Genet. Res., Camb. (2004), **83**, *pp.* 101–111. *With 3 figures.* © 2004 Cambridge University Press
DOI: 10.1017/S001667230400672X Printed in the United Kingdom

High microsatellite diversity and differential structuring among populations of the introduced common brushtail possum, *Trichosurus vulpecula*, in New Zealand

A. C. TAYLOR[1]*, P. E. COWAN[2], B. L. FRICKE[3], S. GEDDES[3], B. D. HANSEN[1], M. LAM[3] AND D. W. COOPER[3]

[1] *Australian Centre for Biodiversity: Assessment, Policy and Management, School of Biological Sciences, Monash University, Victoria 3800, Australia*
[2] *Landcare Research, Private Bag 11052, Palmerston North, New Zealand*
[3] *School of Biological Sciences, Macquarie University, Sydney NSW 2109, Australia*

(*Received 4 August 2003 and in revised form 26 November 2003*)

Summary

An understanding of genetic variation and structure of pest populations has the potential to improve the efficiency of measures to control them. Genetic analysis was undertaken at five microsatellite loci in four native Australian and 14 introduced New Zealand populations of the common brushtail possum *Trichosurus vulpecula* in order to document these parameters. Genetic variation in New Zealand populations, and phylogenetic relationships among Australian and New Zealand populations, were largely predicted by the recorded introduction history. Populations on the two main islands of New Zealand had only slightly lower genetic diversity than did Australian populations, except that allelic richness on the South Is. was significantly lower. Diversity was higher in North Is. than in South Is. populations (although not significantly so) and mainland New Zealand populations as a group were significantly more diverse than offshore islands that represented secondary population size bottlenecks. In phylogenetic analyses South Is. and offshore island populations grouped with Tasmania, while North Is. populations grouped either with mainland Australia or were intermediate between the two Australian sources. This scheme was supported by admixture coefficients showing that North and South Is./offshore island populations were largely mainland Australian and Tasmanian in origin, respectively. Population structure differed markedly between the North and South Islands: populations were typically more genetically differentiated on the former than the latter, which also showed significant isolation-by-distance. Substantial linkage disequilibrium in most sampled New Zealand but no Australian population between microsatellite loci Tv16 and Tv27 suggests they may be physically linked.

1. Introduction

The common brushtail possum (*Trichosurus vulpecula*) is wreaking havoc on New Zealand's agricultural and natural systems, following its introduction from Tasmania and mainland Australia from the mid-1800s to early 1900s (Montague, 2000). Because of the degree of economic threat posed by the possum, substantial effort and money is being devoted to a multi-faceted research programme, with recent emphasis on the development of biological control methods (Cowan, 2000).

Genetic characterization of problem populations can maximize the efficiency of pest control programmes in a number of ways. Genetic differences among source populations may lead to differential responses to a variety of control measures. For example, Tasmanian possums are more resistant at low temperatures to the poison sodium monofluoroacetate (1080) than are mainland Australian ones (McIlroy, 1983). Given that 1080 poisoning is currently the most widely used possum control method, and regional variation in susceptibility is apparent in New Zealand (J. A. Peters, New Zealand Forest Research Institute, unpublished data), an understanding of the genetic origin of different invading populations may lead to enhanced efficiency of 1080 use. In the case of biological control agents, it may be desirable to trial new methods in isolated populations such as those on

* Corresponding author. Tel: (613) 9905 5623. Fax: (613) 9905 5613. e-mail: andrea.taylor@sci.monash.edu.au

islands, in order to minimize the chances of unplanned release. However, due to bottleneck and genetic drift effects, island populations may show a paucity of genetic variation and/or be genetically distinct from mainland populations (e.g. Eldridge *et al*., 1999), and hence may respond very differently to those populations for which the control measure is intended.

In addition to applications in pest control management, genetic analysis may help understand invasions, since the degree of genetic variation may bear on their success. There is evidence that genetic variation (particularly heterozygosity) and fitness may be positively correlated at both the individual and population level, via inbreeding depression (Saccheri *et al*., 1998; Keller, 1998; Coltman *et al*., 1999; Slate *et al*., 2000). The great success of some invasions is therefore perhaps surprising, given that founder effects and genetic drift might be expected to result in low genetic variation in colonizing species. However, significant loss of heterozygosity requires slow expansion from a very small number of individuals – conditions that may not necessarily apply to invading species. Indeed, heterozygosity in invading populations is often maintained at similar or higher levels to that in the species' native range: for example, introduced greenfinches in New Zealand (Merila *et al*., 1996), Nile perch in Lake Victoria (Hauser *et al*., 1998), yellow starthistle in the United States (Sun, 1997) and European rabbits in Australia (Zenger *et al*., 2003). Nonetheless some successful colonizers including vertebrates do exhibit reduced genetic variation: both Bennett's and tammar wallabies introduced to New Zealand have significantly lower microsatellite heterozygosity than populations in their native range (Taylor & Cooper, 1999; Le Page *et al*., 2000). Similarly, cane toads show substantial reductions in mitochondrial DNA diversity in Australia compared with their native South America (Slade & Moritz, 1998). If introductions include multiple differentiated genetic lineages (e.g. subspecies), the resulting 'hybrid' population may well display higher heterozygosity than either parental lineage. Such populations may enjoy even greater fitness than expected on the basis of heterozygosity alone, because heterosis can create favourable combinations of alleles that may not normally be possible (Frankham *et al*., 2002). At least some New Zealand localities received possums of both the Tasmanian (*T. v. fuliginosus*) and mainland Australian (*T. v. vulpecula*) subspecies (Pracy, 1974), raising the possibility that heterosis may have contributed to their success.

Some of these issues were addressed in an allozyme study by Triggs & Green (1989). They found that (a) levels of heterozygosity in New Zealand possum populations were generally not reduced relative to Australian ones, and (b) New Zealand populations with high or low proportions of black individuals were genetically more similar to Tasmanian and mainland Australian populations, respectively (the latter are almost entirely grey, while Tasmanian possums vary greatly in colour). However, that study also found some evidence for selection acting on the level and distribution of allozyme variation, which, along with low diversity displayed by allozyme markers, undermined a full understanding of the invasion history. The current study examines a larger number of populations, and makes use of the availability of highly polymorphic microsatellite markers assumed to be selectively neutral (Taylor & Cooper, 1998).

