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Patterns of differentiation among wild rabbit populations *Oryctolagus cuniculus* L. in arid and semiarid ecosystems of north-eastern Australia

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Abstract

Feral rabbit populations in Australia have generally been managed using localized control procedures. While these procedures may result in local extinctions, persistence of populations will depend on the probability of recolonization. Genetic markers developed using temperature gradient gel electrophoresis (TGGE) combined with heteroduplex analysis (HA) of mitochondrial DNA (mtDNA) were used to characterize the degree of subdivision and extent of gene flow within and among rabbit populations distributed over large distances (up to 1000 km) in southern Queensland (QLD) and north-west New South Wales (NSW), Australia. TGGE analyses revealed significant heterogeneity in mtDNA control region haplotype frequencies. From heterogeneity χ^2 tests, it was evident that the differentiation observed was largely attributable to five sites which were located in the semiarid eastern region, whereas haplotype frequencies were homogeneous throughout the arid western region. These results suggest that there are independent population systems within the study area. The extent of gene flow among local populations within each system is related to the spatial configuration of acceptable habitat patches and the persistence of the populations is determined by the probability of recolonization following local extinction. These data suggest that to provide better overall control of rabbit populations, different management strategies may be necessary in arid and semiarid ecosystems. In arid south-west QLD and north-west NSW, where extensive gene flow occurs over large distances, rabbit populations should be managed at a regional level. In semiarid eastern QLD, where gene flow is restricted and populations are more isolated, localized control procedures may provide effective short-term relief. These results indicate that in nonequilibrium systems with patchy distribution of individuals, the interpretation of migration rate from estimates of gene flow obtained using existing genetic models must include an understanding of the spatial and temporal scales over which population processes operate.

Keywords: genetic differentiation, mtDNA, control region, temperature gradient gel electrophoresis, heteroduplex analysis, local extinction, recolonization

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Introduction

The release of the wild rabbit *Oryctolagus cuniculus* L. in Australia has had considerable environmental and economic impact, particularly in arid ecosystems. In arid and semiarid regions rabbit control is usually based on the management of individual warrens (baiting or strategic destruction of warrens). These control procedures are often ineffective as several studies have found that depopulated warrens are rapidly recolonized by adjacent rabbit populations (Rowley 1968; Parer & Parker 1987). Biological control via myxomatosis is often ineffective because of resistance and, in the arid zone, because of a lack of reliable vectors (mosquito *Culex annulirostris*) for transmission (Rural Lands Protection Board 1987). The result is localized control around the inoculation site

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(warren). Consequently, for all current control methods, effective management will depend on the integrity of the warren as a local population unit, the degree of isolation of each unit and the rate of recolonization of depopulated sites. Genetic markers can provide a quick estimate of the extent of genetic exchange and level of interaction between populations and therefore, the geographical scale at which population differentiation will become evident.

In temperate south-eastern Australia (New South Wales), Daly (1979) investigated the effect of social organization on the genetic structure of feral rabbit populations. Daly found that although rabbit populations exhibited social subdivision within a warren, this did not lead to genetic microdifferentiation and that a deme consisted of several warrens. At a slightly larger scale in New South Wales, Richardson (1980) investigated genetic differentiation between groups of warrens located over distances as little as 1 km and found significant heterogeneity in allele frequencies. Localized control may be appropriate therefore in certain areas of south-eastern Australia. Conversely in arid western Queensland (Australia), Fuller *et al.* (1996) [using allozyme and mitochondrial DNA (mtDNA) markers] demonstrated little genetic differentiation and high levels of gene flow among populations spread over 1600 km^2. These data suggested that in arid western Queensland, rabbit population structure may occur at a different level of scale than that identified in previous research. It is possible that within eastern Australia, the level of structuring exhibited by rabbit populations may be dependent on the type of ecosystem in which they are located. The present study was therefore designed to investigate the level of interaction among rabbit populations in the abutting semiarid and arid ecosystems of eastern Australia, to determine whether multiple population systems exist.

Mitochondrial DNA (mtDNA) has been recognized as a sensitive indicator of population subdivision because it evolves at a relatively fast rate and it has a haploid, matrilineal mode of inheritance (Wilson *et al.* 1985). In the present study, genetic markers were developed using temperature gradient gel electrophoresis (TGGE) and heteroduplex analysis (HA) of a mtDNA control region fragment. TGGE and HA are sensitive techniques capable of detecting allelic variation in DNA sequences (see Lessa & Applebaum 1993) and TGGE can theoretically detect single base changes in genomic DNA (Riesner *et al.* 1989; Wartell *et al.* 1990). The fraction of base mismatches resolved can be improved by using heteroduplex formation in combination with TGGE (Myers *et al.* 1985;Myers & Maniatis 1986). Recently, HA has been applied to haploid loci (mtDNA, Campbell *et al.* 1995).

This study examined the extent of interaction among rabbit populations in semiarid and arid eastern Australia. The specific objectives were to:

1 examine mtDNA differentiation among rabbit populations that inhabit two different ecosystems, using genetic markers developed from TGGE/HA;
2 document the effects of isolation between populations, both in terms of geographical distance and geographical or habitat barriers to gene flow;
3 determine the optimum level of scale for control based on the level of interaction among populations.

