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Sex-biased dispersal and a density-independent mating system in the Australian brushtail possum, as revealed by minisatellite DNA profiling

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Abstract

Natal dispersal can have important effects on mammal population structure and dynamics following a local population crash. Such dispersal is of practical importance when applied to the control of pest species because dispersal may significantly, and undesirably, reduce the population recovery time following a control operation. The relative dispersal rate of the sexes is also critical because that too will affect the rate of population increase. Here, we describe a field experiment in which we reduce the density of two populations of the Australian brushtail possum, and use genetic similarity, as estimated by minisatellite DNA profiles, to investigate dispersal in the original (undisturbed) and recovering populations. Our results show that the genetic similarity within the undisturbed populations was lower between males than between females. Conversely, the genetic similarities between males and females in the two recovering populations were not significantly different, while relatedness among males was significantly higher in the recovering populations when compared with those in the pre-removal populations. These data indicate two important characteristics of dispersal in possums: (i) that dispersal in established populations is sex biased towards males; and (ii) that within the first 3 years following population control, 'the vacuum effect', whereby individuals from areas adjacent to a control area expand their home range and invade the depopulated area, is the most important factor in the re-colonization process for possums. We found no evidence that the mating system, which is polygynous, varied when the density was markedly reduced. These results indicate that drastic reductions in population density by conventional control will not affect the rate of spread of biological control agents that rely on sexual transmission for dissemination.

Keywords: brushtail possum, contact rate, mating system, re-colonization, *Trichosurus vulpecula*.

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Introduction

Natal dispersal, which occurs in most birds and mammals, tends to be sex biased (Greenwood 1980) and has important effects on population demography, social structure and genetic composition (Cheepko-Sade & Tang Halpin 1987; Stenseth & Lidicker 1992). The level and nature of dispersal are especially critical to population recovery following a population crash in part of a species' range. In mammals, the most common short-term recovery after depopulation is through a 'vacuum effect', in which individuals from surrounding areas, attracted by reduced competition for food and den sites, rapidly increase their home ranges and invade the depopulated area. Such re-colonization of depopulated areas by immediate neighbours has been documented in the white-footed mouse, *Peromyscus leucopus* (Stickel 1946); meadow voles, *Microtus pennsylvanicus* (Vleck 1968); Great Basin pocket mouse, *Perognathus parvus* (Verts & Carraway 1986); and red-backed voles, *Clethrionomys rufocanus* (Nakata & Satoh 1994).

An understanding of the nature of dispersal into a

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depopulated area is of particular relevance to situations where the control of pest species is being attempted. For example, if most short-term re-colonization results from range extensions of animals from adjacent areas, then the area for which control needs to be effected will be less than for species in which natal dispersal is the dominant driver of re-colonization. The demographic profile of dispersers (particularly sex ratios and age structure) is also important because it will affect the population rate of increase over and above that achieved by re-colonization alone.

The recent developments in genetic techniques make indirect estimates of dispersal possible. Lehman *et al*. (1992) detected a female-biased short-range dispersal among wolf populations using minisatellite DNA profiling. Favre *et al*. (1997) showed a female-biased dispersal in populations of European shrews, *Crocidura russula*, using microsatellite DNA techniques. Using the same method, Mossman & Waser (1999) detected a male-biased dispersal in white-footed mouse, *Peromyscus leucopus*, although the bias was not extreme.

The brushtail possum (*Trichosurus vulpecula*) is an introduced marsupial pest in New Zealand. It damages forests (Campbell 1990; Cowan 1990), preys on native wildlife (Brown *et al*. 1993) and threatens the farming industry by acting as a reservoir for bovine tuberculosis (Ekdahl & Money 1970; Coleman & Caley 2000). Immunocontraception has been investigated as a potential means of controlling this species (Jolly 1993; Barlow 1994). However, at present, conventional control methods, such as culling and poisoning, are the only methods available for controlling the densities and the associated damage of possums. The long-term effectiveness of a conventional control operation is affected by the rate of population recovery. Therefore, the source and demography of re-colonizing animals and the breeding response of those animals to low population density are important factors in the further management of this pest. Although the 'vacuum effect' has been observed in a recovering brushtail possum population shortly after a depopulation event (Efford *et al*. 2000), most studies of possums have suggested that natal dispersal (young animals leaving their birth place to a new home range) was the main cause of re-colonization of possums (Cowan & Tyndale-Biscoe 1997; Clout & Efford 1984; Green & Coleman 1984).

