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Population genetics

Rapid loss of genetic variation in an endangered possum

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The endangered mountain pygmy possum is the only Australian marsupial that hibernates under snow cover. Most of its alpine habitat was burnt by a rare fire in 2003, and habitat loss and disturbance have also occurred owing to ski resort development. Here we show that there has been a rapid loss of genetic variation following habitat loss associated with resort development, but no detectable loss of alleles or decrease in heterozygosity following the fire.

Keywords: genetic variation; *Burramys*; endangered; inbreeding

1. INTRODUCTION

Endangered animals are threatened by the destruction of habitat, leading to a decrease in food and shelter, fragmented populations, and an increase in foreign predators and competitors. In addition, endangered animals are threatened by ecological catastrophes that result in local extinction and/or by environmental changes that make environments unsuitable (Reed 2004). Both catastrophes and fragmentation can cause local extinction directly or indirectly by making populations more susceptible to genetic, demographic and environmental stochasticity (Shaffer 1981). Loss of genetic variation is expected to occur well before extinction (Spielman *et al.* 2004), but has rarely been documented or linked to specific causes (Frankham 2005).

The mountain pygmy possum (*Burramys parvus*) is an endangered marsupial restricted to the alpine region of Australia. It inhabits periglacial block streams and block fields, collectively known as boulderfields, and exploits seasonally abundant food in summer. The limited availability of boulderfields, predators and other factors restrict *B. parvus* numbers; less than 1800 adults remain and their populations are in decline (Heinze *et al.* 2004). Genetic studies on 2003 samples indicate that populations of *B. parvus* are genetically fragmented, but within populations levels of gene diversity and mean allele number are high except in a southern population at Mt Buller (Mitrovski *et al.* 2007).

The main threats to *B. parvus* populations are thought to be human developments and natural catastrophes (D. A. Heinze & I. Mansergh 2004, personal communication). Boulderfields above the snowline represent ideal areas for the development of ski fields. Between 1980 and 2002, resort activities at Mt Buller have disturbed or eliminated an estimated 80% of this habitat at the main site where *B. parvus* are found (Federation; table 1; figure 1). This loss is apparent from aerial photographs taken over the last 25 years and has occurred owing to the development of ski runs and supporting infrastructure. Only one other area inhabited by *B. parvus* at Mt Buller (Fanny's Finish) is known to support a persistent population; this site has so far escaped damage from development, but has poor quality habitat (see electronic supplementary material) that only supports a small population. Ski resort developments have also been made in two other *B. parvus* sites (East and West Higginbotham) in the Bogong High Plains of Victoria.

In early 2003, a massive fire in the Victorian alpine area destroyed more than 1 Mha of vegetation including vegetation within *B. parvus* habitats. At the Mt McKay site, more than 80% of the *B. parvus* habitat was affected, while there was less damage at three other sites (figure 1; table 1).

These events provide an opportunity to compare the impact of a natural catastrophe with the ongoing impact of human developments on genetic variation in *B. parvus* populations. Therefore, we have examined changes in levels of genetic variation in populations at Mt Buller with changes in areas affected by the fires and other areas.

2. MATERIAL AND METHODS

(a) *Sampling, markers and analysis*

Hair samples from individual *B. parvus* for DNA extraction were collected from eight populations across two regions (electronic supplementary material, table S1) between November 1993 and December 2006. Extraction of DNA from hair samples was performed with Chelex (Bio-Rad) and genetic variation was scored at eight microsatellite loci as in Mitrovski *et al.* (2005).

FSTAT (Goudet 1995) was used to calculate the average number of alleles per locus, allelic richness averaged over loci and F_{IS}. Observed and expected heterozygosities were estimated and deviations from Hardy–Weinberg equilibrium determined by exact tests and permutation in GDA v. 1.1 (Lewis & Zaykin 2001). Genetic diversity between samples was compared using paired *t*-tests after angular transformation. To investigate population differentiation, pairwise measures of F_{ST} between populations were calculated and significance determined using FSTAT.

Effective population size (N_e) was estimated with several approaches. Short-term N_e estimates were calculated directly from sex ratio (Caballero 1994) and indirectly from the temporal-based method (Waples 1989; see electronic supplementary material). Two further indirect methods were used to calculate long-term N_e-estimates based on infinite allele (IAM; Kimura & Crow 1963) and stepwise mutation models (SMM; Ohta & Kimura 1973). To test for recent reductions in N_e for each population, we ran the program BOTTLENECK (Cornuet & Luikart 1996) under a two-phased model (TPM) and reported the results of a Wilcoxon signed-rank test.

