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Variability and differentiation of microsatellites in the genus *Dasyurus* and conservation implications for the large Australian carnivorous marsupials

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Abstract

All four species of Australian quolls (*Dasyurus* species) have declined since European settlement in terms of both range and population numbers. Six highly polymorphic simple sequence repeats (CA_n microsatellites) were used to estimate the genetic variability and population differentiation within and among twenty populations (including museum specimens from six populations), as a preliminary means of assessing population conservation status and relative levels of variability within members of the genus. Overall mean expected heterozygosity (H_E) and corrected allelic diversity (A') were highest among western quolls. Northern quolls, eastern quolls, and tiger quolls were not significantly different from each other in either measure. There were also significant differences in diversity among populations within species. Genetic differentiation was estimated by a number of methods and showed that the microsatellites used here were useful for defining differences both among species and populations. Allele frequency data were summarised by two-dimensional MDS, which was able to partition populations into distinct species clusters. Similarly, the assignment test was able to assign most individuals to both the correct species and population levels. Results of MDS and the assignment test may prove useful in forensic applications. Genetic distance and subdivision between pairs of populations were assessed by two means based on different mutation models for microsatellites: infinite alleles model (Nei's D, F_{ST}) and stepwise mutation model (Goldstein's $\delta\mu^2$, R_{ST}). Pairwise measures of population subdivision indicate that most populations should be conserved as separate management units. We discuss results of these analyses in terms of applications to conservation for each of the four Australian species of quoll and provide a genetic basis for future population monitoring in these species.

Introduction

Genetic variability or diversity has long been recognized as a key component of population and conservation genetics. The loss of genetic variation (either allelic diversity or heterozygosity), due to drift, inbreeding, or other factors can reduce both individual fitness and the ability of populations to adapt to altered environmental conditions (Lacy 1997). Inbreeding may cause decreased levels of heterozygosity in individuals. Inbreeding depression, as a result of close consanguineous matings, may decrease individual fitness via a number of routes (Gall 1987; Ralls et al. 1988; Frankham 1995; Newman and Pilson 1997). The long term viability of populations also may be threatened by the loss of genetic variation due to drift. Loss of variability may affect small populations by decreasing the ability to adapt to changing environments and by increasing the probability of extinction due to stochastic effects (Lacy 1997). Alternatively, relatively high levels of genetic diversity may increase qualities associated with fitness (Allendorf and Leary 1986).

Differentiation between both species and populations is another area of concern to conservationists, as this gives an indication of the evolutionary divergence

between taxa. For conservation purposes, it is vital to ensure that the species in question are indeed different enough that we can easily distinguish between them genetically. There are many examples now in the literature where taxa that are morphologically different are genetically similar and vice versa (e.g. Firestone et al. 1999). Also, lack of genetic differentiation between morphologically distinct species may indicate that the process of hybridization is occurring (e.g. Roy et al. 1994).

At the population level much debate has been focussed on determining what units to conserve, particularly given limited resources (Vane-Wright et al. 1991; Crozier 1992; Rojas 1992; Vogler and DeSalle 1994; Waples 1998). Moritz (1994) and Moritz et al. (1995) suggested that populations that are genetically divergent at both nuclear and mitochondrial loci should be conserved as separate units, i.e. evolutionarily significant units (ESUs) or management units (MUs). Several recent studies have applied these concepts to conservation of endangered taxa (e.g. Pope et al. 1996; Zhu et al. 1998; Firestone et al. 1999).

Analysis of microsatellites has become an important tool in population studies and is useful for estimating both variability and differentiation (e.g. Taylor et al. 1994; FitzSimmons et al. 1995; García-Moreno et al. 1996; Houlden et al. 1996; Brunner et al. 1998). Microsatellites are single-locus, biparentally inherited, and highly variable markers occurring throughout the genome (Tautz 1989). They contain short-length (usually di-, tri-, or tetranucleotide) repeat units which vary in the number of repeats between individuals (Tautz and Renz 1984; Tautz 1989). In addition, microsatellites have proven to be highly informative where other markers have yielded little information when applied to the same species (e.g. Paetkau and Strobeck 1994; Estoup et al. 1998; Goodman 1998).

The six species of quolls (*Dasyurus* spp.) are among the largest of the remaining carnivorous marsupials in Australia and Papua New Guinea. The four species of quolls found in Australia range in size from seven kilograms (some male tiger quolls) to less than 400 g (female northern quolls) (Strahan 1998). Like their placental counterparts, these large marsupial carnivores have faced declines in numbers and distribution throughout their ranges (Figure 1). The reasons for these declines are poorly understood, and are likely to be due to a number of interacting factors. Factors such as habitat loss, the introduction of feral predators and poisonous prey resources, altered fire regimes, disease susceptibility, and continued persecution by humans may all have played different roles in the decline of each species (Maxwell et al. 1996).

Each quoll species is considered to be threatened to some degree and each species has had a different history of decline. The western quoll (*Dasyurus geoffroii*) exhibited a population decline on a continental scale and survives only in one area of southwestern Western Australia (WA). Due to recent successful management efforts, this species has been downgraded from 'endangered' to 'vulnerable' by the World Conservation Union (IUCN) (Serena et al. 1991; Orell and Morris 1994; Maxwell et al. 1996). Eastern quoll (*D. viverrinus*) numbers were decimated around the turn of this century; they are currently presumed extinct on the mainland. This species persists in Tasmania, however, where population numbers are stable. Eastern quolls are currently designated as 'lower risk-near threatened' (IUCN listing; Maxwell et al. 1996). The decline of northern quolls (*D. hallucatus*) is a relatively recent phenomenon. Once widely distributed throughout the northern third of the continent, this species is now restricted to six main population centres (Braithwaite and Griffiths 1994) and is also currently designated as 'lower risk-near threatened' by the IUCN. The southern mainland subspecies of tiger quolls (*D. maculatus maculatus*) declined early this century, however unlike eastern quolls, tiger quolls persisted on the mainland where populations are scattered and numbers are low (Mansergh 1984). Tiger quolls also occur in Tasmania, where populations are thought to be naturally limited by competition from both Tasmanian devils (*Sarcophilus harrisii*) and eastern quolls (Jones 1995). Southern mainland tiger quolls are presently restricted to less than 50% of their range and are listed as 'vulnerable' by the IUCN. The northern mainland subspecies of tiger quoll (*D. m. gracilis*) is centred in a few small localities in north Queensland and is currently considered 'endangered' by the IUCN (Maxwell et al. 1996). Despite these widespread and precipitous declines, little attention has been given to genetic implications for conservation management of these taxa.

The applications of genetic management to quoll conservation are multifaceted. Captive management and breeding of western quolls is part of this species recovery plan and has been successful for a number of years (Orell and Morris 1994), yet determination of the level of diversity of the captive population has not been attempted. This is important in light of the

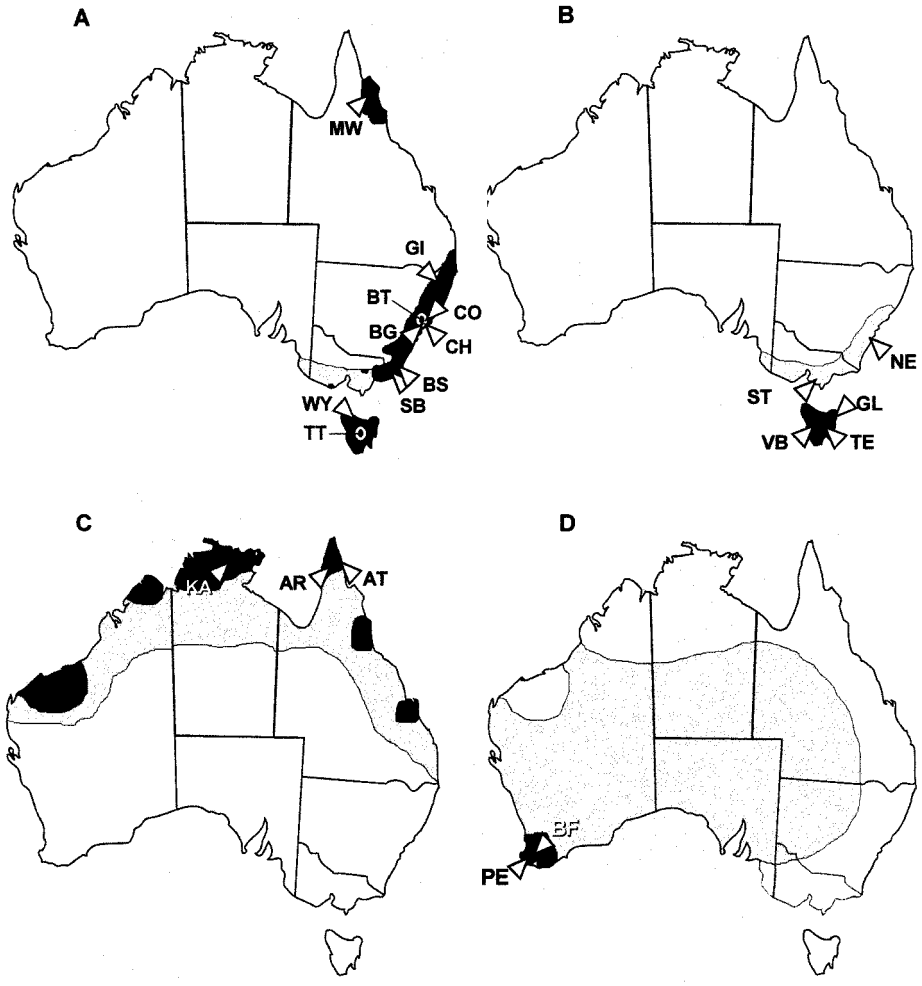

Figure 1. Current (black) and past (grey + black) distribution of the Australian quolls including sample sites. (A) *Dasyurus maculatus*, (B) *D. viverrinus*, (C) *D. hallucatus*, (D) *D. geoffroii*. A, B, and D redrawn from Strahan (1998); C redrawn from Braithwaite and Griffiths (1994). See Table 1 for key to population names.