Recently, a modelling analysis (Bayliss 1999) indicated that biological control of possums may work best when integrated with conventional control. For such integration, information on the origins of potential colonizers and possible changes in the mating patterns resulting from changes in density are critical. Virus-disseminated immunocontraception relies largely on sexual contacts between individuals (Barlow 1994). This means that changes in the mating pattern resulting from reduced density by conventional control may alter contact rates, and thus affect the outcome of the biological control. There have been two published studies of the possum mating system: Sarre *et al*. (2000), using minisatellite DNA profiling, and Taylor *et al*. (2000), using microsatellites. These studies have revealed that possums are not monogamous and exhibit a random mating system. This kind of mating system should facilitate the spread of a virus-vectored immunocontraceptive. However, the behavioural response of possums (especially mating behaviour) to density reduction by control operations is not known.

Hypervariable DNA markers, such as mini- and microsatellites, have been used to study parentage, reproductive success and mating systems of mammals (Ribble 1991; Moritz *et al*. 1997) and birds (Ardern *et al*. 1997b; Millar *et al*. 1997; Currie *et al*. 1998). Although DNA profiling techniques may not provide precise estimates of relatedness between specific pairs of individuals (Lynch 1988), they can estimate mean relatedness from a relatively limited sample of individuals (Reeve *et al*. 1992). Minisatellite DNA band-sharing levels have been shown to correlate with level of kinship (Wetton *et al*. 1987; Descalzi *et al*. 1998) and have been used successfully to study the relatedness among individuals within populations (Wetton *et al*. 1987; Reeve *et al*. 1992) and between populations (Patenaude *et al*. 1994; Ardern *et al*. 1997a). Bandsharing is therefore a useful tool for investigating animal behaviours that can cause a difference in average genetic relatedness within populations.

Genetic relatedness among individuals can be used to investigate two characteristics relevant to population dynamics. First, higher relatedness among one sex relative to the other indicates that the source population of the less related sex is more variable. Hence, differences in relatedness between the sexes is indicative of sex-biased dispersal. Second, relatedness among individuals in a recovering population will be defined by the nature of the subsequent re-colonization. For example, in the case of local range shift or expansion, relatedness within the new population should not be markedly different from that of the original population because re-colonizers are from nearby. On the other hand, if re-colonization mainly results from natal dispersal, we would expect a lower relatedness in the colonizing population than that of the original population.

The issues of re-colonization and density effects on mating systems are clearly of importance for the management of pest species such as brushtail possums in New Zealand. Therefore, we used estimates of genetic relatedness in conjunction with an experimental control operation, to address three key aspects of possum demography relevant to their control: (i) sex differences in dispersal; (ii) the source (from dispersal or 'vacuum effect') of recovery following a population collapse; and (iii) the impact of reduced density on the mating system. In order to achieve this, we simulated control operations by removing pre-existing possum populations from two study sites and recorded the process of population recovery. We then investigated the differences in genetic relatedness and mating patterns between the original and recovering populations. The implications of

our results are discussed in relation to the future control of this pest.

Materials and methods

Field studies

Our study was conducted at two sites: Huapai (36°47′ S, 174°45′ E) and Coatesville (36°44′ S, 174°40′ E), near Auckland, New Zealand (Fig. 1). The sites were each approximately six hectares in area and were partially bordered by agricultural pasture. At each site, a 50m square trapping grid was established in which treadle-operated cage traps were permanently established at each point of the grid. Live trapping commenced at these two sites in early 1995 to investigate the response of possums to female sterilization (Ji *et al*. 2000; Sarre *et al*. 2000) and continued until the end of June 1997. In July 1997, we removed the original populations by kill trapping. Live trapping resumed in August 1997 to investigate the recovery of possums in the two study sites after the control operations.

The cage traps were baited with apples dusted with fruit-flavoured flour. Traps were set for at least four nights each month but more intensively (two nights/week) during the mating and postmating periods (mid-March to the end of April and mid-May to the end of June, respectively) of 1996 and 1997. After the July 1997 kill trapping, two trapping sessions in August and February, respectively, were conducted each year for 3 years to monitor the population recovery. In each session, traps were operated for 2 nights per week for 4 weeks.

Upon capture, possums were anaesthetized with ether and then tagged with two permanent, numbered metal ear tags. Approximately 5–10 mL of whole blood was taken from the caudal vein of every captured possum, mixed with a small quantity of 0.5 M di-potassium ethylene diaminetetraacetic acid, and immediately placed on ice. Sex, maturity, weight and body measurements were recorded before release. In July or August of each year, ear samples were obtained from the pouch young (using a sterile biopsy punch) whilst they were still in the pouch and attached to the teat. These samples were kept on ice until they were frozen at −80 °C. Those pouch young that were large enough were also marked with permanent metal ear tags. All procedures performed were within animal ethics guidelines (Auckland University, Approval Number N420).