(b) *Habitat loss estimation*

The distribution and area of preferred habitat was mapped using aerial photographs incorporated into a GIS system (ARCGIS v. 9.0), coupled with habitat verification in the field (ground-truthing) most recently in 2006 at Mt Buller and in 2003 on the Bogong High Plains (see electronic supplementary material).

3. RESULTS

To test whether genetic variation in populations was influenced by habitat changes at Mt Buller, we

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Table 1. Habitat area (ha), fire damage (%) and anthropogenic related habitat loss (%) in populations of *B. parvus*

	habitat area (ha)				
population	1980	2002	2004	% loss/modification from human activities	% loss from fire
Bogong High Plains					
West Higginbotham	34.3	30.87	30.87	10	62
Mt Loch	27.7	27.44	27	2	44
Mt McKay	21	20.58	20.58	0	87
Timms Spur	15.6	14.82	14.82	5	35
Bundara	15	15	15	0	0
East Higginbotham	30	24	24	20	0
Mt Buller populations					
Federation	90.48	18.09	18.09	80	0
Fanny's Finish	77.32	76.55	76.55	1	0

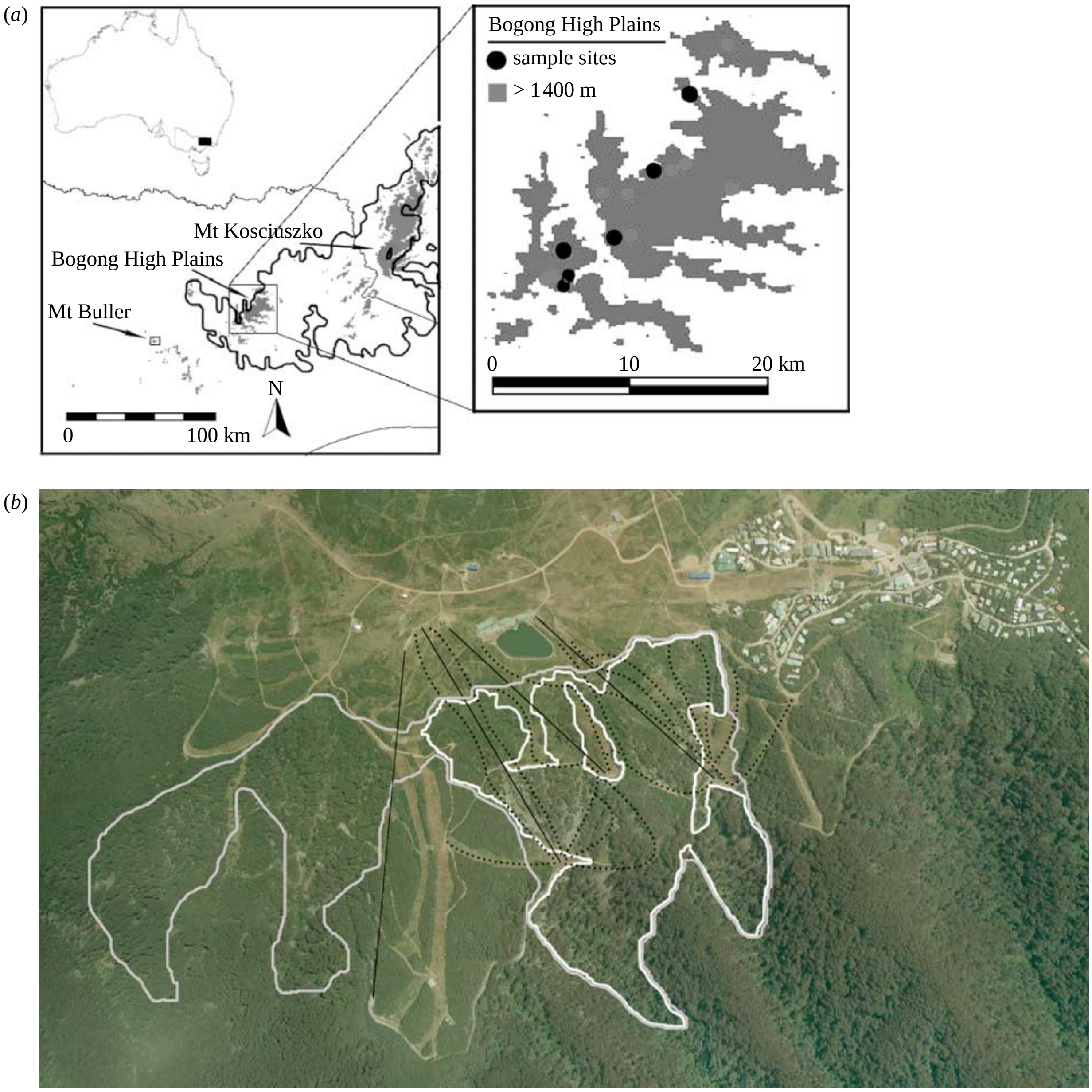

Figure 1. (*a*) Distribution of *Burramys parvus* and area burnt in the 2003 fires (inset; location of populations sampled from the Bogong High Plains). (*b*) Aerial photograph of Federation and Fanny's Finish areas at Mt Buller ski resort in 2006 showing the distribution of *B. parvus* pre-1980 (grey line) when suitable habitat between the populations in this area was continuous, and the current distribution of *B. parvus* at Federation (white line) when habitat is intersected with ski runs (black dotted lines) and lifts (black lines).