Materials and methods

Sample collection

A minimum of 20 adult individuals was collected from 13 sites located over large geographical distances throughout south-western Queensland and north-western New South Wales. In the original design (Fig. 1), six sites were positioned at 25, 250 and 500 km intervals from a central site (Bulloo Downs), and in three directions (north-east, north-west and south-west). Later, six additional sites were included in eastern Queensland. From each individual sampled, a small section of liver was dissected and stored in cryoware vials (Nalgene Co.) under liquid nitrogen. On return to the laboratory, samples were immediately transferred to a – 70 °C freezer.

mtDNA control region analyses

Total genomic DNA was extracted from ≈ 100 mg of liver tissue by grinding to a fine powder in liquid nitrogen and then incubating at 55 °C for 3 h in an extraction buffer [100 mM Tris (tris (hydroxymethyl) aminomethane), 20 mM EDTA (ethylenediamine-tetra-acetic-acid), 100 mM NaCl, 10% SDS (sodium dodecyl sulphate), 2 M DTT (dithiothreitol), 10 mg/mL proteinase K]. The isolation procedure consisted of a phenol extraction followed by a series of phenol/chloroform (1 : 1) extractions, ending in a chloroform extraction. All centrifugation was performed at 12 000 *g* DNA was precipitated in 3 M sodium acetate (pH 5.2) and 100% ethanol at – 70 °C, and then redissolved in 50 µL of TE Buffer (Tris, EDTA, pH 7.5).

A 523 base-pair (bp) region of DNA flanking the tRNA-proline gene in the mtDNA control region was amplified using polymerase chain reaction (PCR; Saiki *et al.* 1988). This portion was amplified using a 22-bp primer (MT15996 L) created by M. S. Elphinstone (Southern Cross University) of sequence 5'CTCCACCATCAGCACCCAAAGC3' and a 20-bp internal primer (MT16498H) of sequence 5'CCTGAAGTAGGAACCAGATG3' created by Meyer *et al.* (1990), located in the central conserved domain of the mammalian control region. The equivalent fragment in other mammalian species has been found to be hypervariable (Saccone *et al.* 1991).

Individual PCR reactions contained final concentra-

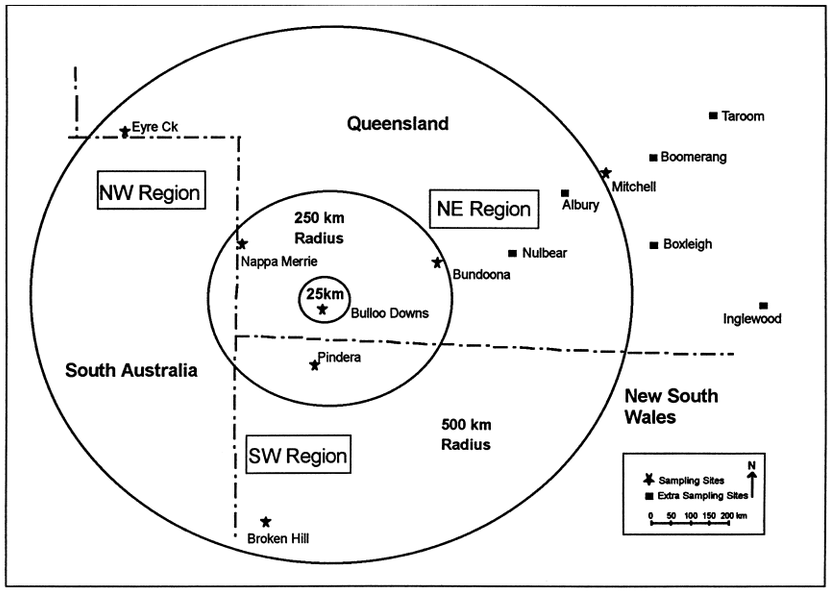

Fig. 1 Location of study sites in southern Queensland and north-west New South Wales.

tions of 100 μM deoxynucleoside triphosphate (Promega), *Taq* 10 × buffer (Boehringer-Mannheim) (containing a final $MgCl_2$ concentration of 1.5 mM), 120 nM of primer 1, 120 nM of primer 2 and 0.5 U of *Taq* polymerase (Boehringer-Mannheim) and 100 ng of template DNA. Temperature cycling was carried out in a programmable 'Minicycler' thermal controller (MJ-Research Inc.) with the following cycle programme: (i) 94 °C for 30 s, (ii) 50 °C for 10 s, (iii) 72 °C for 1 min, (iv) 94 °C for 10 s, (v) 50 °C for 30 s, (vi) cycle to step 3, 34 more times and 72 °C for 5 min.

A horizontal TGGE-system was used for heteroduplex analysis of rabbit DNA samples (TGGE Handbook 1993; DIAGEN GmbH, QIAGEN Inc.). Each PCR product (≈10 ng) was heteroduplexed with a single reference rabbit PCR product. Optimum temperature gradient conditions were determined by electrophoresis (300 V, 20–30 mA, 1.5 h) of a double-stranded product through a 5% polyacrylamide gel, over a perpendicular gradient of temperature from 20 to 60 °C. Subsequent electrophoretic runs were of 4 h duration, using a parallel temperature gradient of 11–46 °C. Internal standards (known sequence variants of differential mobility) were included on every gel. DNA was visualized using silver staining (TGGE Handbook 1993).