reintroduction program taking place as part of this species recovery plan. In addition, there are plans to reintroduce eastern quolls into areas of their former range on the mainland; it is important to evaluate the genetic variation and differentiation between different stocks before reintroducing animals to areas where remnant populations might persist. Without sound knowledge of the genetic diversity within and between the remaining Tasmanian populations of eastern quolls, it is difficult to determine which populations are valuable sources for reintroductions. Analysis of the mitochondrial DNA control region and microsatellites has proven to be very useful in elucidating conservation units among tiger quolls (Firestone et al. 1999) and may be so for northern quolls.

We undertook this study to determine the relative levels of variability and differentiation present both among species and among populations within species, to provide baseline information regarding variability within populations of each species for future population monitoring, and to assist wildlife agencies and managers in making sound conservation decisions regarding these species. In particular, we test the following null hypotheses: (1) genetic diversity is the same among all populations within each species, (2) each species of quoll has the same genetic diversity, (3) there is no genetic differentiation between populations within each species, and (4) there is no genetic differentiation between species of quolls. We examined six highly polymorphic $(CA)_n$ microsatellite

markers and a total of 347 individuals, representing 20 populations and four species, as a means of assessing the genetic variability and differentiation in quolls and as a basis for future population monitoring and conservation breeding programs.

Methods

Study populations, DNA samples, and population screening

Tissue samples were collected from all Australian species of quolls representing 20 different populations (Figure 1, Table 1). Six of these sample populations (PE, AR, NE, ST, GL, and WY) were from dried skins held in museum collections; two populations (BF, GI) were from captive stock bred in captivity for a number of years; and four groups (TE, TT, NE, and BT) were from samples collected opportunistically and represent sites encompassing broader geographic areas. Samples were either fresh tissues (skin, blood, liver, or muscle) from live-trapped or road-killed individuals, or dried preserved skins from museum specimens. DNA from fresh tissues was extracted according to standard protocols by phenol-chloroform extraction followed by ethanol precipitation (Sambrook et al. 1989). DNA from museum specimens was extracted by a modified guanidine thiocyanate method (Boom et al. 1990; Hoss and Pääbo 1993). The microsatellite markers were derived from either a tiger quoll or a mixed tiger/eastern quoll genetic library. The isolation of these markers and amplification procedures used in this study have been described elsewhere (Firestone 1999).

Aliquots of the PCR products were mixed with an equal volume of formamide loading dye, heated to 80 °C, and loaded on a 6% polyacrylamide sequencing gel containing 50% w/v urea. Gels were fixed (10% glacial acetic acid/10% methanol) and dried, then exposed to autoradiographic film (X-OMAT, Kodak or Hyperfilm-HP, Amersham). Alleles were scored by comparison with a size marker (M13 sequence, USB) electrophoresed alongside the samples on each sequencing gel. Eighteen populations were typed at all six loci; ST was typed at four loci while AR was typed at five loci.

Statistical analyses

Deviations from Hardy-Weinberg equilibrium (HWE) were tested in each population by one of two methods as implemented in GENEPOP (Raymond and Rousset 1995): either complete enumeration for loci with up to four alleles (Louis and Dempster 1987) or by a Markov chain method for loci with five or more alleles (Guo and Thompson 1992). Genetic variability of species and populations was measured as the number of alleles per locus (A) and unbiased expected heterozygosity (H_E) for each locus using BIOSYS (Swofford and Selander 1981). Differences in mean H_E between species and populations were tested by analysis of variance (ANOVA; Sokal and Rohlf 1981) and post hoc hypotheses were examined using Scheffe's test. Empirical studies have shown that population size and level of genetic variability are positively correlated (Frankham 1996). Similarly, measures of genetic diversity are likely to be affected by small sample size (e.g. Roy et al. 1994); A more so than H_E (Nei et al. 1975; Bouzat et al. 1998). Sample sizes in this study ranged from three to 58 individuals per population, therefore, we could not discount the effects of limited sampling regimes in assessing allelic diversity. To compare the number of alleles in species that differed in sample size, we calculated the expected number of alleles in an infinite population by Monte-Carlo simulations (Roy et al. 1994). We selected individuals at random without replacement and calculated the cumulative number of alleles until all individuals had been sampled. This procedure was repeated 1000 times for each species and the mean and standard deviation of the number of alleles was calculated as a function of sample size. A quasi-Newton best fit curve was then applied to the means using the equation $y = \alpha x/(x + \beta)$ where y = number of alleles, and x = number of individuals. In this equation α and β are constants, where α represents the number of alleles in an infinite population. In addition, we employed residual analysis as a measure of corrected allelic diversity (A') using ANOVA and Scheffe's post hoc tests, to examine interspecific and interpopulation differences in number of alleles. The data were log-transformed prior to the analysis to accommodate nonlinearity and deviation from the normal distribution. To control for the effect of sample size, we have used residuals generated by linear regression of sample size versus number of alleles.

Genetic differentiation was assessed by a number of methods. First, the number of unique or private alleles found between populations or species may be seen as a measure of genetic differentiation; however similarly to the number of alleles, the number of *unique* alleles is also affected by the extent of the

Table 1. Species and populations sampled

Species	Location	Pop	Map reference East	Map reference South	Status	N	A
Tiger quoll	1. Mount Windsor, Qld	MW	145°02′	16°15′	Wild	12	2.5 (0.2)
Dasyurus maculatus	2. Glenn Innis, NSW	GI	152°04′	29°44′	Captive	5	2.7 (0.4)
	3. Copeland, NSW	CO	151°48′	31°59′	Wild	9	4.3 (0.5)
	4. Chichester State Forest, NSW	CH	151°31′	32°08′	Wild	12	4.7 (0.6)
	5. Barrington Guest House, NSW	BG	151°42′	32°09′	Wild	16	4.7 (0.5)
	6. Barrington Tops area, NSW	BT	Wide area		Wild	11	4.2 (0.9)
	7. Badja State Forest, NSW	BS	149°33′	36°07′	Wild	6	2.5 (0.3)
	8. Suggan Buggan, Vic	SB	148°22′	36°57′	Wild	3	2.5 (0.2)
	9. Wynyard, Tas	WY	145°44′	41°00′	Museum	32	4.3 (1.1)
	10. Central Tasmania	TT	Wide area		Wild	11	3.8 (0.6)
Eastern quoll	11. New South Wales	NE	Wide area		Museum	25	7.0 (1.4)
Dasyurus viverrinus	12. Studley Park, Vic	ST	145°01′	37°48′	Museum	13	2.8 (0.5)*
	13. Gladstone, Tas	GL	148°01′	40°58′	Museum	58	4.8 (0.8)
	14. Vale of Belvoir, Tas	VB	145°53′	41°32′	Wild	21	3.5 (0.8)
	15. Central Tasmania	TE	Wide area		Wild	14	3.7 (0.6)
Northern quoll	16. Kakadu National Park, NT	KA	132°12′	12°44′	Wild	26	9.0 (1.7)
Dasyurus hallucatus	17. Archer River, Qld	AR	142°09′	13°35′	Museum	9	3.6 (0.7)§
	18. Atherton Tableland, Qld	AT	145°35′	17°03′	Wild	6	4.0 (1.0)
Western quoll	19. Perth area, WA	PE	116°10′	32°12′	Museum	23	8.8 (0.8)
Dasyurus geoffroii	20. Batalling State Forest, WA	BF	116°13′	33°14′	Captive	35	9.2 (0.7)

N = number of individuals sampled per population; A = mean uncorrected allelic diversity, standard errors in parentheses. *Four loci analysed. §Five loci analysed. All other populations typed at all six loci.

sampling regime. When closely related species are compared, the number of unique alleles found within each species is a measure of genetic distinction. However, this is strongly influenced by the sample size and geographic scope of the sampling within each taxon. We calculated the expected number of unique alleles for each species in comparison with another species, given different sample sizes, using Monte-Carlo simulations as above.