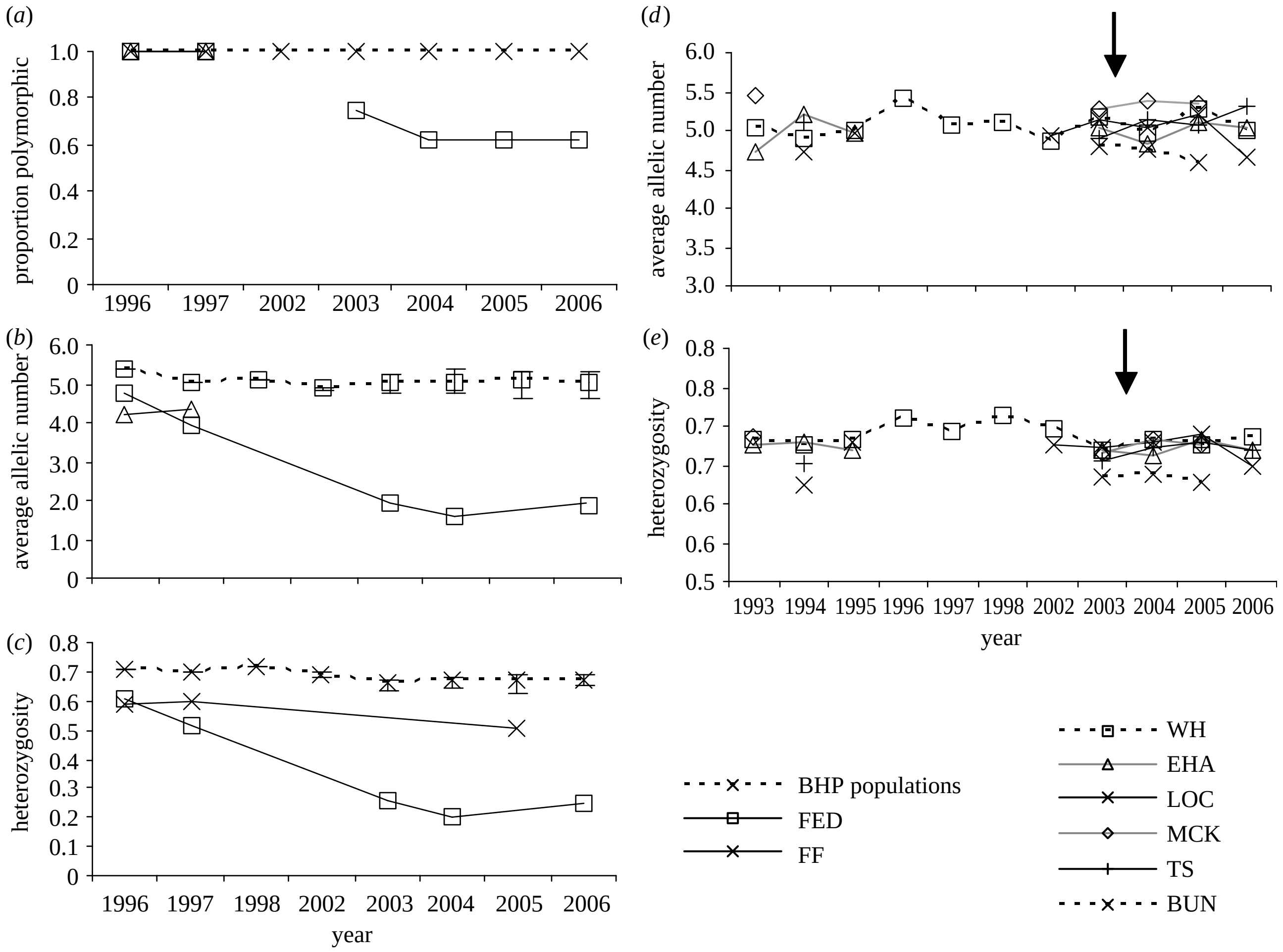

Figure 2. (*a–c*) Temporal changes in proportion of polymorphic loci, mean allelic richness and mean expected heterozygosity from populations of *B. parvus* in the central region (dotted lines with the range of values indicated by error bars) and populations from Mt Buller (solid lines). Crosses indicate populations from the central region, open squares represent the Federation site and open triangles represent the Fanny's Finish population. (*d*,*e*) Temporal changes in mean allelic richness and expected heterozygosity from populations of *B. parvus* in the central region. Dotted lines indicate populations that were not affected by fire, whereas solid lines indicate populations potentially affected by fire. Arrows indicate when the alpine fire occurred.

scored variation at eight microsatellite loci on more than 1500 individuals over 9–12 years (electronic supplementary material, table S1). Levels of genetic variation at Federation in 1996 were similar to those in other *B. parvus* populations around this time (figure 2), although evidence consistent with inbreeding was found; deviations from Hardy–Weinberg equilibrium due to a deficiency of heterozygotes were detected in five out of the eight loci (electronic supplementary material, table S1). After 1996, there was a rapid decline in expected heterozygosity at Federation to approximately 0.2 by 2004. Changes in expected heterozygosity between samples from 1996 to 1997 and from 1997 to 2004 were significant by paired t-tests (1996–1997, $t=2.76$, d.f.$=7$, $p=0.014$; 1997–2004, $t=4.04$, d.f.$=7$, $p=0.002$). This rapid decline in genetic variation was also evident from the loss of alleles (1996–1997, $t=2.49$, d.f.$=7$, $p=0.024$; 1997–2004, $t=5.01$, d.f.