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Genetic divergence in the small Indian mongoose (*Herpestes auropunctatus*), a widely distributed invasive species

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Abstract

The combination of founder events, random drift and new selective forces experienced by introduced species typically lowers genetic variation and induces differentiation from the ancestral population. Here, we investigate microsatellite differentiation between introduced and native populations of the small Indian mongoose (*Herpestes auropunctatus*). Many expectations based on introduction history, such as loss of alleles and relationships among populations, are confirmed. Nevertheless, when applying population assignment methods to our data, we observe a few specimens that are incorrectly assigned and/or appear to have a mixed ancestry, despite estimates of substantial population differentiation. Thus, we suggest that population assignments of individuals should be viewed as tentative and that there should be agreement among different algorithms before assignments are applied in conservation or management. Further, we find no congruence between previously reported morphological differentiation and the sorting of microsatellite variation. Some introduced populations have retained much genetic variation while others have not, irrespective of morphology. Finally, we find alleles from the sympatric grey mongoose (*Herpestes edwardsii*) in one small Indian mongoose within the native range, suggesting an alternative explanation for morphological differentiation involving a shift in female preferences in allopatry.

Keywords: differentiation, founder, *Herpestes*, introduced species, microsatellite, mongoose

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Introduction

Although the role of founder events is much debated, it has long been recognized that they may enhance speciation rates (Mayr 1942; Templeton 1980). Populations of introduced species are often greatly affected by both random and deterministic processes that can cause rapid evolution. Within recently introduced populations, the combination of a small initial number of founders and random drift may cause loss of alleles and reduced genetic variation, and thereby induce genotypic and, possibly, phenotypic differentiation from ancestral populations (Roderick 1992; Roderick & Navajas 2003). Introduced species may also experience altered selection pressures in response to novel physical and biotic environments. However, if the population size of an invader increases very rapidly after introduction, most ancestral genetic variation is likely to be retained, because many of the founders and their primary offspring contribute to the future gene pool (Nei *et al*. 1975). Relaxed selection pressure may also allow survival of genetic recombinants previously selected against and thereby increase the genetic diversity available for natural selection to act upon (Carson & Templeton 1984). In combination, random drift and shifts in selection pressure create a new situation for introduced populations, in which evolution may be accelerated (Huey *et al*. 2000; Simberloff *et al*. 2000).

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Because they are exposed to a variety of evolutionary forces, introduced populations may be useful model organisms for investigations in evolutionary biology and speciation.

Mongooses (Herpestidae) are small, widespread carnivores occupying various habitats from Africa to Southeast Asia. The genus *Herpestes* contains 10 species (Nowak 1999) and is considered the oldest genus within the order Carnivora, dating back approximately 30 million years (Hinton & Dunn 1967). The native distribution of the small Indian mongoose [*Herpestes auropunctatus* (Hodgson 1836)] stretches from Iraq in the west to Myanmar in the east, and from northern Pakistan southwards throughout the Indian subcontinent. East of Myanmar (near the Salween River), the small Indian mongoose is replaced by the Javan mongoose, *Herpestes javanicus* (E. Geoffroy Saint-Hilaire, 1818), which recently has been recognized as a separate species (G. Veron, personal communication). The small Indian mongoose (but not the Javan mongoose) has been introduced to many islands worldwide for control of rats and snakes, mainly in tropical areas, but also to islands in the Adriatic Sea. Moreover, it has been introduced successfully in two continental areas: the northeast coast of South America (Husson 1960) and a Croatian peninsula (Tvrtkovic & Krystufek 1990; Krystufek & Tvrtkovic 1992). Almost all introduced populations arose from very small numbers of founding individuals, and the introduction history is often well documented.