Second, we summarized allele frequencies for populations into two dimensions using multidimensional scaling (MDS), which makes few assumptions of the structure of the data. MDS analysis was performed using a convergence factor of 0.005 and 50 iterations as implemented in STATISTICA (Statsoft, Inc.). Finally, genetic distances and population subdivision among all pairwise comparisons of populations were estimated using methods based on both the step-wise mutations model (SMM) and the infinite alleles model (IAM), since neither mutation model is strictly correct for microsatellites (Primmer et al. 1998). Thus we employed Goldstein's $\delta\mu^2$ distance (SMM) (Goldstein et al. 1995) and Nei's unbiased genetic distance (Nei's D; IAM) (Nei 1972). Subdivision among populations was estimated by both R_{ST} (SMM) (Slatkin 1995; Goodman 1997) and F_{ST} (IAM) (Wright 1951), since R_{ST} includes allele size information while F_{ST} is based only on genetic drift. A Mantel procedure was used to test for correlation both between $\delta\mu^2$ and D and between R_{ST} and F_{ST}. Nei's D was further used to construct a neighbor-joining tree as implemented in the NEIGHBOR program in PHYLIP (Felsenstein 1995). Bootstrap analysis was done by first generating 1000 distance matrices using MICROSAT (Minch et al. 1998); 1000 bootstrapped neighbor-joining trees were then constructed using the NEIGHBOR program and summarized by the CONSENSE program in PHYLIP (Felsenstein 1995). Significance of all pairwise F_{ST} values was assessed by 10,000 iterations as implemented in FSTAT (v. 2.8) not assuming HWE (Goudet 1999). Furthermore, Mantel tests were performed to assess the relationship of genetic differentiation between populations (F_{ST}) to that of geographic distance.

Lastly, an assignment test (available from www.biology.ualberta.ca/jbrzusto/Doh.html) was performed to determine how characteristic an individual's genotype was of both the species and population from which it was sampled (Paetkau et al. 1995; Paetkau et al. 1998). The expected frequency of each individuals' genotype was calculated at both the species and population levels and assigned to the species or population for which the expected frequency was greatest. All frequencies were adjusted to avoid zeros using the method of Titterington et al. (1981).

Results

Genetic variability of microsatellites in quolls

The six microsatellite loci used in this study were highly polymorphic in all species examined, with 14–23 total different alleles per locus (Appendix A). Uncorrected mean A per population ranged from 2.5 (MW, BS, and SB, tiger quolls) to 9.2 (BF, western quolls) and mean H_E ranged from 0.469 (BS, tiger quolls) to 0.883 (PE, western quolls). The samples analysed in both GI and WY populations were monomorphic at a single locus each (locus 1.3 and 3.3.1, respectively); the samples analysed from the AT population were monomorphic at two loci, 1.3 and 4.4.10. Allele frequency distributions were highly skewed at each locus, generally with two or three common alleles and many rare alleles.

Deviations from Hardy Weinberg equilibrium

Some loci deviated from HWE proportions in eleven of the twenty populations. All six loci in the BF population deviated from HWE. Deviations were also found in three loci from NE (1.3, 3.1.2, 4.4.2), and GL (3.1.2, 3.3.1, 3.3.2) populations; two loci from each of TE (3.3.2, 4.4.2), KA (3.1.2, 3.3.2) and PE (1.3, 3.3.1) populations; and one locus from each of the MW (1.3), BS (3.1.2), WY (4.4.2), ST (3.3.1), and VB (3.3.2) populations. No locus was prevalent in deviations from HWE.

We also examined overall HW proportions, combined over all loci, for each population. Of all tiger quoll populations, only one (WY) was significantly deviant from overall HWE at all six loci (χ^2 = 24.2, P = 0.007); this was due to a general heterozygote deficiency. Among eastern quoll populations NE, ST, GL and VB were all significantly different from genotype proportions expected under HWE, over all loci. Significant deviation of GL (χ^2 infinity, P highly significant) was due to heterozygote excesses at two loci (3.3.1, 3.3.2) and heterozygote deficits at one locus (3.1.2). All other significant differences in eastern quolls were due to heterozygote deficits (NE, χ^2 infinity, P highly significant; ST, χ^2 = 19.9, P = 0.0029; VB, χ^2 = 28.2, P = 0.0052). Among northern quolls, KA was the only population significantly different from overall HWE proportions (χ^2 = 35.3, P = 0.0004); this deviation was due to heterozygote deficits. In addition, neither western quoll population was in HWE proportions. Overall differences from HWE in the BF and PE populations (BF, χ^2 = 78.2, $P < 0.0001$; PE, χ^2 infinity, P highly significant) were due to heterozygote deficiencies.

Genetic diversity among species

Monte Carlo simulations of the estimated cumulative alleles for each species are shown in Figure 2. The cumulative number of alleles begins to asymptote between 10–20 samples for most species except for *D. viverrinus*, which begins to asymptote after approximately 25 individuals are sampled. These simulations indicate that our sampling regime was adequate in picking up a substantial proportion of alleles present for most populations, although in populations where only a few individuals were sampled the number of alleles is underestimated.

Uncorrected allelic diversity (A) is shown in Table 1. In general, western quolls had higher numbers of alleles than any other species, while tiger quolls had lower allelic diversity than other species. Measures of genetic diversity after correction for sample size differences are shown in Figure 3. Significant differences were found among species in both corrected allelic diversity (A′; ANOVA F = 10.59; $P \leq 0.0001$) and mean H_E (ANOVA F = 5.70, P = 0.001) (Figure 3a). Post hoc tests revealed that H_E was significantly higher in populations of western quolls than in any other species; western quolls also had a significantly higher allelic diversity than either tiger quolls or eastern quolls, but were not significantly different to northern quolls. Eastern, tiger, and northern quolls were not significantly different from each other in either A′ or mean H_E.

Genetic diversity among populations within species

When each species was examined separately, significant differences in A′ were found among popula-

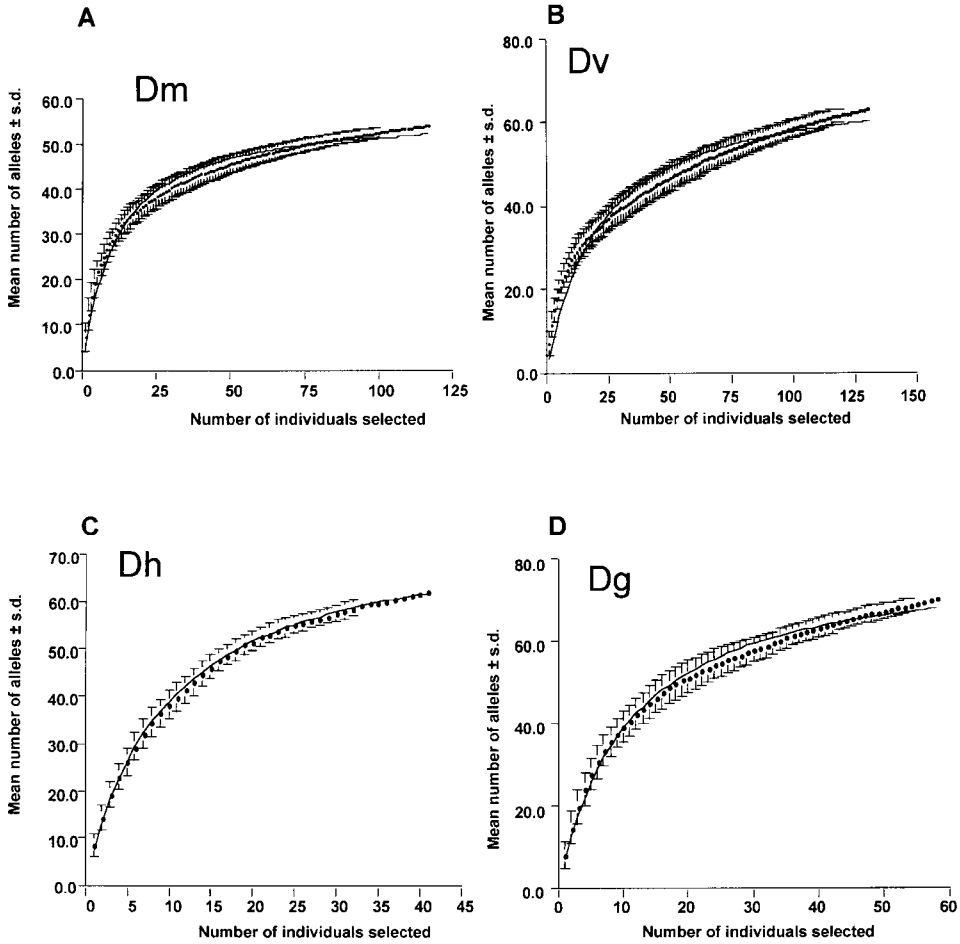

Figure 2. Estimated cumulative number of alleles per sample size for each species by Monte Carlo simulation. Curves were fitted using the equation $y = \alpha\, x/(x + \beta)$ where y = number of alleles and x = number of individuals. In these equations α represents the number of alleles in an infinite population. (A) *D. maculatus*, $\alpha = 57.0$, $\beta = 10.87$, $r^2 = 0.979$; (B) *D. viverrinus*, $\alpha = 69.70$, $\beta = 20.88$, $r^2 = 0.965$; (C) *D. hallucatus*, $\alpha = 76.11$, $\beta = 9.54$, $r^2 = 0.999$; (D) *D. geoffroii*, $\alpha = 80.23$, $\beta = 10.61$, $r^2 = 0.993$. Note that y-axis scales are different between species.

tions of all species except western quolls (Figure 3b). Among tiger quoll populations, GI and SB had significantly lower A′ than MW, BG, TT, CH, and BT (ANOVA $F = 9.83$, $P < 0.0001$). Among eastern quoll populations, GL had significantly higher allelic diversity than TE, NE, and ST; furthermore, ST had significantly lower allelic diversity than GL, VB, and TE (ANOVA $F = 14.18$, $P < 0.0001$). Among northern quoll populations, KA had a significantly higher number of alleles than either AR or AT (ANOVA $F = 11.01$, $P < 0.001$). There were no differences in allelic diversity between the two western quoll populations (unpaired *t*-test, $t = 0.31$; $P = 0.76$).