(i) *Predictions regarding genetic variation in New Zealand possum populations*

Genetic diversity in an introduced population is shaped by complex interactions between the level of diversity in the source population, the number of founders, the rate at which the population increased following the founding bottleneck and subsequent control operations, and the degree of demographic isolation of the population. Most of these parameters are not known in detail for New Zealand possum populations, but some broad patterns of genetic diversity may be predicted for particular groups of populations.

(a) *South Is.*

Historical records suggest that South Is. populations were founded predominantly with Tasmanian possums (often only small numbers of animals at a time) and that establishment of those populations was not always immediately successful, hence the need for repeated introductions of animals in the early stages (Pracy, 1974). Furthermore, Tasmanian possum populations harbour significantly lower allozyme heterozygosity than those on the Australian mainland ($H =$ 0·029 and 0·044, respectively; Triggs & Green, 1989). Hence we might predict that South Is. populations will show reduced microsatellite diversity compared with both Australian and North Is. populations.

(b) *New Zealand offshore islands*

The establishment of possum populations on Codfish, Chatham and Stewart Islands using South Is. founders (Pracy, 1974), coupled with continuing restricted population size and isolation, leads to the prediction that these three island populations will show the lowest genetic diversity of all. Also being an island population, Kawau Is. may show reduced genetic diversity, except that this may be mediated somewhat by founders being introduced directly, and from mainland Australia.

(c) *North Is.*

The North Is. populations were largely established later than South Is. ones, using New-Zealand-bred,

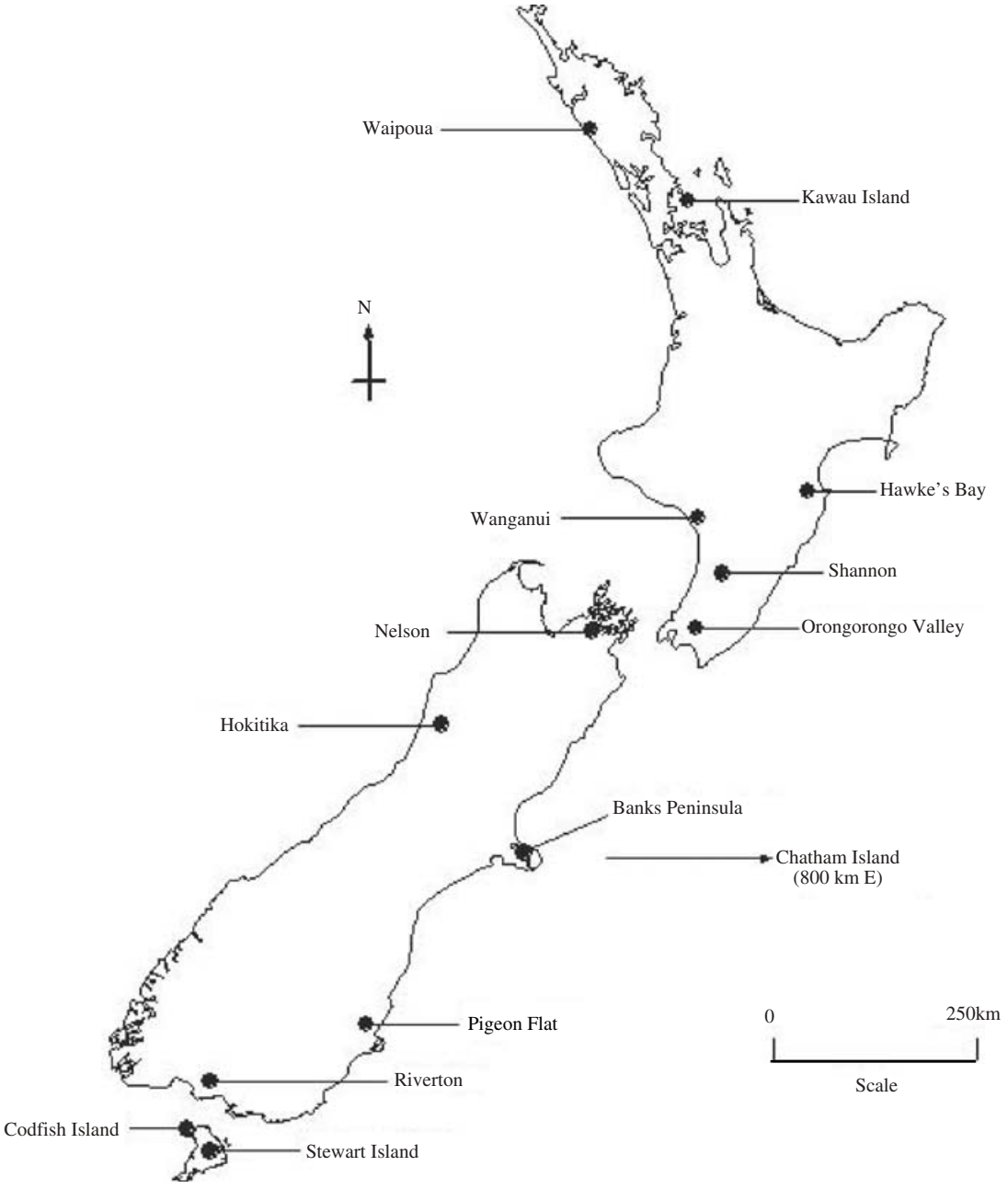

Fig. 1. Map showing the New Zealand possum sampling sites.

Tasmanian and mainland Australian possums, in an attempt to offset the possible effects of inbreeding by introduction of stock from diverse sources (Pracy, 1974). This leads to the prediction that North Is. populations may harbour even higher levels of genetic diversity than Australian ones, as a result of hybridization.