To test if geographical variation in the morphology of the small Indian mongoose is consistent with a hypothesis of evolutionary character displacement and release, Simberloff *et al*. (2000) investigated skull size and upper canine diameter in 467 specimens representing much of the native and introduced range. Simberloff *et al*. also compared degree of sexual dimorphism in these characters. The results show that introduced individuals, allopatric with the slightly larger congeners the grey mongoose (*Herpestes edwardsii*) and/or the ruddy mongoose (*Herpestes smithii*), have larger skull length, larger canine diameter, and greater sexual dimorphism than specimens from areas where these species are sympatric. In spite of substantial morphological changes in introduced small Indian mongoose populations, the coefficients of variation for morphological traits were greatly reduced relative to those of native populations. There are indications of a genetic basis for the observed phenotypic differences, a criterion for true character displacement (cf. Schluter & McPhail 1992). Simberloff *et al*. (2000) argue that the lower coefficients of variation on islands of introduction may reflect an introduction bottleneck and a consequent decrease in genetic variation.

Because of the extreme founder events experienced by most introduced mongoose populations, highly variable markers are needed for investigating genetic variation within them and differentiation between them. Length polymorphisms in autosomal microsatellite motifs are useful for studies of species with low levels of genetic variation (Hughes & Queller 1993). Therefore, to find markers with substantial polymorphism for genetic investigation of the small Indian mongoose, Thulin *et al*. (2002) constructed a microsatellite library and optimized polymerase chain reaction (PCR) conditions for a subset of nine variable loci.

Here we study the extent of genetic differentiation within and between introduced and native populations of the small Indian mongoose. We also investigate how relationships inferred from genetic differentiation relate to the known history of introduction and, in so doing, evaluate the short-term effect of random processes on the degree and sorting of genetic variation. Finally, we hypothesize that there is no association between differentiation of noncoding autosomal microsatellites and the previously documented morphological changes among non-native small Indian mongoose populations.

Materials and methods

Samples

A total of 453 tissue samples (typically muscle) were collected from 13 different geographical areas from the native as well as non-native range (Table 1). Within the native range, we sampled mongooses from Bangladesh and Pakistan. All except three Bangladesh individuals were from the western/central part of the country, typically within 70 km of Calcutta. Thus, these samples may therefore be considered as from the 'Calcutta region', the source for several founder populations (Table 1 and Fig. 1). The three additional specimens were from eastern Bangladesh. They were included to capture as much of the genetic variation present within native populations as possible. Pakistan is near the westernmost limit of the native distribution of the small Indian mongoose. Among the non-native populations, we focused on areas where previous investigations of morphology have been conducted (see Simberloff *et al*. 2000).

We aimed for a minimum of 30 specimens and reached this threshold for eight sampling areas, including Bangladesh (Table 1). From Pakistan, we were able to obtain only 22 specimens. Three additional populations, Guadeloupe, Puerto Rico and Guyana were excluded from the analyses of genetic differentiation, population assignments and simulations to avoid premature conclusions that may stem from small sample sizes (nine, nine and two individuals/population, respectively). For calculations of allelic richness, a measure of allelic variation that takes sample size into account, we excluded the two specimens from Guyana. Four foetuses, obtained from one female from Jamaica, allowed us to test Mendelian inheritance of the

Table 1 The populations sampled and their proposed origin. *N* is the number of samples collected. Origin of populations and, if applicable, year of introduction (Y_I), specimens introduced (N_I), sex of introduced specimens and references

Population	*N*	Proposed origin	Y_I	N_I	Sex	References*
Bangladesh	35	Native	—	—	—	—
Pakistan	22	Native	—	—	—	—
Jamaica	52	Calcutta region	1872	9	4 males, 5 females	1, 2
Puerto Rico	9	Jamaica	1877	*c*. 20	Unknown	2
Guadeloupe (a)	9	Probably Jamaica	1880 –1885	Unknown	Unknown	2
Guadeloupe (b)	—	Probably Jamaica	1888	Unknown	Unknown	3, 4
Fajou	59	Guadeloupe	*c*. 1930	Unknown	Unknown	5
Hawaii (a)	48	Jamaica	1883	72	36 males, 36 females	6
Hawaii (b)	—	Jamaica	1885	215	Unknown	6, 7, 8
Hawaii (c)	—	West Indies	> 1885	Unknown	Unknown	8
Fiji	36	Calcutta region	1883	2	1 male, 1 female	8
Mauritius	35	India	1900	19	16 males, 3 females	9, 10, 11
Amami-Oshima	44	Okinawa	1979	30	Unknown	12, 13, 14
Okinawa (a)	94	Calcutta	Unknown	1–5	Unknown	15
Okinawa (b)	—	Bangladesh	1910	12	6 males, 6 females	13
Guyana	2	Barbados	*c*. 1900	Unknown	Unknown	16
Bangladesh (*Herpestes edwardsii*)	8	Native	—	—	—	—
total	453					