Significant differences in levels of H_E also were found among eastern quoll populations (ANOVA $F = 2.88$, $P = 0.045$) and western quoll populations (ANOVA $F = 8.77$, $P = 0.014$), however post hoc tests of eastern quolls indicated that there were no significant pairwise differences. Among western quoll populations, PE had significantly higher H_E than BF (Figure 3b).

Genetic differentiation among species: unique alleles

Each species possessed only a subset of the total alleles found in the genus (Appendix A). While there was great overlap among species in the alleles present (e.g. alleles 101–107 were found in all four species at locus 3.3.1), there was also substantial partitioning at each locus (e.g. at locus 3.3.1, alleles 91, 93, 97, and

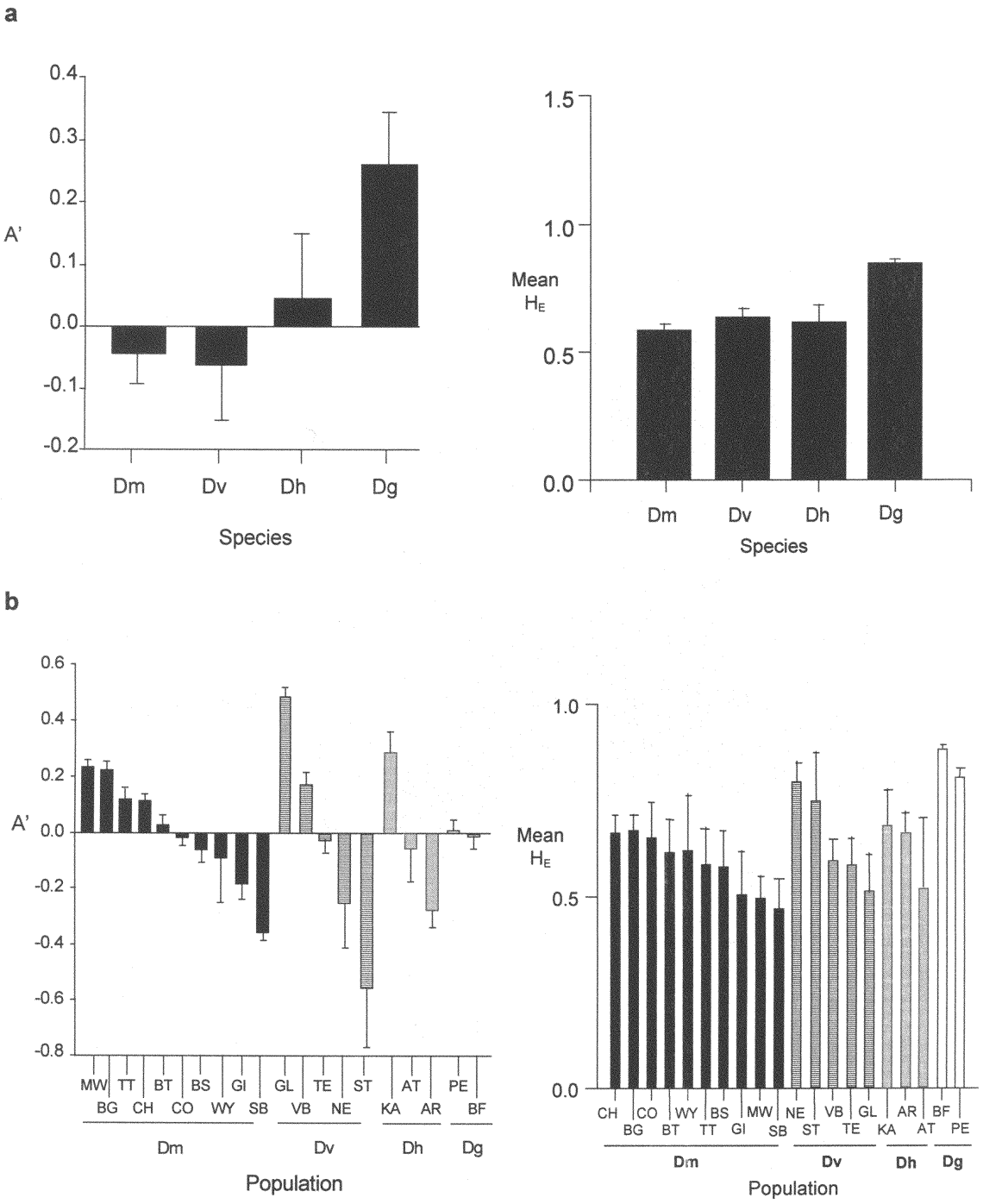

Figure 3. Mean and standard error of corrected allelic diversity (A′) and expected heterozygosity (H_E) at six microsatellite loci (a) among species and (b) within species of quolls. Horizontal bars indicate significant differences among groups by Scheffe's post hoc test. Dm = *D. maculatus*, Dv = *D. viverrinus*, Dh = *D. hallucatus*, Dg = *D. geoffroii*. See Table 1 for key to population names.

Table 2. The number and percentage (in parenthesis) of unique alleles between four species of quolls

	Dm	Dv	Dh	Dg
Dm (54)	–	14 (25.9)	20 (37.0)	20 (37.0)
Dv (63)	23 (36.5)	–	22 (34.9)	22 (34.9)
Dh (62)	28 (45.2)	21 (33.9)	–	26 (41.9)
Dg (70)	36 (51.4)	29 (41.4)	34 (48.6)	–

Total number of alleles in each species is in parenthesis beside the species name at the left column of the table. All 20 populations were included.

117 were unique to northern quolls, whereas alleles 127–145 were found only in western quolls). The effect of our sampling regime on the number of unique alleles observed among species was examined and the proportion of unique alleles estimated by Monte Carlo simulations is shown in Figure 4 and Table 2. In general *D. geoffroii* had the highest number of unique alleles in relation to all other species, whereas *D. maculatus* had the lowest number of unique alleles in relation to all other species. After approximately 30–40 individuals of most species had been sampled the graphs begin to asymptote (e.g. Figure 4a, c, d) indicating that the proportion of unique alleles found in relation to other species has peaked. *D. viverrinus*, however, may require additional samples to reach this asymptote (Figure 4b).

Genetic differentiation among species: allele frequencies

Genetic differentiation was also examined using allele frequency data. The differences in allele frequencies among species and populations of quolls were summarized using MDS (Figure 5) which showed that populations within species generally clustered closely in two-dimensional space. Furthermore, these data fit in two dimensions with low stress (0.10) and with a high proportion of the variance accounted for (r^2 = 0.94), indicating a good fit of the data in multidimensional space.

Genetic differentiation among populations within species: population subdivision

Pairwise genetic differentiation measures among populations were also estimated by both F_{ST} and R_{ST}. A Mantel test showed that F_{ST} and R_{ST} values were highly correlated (r = 0.72; $P \leq 0.001$); therefore only F_{ST} values are shown in Table 3. All pairwise comparisons of F_{ST} values were tested for significance. Most pairwise comparisons showed significant population subdivision both between populations and between species (Table 3). An exception was found among populations of tiger quolls, particularly among the four populations from the Barrington area. None of these pairs of populations showed significant subdivision. Additionally, the SB population was not differentiated from most other populations, but this is probably due to the very low sample size for this population.