$=7$, $p<0.001$) and from changes in the proportion of polymorphic loci (figure 2). Levels of genetic variation fell to approximately one-third of 1996 levels. This decline corresponded to a marked drop in census population size at Federation, particularly in male numbers (table 2). By contrast, only a small and non-significant decrease in heterozygosity and allelic richness was evident at Fanny's Finish only 1 km from Federation (figure 2), although there was evidence of an excess of homozygotes at this site (electronic supplementary material, table S1). Genetic differentiation was detected between all populations as indicated by significant pairwise F_{ST} estimates (range 0.013–0.430), except between Federation and Fanny's Finish (range 0–0.012). However, capture-mark-recapture data from 1996 to 2007 indicate that these populations are likely to be isolated with no dispersal events detected between populations (D. A. Heinze 2006, unpublished data). Genetic differentiation was not detected between samples from the same site.

In populations of *B. parvus* from the Bogong High Plains that included burnt sites, there was no evidence that levels of genetic variation changed across years, with expected heterozygosities all greater than 0.62 (figure 2). Furthermore, trapping rates and animal captures were consistent with those obtained during 1984 to 2003. There was no consistent reduction in population size between the pre-fire and post-fire samples 2005 (table 2). Changes in population size

Table 2. Effective population size estimates of *B. parvus* populations. (n, number of individuals sampled; N_c, adult census estimates; N_e (dir), direct estimates based on sex ratio; N_e (temporal), temporal-based estimates; N_e (IAM) and N_e (SMM) effective population size calculated under the assumptions of an infinite allele model (IAM) and stepwise mutation model (SMM), using mutation rates of 10^{-3}; N_e/N_c, effective population size to census size ratio.)