*, 1. Espeut (1882), 2. Hoagland *et al*. (1989), 3. Pinchon (1967), 4. Pascal *et al*. (1996), 5. Lorvelec *et al*. (2004), 6. Bryan (1938), 7. Laycock (1966), 8. Lever (1983), 9. Mcmillan (1914), 10. Carié (1916), 11. Cheke (1987), 12. Abe *et al*. (1991), 13. Sekiguchi *et al*. (2001), 14. N. Ishii, personal communication, 15. Ogura *et al*. (1998), 16. Husson (1960).

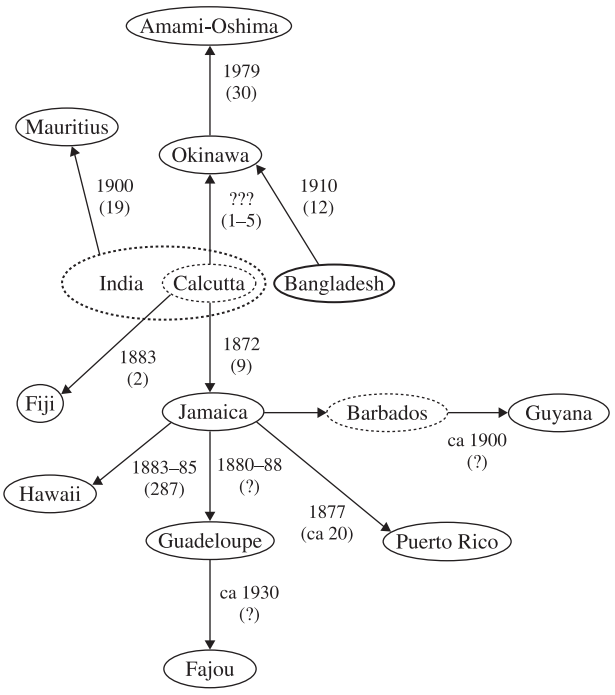

Fig. 1 Schematic drawing of sequential founder events of the small Indian mongoose. Whole circles refer to islands/areas sampled for the present study, while circles with dashed lines are not represented. Bold lines refer to localities within the native range. The numbers given are year of introduction (top) and number of specimens introduced in parenthesis.

microsatellite markers. In addition, we included eight specimens of the grey mongoose (*Herpestes edwardsii*) from Bangladesh as reference and for testing interspecific applicability of microsatellite primers. Samples were transported in vials with 95% ethanol and stored at –20 °C upon arrival. Whole genomic DNA was extracted using a DNeasy Tissue Kit (QIAGEN). Initial DNA concentrations ranged from 1 to 250 ng/μL.

Microsatellite scoring

We used eight previously reported microsatellite primer pairs (Hj14, Hj15, Hj34, Hj35, Hj40, Hj45, Hj51 and Hj56) to score allelic differences (Thulin *et al*. 2002). An additional locus (Hj5) proved monomorphic and was therefore excluded from the analyses. We tagged the upper primer of each pair with fluorescent markers FAM (locus Hj5, Hj14, Hj15, Hj34 and Hj35) or HEX (locus Hj40, Hj45, Hj51 and Hj56). We performed the PCR using three different compositions, each with a 5–50 nanogram template DNA, 1 x PCR buffer (10 mM Tris-HCl pH 9.0 at 25 °C, 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$ and 0.5 U of *Taq* DNA polymerase (Promega) per 10-μL reaction volume. Locus Hj14 was amplified in 10-μL reactions with a final concentration of 400 nM of each primer and 240 μM dNTP. The remaining loci were combined in two different multiplex reactions: Multiplex I consisted of primers Hj5, Hj40 and Hj56 with the respective final concentrations

111 nM, 111 nM and 185 nM, and 500 μM dNTP in a 20-μL final reaction volume. Multiplex II was composed of primers Hj15, Hj34, Hj35, Hj45 and Hj51 with the respective final concentrations 185 nM, 277 nM, 185 nM, 185 nM and 277 nM, and 1 mM dNTP in a 20-μL final reaction volume.