Genetic differentiation among populations within species: genetic distance measures

A Mantel test of Nei's D and Goldstein's $\delta\mu^2$ indicated that these distance measures were significantly correlated (r = 0.64; $P \leq 0.001$). We therefore present pairwise values of Nei's D only (Table 3). Examination of both genetic distances among tiger quoll populations revealed that MW was consistently the most distant from all other tiger quoll populations. Lowest genetic distances were found among the four geographically close populations of tiger quolls (CO, CH, BG, BT) by Nei's D; the results for $\delta\mu^2$ were not consistent with Nei's D, however (data not shown). Among eastern quolls, both Nei's D and $\delta\mu^2$ values indicated that the Tasmanian populations (GL, VB, TE) were closer to each other than to the mainland populations (NE and ST; data not shown). Nei's D also showed that the two northern quoll populations (KA and AT) were more closely related to each other than either was to any other population.

Nei's D was used to build a neighbour joining tree among 15 populations of quolls (Figure 6). These data show that all species form their own clades, although bootstrap support is very low in many cases. Similar to trees based on mtDNA sequences, northern quoll populations are the most distant from other populations based on microsatellites and thus form an early split. The topology of this tree, however, is not consistent with those based on mitochondrial DNA loci (Krajewski et al. 1997; Firestone, in press).

Geographic distance vs. F_{ST}

The relationship of genetic subdivision (F_{ST}) to geographic distance was examined by a Mantel test within each of the three species in which more than two populations were available. There was a significant correlation between geographic distance and F_{ST} for tiger quoll populations (r = 0.615; P = 0.01), indicating that distance explains a substantial amount of the genetic variance observed between populations of

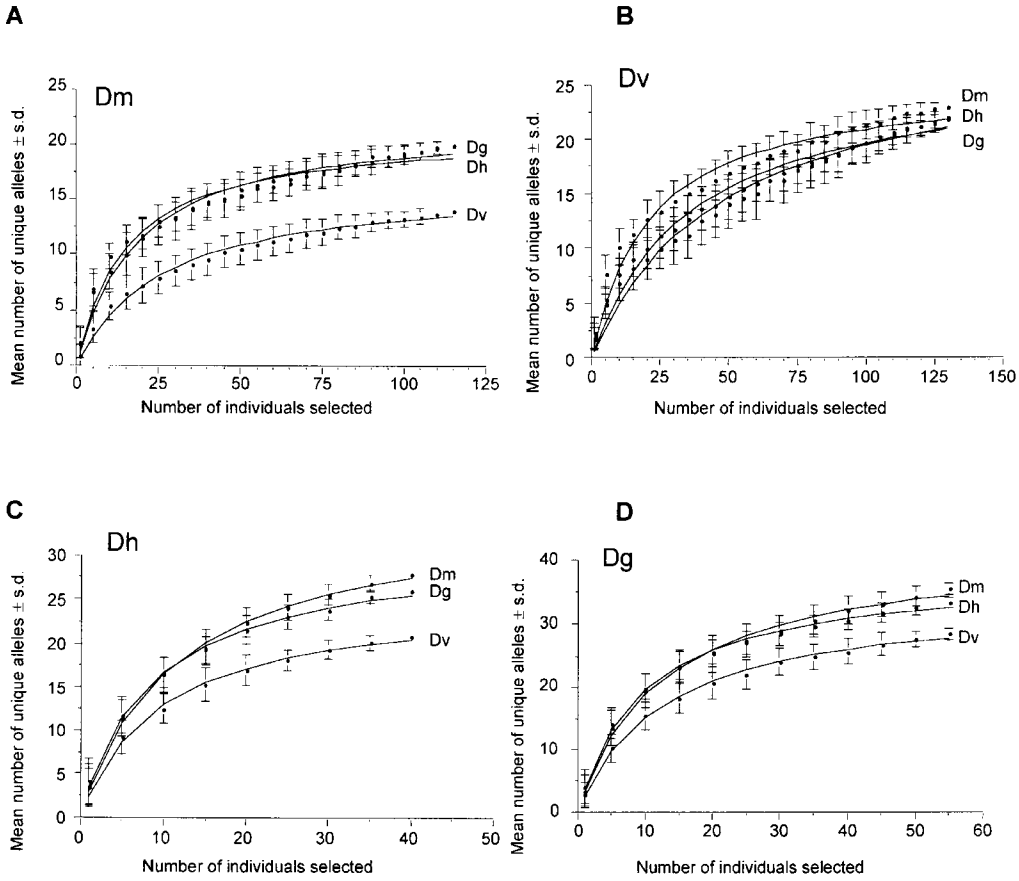

Figure 4. Estimated number of unique alleles for each species by Monte Carlo simulations. Curves were fitted using the equation $y = \alpha x/(x + \beta)$ where y = number of alleles and x = number of individuals. In these equations α represents the number of unique alleles in an infinite population. (A) No. of unique alleles in Dm compared with Dg [α = 22.32, β = 18.51, r^2 = 0.98], Dh [α = 21.22, β = 14.93, r^2 = 0.96], and Dv [α = 16.63, β = 26.44, r^2 = 0.99]. (B) No. of unique alleles in Dv compared with Dm [α = 25.22, β = 20.74, r^2 = 0.95], Dh [α = 26.68, β = 35.72, r^2 = 0.97], and Dg [α = 28.86, β = 48.22, r^2 = 0.97]. (C) No. of unique alleles in Dh compared with Dm [α = 35.02, β = 11.09, r^2 = 0.99], Dg [α = 30.70, β = 8.32, r^2 = 0.99], and Dv [α = 25.46, β = 9.70, r^2 = 0.99]. D) No. of unique alleles in Dg compared with Dm [α = 42.05, β = 12.19, r^2 = 0.99], Dh [α = 38.14, β = 9.40, r^2 = 0.99], and Dv [α = 34.36, β = 12.64, r^2 = 0.99]. Note that y-axis scales are different between species.

this species. This correlation did not hold for either eastern quolls (r = 0.603; P = 0.06) or northern quolls (r = 0.837; P = 0.16). It should be noted, however, that the range of geographic distances and the number of populations available were low in these two species in relation to that of tiger quolls.

Assignment tests

Results from the assignment test show that 252 (98.4%) individuals were correctly assigned to their true species (Table 4). Only 4 animals (1.6%) were misassigned at the species level, each of which was only partially genotyped: one *D. maculatus* individual was misassigned as *D. geoffroii*; two *D. viverrinus* were misassigned as either *D. maculatus* or *D. hallucatus*; and one *D. geoffroii* was misassigned as *D. maculatus*. Results of population assignments showed that 211 (82.4%) animals were correctly assigned to their source populations (Table 5). Of the animals that were misassigned, 33 (12.9%) were misassigned to a geographically close population; 8 (3.1%) were misassigned to more distant populations within the same species and 4 animals (1.6%) were misassigned to a different species. Three of the four individuals misassigned to different species at the population level were the same as those misassigned at the species level (Table 4).

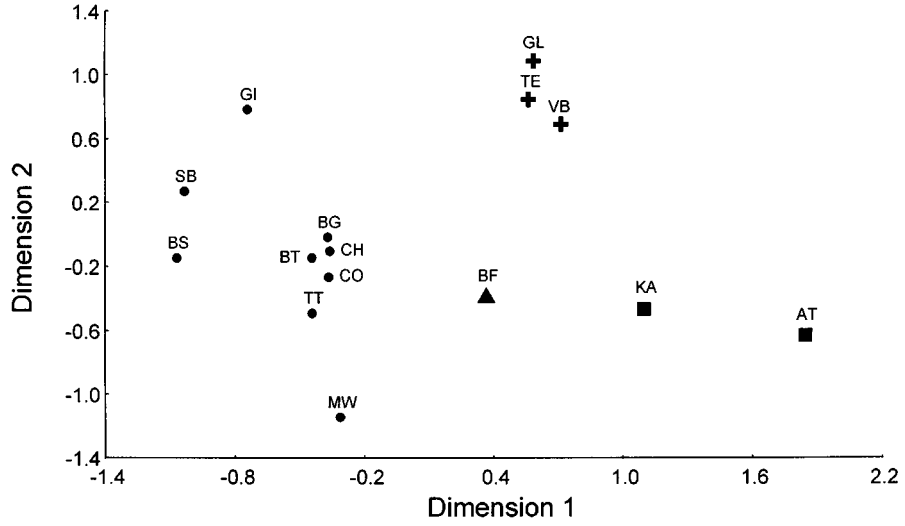

Figure 5. MDS based upon Euclidean distances among 15 populations of quolls. (●) *D. maculatus*, (+) *D. viverrinus*, (■) *D. hallucatus*, (▲) *D. geoffroii*. Stress = 0.10, Rsq = 0.94.

