarily on the Main colony, which also happens to be the largest in this valley (Piggott *et al*. 2005). However, if the smaller colonies are found to be demographically discrete (at least in the short term), this would strengthen the case for population monitoring and conservation management strategies for each of the colonies.

This paper describes the second aspect of a population study on the Wolgan Valley *P. penicillata* colonies. The first component of the study involved estimating the population size of the four known colonies based on a mark–recapture analysis of microsatellite genotypes identified from faecal DNA (Piggott *et al*. 2005). The Wolgan Valley population consists of the Main colony (estimated population size ≈ 70) and three smaller colonies (estimated population sizes ranging from 5 to 20), with two of these being separated from the Main colony by the Wolgan River (Fig. 1). Here, we use the microsatellite genotypes of the individuals identified from faecal DNA to infer population structure and dispersal patterns and provide recommendations for the conservation and management of the Wolgan Valley population. We determine whether the colonies represent genetically differentiated units and whether the Wolgan River presents a boundary to gene flow. We also investigate dispersal patterns with

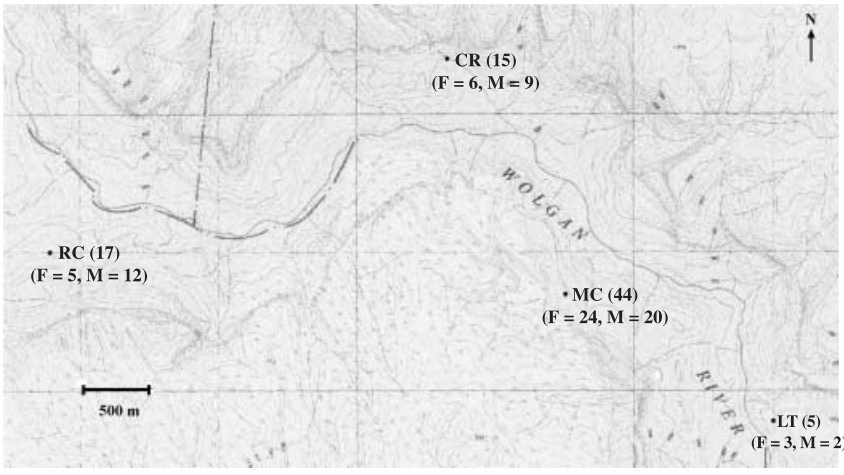


**Fig. 1** Locations and sample sizes of four *Petrogale penicillata* colonies in Wolgan Valley, Wollemi National Park, New South Wales. MC, Main colony; RC, Rocky Creek; CR, Crocodile Rock; and LT, Little Tower.

analyses of multilocus genotypic correlations and assignment tests.

The secondary aim of the study is to provide guidelines for the conservation of genetic diversity among the Wolgan Valley *P. penicillata* colonies. Other *P. penicillata* colonies that have suffered severe population declines have lost much of their genetic diversity and are highly inbred (Browning *et al*. 2001; Eldridge *et al*. 2004). We use the approach of Petit *et al*. (1998) to determine what proportion of the genetic diversity among the four known colonies would be lost if any of the colonies, or all three small colonies, went extinct. In addition to providing specific management recommendations for the studied colonies, information on population structure in undisturbed habitat will provide reference data to assist in the interpretation of population structure of the many other colonies that persist in modified landscapes, in which the native forest between colonies on rocky terrain has been cleared for agriculture (Eldridge 1997).

## Methods

### *Study site and molecular data*

The study site is described in detail in Piggott *et al*. (2005). The genotypic data used in this study are from the 80 individuals identified using noninvasive genetic profiling from faeces collected at the Main, Rocky Creek, Crocodile Rock and Little Tower colonies over a 2-year period from April 2001 to April 2003 (see Piggott *et al*. 2005) and from a dead subadult male found at the Main colony. Location of each colony and overall sample sizes for each of the four *Petrogale penicillata* colonies is provided in Fig. 1. Estimated population sizes for each colony are provided in Table 1.

### *Genetic diversity*

Allele frequencies, the average number of alleles per locus (allelic diversity: *AD*) and expected heterozygosity ($H_E$) were calculated for each colony using the program GENEPOP 3.2 (Raymond & Rousset 1995). Allelic richness (*AR*), a measure of the number of alleles per locus standardized for differences in sample size, was calculated for each colony using FSTAT 2.9.3.2 (Goudet 2001). Differences in *AD*, $H_E$ and *AR* among each pair of colonies were assessed using a Wilcoxon signed rank test with loci as the pairing factor (Sokal & Rohlf 1995). GENEPOP 3.2 was used to test for conformance to Hardy–Weinberg expectations, employing the Markov chain method of Guo & Thompson (1992) at each combination of locus and population, with the resultant *P* values being adjusted for *N*-tests (where *N* is the number of locus/population combinations) via the sequential Bonferroni method (Rice 1989). Multilocus $F_{IS}$ was also calculated for each population using GENEPOP 3.2, then tested by permutation using Weir & Cockerham's estimator (1984).

### *Genetic structure*

To determine the relative amount of genetic variation occurring within and between colonies, and to assess whether the Wolgan River contributes to genetic differentiation between the colonies, a hierarchical analysis of molecular variance (AMOVA) was carried out using ARLEQUIN 2.0 (Schneider *et al*. 2000). The degree of genetic differentiation ($F_{ST}$) between each pair of colonies was evaluated using the estimator θ (Weir & Cockerham 1984).