Approximately 100 ng of purified DNA fragment (QIAquick PCR Purification Preps, QIAGEN Inc.) and 3.2 pmol of primer were sequenced using ABI (Applied Biosystems) automated DNA sequencing. Each DNA fragment was sequenced (in the majority of cases, twice) from both the 3′ and 5′ ends. Sequences were aligned by eye using a sequence editor program (ESEE, Version 1.09D). Replicate sequencing (for each haplotype, $n = 5$) was performed, to confirm that individuals of identical haplotype possessed the same nucleotide sequence. The evolutionary distance between haplotypes was calculated using the Jukes & Cantor (1969) correction in the DNADIST program of PHYLIP 3.5c (Felsenstein 1993).

χ^2 contingency tests were used to determine whether haplotype frequencies varied significantly among sites. Simple cluster analysis techniques (k-means clustering with all haplotypes equally weighted) were applied to identify potential groupings of populations with similar frequencies (Statistica for Windows Version 5.0). The presence of population subdivision was investigated using an analysis of molecular variance (AMOVA) approach as outlined by Excoffier *et al.* (1992). Hierarchical population structure was tested for by analysing genetic variance components; within populations, among populations within groups (identified by cluster analysis) and between groups. The significance of the variance components were estimated using *F*-statistic analogues, designated as Ø-statistics and permutation (1000 random iterations) procedures. $Ø_{st}$ estimates were used to calculate levels of gene flow among populations using the equation, $Nm = 0.5\,[1/Ø_{st} - 1]$, where N is the average deme size and m is the average migration rate among demes in an island model of gene flow (Hudson *et al.* 1992).

Results

Haplotype frequencies at the 13 sites are presented in Fig. 2 and are variable throughout the region. Haplotype A was present in all populations, while haplotype C was found in all populations but one (Mitchell). Haplotype B was present in all sites west of Albury and absent in all sites to the east. Haplotype D was found in only three

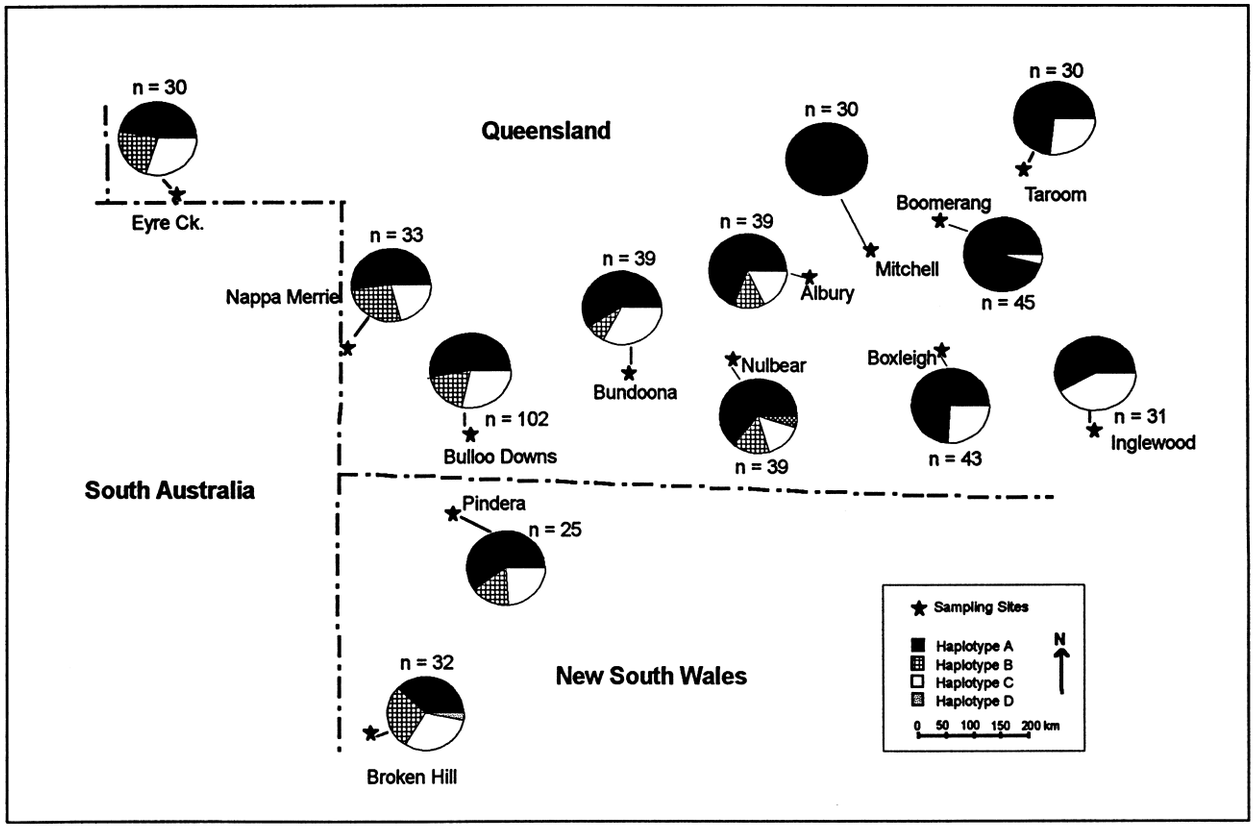

Fig. 2 MtDNA haplotype frequencies at 13 sites located throughout southern Queensland and north-western New South Wales.

individuals from two populations separated by ≈ 700 km. Figure 2 highlights a marked disparity in haplotype frequencies east and west of Albury, although this pattern only reflects the dispersion of females.