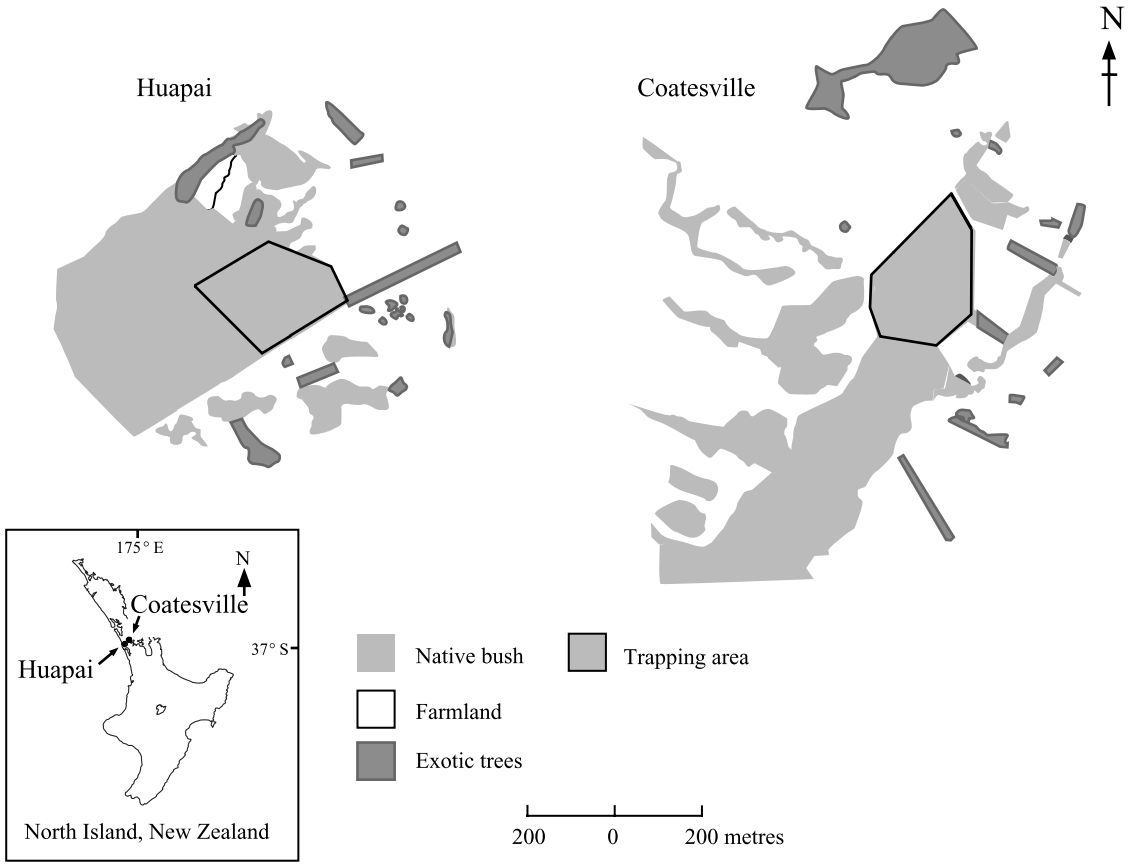

Fig. 1 Maps of the study sites.

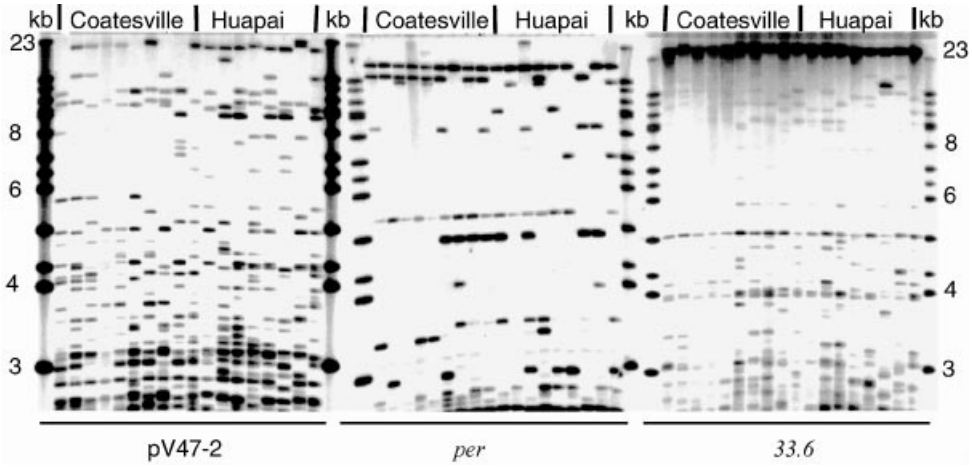

Fig. 2 Examples of DNA fingerprinting profiles of females from the Huapai and Coatesville study sites revealed by probes pV47-2, *per* and 33.6.