The Bayesian clustering method in the program STRUCTURE 2.1 (Pritchard *et al*. 2000) was used as an exploratory analysis to determine whether the population of *P. penicillata* in the Wolgan Valley could be subdivided into genetically distinct groups and whether the structure of these groups reflected the colony structure in the study area. STRUCTURE attempts to find population groupings that minimize Hardy–Weinberg and linkage disequilibrium by assigning individuals to subpopulations on the basis of their genotypes while simultaneously estimating population allele

**Table 1** Number of declared unique *Petrogale penicillata* genotypes and estimated population size for each colony and sample collection using $M_{h\text{-jackknife}}$ estimate for the Main colony and Kohn *et al*. (1999) rarefaction estimates for Crocodile Rock, Rocky Creek and Little Tower colonies (Piggott *et al*. 2005)

| Colony (collection period) | | No. of declared genotypes | No. of males and females (M/F) | Estimated population size (95% CI) |
|---|---|---|---|---|
| Main colony | (1) | 44 | 20/24 | 67 (55–91) |
| Total unique genotypes | | 44 | 20/24 | |
| Crocodile Rock | (1) | 9 | 5/4 | 11 (9–18) |
| | (2) | 10 | 7/3 | 14 (10–22) |
| Total unique genotypes | | 15 | 9/6 | |
| Rocky Creek | (1) | 10 | 8/2 | 14 (11–23) |
| | (2) | 12 | 8/4 | 17 (15–23) |
| Total unique genotypes | | 17 | 12/5 | |
| Little Tower | (1) | 2 | 0/2 | 2 (2–3) |
| | (2) | 4 | 2/2 | 5 (4–12) |
| Total unique genotypes | | 5 | 2/3 | |

frequencies (Pritchard *et al*. 2000). Although this method has been used in other studies as an assignment method to identify putative dispersers (e.g. Eldridge *et al*. 2001; Berry *et al*. 2004), it was not used for that purpose in this study as there may be unsampled populations in the Wolgan Valley. We compared two different ancestry models: admixture and no admixture. The additional parameters used in both models were admixture, correlated allele frequencies, different values of $F_{ST}$ for subpopulations, prior $F_{ST}$ mean (0.1), standard deviation (0.2) and constant lambda valued at 1. No prior information about group membership was provided and we estimated the most likely number of genetic groups ranging from one to six, as this was thought to be a realistic range of groups to test. The length of the initial burn-in period was set at 50 000 iterations followed by a run of 500 000 Markov chain Monte Carlo repetitions. The model (based on admixture or no admixture and the number of groups) with the highest posterior probability was selected. The number of genetic subpopulations was compared to the actual demographic structure of the four colonies.

We then used the multilocus genotypic autocorrelation measure of Smouse & Peakall (1999) to investigate the level of genetic similarity among individuals within the groups designated by STRUCTURE. An individual was 'assigned' to a group if its estimated membership fraction in that group was greater than 0.9. We used GENALEX version 5.1 (Peakall & Smouse 2001) to calculate the autocorrelation coefficient among the genotypes of all individuals assigned to each group and to determine whether the autocorrelation coefficient among individuals within each group was greater than that which would be expected among a random sample of the same number of individuals from all individuals sampled in the Wolgan Valley.

### *Estimation of dispersal patterns using assignment tests*

We used the program GENECLASS 2 (Piry *et al*. 2004) to identify putative first generation migrants and their population of origin. We estimated the likelihood of each genotype in each population using the assignment algorithm of Rannala & Mountain (1997). We compared the likelihood of each individual in its 'home' colony to its likelihood in the most likely of the other colonies. The individual was provisionally assigned as a migrant from the other colony if its likelihood in the other colony was at least 99 times higher than in the colony where it was sampled. This procedure could result in the inaccurate assignment of immigrants from unsampled populations, so we used the gamete-based Monte Carlo resampling procedure of Paetkau *et al*. (2004) to estimate the probability of excluding the individual from each colony with a critical *P* value of 0.99. Individuals that were excluded from all colonies according to this criterion were considered to be immigrants from unsampled colonies. Although our sample sizes for the small colonies were low, the high $F_{ST}$ values between the colonies are likely to ensure that the procedure was reasonably accurate (Cornuet *et al*. 1999; Paetkau *et al*. 2004).

If any putative immigrants could not be assigned as belonging to any of the colonies sampled, we were interested in determining whether such immigrants were likely to have come from a single unknown population or multiple populations. We compared the average genetic distance (Smouse & Peakall 1999) among the individuals from unsampled colonies to a distribution of average genetic distances among randomly sampled nonimmigrants from each of the sampled colonies. For each of the four sampled colonies, we calculated the average genetic distance among these individuals using POPTOOLS 2.6.2 (Hood 2004). This was repeated 1000 times and the proportion of average genetic distances from the random samples that was greater than the average genetic distance among the putative immigrants was calculated. If the genetic distance among the putative immigrants from unsampled colonies was significantly greater than that among the randomly sampled nonimmigrants from each of the four colonies, we considered that the immigrants were most likely to have originated from more than one source population.

### *Inference of sex-specific dispersal patterns from multilocus genotypic correlations*

We used the multilocus genotypic autocorrelation coefficient (*r*) of Smouse & Peakall (1999) to infer sex-specific dispersal patterns in each of the four colonies. This spatial autocorrelation approach has been used to infer restricted dispersal, which is expected to result in significantly higher than random *r* values among spatially proximal individuals due to the spatial clustering of relatives (e.g. Smouse & Peakall 1999; Peakall *et al*. 2003; Hazlitt *et al*. 2004; Banks *et al*. 2005). Different rates of dispersal by males and females are expected to result in stronger spatial autocorrelation among individuals of the more philopatric sex (Peakall *et al*. 2003; Hazlitt *et al*. 2004; Banks *et al*. 2005). We used GENALEX version 5.1 (Peakall & Smouse 2001) to calculate *r* values among males and females within each colony and determine whether the *r* value for each sex in each colony differed significantly from a permuted random sample from all individuals sampled in the Wolgan Valley. We conducted a simple significance test of the difference in *r* between males and females in each colony by comparing the bootstrap 95% confidence intervals around the estimate of *r* for each sex (Peakall *et al*. 2003). To determine if there was an overall difference between males and females in *r* values among individuals in the same colony, we combined the data from the four colonies as described in Peakall *et al*. (2003) and implemented in GENALEX version 5.1 (Peakall & Smouse 2001).