2. Materials and methods

(i) *Sample collection*

Sampling sites were selected to represent broad geographic coverage of populations in New Zealand (Fig. 1) and their potential sources in southeastern Australia. The New Zealand samples came from five South Is., five North Is. locations and four offshore islands. The Australian samples and those from Wanganui, Waipoua and Codfish Is. were collected for the allozyme study of Triggs & Green (1989). The remaining samples were collected during an extensive survey of possum parasites that focused on recorded sites of original liberations (Cowan *et al*., 2000). Methods of collection are described in Stankiewicz *et al*. (1996). The colour of each possum's fur was recorded at the time of collection.

(ii) *Microsatellite analysis*

Genomic DNA was extracted from ear or liver samples following the salting-out procedure described

in Sunnucks & Hales (1996). Population samples were genotyped for five *T. vulpecula* microsatellite loci (Tv16, Tv19, Tv27, Tv58 and Tv64) via the polymerase chain reaction (PCR), incorporating radioactive nucleotides according to Taylor & Cooper (1998), in a PTC-100 thermocycler (MJ Research). For each population, allelic diversity and observed and expected heterozygosity (Nei, 1987) were calculated. Because of substantial disparities in sample sizes among populations (ranging from 12 to 155), allelic richness was also estimated for each population by rarefaction (Hurlbert, 1971; El Mousadik & Petit, 1996), using FSTAT 2.9.3 (Goudet, 2001). The significance of differences in these parameters among various groups of populations was assessed using a randomization procedure, also in FSTAT 2.9.3. Deviations of genotype frequencies from those expected under Hardy–Weinberg equilibrium were tested for each locus in each population using the exact test as implemented in GENEPOP 3.2d (Raymond & Rousset, 1995).

Linkage disequilibrium may be informative about recent population bottlenecks, selection and/or physical marker linkage. LINKDOS (Black & Krafsur, 1985) was used to test for linkage disequilibrium amongst loci within each population sample, and to calculate Ohta's (1982) D statistics, which partition the variance of linkage disequilibrium into within- and between-subpopulation components.

The relative mainland Australian contribution to each New Zealand population's gene pool (mY) was calculated by pairwise comparison (with mainland Australian populations pooled, since information on exact sources is lacking) using the program Admix1.0 (Bertorelle & Excoffier, 1998). mY takes into account the evolutionary distance between microsatellite allele sizes based on a single-step stepwise mutation model (Bertorelle & Excoffier, 1998). Values presented are the averages with standard deviations generated by 1000 bootstrappings.

Microsatellite allele frequencies were subjected to phylogenetic analysis using PHYLIP 3.57 (Felsenstein, 1995). First, 1000 bootstrapped allele frequency tables were generated using the SEQBOOT routine. These were then used to construct a maximum likelihood consensus tree using CONTML followed by CONSENSE, and to calculate Cavalli-Sforza & Edwards (1967) chord distances among populations using the GENDIST routine. This genetic distance, being based on infinite allele models (IAM), was considered more appropriate in the current situation than those assuming stepwise mutation models (SMM; e.g. Goldstein *et al.*, 1995). This is because New Zealand possum population divergence is likely to be a result of founder effect and genetic drift, with little or no influence from mutation given the relatively short time since introduction (<150 years). Distances were used to build trees using the FITCH and NEIGHBOR routines, followed by CONSENSE to obtain consensus trees.

Finally, Cavalli-Sforza & Edwards (1967) chord distances were used to examine isolation-by-distance on the North and South Islands respectively, using the ISOLDE program within GENEPOP.

3. Results

(i) *Levels of genetic variation*

All but three of the sampled populations were polymorphic at all five loci. The exceptions were three offshore New Zealand island populations (Chathams, Stewart and Codfish) that each retained only one allele (136) at locus Tv58. Population rankings of genetic variation based on allelic richness and expected heterozygosity were very similar: the three offshore New Zealand islands had the lowest diversity, followed by the five South Is. and then the five North Is. populations plus Kawau Is. (Table 1). The Australian populations ranked highest in genetic diversity, with very little difference between them. Despite these general trends there was overlap between population groups in level of genetic diversity, and the only group showing significantly lower genetic diversity in randomization tests comparing measured parameters was the offshore versus main New Zealand islands ($P=0{\cdot}007$, $0{\cdot}003$ and $0{\cdot}002$ for AR, Ho and He, respectively). South Is. populations showed a significant reduction only in allelic richness compared with Australian ones ($P=0{\cdot}04$).

The percentage of black individuals in New Zealand populations was strongly negatively correlated with both heterozygosity ($r=-0{\cdot}77$) and allelic richness ($r=-0{\cdot}72$).

(ii) *Hardy–Weinberg and linkage disequilibria*

Many locus/population combinations showed departure from Hardy–Weinberg equilibrium on raw probability scores. However, only three of these (Tv16 in Wanganui, Tv19 in Banks Peninsula and Tv64 in Orongorongo Valley – all due to heterozygote deficits) were significant after Bonferroni correction for multiple tests (Rice, 1989), and there was no indication that any particular locus or population was more affected.

Significant linkage disequilibrium results were seen predominantly for the locus pair Tv16 and Tv27. The magnitude and consistency of deviations of this pair is illustrated by the fact that following Bonferroni correction for multiple tests (Rice, 1989), only seven of 156 locus pair/population combinations showed significant linkage disequilibrium, five of which were for Tv16/Tv27 (Table 2). In fact this pair of loci had *P* values of $<0{\cdot}1$ in all but three New Zealand and no Australian populations (Table 2). While some other

Table 1. *Parameters describing genetic variation in each of 18 sampled possum populations*