The number of possums known to be alive (minimum alive estimation) before the removal at our study sites varied seasonally. The number was higher in autumn and summer, [25–37 (4.2–6/ha) at Coatesville and 48–56 (8–9.3/ha) at Huapai] and lower in winter and spring [21–31 (3.5–5.2/ha) at Coatesville and 41–46 (6.8–7.7/ha) at Huapai]. Forty-three possums at Huapai and 37 at Coatesville were removed during the removal trapping. Six possums from Huapai and two from Coatesville survived the removal trapping and were caught regularly during the subsequent trapping, indicating 95% of trappable possums of the original population in Coatesville and 88% in Huapai were removed.

Minisatellite DNA analysis

In a recent study (Sarre *et al*. 2000), the minisatellite DNA profiles of the pre-removal populations at both Huapai and Coatesville were obtained for investigating possum mating systems. To make the data from the present study directly comparable to those obtained previously, we used minisatellite DNA profiling to estimate relatedness among possums in the recovering populations and followed strictly the methods, conditions and probes (pV47-2, Longmire *et al*. 1990; *per*, Shin *et al*. 1985; 33.6, Jeffreys *et al*. 1985) used by Sarre *et al*. (2000). A single observer scored or re-scored all DNA profiles referred to in the present study to avoid scoring errors resulting from variation between observers (Descalzi *et al*. 1998).

Analysis of minisatellite profiles

Each gel included two molecular weight markers (Fig. 2). Bands less than 12 kb in size were considered to match if their centres differed in mobility by less than 2 mm. For bands greater than 12 kb, a bin size of 3 mm was used to overcome the slippage observed in high molecular weight bands of some of the pouch young (Sarre *et al*. 2000).

The bandsharing coefficient (BC) between two individuals run on the same gel was calculated following the procedure of Wetton *et al*. (1987): $BC = 2N_{ab}/(N_a + N_b)$, where N_{ab} = number of bands of similar intensity and mobility shared between two individuals (a and b), N_a = total number of bands in individual a, N_b = total number of bands in individual b.

The paternity of pouch young was assigned on the basis of paternal bands (i.e. bands present in the pouch young but not in the mother). A male was rejected as the actual father of a pouch young if his minisatellite profile did not contain all paternal bands present in the pouch young. The probability (p) of false inclusion of a male as the father for each pouch young was calculated as:

$$p = \prod_{i=1}^{n} \frac{n_i}{m}$$

Where n_i = the number of males with the i^{th} paternal band and m = the total number of males surveyed for paternal bands. These probabilities were then adjusted to account for multiple comparisons among pouch young by $P = 1 - (1 - p)^m$ (Sarre *et al*. 2000).

To overcome the non-independence of bandsharing data caused by pairwise comparisons, two randomization tests described in detail by Ardern *et al*. (1997a), fixed covariance model (FCM) and independent assortment model (IAM), were used to detect the difference in relatedness between given groups.

We followed the method of Ardern *et al*. (1997a) for within-population comparisons. For between-population comparisons, we modified the methods as follows.

1 Calculate the mean bandsharing coefficienct (BC) between individuals from Coatesville and individuals from Huapai.
2 Generate two pseudo-samples from one population. Calculate the mean BC from these two pseudosamples.

Table 1 Number of bands of each individual for different probes

	Recovering populations (n = 60)				Original populations (n = 31)			
Probe	Per	33.6	pV47-2	Combined	Per	33.6	pV47-2	Combined
Range	3–8	6–22	6–15	17–42	2–12	3–22	4–12	15–38
Mean	5.6	14.1	9.9	29.6	6	14.4	8.4	29.4
Standard deviation	0.39	0.6	0.16	0.81	0.58	0.89	0.33	1.26

Call this mean BC*. Repeat this step 500 times for both the Coatesville and Huapai populations.

3 Build a probability density function (PDF) of mean BC* obtained from both Coatesville and Huapai.

4 If the observed mean BC between individuals of different sites falls outside a critical region of the PDF (set here at 5%), then the difference between within-population bandsharing and between-population bandsharing is significantly different from zero.

For within-population comparisons of females and their pouch young we modified the method as follows.

1 Calculate BC between each female and its pouch young within each study site. Include only one pouch young for each female to ensure that the data are independent.

2 Generate 500 pseudo-samples for females within each population and calculate BC for each pseudo-sample. Call this BC*.

3 Compare BC between females and their pouch young with BC* obtained from both study sites using the Komogorov–Smirnov test. If $P < 0.05$, then the difference in BC between female within population and female-pouch young is significantly different from zero at the 5% level.

We rejected the null hypothesis of no difference between populations when both FCM and IAM models resulted in significant differences. If only one model was significant, then we accepted a strong trend towards significance (Ardern *et al*. 1997a).