The analysis described above was used to determine if there were differences between the sexes in dispersal between colonies. To investigate possible differences between the sexes in the patterns of within-colony dispersal, we conducted a multilocus spatial autocorrelation analysis among males and females in the Main colony. For each individual in the Main colony, a geographical coordinate was assigned based on the mean UTM coordinate (recorded with a GPS within ± 7 m accuracy) of all of the faecal samples collected for that individual (averaging 4.33 faeces per female, SD = 3.63 and 5.37 faeces per male, SD = 4.17). Inter-individual distances were calculated in metres based on the geographical coordinates of each individual and we conducted a spatial autocorrelation analysis among males and females using five distance intervals of 100 m. This interval size represented the best compromise between within-interval sample size and precision. We expected that if females disperse less than males within the colony, as reported by Hazlitt *et al*. (2004), we would observe higher *r* values among females than males in the first distance interval. We repeated this analysis for the Rocky Creek and Crocodile Rock colonies to determine if the patterns of within-colony spatial genotypic structure were consistent between colonies. This analysis was not carried out for Little Tower due to the very small sample size. The patterns may differ because these colonies cover a much smaller area so the potential for within-colony dispersal may be limited. The analyses were conducted among males and females in each colony using two distance intervals: 0–100 m and 100–200 m. The differences between the sexes were compared for the analyses to infer dispersal at the two scales: intracolony dispersal and between-colony dispersal.

### *Contributions of populations to total genetic diversity*

The contribution of each individual colony as well as the contribution of the Main colony alone and the three other colonies combined to the overall *AD* and $H_E$ was measured using the approach of Petit *et al*. (1998) (substituting allelic diversity for allelic richness). Petit *et al*. (1998) considered that the number of alleles (allelic diversity) was the most appropriate measure of a population's contribution to total genetic diversity. In this study, the number of individuals sampled in each colony was on average 75% of the estimated population size of that colony (Piggott *et al*. 2005). The observed correlation between sample size and estimated population size was 0.9997 (Pearson correlation coefficient). Therefore, we considered that the relative number of alleles detected in each colony was not biased due to sampling effect. The contribution of a given population to total diversity is measured as the difference between the total diversity of all populations and the diversity of all populations excluding the one in question (Petit *et al*. 1998). This contribution of each population to total diversity can be subdivided into two further components: the contribution due to the diversity of the population and the contribution due to its divergence from the other populations (Petit *et al*. 1998). Contributions can be positive (i.e. the contribution is greater than the mean) or negative (i.e. the contribution is lower than the mean).

## Results

### *Genetic variation*

The total number of alleles in each population ranged from 29 in Little Tower to 36 in Rocky Creek and Crocodile Rock (Table 2). Alleles only found in a single population were present in all colonies, with the Crocodile Rock colony having the highest number (Table 2). A total of 13 alleles were present in all but one colony and most of them ($n = 7$) were missing from Crocodile Rock (Table 2).

Wilcoxon signed rank tests revealed no significant differences in *AD*, *AR* or $H_E$ between populations. Tests for Hardy–Weinberg disequilibrium revealed a significant heterozygote deficit at just one locus in the Crocodile Rock colony and a significant heterozygote excess at one locus in the Main colony (Table 3). Over all loci, each colony showed a heterozygote excess, although this was only significant for one locus at the Main colony (Table 3).

### *Genetic structure*

The four colonies showed moderate levels of differentiation (average $F_{ST} = 0.115 \pm 0.017$) and all pairwise $F_{ST}$ values were highly significant ($P < 0.001$). Subdivision apparently imposed by the Wolgan River was a significant but relatively minor component of the variance between colonies (Table 4).

The most likely number of distinct genetic groups for the admixture and no admixture models was four (posterior probability ≥ 0.99999). The most likely model of population structure inferred from the posterior probabilities of all models tested was the no admixture model with four genetic groups, as the posterior probability was 6 582 992.585 times more likely than the admixture model with four genetic groups. The individuals assigned to each of these groups (> 0.9) occurred predominantly (minimum of 65% of individuals) within a single colony, which suggests that the colonies consist largely of genetically distinct groups of individuals (Fig. 2).

Individuals assigned to each of the STRUCTURE groups were more genetically similar than random (Table 5). Although the genetic similarity of individuals assigned to Group 4 fell within the 95% confidence interval, the probability value for a one-tailed test was not as significant as for individuals assigned to the other STRUCTURE groups ($P = 0.042$ vs. $P = 0.001$ for all other groups, Table 5).

**Table 2** Allelic frequencies (percentage) at seven polymorphic microsatellite loci among four *Petrogale penicillata* colonies. The total number of alleles is presented for each colony