Over all sampling locations, haplotype distribution was dependent on site ($\chi^2 = 90$, d.f. = 24, $P < 0.001$). Within a 250-km radius from Bulloo Downs (see Fig. 1 for details) there were no significant differences observed in haplotype frequencies ($\chi^2 = 5.5$, d.f. = 6, $P = 0.482$), while within a 500-km radius the Mitchell sample caused a significant disruption to the homogeneity of haplotype frequencies ($\chi^2 = 23.6$, d.f. = 6, $P = 0.010$). To examine heterogeneity further, frequencies were compared by excluding sites that were deficient in haplotype B and, progressively, those with increasing frequency of haplotype C, until homogeneity of frequencies ($P > 0.10$) was achieved (Table 1). These analyses indicated that the five most easterly sites had a significant impact on the homogeneity of frequencies ($P < 0.10$, Table 1).

DNA sequence data for the four haplotypes are provided in Table 2 and have been submitted to GenBank under the following accession numbers (U62924, U62925, U62926 and U62927). Sequence divergence between haplotypes was found to range between 0.8 and 2.2% (Table 3).

Haplotype frequencies were used to calculate intra and interpopulation divergence. Based on an equilibrium model, overall population subdivision ($Ø_{st}$) was estimated to be 0.08, indicating that there was little (8%) genetic differentiation among subpopulations. This $Ø_{st}$ equates to a gene flow (Nm) estimate of approximately six migrants per generation. A matrix of pairwise $Ø_{st}$ estimates and associated probabilities that the random distance based on 1000 iterations is greater than the observed distance, are given in Table 4. It was demonstrated in 40% of the pairwise comparisons that the observed $Ø_{st}$ estimate was greater than that expected by chance ($P < 0.05$). Therefore, effectively 60% of the pairwise comparisons demonstrated a lack of genetic subdivision between sites. On closer examination it was obvious that in the majority of pairwise comparisons between western sites, distance estimates were approximately zero, indicating genetic homogeneity. This was further supported by there being no association between interpopulation genetic distance and geographical distance (Mantel test, $g = 1.26$, $P > 0.05$), indicating no isolation-by-distance effect.

Simple cluster analysis (k-means clustering with all haplotypes equally weighted) distinguished two tight clusters of sites with small distances between cluster

members (mean distance of members from respective cluster centre: cluster 1, 0.33; cluster 2, 0.33) and a large euclidean distance (0.974) between clusters. Cluster 1 was comprised of the western sites (Broken Hill, Eyre Ck., Nappa Merrie, Pindera, Bulloo, Nulbear, Albury and Bundoona), while cluster 2 was composed of the eastern sites (Mitchell, Boxleigh, Boomerang, Taroom and Inglewood). On the basis of these clusters, an hierarchical population subdivision analysis was performed, resulting in a divergence estimate among populations (within the total) of $\emptyset_{st} = 0.119$ ($Nm = 3.7$). As predicted from the previous analyses, divergence between the two clusters (relative to the total) was higher ($\emptyset_{ct} = 0.086$, $Nm = 5.3$) than the divergence among populations within each cluster ($\emptyset_{sc} = 0.036$, $Nm = 13$). The significance of each of these variance components was calculated by estimating the probability of obtaining more extreme random values from 1000 permutation tests. All variance components were significantly different from that found for a random distribution of individuals; $\emptyset_{st}$ ($P < 0.001$), $\emptyset_{sc}$ ($P = 0.001$) and $\emptyset_{ct}$ ($P = 0.001$). In total, these results suggest a clear division between the eastern and western sites. This dichotomy does not appear to coincide with an obvious geographical barrier, but more loosely conforms to a shift from an arid to a semiarid ecosystem.

Discussion

Effective rabbit control can only be achieved if an appropriate geographical scale for management is identified. Connectivity between populations and the boundaries of demographically independent local populations (or management units, Moritz 1994), need to be defined. In temperate ecosystems, the appropriate geographical scale for management of rabbit populations may be at the level of the deme (groups of warrens found within a localized area, generally consisting of 50–400 individuals, Richardson 1981). However, results from Fuller *et al.* (1996) have revealed that a regional perspective may be necessary to recognize potential boundaries between management units in the arid ecosystems of eastern Australia. In the present study, the examination of Control Region haplotype and sequence data from sites located over vast regions of arid and semiarid Australia, has allowed the investigation of both phylogeographic population structure (Avise *et al.* 1987) and frequency-based population structure.