Table 3. Pairwise comparison of Nei's D (below the diagonal) and F_{ST} (above the diagonal)

Species/population	DM									DV			DH		DG
	MW	GI	CO	CH	BG	BT	BS	SB	TT	GL	VB	TE	KA	AT	BF
Tiger quoll															
MW	–	0.445*	0.156*	0.221*	0.221*	0.275*	0.399*	0.429*	0.304*	0.451*	0.385*	0.389*	0.378*	0.486*	0.242*
GI	1.59	–	0.242	0.183*	0.169*	0.218*	0.310	0.207	0.327*	0.377*	0.342*	0.351*	0.361*	0.472	0.256*
CO	0.27	0.64	–	0.011	0.047	0.000	0.167*	0.184*	0.125*	0.393*	0.327*	0.322*	0.313*	0.385*	0.161*
CH	0.48	0.42	0.03	–	0.029	0.019	0.117*	0.136	0.156*	0.376*	0.319*	0.312*	0.309*	0.374*	0.174*
BG	0.51	0.39	0.11	0.07	–	0.058	0.143*	0.086	0.156*	0.356*	0.307*	0.299*	0.299*	0.370*	0.185*
BT	0.64	0.46	0.01	0.03	0.12	–	0.124*	0.110	0.128*	0.393*	0.332*	0.331*	0.323*	0.394*	0.196*
BS	1.01	0.60	0.32	0.20	0.27	0.20	–	0.117	0.257*	0.441*	0.390*	0.396*	0.370*	0.462	0.272*
SB	1.76	0.36	0.50	0.33	0.20	0.23	0.16	–	0.222	0.411*	0.347*	0.356	0.329*	0.432	0.250*
TT	0.69	0.97	0.25	0.36	0.38	0.26	0.53	0.54	–	0.416*	0.350*	0.349*	0.340*	0.425*	0.221*
Eastern quoll															
GL	1.80	0.97	1.66	1.51	1.31	1.52	1.54	1.46	1.71	–	0.222*	0.109*	0.384*	0.440*	0.329*
VB	1.50	1.19	1.63	1.57	1.44	1.55	1.64	1.59	1.56	0.41	–	0.078*	0.323*	0.385*	0.273*
TE	1.40	1.17	1.46	1.39	1.29	1.43	1.53	1.56	1.43	0.14	0.15	–	0.329*	0.399*	0.269*
Northern quoll															
KA	2.83	3.08	3.00	2.99	2.47	2.88	2.69	2.83	2.91	2.12	2.03	2.12	–	0.223*	0.225*
AT	3.53	2.92	2.54	2.48	2.65	2.28	1.98	2.38	2.93	1.76	1.82	1.90	0.67*	–	0.266*
Western quoll															
BF	1.04	1.65	0.81	0.97	1.11	1.05	1.65	2.73	1.20	2.21	2.51	2.28	2.08	1.93	–

Shaded areas denote major groups. Asterisks indicate significant differentiation between pairs of populations after Bonferroni correction for multiple comparisons.

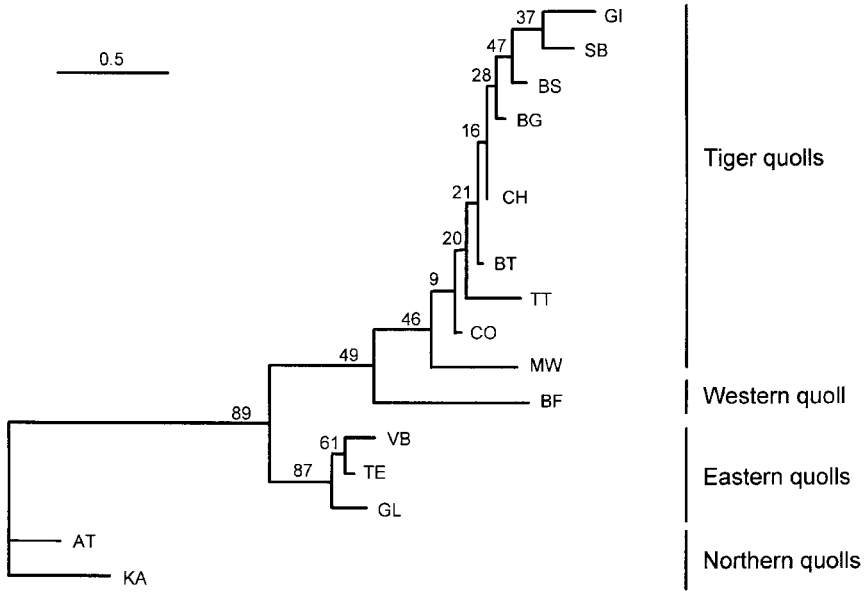

Figure 6. Neighbor-joining tree of 15 populations of quolls using Nei's D. Numbers at nodes indicate the percent of trees from 1000 bootstrap replicates that have the branch to the right.

Table 4. Species assignment test

Source species	Assigned species				% success
	Dm	Dv	Dh	Dg	
Dm (96)	95	–	–	1	99
Dv (93)	1	91	1	–	98
Dh (32)	–	–	32	–	100
Dg (35)	1	–	–	34	97

Individuals were assigned to the species from which their genotypes were most likely to occur. Number of assignments from species i (row) to species j (column).

Discussion

The microsatellites used here were extremely useful for assessing variability and differentiation at all levels (within populations, among populations within species, and among species). Quolls generally exhibited levels of variability within the range of that found in other species, although western quolls had higher heterozygosity than that reported from some other species (e.g. Roy et al. 1994; Houlden et al. 1996; O'Ryan et al. 1998).

Genetic diversity among and within species

Genetic diversity was significantly different among the four Australian quoll species and was not as expected on the basis of either primer origin or population history. Higher levels of allelic diversity and heterozygosity were found among western quolls than any other species. Western quolls had more than twice the mean number of alleles than tiger quolls, yet only half the number of individuals were sampled. This high level of diversity among western quolls was particularly surprising given the widespread and long-term decline of this species. This finding is also notable in light of the microsatellite primers having been designed from tiger quolls and eastern quolls. There is a possibility that mutations arising in the priming site for microsatellites may lead to the presence of null alleles, and thereby, lower levels of heterozygosity in species where heterologous primers are employed. It has been shown previously that polymorphism at microsatellite loci may decline with increasing phylogenetic distance from the species for which the primers were originally characterized (Moore et al. 1991; FitzSimmons et al. 1995). However, this was not the case with western quolls. The high levels of genetic diversity within the BF population of western quolls may be an artefact of the captive breeding program although the historic population (PE) also had high genetic diversity. Maintaining these high levels of diversity in the BF population is important in light of the intensive captive breeding and reintroduction program instigated to assist conservation of

Table 5. Population assignment test for 15 populations of quolls

Source pop	Assigned population															
	Dm									Dv			Dh		Dg	
	MW	GI	CO	CH	BG	BT	BS	SB	TT	GL	VB	TE	KA	AT	BF	% success
MW (12)	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	100
GI (5)	–	4	–	1	–	–	–	–	–	–	–	–	–	–	–	80
CO (9)	–	–	1	4	1	3	–	–	–	–	–	–	–	–	–	11
CH (17)	–	–	1	13	2	1	–	–	–	–	–	–	–	–	–	76
BG (22)	–	–	2	3	15	1	–	1	–	–	–	–	–	–	–	68
BT (11)	–	–	4	1	–	3	–	–	2	–	–	–	–	–	1	27
BS (6)	–	–	1	–	–	–	5	–	–	–	–	–	–	–	–	83
SB (3)	–	–	–	–	–	1	–	2	–	–	–	–	–	–	–	75
TT (11)	–	–	1	–	–	–	–	–	10	–	–	–	–	–	–	91
GL (58)	–	–	–	–	–	–	1	–	–	53	–	4	–	–	–	91
VB (21)	–	–	–	–	–	–	–	–	–	–	20	1	–	–	–	95
TE (14)	–	–	–	–	–	–	–	–	–	3	2	9	–	–	–	64
KA (26)	–	–	–	–	–	–	–	–	–	–	–	–	26	–	–	100
AT (6)	–	–	–	–	–	–	–	–	–	–	–	–	1	5	–	83
BF (35)	–	–	1	–	1	–	–	–	–	–	–	–	–	–	33	94

Individuals were assigned to the populations from which their genotypes were most likely to occur. Number of assignments from population i (row) to population j (column) are indicated. Shaded areas denote populations within species that are in close geographic proximity.

this species. In addition, high levels of microsatellite variability may be useful for tracking paternity and reproductive success within that colony. Northern quolls, tiger quolls and eastern quolls were not significantly different from one another in either measure of diversity, indicating that the same genetic processes may be operating within these three species (i.e. drift, mutation, migration).

Differences in variability within species were also apparent and were as expected (i.e. low variation was found in small or isolated populations). The GI and SB populations of tiger quolls had lower corrected allelic diversity than that of either the MW, BG, TT, CH, or BT populations. Both the GI and SB populations are small: GI has been a captive bred colony for approximately 18 years with little genetic exchange (Bruce Kubbere, pers. comm.) and the SB population is from an area in Victoria where there have been very few sightings or records over the last decade. In contrast, the MW, BG, CH, and BT populations are wild populations with relatively high numbers of individuals. Similarly, there were differences in allelic diversity among populations of eastern quolls and among populations of northern quolls. The mainland populations of eastern quolls (NE, ST) had significantly lower allelic diversity than populations in Tasmania (GL, VB, TE). This result might be surprising given that theory predicts low genetic diversity among island populations when compared to mainland populations (Frankham 1997). Two possible explanations exist: lower levels of diversity may be an artefact of the difficulties in amplifying DNA from museum tissues, or the mainland populations may have been on the brink of extinction when these samples were collected. Within northern quolls, the KA population had higher allelic diversity than either AT or AR. Again, the KA population is in a relatively stable state, with large population numbers extended over a wide area. It is thought that the AT population is now isolated and in decline and the AR population is extinct.