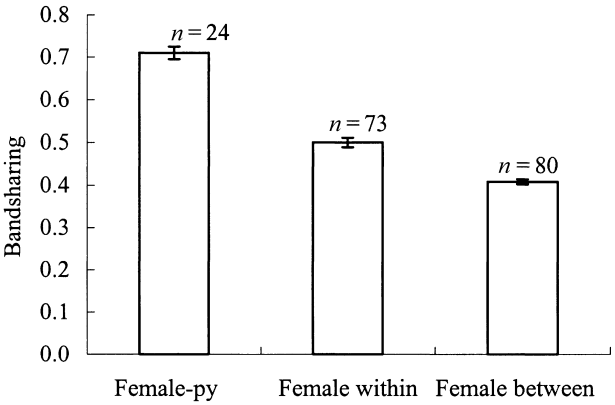

Fig. 3 Mean bandsharing (with standard error) of groups with different degrees of relatedness. Female-py: between females and their pouch young; female within: between females within a population; female between: between females from different study sites; n: number of pairwise comparisons.

Table 2 Results of statistical tests of bandsharing between possums of different degree of relatedness

Population compared	FCM	IAM	Statistical significance
Female-pouch young vs. vs. Female within population	ks = 2.64; $P < 0.001$	ks = 3.45 $P < 0.001$	***
Female within population vs. female between populations	$P < 0.001$	$P < 0.001$	***

ks, Komogorov-Smirnov statistic; ***significant.

Results

Minisatellite profiling

The average number of bands for each individual varied with different probes, being highest in 33.6 profiles and lowest in *per* (Table 1). The average number of bands of the three probes combined did not differ between individuals from the pre-removal Huapai population and those from the recovering Huapai population (Mann–Whitney test, $z = 0.14$, $P = 0.84$, $n = 22.42$).

Significant differences were detected in DNA profiles between groups expected to exhibit differences in relatedness (Fig. 3, Table 2) such that bandsharing was highest between females and their pouch young and lowest between females from different study sites (Table 2).

The mean bandsharing between females was higher than that between males (Table 3) in the pre-removal populations at both study sites. The results of statistical tests on the difference between male relatedness and female relatedness showed a strong trend toward significance at both study sites (Table 4). However, in the recovering populations at both study sites, the bandsharing between

Table 3 Band-sharing (and standard errors) between possums within the original (OP) and re-colonizing (RP) populations

Population	Site	Sex	Probes				No. of pairwise comparisons
			33.6	pV47-2	*per*	combined	
Huapai	OP	M	0.466 (0.016)	0.366 (0.016)	0.354 (0.017)	0.409 (0.010)	78
		F	0.525 (0.017)	0.328 (0.021)	0.560 (0.020)	0.476 (0.012)	36
	RP	M	0.586 (0.012)	0.350 (0.020)	0.716 (0.014)	0.543 (0.009)	66
		F	0.577 (0.017)	0.363 (0.032)	0.453 (0.035)	0.497 (0.015)	28
Coatesville	OP	M	0.505 (0.019)	0.279 (0.044)	0.483 (0.033)	0.423 (0.018)	21
		F	0.546 (0.014)	0.383 (0.019)	0.589 (0.019)	0.491 (0.01)	55
	RP	M	0.567 (0.013)	0.416 (0.017)	0.631 (0.017)	0.525 (0.009)	48
		F	0.557 (0.018)	0.357 (0.021)	0.596 (0.024)	0.512 (0.015)	45

Table 4 Statistical significance of difference in relatedness within and between possum populations

Population compared	Study site	*P*-values		Statistical significance
		FCM	IAM	
Female-male OP	Huapai	0.15	0.012	*
	Coatesville	0.16	0.016	*
Female-male RP	Huapai	0.29	0.10	NS
	Coatesville	0.51	0.37	NS
Female OP-female RP	Huapai	0.66	0.45	NS
	Coatesville	0.76	0.67	NS
Male OP-male RP	Huapai	0.0009	0	***
	Coatesville	0.06	0.0004	*

OP, original population; RP, recovering population; NS, not significant; * strong trend towards significant; *** significant.

females did not differ significantly from that between males (Tables 3, 4).

Comparisons between the original and the recovering populations revealed that there were no significant differences in bandsharing between females (Table 4). However, the bandsharing among males in the recovering populations was significantly higher than among those of the original populations. There were no significant differences in the frequency distribution of bandsharing of females between the original and recovering populations (Huapai: $\chi^2 = 1.25$, d.f. = 1, $P > 0.1$; Coatesville: $\chi^2 = 0.45$, d.f. = 1, $P > 0.5$). However, the frequency distribution of bandsharing of males between the pre-removal and recovering populations was significantly different, with relatedness being higher among males in the new populations compared with those from the original populations (Huapai: $\chi^2 = 46.2$, d.f. = 1, $P < 0.001$; Coatesville: $\chi^2 = 11.98$, d.f. = 1, $P < 0.001$) (Fig. 4).