| Locus | Colony (*n*) | | | |
|---|---|---|---|---|
| | Main colony (44) | Crocodile Rock (15) | Rocky Creek (17) | Little Tower (5) |
| Pa 597 | | | | |
| 94 | 0.00 | 6.67* | 0.00 | 0.00 |
| 100 | 2.27 | 6.67 | 0.00 | 0.00 |
| 102 | 39.77 | 46.67 | 29.41 | 10.00* |
| 104 | 0.00 | 0.00 | 0.00 | 10.00* |
| 108 | 0.00 | 0.00 | 0.00 | 10.00 |
| 112 | 22.73 | 0.00 | 5.88 | 0.00 |
| 114 | 0.00 | 0.00 | 0.00 | 10.00* |
| 118 | 1.14* | 0.00 | 0.00 | 0.00 |
| 120 | 0.00 | 0.00 | 2.94* | 0.00 |
| 126 | 2.27 | 0.00 | 23.53 | 0.00 |
| 132 | 22.73 | 40.00 | 20.59 | 40.00 |
| 136 | 9.09 | 0.00† | 17.65 | 20.00 |
| Pa 297 | | | | |
| 120 | 38.64 | 0.00† | 8.82 | 10.00 |
| 122 | 0.00 | 10.00 | 0.00 | 30.00 |
| 124 | 0.00 | 6.67* | 0.00 | 0.00 |
| 126 | 0.00 | 0.00 | 29.41* | 0.00 |
| 128 | 0.00 | 20.00 | 0.00 | 30.00 |
| 130 | 10.23 | 0.00† | 29.41 | 20.00 |
| 132 | 0.00 | 6.67 | 8.82 | 0.00 |
| 134 | 48.86 | 56.67 | 11.76 | 10.00 |
| 136 | 2.27 | 0.00 | 11.76 | 0.00 |
| Pa 593 | | | | |
| 117 | 0.00 | 3.33* | 0.00 | 0.00 |
| 119 | 0.00 | 3.33* | 0.00 | 0.00 |
| 123 | 0.00 | 3.33* | 0.00 | 0.00 |
| 127 | 0.00 | 13.33 | 20.59 | 0.00 |
| 129 | 31.82 | 30.00 | 38.24 | 0.00† |
| 133 | 20.45 | 0.00 | 8.82 | 0.00 |
| 135 | 5.68 | 16.67 | 0.00† | 10.00 |
| 137 | 40.91 | 0.00† | 23.53 | 60.00 |
| 139 | 1.14 | 20.00 | 2.94 | 10.00 |
| 149 | 0.00† | 10.00 | 5.88 | 20.00 |
| Pa 385 | | | | |
| 155 | 37.50 | 76.67 | 17.65 | 50.00 |
| 157 | 4.55 | 10.00 | 5.88 | 30.00 |
| 159 | 34.09 | 13.33 | 35.29 | 10.00 |
| 161 | 0.00 | 0.00 | 5.88* | 0.00 |
| 163 | 23.86 | 0.00† | 35.29 | 10.00 |
| Pa 55 | | | | |
| 151 | 70.45 | 33.33 | 55.88 | 60.00 |
| 153 | 3.41* | 0.00 | 0.00 | 0.00 |
| 155 | 26.14 | 50.00 | 32.35 | 40.00 |
| 159 | 0.00 | 6.67 | 5.88 | 0.00 |
| 161 | 0.00 | 0.00 | 5.88* | 0.00 |
| 163 | 0.00 | 6.67* | 0.00 | 0.00 |
| 169 | 0.00 | 3.33* | 0.00 | 0.00 |
| Me 17 | | | | |
| 135 | 4.55 | 0.00 | 2.94 | 0.00 |
| 137 | 61.36 | 50.00 | 88.24 | 40.00 |
| 139 | 23.86 | 16.67 | 0.00† | 40.00 |
| 141 | 0.00 | 3.33* | 0.00 | 0.00 |
| 147 | 0.00 | 0.00 | 0.00 | 10.00* |
| 149 | 0.00 | 13.33 | 2.94 | 0.00 |
| 151 | 9.09 | 0.00† | 5.88 | 10.00 |
| 157 | 0.00 | 16.67* | 0.00 | 0.00 |
| 159 | 1.14* | 0.00 | 0.00 | 0.00 |
| IL5 | | | | |
| 169 | 22.73 | 3.33 | 11.76 | 0.00† |
| 177 | 0.00 | 0.00 | 0.00 | 20.00* |
| 179 | 21.59 | 46.67 | 26.47 | 0.00† |
| 189 | 18.18 | 0.00† | 2.94 | 30.00 |
| 191 | 11.36 | 20.00 | 52.94 | 10.00 |
| 193 | 3.41 | 0.00 | 5.88 | 0.00 |
| 197 | 0.00 | 3.33 | 0.00 | 40.00 |
| 199 | 22.73 | 6.67 | 0.00 | 0.00 |
| 201 | 0.00 | 20.00* | 0.00 | 0.00 |
| Total no. of alleles | 34 | 36 | 36 | 29 |

*Allele present only in that population; †allele absent only in that population.

**Table 3** Estimates of genetic variation at seven microsatellite loci in four *Petrogale penicillata* colonies in the Wolgan Valley

| | Main colony | | | | Crocodile Rock | | | | Rocky Creek | | | | Little Tower | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | *AD* | *AR* | $H_E$ | $F_{IS}$ | *AD* | *AR* | $H_E$ | $F_{IS}$ | *AD* | *AR* | $H_E$ | $F_{IS}$ | *AD* | *AR* | $H_E$ | $F_{IS}$ |
| Pa 597 | 7 | 4.12 | 0.74 | +0.11 | 4 | 3.12 | 0.63 | −0.05 | 6 | 4.60 | 0.80 | −0.10 | 6 | 6 | 0.85 | +0.06 |
| Pa 593 | 5 | 3.60 | 0.69 | −0.21* | 8 | 5.36 | 0.84 | −0.12 | 6 | 4.30 | 0.77 | 0.00 | 4 | 4 | 0.68 | +0.41 |
| Pa 385 | 4 | 3.38 | 0.69 | −0.18 | 3 | 2.54 | 0.40 | +0.17 | 5 | 3.97 | 0.74 | +0.12 | 4 | 4 | 0.70 | −0.14 |
| Pa 297 | 4 | 2.89 | 0.61 | −0.23 | 5 | 3.78 | 0.64 | +0.07 | 6 | 4.84 | 0.80 | −0.17 | 5 | 5 | 0.85 | +0.06 |
| Pa 55 | 3 | 2.30 | 0.44 | +0.28 | 5 | 3.45 | 0.65 | −0.02 | 4 | 3.06 | 0.60 | −0.30 | 2 | 2 | 0.50 | −0.60 |
| Me 17 | 5 | 3.39 | 0.56 | +0.07 | 5 | 3.94 | 0.70 | +0.05* | 4 | 2.16 | 0.23 | +0.21 | 4 | 4 | 0.70 | −0.43 |
| IL5 | 6 | 4.65 | 0.81 | −0.06 | 6 | 4.10 | 0.71 | −0.21 | 5 | 3.62 | 0.65 | +0.19 | 4 | 4 | 0.75 | −0.33 |
| Average | 4.86 | 3.40 | 0.65 | −0.03 | 5.14 | 3.76 | 0.65 | −0.02 | 5.14 | 3.76 | 0.65 | −0.01 | 4.14 | 4.14 | 0.72 | −0.14 |

Allelic diversity (*AD*), allelic richness (*AR*), expected heterozygosity ($H_E$) and inbreeding coefficient ($F_{IS}$) are presented for each locus. Loci with a significant deviation from Hardy–Weinberg expectations are indicated with an asterisk (Bonferroni-corrected for seven tests, $P < 0.01$).