Population	% black	N	P	AD	AR	He	Ho
Australia							
Stonehenge, Tasmania	42	45	100	9·2 (3·1)	6·1 (1·7)	0·77 (0·15)	0·80 (0·12)
Adelaide, South Australia	0	14	100	7·8 (2·3)	6·7 (1·5)	0·83 (0·06)	0·79 (0·17)
Healesville, Victoria	0	39	100	10·6 (3·2)	6·8 (1·3)	0·83 (0·05)	0·74 (0·05)
Sydney, New South Wales	0	12	100	8·4 (2·7)	7·2 (2·0)	0·84 (0·09)	0·74 (0·20)
North Island, New Zealand							
Wanganui, Taranaki	2	49	100	6·4 (3·2)	4·6 (1·4)	0·72 (0·08)	0·69 (0·20)
Waipoua, North Auckland	0	33	100	7·2 (1·9)	5·6 (0·8)	0·78 (0·06)	0·73 (0·04)
Shannon, Wellington	63	45	100	9·2 (1·6)	6·1 (0·8)	0·78 (0·07)	0·79 (0·05)
Orongorongo V., Wellington	36	69	100	11·0 (4·4)	6·2 (1·6)	0·76 (0·13)	0·69 (0·09)
Hawke's Bay, Gisborne	17	155	100	10·8 (2·9)	6·5 (1·1)	0·83 (0·05)	0·78 (0·07)
South Island, New Zealand							
Riverton, Southland	59	46	100	5·4 (1·8)	3·6 (1·2)	0·53 (0·26)	0·54 (0·28)
Banks Peninsula, Canterbury	53	34	100	5·6 (2·7)	4·0 (1·7)	0·59 (0·27)	0·59 (0·29)
Nelson, Marlborough	62	36	100	5·4 (1·1)	4·4 (0·7)	0·69 (0·12)	0·72 (0·24)
Hokitika, Westland	98	36	100	6·4 (2·9)	5·1 (2·2)	0·65 (0·29)	0·62 (0·26)
Pigeon Flat, Otago	17	106	100	8·0 (2·2)	5·1 (1·3)	0·72 (0·10)	0·74 (0·11)
Offshore NZ islands							
Kawau Is.	0	37	100	7·0 (2·9)	5·2 (1·5)	0·72 (0·16)	0·73 (0·10)
Codfish Is.	100	34	80	2·2 (0·8)	1·9 (0·7)	0·23 (0·17)	0·23 (0·16)
Stewart Is.	73	25	80	2·6 (1·1)	2·1 (0·9)	0·30 (0·27)	0·32 (0·31)
Chatham Is.	100	51	80	2·2 (0·8)	2·2 (0·8)	0·38 (0·27)	0·42 (0·29)

% black, percentage of black possums; N, number of sampled individuals; P, percentage of loci polymorphic; AD, allelic diversity; AR, allelic richness; He, expected heterozygosity; Ho, observed heterozygosity, calculated as described in the text.

Table 2. *Significance* (*prior to correction for multiple tests*) *of linkage disequilibrium amongst pairs of microsatellite loci* (*e.g. 16/19, names given without 'Tv' prefix*) *in 18 possum populations*

	16/19	16/27	16/58	16/64	19/27	19/58	19/64	27/58	27/64	58/64
Tasmania										
Adelaide				ND			ND		ND	ND
Healesville										
Sydney		ND		ND	ND		ND	ND	ND	ND
Wanganui										
Waipoua										
Shannon										
Orongorongo V.										
Hawke's B.										
Riverton										
Banks P.										
Nelson										
Hokitika										
Pigeon F.										
Kawau Is.										
Codfish Is.			ND			ND		ND		ND
Stewart Is.		ND	ND			ND		ND		ND
Chathams			ND			ND		ND		ND

Black shading indicates $P<0{\cdot}000001$, grey indicates $0{\cdot}000001<P<0{\cdot}1$ and ND indicates a test was not done because of insufficient sample size or monomorphism (at Tv58).

P values $<0{\cdot}1$ were scattered throughout Table 2, no other pair of loci shows such a consistent pattern of linkage disequilibrium, suggesting putative linkage of loci Tv16 and Tv27. The Hawke's Bay population showed some evidence of generalized linkage disequilibrium across a number of loci (four of 10 pairs had $P<0{\cdot}1$, including two with $P<0{\cdot}000001$). This may be due to the fact that the sample consists of the

Table 3. *Variance components (Ohta, 1982) of linkage disequilibrium amongst pairs of microsatellite loci in 18 possum populations*

Locus pair	Within subpopulation		Between subpopulation		Total
	D(IS)	D″(IS)	D(ST)	D″(ST)	D(IT)
Tv16–Tv19	0·016	0·373	0·110	0·009	0·382
Tv16–Tv27	0·045	0·308	0·097	0·022	0·330
Tv16–Tv58	0·012	0·487	0·174	0·014	0·501
Tv16–Tv64	0·016	0·315	0·107	0·008	0·323
Tv19–Tv27	0·015	0·271	0·076	0·008	0·279
Tv19–Tv58	0·011	0·553	0·150	0·012	0·565
Tv19–Tv64	0·017	0·325	0·090	0·011	0·336
Tv27–Tv58	0·012	0·370	0·120	0·009	0·378
Tv27–Tv64	0·018	0·260	0·078	0·009	0·269
Tv58–Tv64	0·012	0·400	0·127	0·025	0·426

majority of adults in the population, which is subject to regular and severe control (see Taylor *et al*., 2000).

The variance components of linkage disequilibrium provide further support for linkage of Tv16 and Tv27: D(IS) ranged from 0·011 to 0·018 for all locus pairs except Tv16/Tv27, for which the value was 0·045 (Table 3). This suggests that the co-occurrence within individuals of particular alleles at Tv16 and Tv27 was substantially higher than for other locus pairs. Variance components for apparently unlinked locus pairs followed a pattern whereby D(IS) < D(ST), and D″(IS) > D″(ST), suggesting subpopulations are differentiated and allele combinations have been established by drift rather than selection (Ohta, 1982).

(iii) *Admixture*

Admixture coefficient values suggest negligible contributions of mainland Australian genes to most South Is. and offshore island populations, apart from Kawau Is. (Table 4). The latter, consisting entirely of grey possums, has the highest mainland contribution of any New Zealand population, followed by Wanganui and Hawke's Bay. The percentage of black individuals in New Zealand populations was strongly negatively correlated with mainland Australian genetic contribution ($r = -0{\cdot}83$).