Paternity

The profiles of all three probes were used for the identification of paternity. Of the 31 pouch young examined, paternity of 19 pouch young (14 at Coatesville and five at Huapai) could be assigned to specific males to the exclusion of all others. The probabilities of false inclusion of males were low (< 0.05) for most of the sires (Table 5). Among the pouch young recorded in the first year after the depopulation, only one of six in Coatesville and none of six in Huapai could be assigned to a male that we caught. However, paternity could be assigned in all 13 pouch young from Coatesville and six of the seven pouch young from Huapai caught in the second and third years after the depopulation (Table 5).

A large proportion of males recorded in the pre-removal (13 of 20 at Coatesville and seven of 23 at Huapai; Sarre *et al*. 2000) and recovering (five of 11 males at Coatesville and nine of 12 at Huapai) populations were not recorded as sires (Fig. 5). In the recovering populations, the numbers of young sired by each individual male were unevenly distributed (Table 6), but not significantly different from a Poisson (random) distribution ($\chi^2 = 7.87$, $0.1 > P > 0.05$). Those males for which multiple paternity was recorded, either within or between years, in the recovering populations, sired different young with different females (Table 5), indicating that as with studies conducted on stable populations, the mating system is polygynous with no evidence of pair bonding. There was no evidence that the paternity

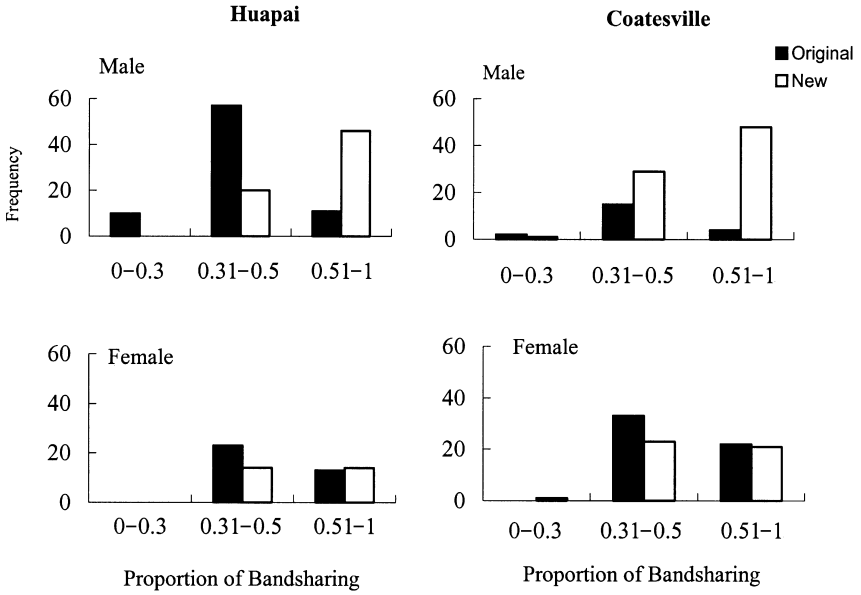

Fig. 4 Frequency distributions of within-population bandsharing in the recovering and the original populations at two study sites.

Table 5 Distribution of the paternal bands for all pouch young

Year	Mother	No. of paternal bands/py	Probability of false inclusion	Assigned sire
1997	6083	12	0.00008	5992
1998	5803	15	0.00006	5855
	5807	13	0.001	5855
	5834	14	0.00002	5855
	5869	12	0.0008	5880
	5877	8	0.006	5882
	5896	7	0.008	5882
	5811	5	0.045	5890
	5861	11	0.0008	5890
	5851	6	0.001	5992
1999	5824	13	0.006	5817
	5861	12	0.001	5826
	5869	8	0.003	5826
	5830	7	0.175	5859
	5801	8	0.017	5882
	5807	11	0.0002	5890
	5811	7	0.007	6910
	5811	8	0.009	6910

Only those pouch young a father could be assigned are shown. Paternal bands are defined as those present in the pouch young but absent in the mother.