### *Estimation of dispersal patterns using assignment tests*

We identified one putative migrant among the four sampled colonies (from the Main colony to Rocky Creek) and three putative immigrants from unsampled colonies (Table 6). The putative immigrants comprised three males and one female. The putative immigrants from unsampled colonies were all excluded from the four sampled colonies with probabilities greater than 0.998. We considered it unlikely that we had underestimated the number of immigrants from sampled or unsampled colonies as no other individuals had *P* values of less than 0.04 for either of the assignment criteria. Assignments to the Little Tower colony were likely to be the least accurate because of the small sample size from this colony. However, none of the individuals sampled at Little Tower were identified as immigrants according to either criterion. It is possible that some individuals identified as putative immigrants from unsampled colonies were falsely excluded from Little Tower. However, all individuals that were excluded from their 'home' colony were also very confidently ($P < 0.001$) excluded from Little Tower, so we considered this unlikely. In any case, these individuals were all confidently excluded from their 'home' colonies, so the overall proportion of migrants would not change.

We found that the mean genetic distance among the three putative immigrants from unsampled colonies (mean GD = 13) was significantly greater than among three randomly sampled nonimmigrants from the Main colony (mean GD = 8.50, $P = 0.002$), Crocodile Rock (7.63, $P = 0.000$), Rocky Creek (7.49, $P = 0.000$) and Little Tower (8.85, $P = 0.000$). This suggests that the three immigrants from unsampled colonies originated in more than one source population.

| Source of variation | Variance | % total | $P^a$ | *F*-statistics |
|---|---|---|---|---|
| Among groups | 0.050 | 1.96 | < 0.0001 | 0.020 |
| Among colonies within groups | 0.244 | 9.44 | < 0.0001 | 0.096 |
| Within colonies | 2.299 | 88.60 | < 0.0001 | 0.114 |

[a]Probability of having a significantly higher variance component and *F*-statistic than the observed values by chance alone.

**Table 4** Hierarchical analysis of molecular variance (AMOVA) for four *Petrogale penicillata* colonies. The amount of variance was assessed among groups (i.e. pairs of colonies on either side of the river), among colonies within groups (i.e. within groups defined by the river) and within colonies

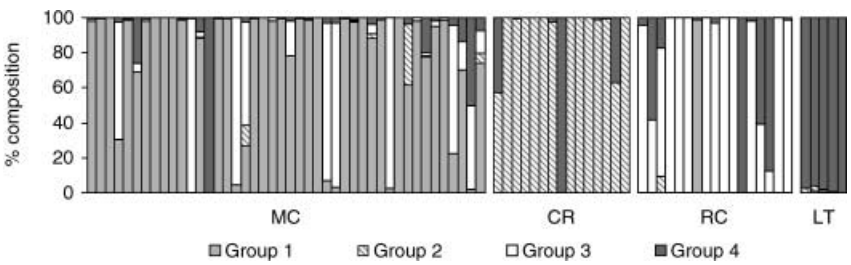


**Fig. 2** Proportion of each genetic group in each colony based on the results from STRUCTURE. Each column represents an individual brush-tailed rock-wallaby sampled in each of the colonies (MC, Main colony; RC, Rocky Creek; CR, Crocodile Rock; and LT, Little Tower) and the percentage composition refers to the probability of an individual belonging to each genetic group.

**Table 5** Autocorrelation coefficients (*r*) among multilocus microsatellite genotypes of individuals assigned with greater than 90% confidence to the four population subgroups identified by the STRUCTURE program

| | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| *r* | 0.189 | 0.329 | 0.403 | 0.132 |
| 97.5% CI | 0.022 | 0.059 | 0.064 | 0.153 |
| 2.5% CI | –0.014 | –0.039 | –0.043 | –0.116 |
| Prob *r* > permuted *r* | 0.001 | 0.001 | 0.001 | 0.042 |

The 95% confidence interval and probability values are for the test that the *r* values for each group are not significantly different to a random sample of individuals from among all those sampled in the Wolgan Valley.

**Table 6** *Petrogale penicillata* individuals assigned using the Bayesian exclusion method to colonies other than those in which they were detected

| Colony | Individual ID | Gender | Probable source population |
|---|---|---|---|
| Main colony | MC14 | M | Unknown |
| Crocodile Rock | CR08 | F | Unknown |
| Rocky Creek | RC07 | M | Main Colony |
| | RC12 | M | Unknown |

### *Inference of sex-specific dispersal patterns from multilocus genotypic correlations*

The multilocus autocorrelation coefficients among males and females within each colony are presented in Fig. 3. In the three larger colonies (Main colony, Rocky Creek and Crocodile Rock), there was significant positive autocorrelation among individuals of each sex sampled within the same colony (the *r* values for each category were outside the 95% confidence interval for a permuted random sample from the whole Wolgan Valley). A comparison of the bootstrap 95% confidence intervals around the *r* estimates for males and females revealed no significant differences in *r* values among males and females in each colony. For the smallest colony, Little Tower, the *r* values were not outside the random 95% confidence interval. Although we could not obtain a bootstrap confidence interval for the *r* estimate for males, since there was only a single pair of males at the colony, the *r* value for males was within the bootstrap 95% CI of the estimate of *r* for females. When the *r* values for males and females were combined over all sites, there was no significant difference between males and females (Fig. 3).