Avise *et al.* (1987) proposed that populations can be categorized according to their intraspecific phylogeographic structure on the basis of phylogenetic relatedness and geographical structuring of mtDNA haplotypes. In the current study, haplotypes were phylogenetically quite divergent (average nucleotide divergence = 1.58%) and geographically widespread. The absence of haplotypes unique to particular sites indicates a lack of phylogenetic population structure (Slatkin & Maddison 1989) and is probably an effect of the original broad colonizing spread of the rabbit and the lack of sufficient time for phylogeographic divergence since introduction to Australia ≈ 200 years ago.

Results from the present study indicate that where the three major haplotypes were found in similar frequencies (western sites), there was a lack of genetic subdivision, indicating high gene flow among sites. Throughout the eastern region, however, genetic variability was reduced and there was substantial differentiation among sites. There was no obvious geographical discontinuity between the western and eastern regions which could impede gene flow. It seems unlikely that this division represents part of an overall west to east cline, as haplotype frequencies (including haplotype B which is absent in the east) were homogeneous over very large geographical distances

Table 1 χ^2 and probability values for the comparison of haplotype frequencies among sites, following a progressive exclusion of those sites deficient in haplotype B and with increasing frequency of haplotype C

Comparison		Freq. C	χ^2 value	d.f.	P -value
12 sites	- Mitchell	0.000	69.65	22	< 0.001
11 sites	- Mitchell				
	- Boomerang	0.044	43.07	20	0.002
10 sites	- Mitchell				
	- Boomerang				
	- Boxleigh	0.256	32.38	18	0.020
9 sites	- Mitchell				
	- Boomerang				
	- Boxleigh				
	- Taroom	0.267	24.84	16	0.073
8 sites	- Mitchell				
	- Boomerang				
	- Boxleigh				
	- Taroom				
	- Inglewood	0.419	15.99	14	0.313

Table 2 DNA sequence alignment of 518 bp of the mtDNA Control Region fragment, for the four haplotypes (A, B, C and D) found in the wild rabbit.

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HAP A  MT15996L TGATATTCTACTTAAACTACCCTCTGCTCTTTTACTTTAATAAAACTCAAG
HAP B           N.................................................
HAP C           N.................................................
HAP D           N.................................................

HAP A  TACTTCATCAGTACTGACAAATTACTAACACACTATGTAATTCGTGCATTAATGCTCGTC
HAP B  ..........................................................C.
HAP C  ..........................................................
HAP D  ..........................................................

HAP A  CCCATTAAAATGTATTACAACAATAAATTCATAACCAACATTTAACATATTATGTTTAAT
HAP B  ..................................................C.........
HAP C  ..................................................C.........
HAP D  ..................................................C.....C.....

HAP A  CGTGCATAAATTCCTCATCCCCATGAATAATAAGCTAGTACATTACTGCTTGATTGGACA
HAP B  ..........................................................
HAP C  ...A.....G................................................
HAP D  ...A......................................................

HAP A  TAATCCACCTAATACATCACACATAATCCAACAAAAAATTGACCCAAACATGAATATTCT
HAP B  ........T.................................................
HAP C  ......................C...T.....C.....C...................
HAP D  ........T.................T.....C.....C...................

HAP A  CACCAAAAATCTAATGATTGACTTGACATTAGACATCAATTCCATAATTAAACATAGACC
HAP B  ..........................C.......................C.......
HAP C  ..........................................................
HAP D  ..........................G.....T.......................

HAP A  ATCAAATCTACACACACCACTCAACTCTTACCCATACGACTATCCCTCTCCCCCAGTCCT
HAP B  ..........................................................
HAP C  ........................C.................................
HAP D  ........................C.................................

HAP A  CTCACAACTTACCATCCTCCGTGAAACCAACAACCCGCCCACCAAGGATCCCTCTTCTCG
HAP B  ..........................................................
HAP C  ..........................................................
HAP D  ..........................................................

HAP A  CTCCGGGCCCATAAAACTTGGGGGTTTCTAATATGAAACTATAACTG MT16498H
HAP B  ..................................................
HAP C  ..................................NNNN....
HAP D  ..................................NN....
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Table 3 Matrix of pairwise percentage nucleotide divergence estimates (below diagonal) and absolute distance estimates (above diagonal) for the four mtDNA haplotypes found in the wild rabbit

Haplotype	A	B	C	D
A	–	4	7	9
B	0.78%	–	11	11
C	1.38%	2.18%	–	6
D	1.77%	2.17%	1.18%	–

throughout the arid western region of this study, and therefore do not provide support for a clinal hypothesis.

If male–female migration rates are equal, Birky *et al.* (1983) proposed for organellar genes that population differentiation would be prevented when there is an effective migration rate of at least four individuals per generation. In this study, gene flow calculated from $Ø_{st}$ resulted in an *Nm* of six, however, this estimate assumes that rabbit populations are at migration-drift equilibrium and are structured according to Wright's (1931) infinite-island model, where migrants are drawn at random from a common pool. While it is possible to obtain low population subdivision estimates and consequently, high levels of gene flow as a result of recent common ancestry and insufficient variation, if differentiation (in the form of significant frequency differences) is observed, then structuring is present within the population.