Genetic differentiation among and within species

Each species possessed some unique alleles (Table 2), which were useful in defining species clusters. Phylogenetic analysis, based on distance values among populations, (Nei's D, Table 3; Figure 6) was able to partition species into distinct clades, although bootstrap support was limited. In phylogenetic reconstructions based on genetic distances between mtDNA sequences, eastern and western quolls are sister species (Krajewski et al. 1997; Firestone, in press); in the reconstruction based on distances between

microsatellite alleles, tiger and western quolls are sister species (Figure 6). Phylogenetic reconstructions based on microsatellites are less sensitive to shared ancestral polymorphisms than those based on mtDNA due to the extremely high mutation rate of microsatellites and homoplasy of alleles (similarity in phenotype, but not identity by descent) (Estoup et al. 1995). Similarly, MDS analysis was able to partition populations into species clusters based on the presence of unique alleles and differences in allele frequencies (Figure 5). The positioning of populations into distinct species clusters or clades indicates that the microsatellites used here are potentially useful for identifying different species of quolls in forensic tests. The assignment test (Table 4) and analysis of the mitochondrial DNA control region (Firestone in press) also may serve this function and may be even more useful when individuals are considered.

Most population pairs within species were significantly differentiated from one another based on allele frequencies (pairwise F_{ST} values; Table 3). This indicates that most populations should be considered as separate management units (MUs) according to recommendations by Moritz (1994). One notable exception is found among tiger quoll populations. The four populations from the Barrington Tops region (CO, CH, BG, BT) are all located within a radius of 50 km, and were not significantly subdivided based on microsatellite loci; similarly, there were difficulties in assigning individuals correctly among these populations (Table 5). However, studies of allele frequencies of the mtDNA control region have shown that some of these populations are actually differentiated (Firestone et al. 1999). Another exception may be found among many of the pairwise comparisons of SB; the lack of genetic differentiation between SB and these other populations may be due to the very small sample size of this population; the same may hold true for the GI and AT populations (Table 3).

Conservation implications

Western quolls

Western quolls possessed greater allelic diversity and levels of heterozygosity than the other species. In addition, western quolls also possessed the greatest number of unique alleles in relation to other species. The recovery plan for western quolls was begun in 1991 with the breeding colony consisting of 20 captive founders (3 males, 5 females, and 12 young from two litters) and additional wild caught young used to augment the captive population (Serena et al. 1991). Supplemental wild-caught males have been periodically introduced to the captive colony for breeding purposes, and surplus young have been routinely released to one of several different unoccupied translocation sites (Serena et al. 1991; Orell and Morris 1994). Maintaining high levels of genetic variability within the captive colony of western quolls is important to the long term viability of the translocated wild populations. Current estimates of variability in the extant population (BF) compared to a extinct population (PE) show no differences in the mean number of alleles but higher levels of heterozygosity in the extant population. The high levels of diversity and heterozygosity in the BF population may be a manifestation of non-random breeding, due to active management of the captive population whereas the high levels of heterozygosity in the PE population may be a manifestation of changing genotypic structure over an extended sampling period.

Breeding programs may greatly influence the levels of diversity within a captive population and the results presented here could indicate that the captive breeding program has been successful in maintaining high levels of diversity. Due to the success of the recovery program, including wide spread fox baiting, this species has been downgraded from ‘endangered’ to ‘vulnerable’ by the IUCN. Continued genetic monitoring of the captive and translocated populations is recommended as a means of assessing inbreeding or founder effects in recolonized areas.

Tiger quolls

Tiger quolls had low numbers of alleles in comparison with other species, however when this was corrected for sample size, there was no significant difference between tiger, eastern, or northern quolls in levels of H_E or in A', indicating that the same evolutionary forces (drift, mutation, migration) may be operating on these species.

Genetic subdivision shows that many populations of tiger quolls are separate MUs for conservation purposes (F_{ST} values; Table 3) although microsatellite data did not show subdivision amongst the populations from the Barrington Tops region (CH, CO, BG, BT). However, other studies have shown that the Barrington region populations are actually subdivided based on frequency differences in mtDNA, which is likely due to sex biased dispersal in these populations (Firestone et al. 1999).

In addition, the MW population (ascribed to *D. maculatus gracilis*) has been shown to be part of the mainland ESU, but the TT population forms a separate ESU to that on the mainland (Firestone et al. 1999). The TT population should be managed as a separate taxon to all other populations, whereas translocations between different MUs belonging to the same ESU may be advisable in cases where population numbers have dropped to low levels. However, analysis of F_{ST} versus geographic distance was significant for this species, indicating that distance itself explains much of the genetic variance observed between populations of tiger quolls. This implies that these animals are quite stationary, and dispersal distances are rather short relative to the large distances between sample localities. For this reason alone, it is important not to mix populations by reintroducing individuals from one site to another unless they are in close proximity.

Eastern quolls

Among the populations sampled, eastern quolls from Tasmania (GL, VB, TE) had higher allelic diversity than those from the mainland (NE, ST; Figure 3). Furthermore, significant differentiation exists between the three Tasmanian populations of eastern quolls at the microsatellite loci examined (pairwise F_{ST} values; Table 3) indicating that each of these populations should be considered as separate MUs.

Eastern quolls from the mainland are currently presumed extinct; the last confirmed sighting of a mainland eastern quoll was in 1963 (Australian Museum records). The reintroduction of eastern quolls from Tasmania to the mainland has been proposed in the past. However, there are still occasional reported sightings of eastern quolls from various mainland sites, and previously 'extinct' species have been resurrected in the past (e.g. Sinclair et al. 1996). If remnant populations of eastern quolls do still exist in remote areas of the mainland, then mixing of potentially different genetic units could prove to be deleterious. Although extinct populations from the mainland were analysed, no conclusions can be drawn regarding differentiation of mainland and island populations due to missing data. Thus it is not clear whether relocations from Tasmania to the mainland would alter or reduce genetic variation of a remnant population, but it would be wise for such relocations to be postponed until the species is no longer just 'presumed' extinct on the mainland.

Northern quolls

In the past, four subspecies of *D. hallucatus* were recognized on the basis of morphological differences and geographical location (Gould 1842; Thomas 1909; Thomas 1926): *D. h. hallucatus* (Northern Territory; including the KA population), *D. h. nesaeus* (Groote Eylandt), *D. h. exilis* (Western Australia), and *D. h. predator* (Cape York Peninsula, Queensland; including the AR and AT populations). However these trinomials are no longer in current use (e.g. Strahan 1998; Maxwell et al. 1996). Furthermore, the lack of taxonomic clarity has proven to be a major stumbling block in the conservation of many species (e.g. Daugherty et al. 1990; Zink and Kale 1995).

Preliminary genetic analysis of northern quolls has shown that the KA and AT populations are separate MUs based on significant differences in allele frequencies (Table 3); no conclusions about population subdivision of the AR population could be drawn however, due to missing data. Other studies examining mtDNA (Firestone in press) have shown that there are two reciprocally monophyletic clades within northern quolls (Northern Territory versus Queensland clades) corresponding to separations between the KA and AT populations in microsatellite allele frequencies shown here. Preliminary results suggest that these two populations may thus represent two distinct ESUs as well as different MUs. The detection of distinct ESUs implies historic separation and divergence between groups; therefore their separate management is recommended, to allow for continued divergence and evolution of the ESUs. The KA population from the Northern Territory should be recognized as a distinct conservation unit separate to the AT population from Queensland. The taxonomy should reflect this and the subspecific designations for these two groups should be resurrected.

We were able to examine only a few populations of northern quolls, however, and only a few individuals from two of those populations. We plan further studies to include the remaining geographically disjunct populations of northern quolls and additional markers (e.g. the mtDNA control region) to more thoroughly assess diversity and differentiation within this species.

In conclusion, the use of microsatellite markers has proven to be very effective in determining both levels of genetic variability and the degree of differentiation amongst all Australian species of quolls. Results presented here provide a genetic basis for future population monitoring and should prove useful

to conservation managers and agencies in decision making processes related to the conservation of these species.