The spatial autocorrelation analyses among males and females sampled within the Main colony (Fig. 4) revealed significant positive spatial autocorrelation among females. The *r* value among females was significantly positive for the first distance interval (0–100 m: *r* > 98.1% of randomizations), significantly negative for the last interval (> 400 m: *r* < 99.3% of randomizations) and nonsignificant in between. We did not detect significant spatial genotypic structure among males, although the correlogram suggests that spatially proximal males are more genotypically similar than more distant pairs of males within the Main colony (*r* among males separated by 0–100 m was greater than 97.1% of random permuted values). There was no significant negative autocorrelation among males in the largest distance class (> 400 m, *r* < 29.2% of randomizations). In comparison to the Main colony, no significant spatial genotypic structure was detected among males or females in the Rocky Creek or Crocodile Rock colonies (Table 7).

One possible reason that males may not show positive correlation is that their home range may be larger than females and if the scale of the study is not large enough then it may not be possible to detect significant patterns of spatial genetic autocorrelation if they exist. We therefore compared the maximum distance between locations of scats collected from individuals for males and females in the Main colony. Although the average maximum distance between locations of scats was higher for males (373.82 m, SD = 482.47) than females (257.16 m, SD = 387.96 m), this was not significant (*T* test, *P* = 0.397).

### *The contribution of each colony to total genetic diversity among the study sites*

The contributions to total $H_E$ and *AD* in the Wolgan Valley population were higher for the three small colonies combined

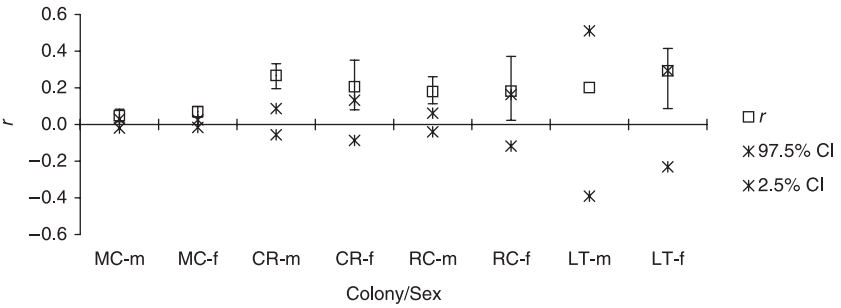


**Fig. 3** The genetic correlation coefficient (*r*) for each sex within each *Petrogale penicillata* colony. The 95% confidence interval and probability values are for the test that the *r* values for each sex are not significantly different to a random sample.

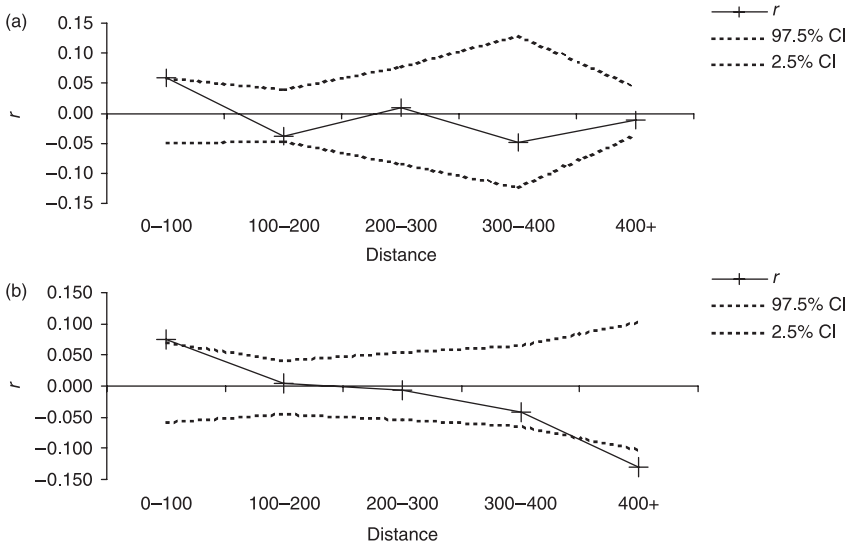


**Fig. 4** Spatial genetic structure autocorrelograms for the Main colony showing the genetic correlation coefficient (*r*) as a function of geographical distance (100 m distance classes) with 95% confidence interval showing a random spatial genetic structure for (a) male brush-tailed rock-wallabies and (b) female brush-tailed rock-wallabies.

**Table 7** Autocorrelation coefficients (*r*) among multilocus microsatellite genotypes of males and females for Rocky Creek and Crocodile Rock colonies as a function of geographical distance (100 and 200 m distance classes)

| | Crocodile Rock | | | | Rocky Creek | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | |
| Distance (metres) | 100 | 200 | 100 | 200 | 100 | 200 | 100 | 200 |
| *r* | 0.011 | –0.020 | –0.019 | 0.000 | 0.009 | –0.030 | –0.008 | –0.006 |
| 97.5% CI | 0.064 | 0.081 | 0.021 | 0.000 | 0.042 | 0.123 | 0.038 | 0.256 |
| 2.5% CI | –0.040 | –0.102 | –0.026 | 0.000 | –0.036 | –0.129 | –0.036 | –0.236 |
| Prob *r* > permuted *r* | 0.292 | 0.707 | 0.834 | 1.000 | 0.305 | 0.697 | 0.686 | 0.681 |

The 95% confidence interval and probability values are for the test that the *r* values for each sex at each distance class are not significantly different to a random sample.