(iv) *Genetic distance analysis*

South Is. populations were generally more genetically similar to one another than were North Is. ones (range 0·04–0·09 and 0·07–1·12, respectively; Cavalli-Sforza & Edwards (1967) chord distances) (Fig. 2). The relationship between genetic distance and geographic distance for pairs of populations was examined separately for the North and South Islands (Fig. 2). Mantel tests revealed that this relationship was significant for the South Is. ($P = 0{\cdot}021$) but not for the North Is. ($P = 0{\cdot}318$).

Table 4. *Values for mY, the admixture coefficient of Bertorelli & Excoffier (1998), quantifying the mainland Australian genetic contribution to 14 New Zealand populations, computed as average and standard deviation over 1000 random bootstrap samples*

'Hybrid' population	mY	
	Average	SD
North Is.		
Wanganui	0·71	0·11
Waipoua	0·41	0·08
Shannon	0·22	0·06
Orongorongo V.	0·23	0·05
Hawke's Bay	0·66	0·08
South Is.		
Nelson	0·06	0·05
Pigeon Flat	0·18	0·05
Riverton	0·17	0·06
Banks Pen.	0·10	0·06
Westland	−0·10	0·04
Offshore islands		
Kawau Is.	0·75	0·10
Codfish Is.	0·01	0·04
Stewart Is.	0·03	0·07
Chatham Is.	0·15	0·07

There was a high level of agreement between the topologies of phylogenetic networks produced by all tree-building methods and distance measures, so only one is shown here (Fig. 3). In all networks, Waipoua and Kawau Is. grouped with mainland Australian populations (specifically Sydney and Healesville) at one end, while Hokitika, Stewart Is., Banks Peninsula, and Nelson grouped together with Tasmania at the other end. Similarly Riverton, Chatham Is. and Codfish Is., amongst which the smallest genetic distance values were observed, formed a robust grouping (bootstrap support 58%), as did Hawke's Bay and Wanganui (74%). The Orongorongo Valley and

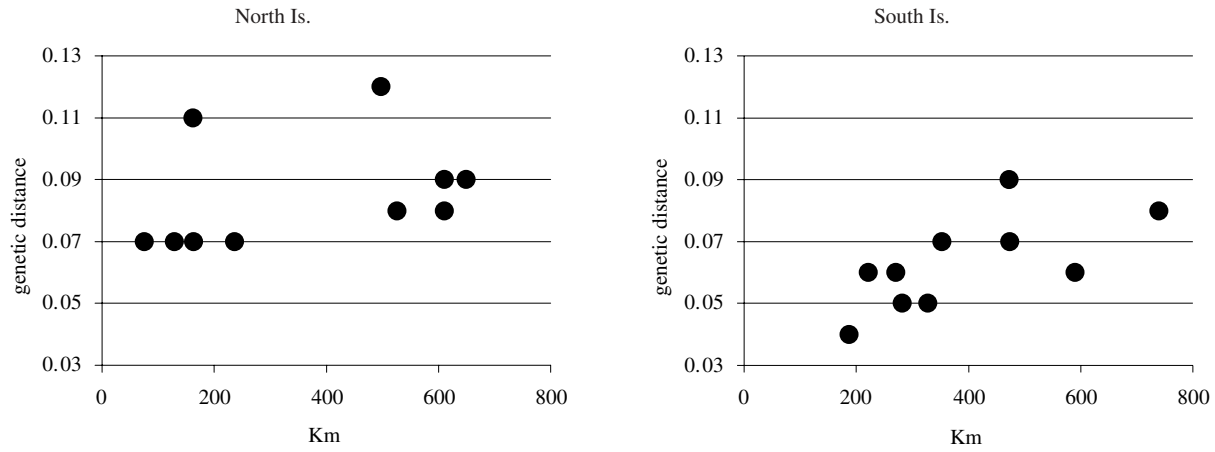

Fig. 2. Relationship between genetic (Cavalli-Sforza & Edwards' (1967) chord) and geographic distance among five possum populations on the North Island (left) and five on the South Island (right) of New Zealand.

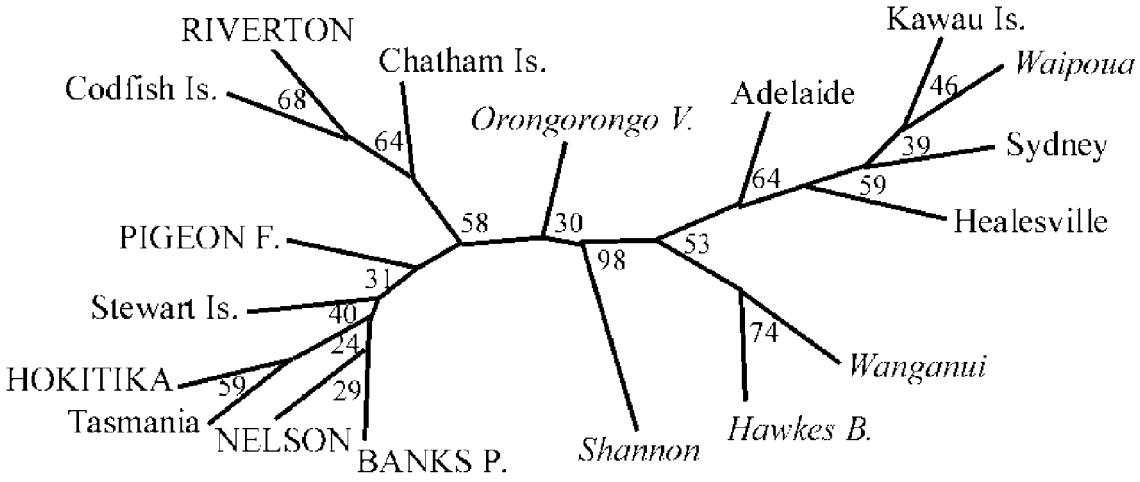

Fig. 3. Consensus of 1000 neighbour-joining trees based on Cavalli-Sforza & Edwards' (1967) chord distance, showing relationships among 18 Australian and New Zealand possum populations (italics denote North Is. and capitals, South Is. populations). Nodal values are the percentage of bootstraps in which the relevant grouping occurred.