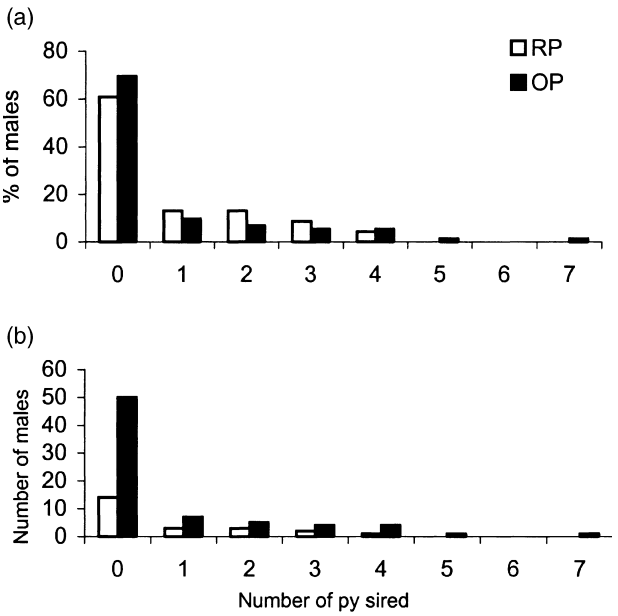

Fig. 5 Percentage (a) and frequency (b) distributions of paternity in the original (OP) and recovering (RP) populations.

distributions in the recovering populations, which had lower densities, were different from those of the original populations (Fig. 5; Fishers Exact test $P = 0.65$, data from two sites are pooled — recovering population: $\chi^2 = 7.2$, d.f. = 5, $0.25 > P > 0.1$; pre-removal population: $\chi^2 = 1.1$, d.f. = 5, $P > 0.95$).

Discussion

Relatedness and dispersal

The research presented here shows clearly that minisatellite DNA profiling can discriminate successfully between groups of possums of varying degrees of relatedness, with the highest bandsharing between closely related individuals (mothers and their pouch young) and the lowest between

Table 6 Paternity of possum pouch young in the recovering populations at Coatesville and Huapai as assigned through unattributable paternal bands

		Male					
Year	Female	5880	5890	5855	5992	5826	5910
Coatesville							
1997	6083				X		
1998	5803			X			
	5807			X			
	5811		X				
	5869	X					
	5861		X				
	5851				X		
	5834			X			
1999	5807		X				
	5803		X				
	5811						XX*
	5869					X	
	5861					X	
Huapai							
		5882	5859	5817			
1998	5896	X					
	5877	X					
1999	5801	X					
	5824			X			
	5830		X				

*Twins.

individuals from different locations. Thus, like a number of other minisatellite DNA profiling studies (e.g. Reeve *et al*. 1992; Finch & Lambert 1996; Marfori *et al*. 1997; Millar *et al*. 1997), the relatedness among individuals as estimated by minisatellite DNA profiling can be used to make inferences about aspects of social structure. Of most significance here are the insights that can be gained into the nature of dispersal in this species.

The differences between the relatedness of males and that of females before the depopulation showed strong trends towards significance at both study sites. Therefore we accept that average genetic similarity between males was lower than that between females. Such a result indicates that in a stable possum population, more males come from different sources than females. There are three possible causes of this outcome. Firstly, male-biased dispersal, where more males than females emigrate from their natal area so that the immigrants to a local population are predominantly males. Secondly, female-biased mortality among emigrants. In this case, more females than males die during the dispersal so that although dispersal is not sex-biased, the immigrants of a local population are predominantly males. Thirdly, sex bias in dispersal distance. In this case, males would disperse further than females so that in a local population, male immigrants come from more remote sources than female immigrants. Previous demographic studies of possums indicate that males disperse more than females (Dunnet 1964; Clout & Efford 1984; Efford 1991), and females tend to settle within or adjacent to their maternal home range (Crawley 1973; Clout & Efford 1984). Field studies have also shown that although females disperse much less frequently, they tend to move further and make more moves before settling (Efford 1991). In addition, mortality among males is usually greater than that among females (Crawley 1973), making it unlikely that the observed differential levels of relatedness occur because of greater mortality among female dispersers. Therefore, the lower degree of relatedness among male than female possums observed in this study is most likely a result of male-biased dispersal.

Our data show that males and females did not differ from each other in their relatedness in the recovering populations, nor did the degree of genetic similarity between females in the recovering populations differ significantly from that in the pre-removal populations. However, the relatedness of males in the recovering populations was higher than that of males in the pre-removal population. This indicates that re-colonizers may have come mainly from the immediate surrounding area ('vacuum effect') and that such short-distance dispersal may be kin-biased. Among the eight possums which survived the removal trapping and were frequently caught thereafter, six (four males and two females) were animals caught only occasionally on the very edge of the trapping grid before the removal. This suggests that they were animals that had ranges only slightly overlapping the trapping grid before the removal and which had expanded/shifted following the removal. Some previous studies have suggested that the recovery of possum populations after control operations must include a 'vacuum effect', because the recovery from depopulation was faster than could be explained by *in situ* breeding and natal dispersal (Green & Coleman 1984). Other studies have indicated that initial recovery is primarily caused by natal dispersal because the re-colonizing possums were mainly young animals (Clout & Efford 1984; Green & Coleman 1984; Cowan & Tyndale-Biscoe 1997). In a radio-telemetry study of home-range changes by adjacent possums after a local depopulation operation, Efford *et al*. (2000) showed that 29% of possums within 100 m of the boundary of the depopulated area shifted their range centre at least 50 m towards the depopulated area. This indicates that in that study, local range shift was a significant factor in the rapid re-population of an area following control. No sexual differences were detected in the observed local range shift. The molecular data from the study described here suggest a response similar to that observed by Efford.