The genetic data obtained in the present study, has led to the identification of two different rabbit population systems within the study area. One system encompasses the western arid sites and the second includes the eastern semiarid sites. The current rabbit distribution is a function of the ecological and behavioural flexibility of rabbits to the different environments in the arid (western sites) and semiarid (eastern sites) ecosystems. The type of population system which develops will ultimately be determined by those habitat attributes that influence the local distribution of rabbit populations. Factors such as the degree of isolation between favourable habitat patches and the probability of recolonization following local extinction need to be considered when characterizing gene flow.

The spatial and temporal heterogeneity of the environment has an important influence on rabbit populations, for example different habitats may become favourable according to variation in the weather. A mosaic of different habitats will buffer the population against irregular changes in weather patterns (Daly 1979). Daly (1979) reported that depending on local habitat heterogeneity, gene flow between social groups may increase in two ways; (i) a subpopulation can expand in size and area if the habitat becomes more suitable after a change in rainfall patterns and (ii) subadults can disperse successfully into recently occupied areas after a population crash.

The favourability of a patch of habitat to rabbits will depend primarily on temperature, presence of green vegetation and soil type. Environmental heterogeneity (both spatial and temporal) will dictate the probability of interaction (migration and recolonization among warrens) and consequently, gene flow among populations. The arid western region, although located in one of the driest areas of Australia, is capable of sustaining high density local populations (Myers & Parker 1965). This can be attributed to the predominance of green vegetation during the cooler months (high temperatures inhibit reproduction, Cooke 1977) and the existence of broad and continuous areas of favourable burrowing substrate, interspersed with drainage areas that provide food (communal feeding grounds). The semiarid eastern region is more heavily vegetated than in the west, however, pasture growth coincides with summer rainfall when temperatures are high and rabbit breeding success is limited. This region is also comprised of predominantly unfavourable soils, with a few small and isolated patches of substrate suitable for burrowing (Parer 1987).

Rabbit populations in both the arid and semiarid ecosystems of eastern Australia are susceptible to considerable temporal variation. The rabbit is not well adapted physiologically to these harsh environments and therefore populations fluctuate in size over time. In particular, the

Table 4 Matrix of $Ø_{st}$ distances between pairs of populations and associated probability that the random distance based on 1000 iterations is greater than the observed distance for the 13 sites.

Site	Boo.	Box.	Alb.	Nul.	Mit.	Tar.	Ing.	Nap.	Pin.	Bun.	Eyr.	Bul.	Bro.
Boomerang	–												
Boxleigh	0.14**	–											
Albury	0.14**	0.00	–										
Nulbear	0.17**	0.03	0.00	–									
Mitchell	0.01	0.20**	0.19**	0.20**	–								
Taroom	0.17**	0.00	0.00	0.02	0.24**	–							
Inglewood	0.34**	0.03	0.06	0.06*	0.40**	0.02	–						
Nappa Merrie	0.31**	0.09**	0.02	0.00	0.33**	0.08*	0.07	–					
Pindera	0.25**	0.02	0.00	0.00	0.30**	0.09	0.02	0.00	–				
Bundoona	0.26**	0.01	0.01	0.01	0.30**	0.00	0.00	0.02	0.00	–			
Eyre Ck.	0.35**	0.10*	0.04	0.02	0.38**	0.08	0.04	0.00	0.00	0.01	–		
Bulloo	0.22**	0.06**	0.02	0.01	0.25**	0.04	0.03	0.00	0.00	0.00	0.00	–	
Broken Hill	0.41**	0.15**	0.09**	0.06	0.43**	0.14*	0.07*	0.00	0.02	0.04	0.00	0.01	–

* Significance at $P < 0.05$
** Significance at $P < 0.01$

arid western region suffers considerable environmental and catastrophic stochasticity, thus elevating the extinction probability for local rabbit populations. Erratic rainfall and pasture growth will cause population sizes to oscillate; fluctuating without limit (Cooke 1981) and then crashing during drought conditions. Myers & Parker (1965, 1975a, b) have documented the regular occurrence of local rabbit population extinctions over large areas in arid New South Wales, as a result of severe drought. In these instances, local populations survive only in extinction-resistant or refuge areas, until periods of above average rainfall. After such times, refuge areas provide colonists for other areas (Myers & Parker 1975a,b). Additionally, continual localized control would result in local extinctions and the repeated recolonization of depopulated areas (Rowley 1968). The combined turnover of many local populations in the arid zone may result in high gene flow among local populations and explain the observed genetic homogeneity over the very large geographical area (at least 750 000 km^2 in the western region) sampled in the present study.