Shannon populations always appeared at positions intermediate along the network. The affinities of Pigeon Flat were less certain, as it appeared variously with the Tasmanian group or intermediate along the network.

4. Discussion

(i) *Genetic diversity in New Zealand possum populations*

Microsatellite diversity was only slightly higher in Australian than New Zealand populations with the exception of the offshore islands, which had significantly lower allelic diversity and heterozygosity than all other populations. An earlier allozyme study similarly failed to demonstrate substantial reduction in genetic variation of New Zealand possums overall in comparison with Australian ones (Triggs & Green, 1989). This suggests that on the whole, processes leading to the establishment of the possum in New Zealand did not constitute particularly severe genetic bottlenecks, i.e. populations were founded with reasonable numbers of animals and did not spend many generations at small population sizes.

Levels of microsatellite variation in a range of New Zealand possum populations were largely as predicted from their history, in that the highest diversity was observed on the North Is. and the lowest on offshore islands, with South Is. populations showing intermediate levels. This is concordant with the recorded history of introduction of possums to New Zealand in Pracy (1974), as summarized in the introduction. It is interesting to note that reduced genetic diversity on offshore islands and the South Is. is matched by reduced parasite fauna in these populations (Cowan *et al.*, 2000). We now discuss genetic diversity results for each of the population groupings separately.

(a) *South Is.*

The range of heterozygosity values within the South Is. possum population group was quite large (0·53 to 0·72) with Riverton, the population at the site of the first introductions from Tasmania, harbouring the least. As a group, however, South Is. possum populations did not exhibit significantly reduced heterozygosity, although they do have significantly lower allelic richness (range 3·6–5·1) compared with Australia (6·1–7·2). There are several potential explanations for this. First, founders may have come from low-diversity source populations. Historic records and other genetic analyses discussed below suggest the source was predominantly Tasmania, which did indeed exhibit lower microsatellite diversity (albeit only slightly so) than mainland Australian populations. However, the relatively small numbers of animals involved in each South Is. introduction, and the initial slow growth rate of the founding populations, could have created a genetic bottleneck that further reduced variation in the South Is. group. The need for repeated introductions at Riverton, for example (Pracy, 1974), would tend to corroborate this explanation since that population shows particularly marked reduction in genetic variation. The greater reduction of allelic diversity than of heterozygosity under population size bottlenecking is a long-established population genetic principle (Nei *et al*., 1975).

(b) *Offshore islands*

According to Pracy (1974), populations on Chatham, Stewart and Codfish Islands were established by introduction of animals taken from the South Is., so were secondarily bottlenecked. They accordingly exhibit significantly lower heterozygosity and allelic richness than other populations examined in this study. In particular they all possess the same single allele (136, the most common allele in Tasmania) at the Tv58 locus, which is variable in all other populations. In contrast, the Kawau Is. population has retained high levels of genetic variation, suggesting it may have expanded rapidly following the initial founding event.

(c) *North Is.*

The generally higher levels of diversity observed in populations on the North Is. than the South Is. is most probably due to extensive mixing by introduction of possums from mainland Australia and Tasmania, as well as other New Zealand localities (Pracy, 1974). It is also possible that populations established more quickly than those on the South Is., hence suffering minimal genetic drift.

(ii) *Heterozygosity and coat colour*

Both microsatellite and allozyme (Triggs & Green, 1989) data sets demonstrate strong negative correlations between heterozygosity and the percentage of black individuals for New Zealand populations ($r = -0{\cdot}43$ for allozymes, $-0{\cdot}77$ for microsatellites). Triggs & Green (1989) suggested this trend reflects the fact that New Zealand populations with predominantly black individuals were established with possums from Tasmania, where heterozygosity is lower than that seen in mainland Australia (He = 0·44 versus 0·29 for allozymes; 0·83 versus 0·77 for microsatellites). However, the percentage of black possums may also reflect the degree of genetic bottlenecking and mixing undergone by different introduced populations, as discussed above.

(iii) *Population relationships*

Admixture and genetic distance analyses produced results broadly concordant with the introduction scheme summarized above. Two New Zealand populations (Waipoua and Kawau Is.) consistently cluster with mainland Australian ones, suggesting they were founded largely or exclusively with mainland animals. This is supported by the fact that both populations consist entirely of grey animals, and by historic records showing that Kawau Is. at least was established with mainland stock, with no record of further introductions. Hawke's Bay and Wanganui, with 17% and 2% black possums respectively, form a secondary grouping that is also closely associated with Waipoua and Kawau Is., suggesting they also consist predominantly of mainland genetic stock. Historic records show this to be the case at least for Wanganui, where the first possums released were Australian greys (Pracy, 1974).

The very close relationship among Riverton, Chatham Is. and Codfish Is. suggests the two island populations may have been established with animals from Riverton, the site of the first liberations of possums in New Zealand, but records appear incomplete or absent. Pigeon Flat, Orongorongo Valley and Shannon are known to have received both black and grey possums (Pracy, 1974), and all three clearly represent genetic mixtures of Tasmanian and mainland stocks.

(iv) *Differential structuring on the North and South Islands*

Genetic distances amongst South Is. populations tended to be lower than those among North Is. populations (see Fig. 2). There are several possible explanations. First, because North Is. populations have both Tasmanian and mainland Australian origins, distance values may to some degree reflect pre-existing genetic differences related to differing proportions

of mainland and Tasmanian animals. Admixture coefficients provide some support for this idea, in that they cover a much greater range for North Is. (0·22–0·71) than for South Is. populations (−0·10 to 0·18) (Table 4). Second, the earlier establishment time of South Is. populations may have given them more time to expand and exchange migrants, thus decreasing genetic distances. The significant isolation-by-distance recorded for the South Is. suggests these populations may currently be exchanging migrants at a rate dependent on their proximity. By contrast, the more recent founding of North Is. populations may mean they are not yet experiencing natural gene flow, such that genetic differentiation has not yet broken down. Given that the Kawau Is. population, despite its total isolation, is no more genetically distant from North Is. populations than the latter are from one another it may be that contemporary New Zealand possum population structure has been more influenced by admixture than by gene flow. Greater insight into this issue might be provided by a more thorough analysis of current rates of dispersal and gene flow between populations separated by smaller distances than those examined here.