Mating system

The densities of the recovering populations at our study sites reached only one-third (at Huapai) and about half (at Coatesville) of the original densities 3 years after the removal (Ji, unpublished results). Our results indicate that the mating system in the recovering populations did not differ significantly from the polygynous promiscuous system observed in the original populations (Sarre *et al*. 2000). Although densities were much lower than in the original populations, there was no evidence that the distribution of the number of pouch young sired by males differed in the recovering populations, with a high proportion of males siring no pouch young and a small number of males siring a high number. A study on a possum population in New Zealand farmland (Taylor *et al*. 2000) showed a similar finding, that the distribution of number of offspring for males was L-shaped, with more than half of the mature males fathering no pouch young.

Whether the polygynous mating system found in New Zealand possum populations with different densities is also representative of this species in Australia is unknown. The results of the study described here and of those by Sarre *et al*. (2000) and Taylor *et al*. (2000) did not confirm field observations by Winter (1976) in a low-density possum population in Queensland, Australia. Winter suggested that a temporary monogamous mating system developed where males formed a 'consort' relationship with females before and during the mating period, involving mate defence by males. The densities of possum in Australia (0.44–2.16/ha, reviewed by Green 1984) are much lower than those found in New Zealand broadleaf/podocarp forest and farmland [ranging from 2.5/ha in the recovering populations at our study sites to as high as 25.4/ha in established populations (Coleman *et al*. 1980)]. This marked difference in population density, as well as in habitat structure, may result in a difference in mating systems. Such differences have been found in other mammals. For example, the mating system of European rabbits (*Oryctolagus cuniculus*) varies from largely monogamy to polygyny/promiscuity, depending on habitat structure (Cowan & Garson 1985).

Implications for management

Any virus-vectored biological control methods will depend largely on the contact rates between animals (Barlow 1994). The polygynous mating system of possums will facilitate the spreading of such control agents. Our findings indicate that this mating pattern, in which both females and males are promiscuous, did not change when the density was markedly reduced by conventional removal control. Our results also showed no mate fidelity within 3 years after the depopulation operation. Taylor *et al*. (2000) found that sequential mating of a female with the same male was uncommon even in the same year when a second oestrous was induced by removal of pouch young. These findings suggest that rate of spread for biological control agents will not be largely affected by density reduction. This will also facilitate the integrated management involving both conventional removal control and biological control (Bayliss 1999).

Conclusion

In this analysis of relatedness among possums in established and re-colonizing populations, we have determined that minisatellite DNA profiling can provide sensitive tests for two critical demographic characteristics: sex bias in dispersal and the proximity of re-colonizers following population control. We have shown that in established populations where control has been absent for some years, possums exhibit clear evidence of female philopatry in contrast to the patterns exhibited by males. The ability to detect such differences is an important component in establishing the generality of sex-biased dispersal behaviour in possums, which in turn is important in determining the applicability of possum control measures. Although not quantitative, the minisatellite tests described here can be used for single sample tests for sex-biased dispersal, negating the need for time (and personnel) intensive, mark–recapture and radiotelemetry field studies. It is also clear from our data that the re-colonizers of control populations at both study sites were derived largely from the expansion of adjacent individuals into the depopulated area. This confirms field observations that long-distance, male-based dispersal was not a critical factor in the initial stages of the re-population process. If such trends were found to be the normal behavioural response of possums to a depopulation event, then the control area required to protect a core area of high conservation value need not be large in the short term.

In addition, our analysis of paternity in the post-removal populations demonstrates clearly that the mating system of possums does not vary significantly with reductions in density. This lack of density dependence in mating systems is important because it indicates that the sexual contact rate, a characteristic that is integral to the success of sexually transmitted biological control agents (Barlow 1994), may not be greatly affected by reductions in population density. Thus, models of possum control by sexually transmitted diseases (not including those that affect fertility) need not include density-dependent effects on mating systems.

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The study presented in this paper is part of a multidisciplinary project investigating the behavioural response of possums to density reduction. We have combined the molecular methods, described in this paper, with field experiments using mark–recapture and radio-telemetry techniques. Weihong Ji is currently a postdoctoral fellow on possum behaviour. Stephen Sarre and Niccy Aitken are members of the Molecular Ecology Research Group at Massey University. Robin Hankin is a lecturer with an interest in statistical analysis and Mick Clout is an Associate Professor in Ecology at the University of Aukland.