**Table 8** Contribution to total heterozygosity and allelic diversity for the Main *Petrogale penicillata* colony and the three smaller colonies combined (SC), and for each *Petrogale penicillata* colony (MC, Main colony; RC, Rocky Creek; CR, Crocodile Rock; and LT, Little Tower) separately, subdivided into diversity and differentiation components

| | | | | Colony | | | |
|---|---|---|---|---|---|---|---|
| Genetic diversity measures | Component | MC | SC | MC | RC | CR | LT |
| Heterozygosity | Total (%) | 0.31 | 11.17 | –2.69 | –0.08 | 0.23 | 2.54 |
| | Diversity (%) | –5.41 | 5.41 | –1.02 | –0.56 | –0.80 | 2.39 |
| | Differentiation (%) | 5.73 | 5.73 | –1.67 | 0.48 | 1.04 | 0.15 |
| Allelic diversity | Total (%) | 5.55 | 50.00 | 5.55 | 7.41 | 18.52 | 9.26 |
| | Diversity (%) | –22.22 | 22.22 | 0.15 | 1.39 | 1.39 | –2.93 |
| | Differentiation (%) | 27.78 | 27.78 | 5.40 | 6.02 | 17.13 | 12.19 |

than for the Main colony alone (Table 8). The diversity of the Main colony compared to the small colonies combined was lower than the mean for both $H_E$ and *AD* and thus its net contribution to diversity is negative. The three small colonies all contain alleles not found in the Main colony and this reflects that these alleles would be lost from the valley population if these three colonies became extinct. Generally, $H_E$ was less affected by the loss of a single colony than was *AD* (Table 8). All colonies contributed to the total *AD* due to the fact that each colony was differentiated from one another, i.e. unique alleles would be lost if any colony went extinct (Table 8).

## Discussion

### *Genetic population structure of the Wolgan Valley rock-wallaby colonies*

We detected significant genetic differentiation between the four *Petrogale penicillata* colonies in the Wolgan Valley, indicating that they do not form a panmictic breeding unit. This is consistent with the low proportion of dispersers detected among the sampled individuals in the four colonies. The Wolgan River valley, which divides the four colonies into two groups, contributes a small, but significant, proportion of the overall genetic differentiation. It is possible that the river itself poses a barrier to dispersal or that wallabies prefer not to disperse across large areas that do not provide rock shelters (i.e. the river valley). The high level of genetic differentiation among *P. penicillata* colonies in this study is consistent with the results of studies of other rock-wallaby species (e.g. *Petrogale lateralis*: Eldridge *et al*. 2001 and *Petrogale xanthopus*: Pope *et al*. 1996). High levels of genetic structuring are also seen in other species that occur in naturally fragmented habitat, particularly in rocky areas (e.g. Kim *et al*. 1998; Stow *et al*. 2001; Berry *et al*. 2004).

### *Population structure identified by clustering of individual genotypes*

STRUCTURE clustered individual *P. penicillata* into four discrete genetic groups, each of which was largely restricted to a single colony. This supports the AMOVA results, in suggesting that the colonies are genetically discrete populations. This conclusion is further supported by the high genetic similarity (significantly greater than random) among individuals in each colony. Some individuals were clearly identified as immigrants from unsampled colonies by the assignment tests. In the STRUCTURE analysis, these were clustered with most of the individuals in Little Tower (in Group 4), but since the genetic similarity among individuals in Group 4 was lower than in any of the other groups, we considered it unlikely that these individuals were emigrants from Little Tower. Rather they may have been clustered with the Little Tower individuals because STRUCTURE assumes all colonies have been sampled, and the Little Tower individuals may have been the most genetically similar to these immigrants.

### *Dispersal between colonies*

Four individuals (three males and one female) were detected as probable dispersers into the Main, Rocky Creek and Crocodile Rock colonies. It is difficult to determine accurately the number of immigrants into Little Tower due to the small sample size of this colony. Therefore, not including individuals sampled at Little Tower, approximately 5% of individuals identified in this study are probable dispersers. The dispersal rate detected in this study is higher than that detected between colonies in other studies of rock-wallabies. Eldridge *et al*. (2001) used assignment tests to conclude that a small black-footed (black-flanked) rock-wallaby (*P. lateralis*) colony ($n = 6$) was descended from a limited number of dispersers from another colony 8 km away and was most likely founded by a single female with a pouched young and quiescent embryo. Sharp (1997) observed a juvenile male yellow-footed rock-wallaby (*P. xanthopus*) dispersing 600 m. Therefore, the dispersal rate for these studies was 0.5–3.3% and 0.8%, respectively.

Three of the four probable dispersers identified by GENECLASS were excluded from all four colonies and are possibly immigrants from unknown populations. Only one individual was detected as a probable disperser between two of the sampled colonies, which were located on the same side of the river (Main colony to Rocky Creek). This is consistent with AMOVA results that suggest the Wolgan River valley may act as a barrier to dispersal. Locating and sampling the unknown source populations would allow a more thorough analysis of whether dispersing individuals are crossing the river valley. A possible explanation for why few immigration events were detected between the four sampled colonies is that assignment tests may not have the power to identify dispersers between these colonies. In particular, sample sizes were small for three of the colonies and only seven microsatellite loci were used. Increasing the number of loci and samples is likely to improve the accuracy of assignment tests used in this study (Cornuet *et al*. 1999; Bernatchez & Duchesne 2000).

If the low level of dispersal detected between the four studied colonies is real, it may have resulted from high dispersal-related mortality (for example through predation). Feral predators including foxes are thought to use roads for movement and foraging, particularly into forest environments previously inaccessible to them (May & Norton 1996). Foxes may be able to easily access the sampled colonies via a road used by National Parks staff for population monitoring. However, this road terminates near the three larger colonies, so it is possible that the lack of access that prevents the discovery of further colonies may be the very factor that protects them and their dispersing individuals from fox predation.

*Petrogale* are generally considered to be relatively sedentary, displaying infrequent dispersal between patches (Horsup 1994; Pope *et al*. 1996; Delaney 1997; Jarman & Bayne 1997; Sharp 1997; Spencer *et al*. 1998). Most studies have relied on trapping and radiotelemetry to detect dispersal (which can be problematic, particularly if juveniles are difficult to trap; Sharp 1997), and this may be responsible for the low numbers of dispersers detected (Eldridge *et al*. 2001). The high levels of genetic diversity typically displayed by rock-wallaby colonies would tend

to suggest intercolony dispersal must occur more frequently than observed (Spencer *et al*. 1997; Eldridge *et al*. 2001). Indeed no individual was 'observed' to have dispersed among these four colonies based on the locations of the faeces collected from each individual during the mark–recapture sampling period for this study, yet genetic analysis of the same colonies lead to the identification of four dispersal events.