(v) *Apparent linkage between two microsatellite loci*

Only a limited subset of all possible haplotypic combinations will be sampled during the establishment of new populations. The resulting linkage disequilibrium will be transient amongst unlinked loci, while non-random associations of alleles amongst linked loci are expected to persist for much longer periods of time (Hill, 1981). Thus the finding of significant linkage disequilibrium between one locus pair (Tv16 and Tv27) to the general exclusion of others in most New Zealand but no Australian populations strongly suggests these loci are closely linked. Breeding data would be useful in establishing how close the linkage is between the two loci, but in any case they provide a potentially useful new tool in identifying recent bottlenecks in brushtail possum populations (see Tishkoff *et al*., 1996). This could be usefully applied in diagnosing introduced populations, or ones that may be of conservation concern (e.g. those in central Australia; Taylor & Foulkes, 2004).

(vi) *Implications for possum establishment and control*

This study has established that New Zealand possum populations harbour high levels of neutral genetic variation. Although this does not prove the existence of high levels of quantitative variation, proposed explanations for the low meta-analysis correlation between neutral marker and quantitative trait variation mostly lead to the expectation that polygenic traits should retain more genetic variability than marker loci, not less (Reed & Frankham, 2001). While there is no direct evidence that possums would have been less successful at invading New Zealand had they been less genetically diverse, it is reasonable to suspect that the high level of genetic variation could have been beneficial. Possum populations would not have suffered inbreeding depression that might limit the growth rate of a less diverse population in its early stages, and they would have been able to rapidly evolve traits necessary for exploiting novel environments (Sakai *et al*., 2001). Similarly, they may quickly evolve resistance to control measures (Sakai *et al*., 2001). Recent experiments have shown that ability to evolve in the face of new threats is stronger in larger populations and that extinction tends to occur at lower rates in populations with more genetic variation (Frankham *et al*., 1999). In addition it is known for introduced mammals that individuals with high microsatellite heterozygosity can be at great advantage in the face of threats including parasite attack (e.g. Coltman *et al*., 1999). In light of these comments, we would recommend against using the genetically depauperate (at least for marker variation) offshore New Zealand island populations as testing grounds for biological control strategies that may be developed in the future, since they may succumb more easily than more genetically variable populations. In fact mixed-strategy control operations have recently been successful in eradicating possum populations from all offshore islands apart from two that were excluded from the programme (Brown & Sherley, 2002).

Cost-effective use of 1080 baits for possums relies on applying a toxic loading low enough to avoid bait refusal but high enough to kill possums at a rate sufficient to achieve population density goals. However, appropriate levels may vary among populations showing different degrees of susceptibility. The significantly lower tolerance of mainland than Tasmanian possums to 1080 at low temperature (McIlroy, 1983) predicts that New Zealand populations with large contributions from mainland genetic stock should be more susceptible to 1080 baiting. Further, the strong negative correlation ($r = -0{\cdot}83$) observed here between the percentage of black possums in a population and the proportion of its gene pool that came from mainland Australia, makes coat colour proportion a useful indicator of the degree of genetic admixture in a given population. This admixture may explain observations of higher 1080 susceptibility of 'grey' New Zealand possum populations compared with mixed or 'brown' ones (J. A. Peters, New Zealand Forest Research Institute, unpublished data), although the genetic affinities of the populations examined in that study are not known. Alternatively, admixture may be irrelevant if coat colour and resistance genes are linked. Although this latter explanation is favoured in the literature on

the subject, the genetic basis of neither coat colour nor resistance in possums is known, and existing data do not allow the issue of admixture versus linkage to be addressed. In this sense, trials specifically comparing susceptibility between black and grey Tasmanian individuals may be illuminating.

Biological control agent release protocols may benefit from knowledge of population structure. For example, possum-mediated transmission of agents among populations may generally occur more slowly on the North than on the South Is., if the observed isolation-by-distance on the latter is due to higher levels of gene flow or 'connectedness' among populations. However, the genetic structure difference between the North and South Is. will also have been influenced by admixture to an unknown extent, so more direct estimates of dispersal rates are required. This may be facilitated by use of genetic assignment tests in populations sampled over a smaller geographic scale than in the present study (e.g. Paetkau *et al*., 1995).

We are grateful to all field staff and other researchers who assisted with the sample collection. A.C.T. and laboratory costs were funded by a grant to D.W.C. from the Australian Research Council. P.E.C.'s involvement was funded by the NZ Foundation for Research, Science & Technology (Contract nos. C09801 and C09X0009) and the Cooperative Research Centre for Conservation & Management of Marsupials. We wish to thank Paul Sunnucks for constructive comments on the manuscript.

References

Bertorelli, G. & Excoffier, L. (1998). Inferring admixture proportions from molecular data. *Molecular Biology & Evolution* **15**, 1298–1311.

Black, W. C. I. & Krafsur, E. S. (1985). A FORTRAN program for the calculation and analysis of two-locus linkage disequilibrium coefficients. *Theoretical and Applied Genetics* **70**, 491–496.

Brown, K. P. & Sherley, G. H. (2002). The eradication of possums from Kapiti Island, New Zealand. In *Turning the Tide: The Eradication of Invasive Species*. Occasional paper of the IUCN Species Survival Commission No. 27 (ed. C. R. Veitch & M. N. Clout), pp. 46–52. Auckland: Holland Printing.

Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. *Evolution* **32**, 550–570.

Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J. M. (1999). Parasite-mediated selection against inbred soay sheep in a free-living, island population. *Evolution* **53**, 1259–1267.

Cowan, P. E. (2000). Biological control of possums: prospects for the future. In *The Brushtail Possum: Biology, Impact and Management of an Introduced Marsupial* (ed. T. Montague), pp. 262–270. Lincoln: Manaaki Whenua Press.