population	year	n	sex ratio (male : female)	N_c	N_e (dir)	N_e (temporal)	N_e (IAM)	N_e (SMM)	N_e/N_c	bottleneck^{TPM} (p value)
West Higginbotham	1993	54	1 : 4	108	70		542	1255	0.64	0.191
	2004	24	1 : 3	48	36	62.2	534	1230	0.75	0.527
East Higginbotham	1993	26	1 : 4	52	32		519	1181	0.64	0.156
	2004	31	1 : 3	62	48	30.1	489	1091	0.75	0.527
Mt Loch	1995	15	1 : 3	~120	90		531	1218	0.75	0.320
	2004	140	1 : 8	280	110	53.7	532	1224	0.4	0.321
Bundara	1994	42	1 : 4	84	54		414	881	0.64	0.578
	2004	93	1 : 5	102	57	∞	446	970	0.75	0.578
Mt McKay	1993	28	1 : 5	56	30		550	1280	0.56	0.191
	2004	18	1 : 4	36	23	23.3	536	1235	0.64	0.421
Timms Spur	1994	68	1 : 4	136	87		471	1040	0.75	0.769
	2004	52	1 : 3	104	78	75.3	510	1155	0.89	0.527
Federation	1996	25	1 : 12	170	48		392	824	0.28	0.990
	2004	15	1 : 5	38	20	9.4	65	198	0.56	0.109
Fanny's Finish	1996	11	1 : 12	36	11		359	743	0.28	0.312
	2004	6	1 : 4	12	7	303.2	258	515	0.64	0.843

estimated from genetic data ranged from an increase or a decrease in size of less than 10%.

Effective population size estimates for Buller populations were small and similar in most cases (table 2). Both direct and indirect short-term estimates were low but varied among methods and temporally. Changes in effective population size for all populations of *B. parvus* were best described from long-term estimates using the IAM and SMM methods (Kimura & Crow 1963; Ohta & Kimura 1973). These indicated a decrease of 77% in Federation under the IAM or 67% under the SMM, compared with 30% under both models at the Fanny's Finish site (table 2). Loss of genetic variability in the Mt Buller site is likely to reflect a sharp drop in population size compared with other populations. No recent bottleneck signature was detected in any population under the TPM (table 2).

4. DISCUSSION

This is the most rapid loss of genetic diversity in a mammal ever documented. By comparison, in a longitudinal study of a Scandinavian wolf population (Flagstad *et al.* 2003), 30% of gene diversity was lost in just over a century. The rapid loss of genetic diversity in *B. parvus* is likely to reduce evolutionary potential and reproductive fitness, as well as elevate extinction risk (Spielman *et al.* 2004). *Burramys* populations are particularly susceptible to extinction from loss of genetic variation due to their polygynous mating system. Owing to matriarchal defence of optimal habitats (normally the higher altitudes of boulderfields), males are forced to lower suboptimal habitats that contain fewer hibernacula and food resources, and have greater levels of temperature variability. Habitat fragmentation is likely to decrease food availability and increase predator risk in these areas, skewing the sex ratio. During the 2005/2006 season, the sex ratio at Federation was estimated from captured animals as 1 male : 22 females; no males were captured, but some females contained pouch young suggesting a minimum of one breeding male. This skewed sex ratio will decrease the effective population size well below census size. Moreover, asynchronous breeding behaviour was observed for the first time in this species. Second litters were detected in several females late in the season which are likely to be lethal for both the mother and young during hibernation because possums have insufficient time to accumulate adequate fat reserves (D. A. Heinze 2006, unpublished data). This phenomenon, the first time detected at Federation, may reflect deleterious genes influencing viability of the first litter. By contrast, the large drop in census estimates at Fanny's Finish is likely to be a recent occurrence because genetic diversity is maintained. This may reflect recent unfavourable environmental conditions and poor quality habitat and is supported by a lack of dispersal events between Federation and Fanny's Finish in the last 10 years (D. A. Heinze 2006, unpublished data).

In comparison, populations burnt by the unusual fire event were relatively unaffected and census estimates were at pre-fire levels within a season. No loss of rare alleles was detected, so that genetic variation was maintained (figure 2). The genetic data suggest that rare natural catastrophes like the alpine fires do not represent the main threat to *B. parvus* and perhaps other endangered mammals.

Encroachment of development on *Burramys* habitat represents an important long-term threat. Ski field developments continue in the alpine area and *B. parvus* habitat is threatened at several resorts. The data presented here suggest that such developments should proceed in a sensitive manner that preserves all *Burramys* habitat intact. Recovery of the Federation population will take some time even if the boulderfield habitat is restored. Because inbreeding and loss of adaptive potential threaten long-term persistence of populations (Saccheri *et al.* 1998; Frankham 2005), an immediate goal at Mt Buller should be to increase levels of genetic variation in populations. A target for long-term viability of *Burramys* populations is a heterozygosity of 0.65 or higher. Individuals from other populations will need to be introduced to achieve this target.

This work was carried out with ethics approval (AEC04/4(BG)).

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