In semiarid Queensland, especially north of 25 °S where the reliability of winter herb production progressively decreases with increasing summer dominant rainfall, rabbits are restricted to small and scattered local populations (Parer 1987). In this environment there is always the possibility of local extinction, however, the probability of recolonization is relatively low. In particular, recolonization would be reduced in those areas where soils are generally unfavourable, the distance between patches of suitable soil is large and predation on dispersing rabbits is high. Regular local extinctions without recolonization may occur as a result of drought, rabbit control programmes and natural population fluctuations. In a metapopulation, fluctuations in local populations may result in bottleneck effects and a resultant reduction in genetic diversity (Gilpin 1991). This is likely to affect mtDNA variability more rapidly than nuclear DNA (Grant & Leslie 1993). A combination of the above factors, leading to reduced gene flow among populations, offers the most plausible explanation for the relatively high genetic differentiation observed among the five sites in the eastern region of this study. Environmental heterogeneity resulting in a discontinuous distribution of rabbits, and a high probability of local extinction without recolonization has been documented for Mitchell (Myers & Parker 1965; Parker *et al.* 1976; Parer 1987) and most probably applies to other eastern sites as well.

Habitat heterogeneity will also influence the extent of social and spatial structuring within local rabbit populations. Cowan & Garson (1985) reported that when nest sites were clumped (commonly on heavy burrowing substrate) and social groups formed, both burrows and mates were limiting resources. A resource-defence mating system and more rigid social organization may lead to low levels of gene flow among local populations. However, where nest sites were sparsely distributed (on light sandy soil) and food was a major limiting factor, social organization was more relaxed. This may be a likely scenario in arid Australia where food is the major limiting resource, leading to increased gene flow between areas.

In conclusion, these data suggest that current management strategies for rabbit populations in arid Australia (western region) may not achieve effective control if undertaken at a local scale because extensive gene flow occurs over very large distances. Consequently, management strategies for rabbit populations in arid Australia would probably be better directed towards control at a regional scale. Conversely, in the semiarid eastern region

where gene flow is very reduced between sites, current localized control procedures may provide effective short-term relief.

An important theoretical issue that has been highlighted by this study is that haplotype frequencies may be spatially heterogeneous indicating subdivision, while population subdivision estimates ($Ø_{st}$) may result in a high level of gene flow over all sites. Similar conflicting results have been described previously by Sarre (1995) who found that in gecko metapopulations, population subdivision was underestimated and consequently, *Nm* was overestimated even though populations were found to be otherwise independent. In nonequilibrium systems, the validity of calculating *Nm* based on Wright's (1931) infinite-island model of population structure is questionable. For example, in nonequilibrium metapopulation systems, traditional genetic models may not give a particularly accurate representation of population subdivision and gene flow.

Nonequilibrium population structures, in which populations are not constant and gene frequencies among populations have not reached an equilibrium, may have disparate consequences for population genetic structure. Population fluctuations may enhance the rate of genetic differentiation among local populations if recolonization leads to founder effects (Wright 1940), or may reduce differentiation if recolonization results in homogenizing gene flow (Slatkin 1977, 1985). Wade & McCauley (1988) reported that only in certain ecological situations was it possible for the homogenizing effects of gene flow to be enhanced and this was primarily dependent on the circumstances of recolonization.

To determine the effect of extinction and recolonization on population differentiation it is necessary therefore to understand the mechanisms of population foundation and whether colonization is a behaviour distinct from migration (Wade & McCauley 1988). It is also important to have an estimate of the rate of local extinction as this will determine the exact degree to which subdivision will be increased or decreased (McCauley 1991). To date, there is no way of estimating extinction and recolonization rates from genetic data, especially as population turnover may produce a similar pattern to gene flow (Milligan *et al.* 1994; Hastings & Harrison 1994). Gene frequency data still have a major role to play in elucidating ecological processes, however, they must be accompanied by a careful assessment of the spatial and temporal scales over which population processes operate. Moreover, it may be necessary for researchers to reappraise the application of traditional genetic algorithms in nonequilibrium systems with patchy distribution of individuals otherwise conflicting data and more importantly, false interpretations of critical population processes (such as migration) may occur.

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References

Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

Birky CW, Maruyama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics*, **103**, 513–527.

Campbell NJH, Harriss FC, Elphinstone MS, Baverstock PR (1995) Outgroup heteroduplex analysis using temperature gradient gel electrophoresis: high resolution, large scale, screening of DNA variation in the mitochondrial DNA control region. *Molecular Ecology*, **4**, 407–418.

Cooke BD (1977) Factors limiting the distribution of the wild rabbit in Australia. *Proceedings of the Ecological Society of Australia*, **10**, 113– 120.

Cooke BD (1981) Food and dynamics of rabbit populations in inland Australia. In: *Proceedings of the World Lagomorph Conference* (ed. Myers K, MacInnes CD), pp. 633–647. University of Guelph, Ontario.

Cowan DP, Garson PJ (1985) Variation in the social structure of rabbit populations: causes and demographic consequences. In: *Behavioural Ecology: Ecological Consequences of Adaptive Behaviour* (ed. Sibley RM, Smith RH), pp. 537–555. Blackwell Scientific Publications, Oxford.

Daly J (1979) *The ecological genetics of the European wild rabbit (Oryctolagus cuniculus (L.) in Australia*. PhD thesis, Australian National University.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Felsenstein J (1993) *PHYLIP (Phylogeny Inference Package) Version 3.5c*. University of Washington.