Cowan, P. E., Clark, J. M., Heath, D. D., Stankiewicz, M. & Meers, J. (2000). Predators, parasites and diseases of possums. In *The Brushtail Possum: Biology, Impact and Management of an Introduced Marsupial* (ed. T. Montague), pp. 82–91. Lincoln: Manaaki Whenua Press.

Eldridge, M. D. B., King, J. M., Loupis, A. K., Spencer, P. B. S., Taylor, A. C., Pope, L. C. & Hall, G. P. (1999). Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby *Petrogale lateralis*. *Conservation Biology* **13**, 531–541.

El Mousadik, A. & Petit, R. J. (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical & Applied Genetics* **92**, 832–839.

Felsenstein, J. (1995). *PHYLIP* (*Phylogeny Inference Package*), version 3.57c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Frankham, R., Lees, K., Montgomery, M. E., England, P. R., Lowe, E. & Briscoe, D. A. (1999). Do population size bottlenecks reduce evolutionary potential? *Animal Conservation* **2**, 255–260.

Frankham, R., Ballou, J. D. & Briscoe, D. A. (2002). *Introduction to Conservation Genetics*. Cambridge: Cambridge University Press.

Goldstein, D. B., Linares, A. R., Cavalli-Sforza, L. L. & Feldman, M. W. (1995). An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463–471.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hauser, L., Carvalho, G. R., Pitcher, T. J. & Ogutu-Ohwayo R. (1998). Genetic affinities of an introduced predator: Nile perch in Lake Victoria, East Africa. *Molecular Ecology* **7**, 849–857.

Hill, W. G. (1981). Estimation of effective population size from data on linkage disequilibrium. *Genetical Research* **38**, 209–216.

Hurlbert, S. H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**, 577–586.

Keller, L. F. (1998). Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* **52**, 240–250.

Le Page, S. L., Livermore, R. A., Taylor, A. C. & Cooper, D. W. (2000). Genetic analysis of a documented population bottleneck: introduced Bennett's wallabies (*Macropus rufogriseus rufogriseus*) in New Zealand. *Molecular Ecology* **9**, 753–763.

McIlroy, J. C. (1983). The sensitivity of the brushtail possum (*Trichosurus vulpecula*) to 1080 poison. *New Zealand Journal of Ecology* **6**, 125–131.

Merila, J., Bjorklund, M. & Baker, A. J. (1996). The successful founder: genetics of introduced *Carduelis chloris* (greenfinch) populations in New Zealand. *Heredity* **77**, 410–422.

Montague, T. L. (2000). *The Brushtail Possum: Biology, Impact and Management of an Introduced Marsupial*. Lincoln: Manaaki Whenua Press.

Nei, M. (1987). *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Nei, M., Maruyama, T. & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10.

Ohta, T. (1982). Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proceedings of the National Academy of Sciences of the USA* **79**, 1940–1944.

Paetkau, D., Calvert, W., Stirling, I. & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* **4**, 347–354.

Pracy, L. T. (1974). *Introduction and Liberation of the Opossum into New Zealand*. Wellington: New Zealand Forest Service.

Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Reed, D. H. & Frankham, R. (2001). How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* **55**, 1095–1103.

Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution* **43**, 223–225.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., *et al*. (2001). The population biology of invasive species. *Annual Review of Ecology & Systematics* **32**, 305–332.

Slade, R. W. & Moritz, C. (1998). Phylogeography of *Bufo marinus* from its natural and introduced ranges. *Proceedings of the Royal Society of London* **B 265**, 769–777.

Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M. & Clutton-Brock, T. H. (2000). Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proceedings of the Royal Society of London* **B 267**, 1–6.

Stankiewicz, M., Cowan, P. E., Jowett, G. H., Clark, J. M., Jowett, J., Roberts, M. G., Charleston, W. A. G. & Heath, D. D. (1996). Internal and external parasites of possums (*Trichosurus vulpecula*) from forest and farmland, Wanganui, New Zealand. *New Zealand Journal of Zoology* **23**, 345–353.

Sun, M. (1997). Population genetic structure of yellow starthistle (*Centaurea solstitialis*), a colonizing weed in the western United States. *Canadian Journal of Botany* **75**, 1470–1478.

Sunnucks, P. & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera, Aphididae). *Molecular Biology & Evolution* **13**, 510–524.

Taylor, A. C. & Cooper, D. W. (1998). Microsatellite markers for the Phalangerid marsupial, the Common Brushtail Possum (Trichosurus vulpecula). *Molecular Ecology* **7**, 1780–1782.

Taylor, A. C. & Cooper, D. W. (1999). Microsatellites identify introduced New Zealand tammar wallabies (*Macropus eugenii*) as an 'extinct' taxon. *Animal Conservation* **2**, 41–49.

Taylor, A. C., Cowan, P. E., Fricke, B. L. & Cooper, D. W. (2000). Genetic analysis of the mating system of the common brushtail possum (*Trichosurus vulpecula*) in New Zealand farmland. *Molecular Ecology* **9**, 869–879.

Taylor, A. C. & Foulkes, J. (2004). Molecules and morphology: a taxonomic analysis of the common brushtail possum *Trichosurus vulpecula*, with an emphasis on the central Australian form. In *The Biology of Australian Possums and Gliders* (ed. R. Goldingay & S. M. Jackson). Lismore: Southern Cross University Press, in press.

Tishkoff, S. A., Dietzsch, E., Speed, W., Pakstis, A. J., Kidd, J. R., Cheung K., *et al*. (1996). Global patterns of linkage disequilibrium at the CD4 locus and modern human origins. *Science* **271**, 1380–1387.

Triggs, S. J. & Green, W. Q. (1989). Geographic patterns of genetic variation in brushtail possums *Trichosurus vulpecula* and implications for pest control. *New Zealand Journal of Ecology* **12**, 1–10.

Zenger, K. R., Richardson, B. J. & Vachot-Griffin, A. M. (2003). A rapid population expansion retains genetic diversity within European rabbits in Australia. *Molecular Ecology* **12**, 789–794.