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Appendix A. Allele frequency distributions for microsatellite loci in four species (20 populations) of quolls. (N) = sample size

	Population																			
Locus	MW	GI	CO	CH	BG	BT	BS	SB	WY	TT	NE	ST	GL	VB	TE	KA	AR	AT	PE	BF
1.3																				
(N)	11	5	8	12	16	10	6	3	6	11	15	9	51	21	14	26	4	6	15	34
80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000
82	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.712	0.250	0.000	0.000	0.000
84	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.096	0.000	1.000	0.000	0.000
86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.067	0.000	0.000	0.000	0.000	0.154	0.000	0.000	0.000	0.000
88	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.191
92	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.225	0.548	0.393	0.000	0.000	0.000	0.067	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.033	0.333	0.020	0.000	0.000	0.000	0.000	0.000	0.133	0.088
96	0.000	1.000	0.188	0.250	0.406	0.250	0.333	0.333	0.250	0.091	0.367	0.667	0.755	0.452	0.500	0.000	0.625	0.000	0.300	0.044
98	0.545	0.000	0.750	0.667	0.563	0.700	0.667	0.333	0.000	0.909	0.300	0.000	0.000	0.000	0.107	0.000	0.125	0.000	0.133	0.235
100	0.364	0.000	0.063	0.083	0.031	0.050	0.000	0.333	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.015
102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.074
104	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.221
106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.132
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000
3.1.2																				
(N)	12	4	9	12	16	10	6	3	4	11	8	1	52	20	14	26	3	6	9	31
143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000
145	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.096	0.333	0.000	0.056	0.000
147	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.025	0.000	0.250	0.167	0.417	0.167	0.065
149	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.000	0.904	0.550	0.714	0.173	0.500	0.417	0.000	0.000
151	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.077	0.425	0.286	0.135	0.000	0.083	0.111	0.065
153	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.375	0.045	0.063	0.000	0.000	0.000	0.000	0.096	0.000	0.083	0.167	0.065
155	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000	0.111	0.048
157	0.792	0.125	0.778	0.458	0.250	0.500	0.333	0.000	0.125	0.045	0.063	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.403
159	0.000	0.625	0.167	0.375	0.563	0.200	0.667	0.833	0.000	0.227	0.000	0.500	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000
161	0.208	0.000	0.000	0.042	0.063	0.100	0.000	0.000	0.000	0.409	0.000	0.000	0.019	0.000	0.000	0.038	0.000	0.000	0.056	0.339
163	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.273	0.125	0.000	0.000	0.000	0.000	0.058	0.000	0.000	0.000	0.000
165	0.000	0.250	0.056	0.083	0.125	0.200	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.016
167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3.3.1																				
(N)	12	5	9	12	15	10	6	3	11	11	24	6	47	21	14	26	4	6	14	30
91	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.615	0.000	0.000	0.000	0.000
93	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.000	0.250	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.038	0.125	0.083	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000	0.000	0.000
99	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.083	0.167	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000
101	0.000	0.100	0.000	0.000	0.000	0.000	0.083	0.167	0.000	0.045	0.313	0.083	0.149	0.000	0.000	0.038	0.250	0.000	0.179	0.000
103	0.208	0.600	0.389	0.542	0.467	0.650	0.917	0.833	0.000	0.500	0.375	0.250	0.426	0.429	0.393	0.019	0.500	0.000	0.214	0.000
105	0.792	0.200	0.333	0.083	0.233	0.200	0.000	0.000	0.000	0.455	0.125	0.500	0.426	0.571	0.571	0.000	0.000	0.083	0.036	0.000
107	0.000	0.100	0.167	0.292	0.233	0.150	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.036	0.019	0.000	0.000	0.000	0.117
109	0.000	0.000	0.056	0.083	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.036	0.033
111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.125	0.167	0.036	0.167
113	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.167	0.179	0.017
117	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000
119	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.036	0.000
127	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
131	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.183
133	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
135	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.167
139	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.107	0.017
141	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.050
143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167
145	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017

Appendix A. Continued

	Population																			
Locus	MW	GI	CO	CH	BG	BT	BS	SB	WY	TT	NE	ST	GL	VB	TE	KA	AR	AT	PE	BF
3.3.2																				
(N)	11	4	8	12	16	5	5	3	6	10	23	3	51	21	14	24	2	6	9	35
108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.271
114	0.545	0.000	0.125	0.000	0.156	0.000	0.200	0.000	0.000	0.050	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
116	0.000	0.500	0.500	0.542	0.438	0.800	0.600	0.500	0.000	0.200	0.087	0.000	0.000	0.000	0.000	0.042	0.000	0.167	0.000	0.000
118	0.000	0.500	0.063	0.000	0.000	0.000	0.100	0.333	0.000	0.250	0.261	0.333	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.014
120	0.455	0.000	0.313	0.292	0.313	0.200	0.100	0.000	0.000	0.400	0.565	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.043
124	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000
126	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.143	0.000	0.125	0.000	0.083	0.000	0.014
128	0.000	0.000	0.000	0.042	0.063	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.024	0.000	0.292	0.000	0.000	0.000	0.000
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.225	0.024	0.000	0.000	0.750	0.083	0.056	0.014
132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.667	0.000	0.000	0.333	0.000	0.000	0.000	0.042	0.000	0.500	0.000	0.271
134	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.235	0.167	0.214	0.021	0.250	0.000	0.167	0.086
136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.049	0.000	0.000	0.083	0.000	0.000	0.167	0.000
138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.147	0.310	0.321	0.104	0.000	0.083	0.167	0.200
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.137	0.262	0.321	0.021	0.000	0.000	0.000	0.000
142	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.196	0.071	0.071	0.063	0.000	0.083	0.111	0.000
144	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.071	0.021	0.000	0.000	0.056	0.071
146	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.021	0.000	0.000	0.056	0.000
148	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.056	0.014
4.4.2																				
(N)	12	5	9	12	16	11	6	3	26	11	18	0	55	21	13	26	9	6	9	34
70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	–	0.000	0.000	0.000	0.154	0.000	0.000	0.000	0.000
74	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	–	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
76	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.333	0.058	0.000	0.028	–	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
78	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.019	0.000	0.056	–	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.147
80	0.458	0.200	0.222	0.208	0.219	0.045	0.000	0.000	0.019	0.000	0.056	–	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.029
82	0.000	0.700	0.222	0.375	0.156	0.182	0.000	0.000	0.250	0.091	0.083	–	0.000	0.000	0.000	0.000	0.167	0.083	0.167	0.250
84	0.000	0.000	0.167	0.083	0.094	0.136	0.250	0.000	0.385	0.545	0.222	–	0.000	0.000	0.000	0.038	0.500	0.000	0.278	0.147
86	0.000	0.000	0.167	0.250	0.125	0.318	0.167	0.500	0.115	0.364	0.000	–	0.000	0.000	0.000	0.019	0.000	0.167	0.056	0.000
88	0.000	0.000	0.056	0.000	0.063	0.091	0.583	0.167	0.000	0.000	0.083	–	0.000	0.000	0.000	0.154	0.000	0.500	0.111	0.029
90	0.292	0.100	0.167	0.083	0.344	0.136	0.000	0.000	0.000	0.000	0.056	–	0.109	0.000	0.000	0.231	0.056	0.000	0.167	0.338
92	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	–	0.018	0.071	0.038	0.250	0.000	0.083	0.111	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	–	0.209	0.333	0.000	0.038	0.056	0.167	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.135	0.000	0.111	–	0.609	0.167	0.769	0.038	0.056	0.000	0.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	–	0.018	0.429	0.192	0.019	0.000	0.000	0.056	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	–	0.000	0.000	0.000	0.058	0.000	0.000	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.015
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044
4.4.10																				
(N)	12	4	9	12	15	10	6	3	2	11	2	0	54	21	14	25	0	6	9	33
179	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.720	–	1.000	0.000	0.000
181	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.260	–	0.000	0.000	0.000
183	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.020	–	0.000	0.000	0.000
187	0.000	0.000	0.111	0.167	0.033	0.150	0.750	0.333	0.500	0.000	0.000	–	0.000	0.000	0.000	0.000	–	0.000	0.056	0.000
189	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	–	0.009	0.000	0.036	0.000	–	0.000	0.000	0.000
191	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.250	–	0.009	0.524	0.143	0.000	–	0.000	0.000	0.000
193	0.667	0.000	0.167	0.417	0.333	0.050	0.250	0.000	0.250	0.000	0.000	–	0.009	0.000	0.071	0.000	–	0.000	0.000	0.000
195	0.042	0.000	0.278	0.083	0.033	0.150	0.000	0.000	0.000	0.500	0.500	–	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000
197	0.000	0.125	0.278	0.083	0.467	0.300	0.000	0.667	0.250	0.227	0.000	–	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000
199	0.292	0.375	0.167	0.167	0.033	0.350	0.000	0.000	0.000	0.227	0.250	–	0.000	0.000	0.000	0.000	–	0.000	0.000	0.015
201	0.000	0.500	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000	–	0.000	0.167	0.030
203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	–	0.000	0.000	0.000	0.000	–	0.000	0.000	0.091
205	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000	–	0.000	0.278	0.288
207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000	–	0.000	0.167	0.152
209	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.009	0.000	0.000	0.000	–	0.000	0.111	0.212
211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.269	0.000	0.000	0.000	–	0.000	0.111	0.152
213	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.667	0.024	0.286	0.000	–	0.000	0.111	0.030
215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.028	0.452	0.393	0.000	–	0.000	0.000	0.015
217	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.071	0.000	–	0.000	0.000	0.015