Fuller SJ, Mather PB, Wilson JC (1996) Limited genetic differentiation among wild *Oryctolagus cuniculus* L. (rabbit) populations in arid eastern Australia. *Heredity*, **77**, 138–145

Gilpin M (1991) The genetic effective size of a metapopulation. *Biological Journal Linnaean Society*, **42**, 165–175.

Grant WS, Leslie RW (1993) Effect of metapopulation structure on nuclear and organellar DNA variability in semi-arid environments of southern Africa. *South African Journal of Science*, **89**, 287–293.

Hastings A, Harrison S (1994) Metapopulation dynamics and genetics. *Annual Review of Ecology and Systematics*, **25**, 167–188.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583–589.

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In:

Mammalian Protein Metabolism (ed. Munro HN), pp. 21–123. Academic Press, New York.

Lessa EP, Applebaum G (1993) Screening techniques for detecting allelic variation in DNA sequences. *Molecular Ecology*, **2**, 119–129.

McCauley DE (1991) Genetic consequences of local population extinction and recolonisation. *Trends in Ecology and Evolution*, **6**, 5–8.

Meyer A, Kocher TD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Victoria cichlid fish suggested by mitochondrial DNA sequences. *Nature*, **347**, 550–553.

Milligan BG, Leebens-Mack J, Strand AE (1994) Conservation genetics: beyond the maintenance of marker diversity. *Molecular Ecology*, **3**, 423–435.

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, **3**, 401–411.

Myers K, Parker BS (1965) A study of the biology of the wild rabbit in climatically different regions in eastern Australia. I. Patterns of distribution. *CSIRO Wildlife Research*, **10**, 1–32.

Myers K, Parker BS (1975a) A study of the biology of the wild rabbit in climatically different regions in eastern Australia. VI. Changes in numbers and distribution related to climate and land systems in semiarid north-western New South Wales. *Australian Wildlife Research*, **2**, 11–32.

Myers K, Parker BS (1975b) Effect of severe drought on rabbit numbers and distribution in a refuge area in semi-arid north-western New South Wales. *Australian Wildlife Research*, **2**, 103–120.

Myers RM, Lumelsky N, Lerman LS, Maniatis T (1985) Detection of single base substitutions in total genomic DNA. *Nature*, **313**, 495–498.

Myers RM, Maniatis T (1986) Recent advances in the development of methods for detecting single-base substitutions associated with human genetic diseases. *Cold Spring Harbour Symposium on Quantitative Biology*, **51**, 275–284.

Parer I (1987) Factors influencing the distribution and abundance of rabbits (*Oryctolagus cuniculus*) in Queensland. *Proceedings of the Royal Society of Queensland*, **98**, 73–82.

Parer I, Parker BS (1987) Recolonisation by rabbits (*Oryctolagus cuniculus*) after warren destruction in western New South Wales. *Australian Rangeland Journal*, **8**, 150–152.

Parker BS, Hall LS, Myers K, Fullagar PJ (1976) The distribution of rabbit warrens at Mitchell, Queensland, in relation to soil and vegetation characteristics. *Australian Wildlife Research*, **3**, 129–148.

Richardson BJ (1980) Ecological genetics of the wild rabbit in Australia. III. Comparison of the microgeographical distribution of alleles in two different environments. *Australian Journal of Biological Science*, **33**, 385–391.

Richardson BJ (1981) The genetic structure of rabbit populations. In: *Proceedings of the World Lagomorph Conference* (eds Myers K, MacInnes CD), pp. 37–52. University of Guelph, Guelph, Ontario.

Riesner D, Steger G, Zimmat R *et al.* (1989) Temperature-gradient gel electrophoresis of nucleic acids: analysis of conformational transitions, sequence variations, and protein-nucleic acid interactions. *Electrophoresis*, **10**, 377–389.

Rowley I (1968) Studies on the resurgence of rabbit populations after poisoning. *CSIRO Wildlife Research*, **13**, 59–69.

Rural Lands Protection Board (1987) *Rabbits and their control in Queensland.* Queensland Government, Australia.

Saccone C, Pesole G, Sbisa E (1991) The main regulatory region of mammalian mitochondrial DNA: structure-function model and evolutionary pattern. *Journal of Molecular Evolution*, **33**, 83–91.

Saiki RK, Gelfand DH, Stoffel S *et al.* (1988) Primer-directed enzymic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487–491.

Sarre S (1995) Mitochondrial DNA variation among populations of *Oedura reticulata* (Gekkonidae) in remnant vegetation: implications for metapopulation structure and population decline. *Molecular Ecology*, **4**, 395–405.

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Slatkin M, Maddison WP (1989) A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics*, **123**, 603–613.

TGGE Handbook (1993) *DIAGEN*. GmbH, Germany.

Wade MJ, McCauley DE (1988) Extinction and recolonisation: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

Wartell RM, Hosseini SH, Moran CP (1990) Detecting base pair substitutions in DNA fragments by temperature-gradient gel electrophoresis. *Nucleic Acids Research*, **18**, 2699–2705.

Wilson AC, Cann RL, Carr SM *et al.* (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnaean Society*, **26**, 375–400.

Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

Wright S (1940) Breeding structure of populations in relation to speciation. *American Naturalist*, **74**, 232–248.

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