### *Sex-specific dispersal patterns*

Although both male and female dispersal was detected between colonies and there was no significant difference between male and female genotypic similarity in any colony, the number of dispersers is too low to assess intercolony sex-biased dispersal. However, there was some evidence of sex-biased dispersal within the largest colony in the valley. As reported by Hazlitt *et al*. (2004), spatial autocorrelation of multilocus genotypes provided evidence of female philopatry within the largest colony in the valley. There was no significant positive genotypic spatial autocorrelation among males, although males in close proximity were generally more genetically similar than those separated by larger distances. Spatial autocorrelation was not detected among the genotypes of females in the Crocodile Rock or Rocky Creek colonies, which probably reflects the smaller size of these colonies in relation to the Main colony. It is likely that dispersal within colonies is slightly male biased in brush-tailed rock-wallabies. Male-biased dispersal within colonies has also been observed in yellow-footed rock-wallabies (Sharp 1997). As discussed in Hazlitt *et al*. (2004), as rock-wallabies appear to display infrequent between colony dispersal, within-colony dispersal by males and female philopatry may assist in inbreeding avoidance.

### *Conservation of genetic diversity in the Wolgan Valley*

All of the four sampled colonies were found to contain distinct components of the genetic diversity of the Wolgan Valley *P. penicillata* population. Managing and protecting only the largest colony in the valley (Main colony) may not be sufficient to conserve the genetic diversity of the Wolgan Valley population. The loss of any colony (including unsampled ones) is likely to result in the loss of unique alleles.

The dispersal results suggest there may be unsampled colonies in the Wolgan Valley. These colonies are also likely to contain unique components of the genetic diversity of the Wolgan Valley population. Locating and protecting the unknown source colonies may also be an important strategy for conserving genetic diversity in this valley. Some of the critically endangered populations of *P. penicillata* are isolated and have lost a large proportion of their genetic diversity (Browning *et al*. 2001; Eldridge *et al*. 2004). This study suggests that maintaining connectivity between local networks of protected colonies is important for conserving the genetic diversity of *P. penicillata* populations.

### *Potential effects of genotyping errors on population structure analyses*

The potential for genotyping errors in noninvasive sampling studies may result in false interpretations of data (Taberlet *et al*. 1999; Piggott & Taylor 2003b), and this possibility should be considered in the current context. Undetected high levels of allelic dropout resulting in erroneously low observed heterozygosity levels will lead to false interpretations with regard to inbreeding and population structure (Taberlet *et al*. 1999). In particular, low observed heterozygosity can generate false Wahlund effects leading to an erroneous conclusion that substructure exists. Applications requiring assessment of relatedness and kinship, and assigning individuals to populations as in this study, may also be severely affected (Taberlet *et al*. 1999). False individuals would likely appear highly related to another sampled individual, as their genotypes would differ at only a small number of alleles. Such an outcome would have implications for inferences regarding both relatedness structure and the occurrence of inbreeding. Genotyping errors may lead to the assignment of individuals to the wrong population of origin resulting in erroneous estimates of migration and sex-biased dispersal (Taberlet *et al*. 1999).

Methods in this study were thoroughly optimized to ensure a low risk of false individuals (Piggott & Taylor 2003a; Piggott 2004; Piggott *et al*. 2005). The error rate for single polymerase chain reactions (PCRs) in the initial pilot study was relatively low (4.26%) and the replication strategy employed was stringent (Piggott *et al*. 2005). We also carried out a retrospective error-checking analysis using a pairwise comparison of the percentage of matching alleles. False individuals would probably appear highly related to another sampled individual, as their genotypes would differ at only a few alleles. There was no evidence for a higher proportion of similar genotypes among those sampled in this study compared to those sampled by trapping (Eldridge *et al*. 2004). A lower proportion of pairs in this study differed at one locus in their genotype compared to four other *P. penicillata* populations genotyped using biopsy samples (Piggott *et al*. 2005). Further, the pattern of spatial genetic autocorrelation in this study (using genotypes obtained from faeces) was the same as in a study by Hazlitt *et al*. (2004), which relied on genotypes and home range data from trapped animals.

### *Conclusions and implications for management*

This study has shown that analyses of genetic data obtained from noninvasive genotyping can provide vital information on population structure and dispersal patterns, which can

assist in the conservation and management of an endangered species. As concluded for yellow-footed rock-wallabies *Petrogale xanthopus* (Pope *et al*. 1996), each of the *P. penicillata* colonies in the Wolgan Valley should be regarded as an independent management unit. This is not to say that the colonies represent totally independent populations, but that management plans should recognize that the colonies represent genetically differentiated discrete subpopulations. A specific management issue for the four colonies studied here relates to the appropriate scale of predator control programs. The introduced red fox (*Vulpes vulpes*) is considered the major threat to the survival of most populations of the species (Wakefield 1961; Dovey *et al*. 1997; Kinnear *et al*. 1998; Eldridge *et al*. 2001), so active fox baiting is carried out in the Wolgan Valley. Until recently, only the largest colony (Main colony) has been protected, with the perimeter being regularly fox-baited since 1998. Recently, a fox control programme to target all colonies in the Wolgan Valley has been implemented. Our findings support that decision, in that if one of the smaller colonies became extinct due to fox predation, it may not be rapidly recolonized by regular dispersal from the Main colony. Further, the conservation of each of the colonies is a more appropriate strategy for maintaining genetic diversity compared to protecting only the largest colony in the Wolgan Valley.

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This study forms part of Maxine Piggott's PhD thesis on development and application of non-invasive genotyping for the conservation of Australian mammals. Sam Banks is interested in applying molecular techniques to conservation issues, including non-invasive sampling, impacts of habitat fragmentation on wildlife populations, and presently, dispersal patterns in marine invertebrates. Andrea Taylor applies genetic marker technology to a diverse range of molecular ecology and conservation issues, primarily in marsupials.

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