We used touchdown cycles for the reactions. PCR was performed in a Mastercycler Gradient (Eppendorf) and a TGradient Cycler (Biometra), with an initial denaturation step at 95 °C for 3 min, then 20 cycles with 30 s denaturation at 94 °C, 30 s annealing at 55–45(Hj14)/60–50(I & II) °C (lowered 0.5°/cycle), and 45 s elongation at 72 °C. We repeated the last cycle [e.g. with annealing at 45(Hj14)/50(I & II) °C] 10 times. All loci included in multiplex I & II can also be run separately under the same conditions as for locus Hj14, but with touchdown annealing temperatures of 60 °C– 50 °C. The lengths of the PCR products were obtained from an ABI PRISM 310 Genetic Analyser, using the ROX size standard and GENESCAN ANALYSIS 3.1 software (Applied Biosystems). We combined locus Hj14 and Multiplex I and ran Multiplex II separately.

Genetic analyses

Our analyses had two main goals, to estimate genetic differentiation among populations and to investigate genetic structuring within populations using population assignment tests. First, we scored alleles and genotypes with the computer program GENOTYPER 2.0 (Applied Biosystems). Number of alleles, allele frequencies, exact tests of Hardy–Weinberg equilibrium, and inbreeding coefficient (F_{IS}) were calculated in GENEPOP on the Web (http://wbiomed.curtin.edu.au/genepop/), versions 3.1c-3.4 (Raymond & Rousset 1995). We also calculated allelic richness (R) using the software FSTAT 2.932 (Goudet 1995). To measure degree of population differentiation we calculated F_{ST} (Weir & Cockerham 1984), also in GENEPOP. In addition to F_{ST}, we calculated Nei's unbiased genetic distance (Nei's D, Nei 1978; Takezaki & Nei 1996) using the software GENETIX 4.02 (Belkhir *et al*. 2000). We also used GENETIX 4.02 to calculate observed (H_O) and expected (H_E) heterozygosities and to perform a significance test of 10 000 permutations of the obtained estimates of F_{ST} and Nei's D.

Population assignment and exclusion tests, including calculations of probability of origin for each individual included in our study, were calculated using the software GENECLASS2 (Piry *et al*. 2004). In addition, we tested our data for population genetic structuring using the software STRUCTURE 2.1 (Pritchard *et al*. 2000) and BAPS 3.2 (Corander *et al*. 2003; Corander *et al*., in press), which apply partition-based Bayesian statistics for identification of genetic mixtures without baseline information of population origin. BAPS 3.2 also use an analytical integration strategy combined with stochastic optimization methods to make Bayesian estimation more feasible, in particular for mixed populations with individuals of unknown origin (Corander *et al*., in press).

For the calculations in GENECLASS2, we used the standard criterion described by Rannala & Mountain (1997), which applies Bayesian statistics to compute probabilities. In addition, we used the simulation algorithm for population assignment described by Paetkau *et al*. (2004). We simulated 10 000 genotypes for each population and applied an arbitrary threshold probability value of 0.05 or greater to determine origin. The calculations in STRUCTURE 2.1 were performed using admixture as well as nonadmixture models and with allele frequencies correlated and uncorrelated, respectively. The burn period was set for 10 000, with 100 000 Markov chain Monte Carlo repetitions. Probabilities Pr(K) for the inferred number of populations (K) were calculated as described in the manual (Pritchard & Wen 2003). K was set between 1 and 25 with five iterations for each setting, and after initial simulations, we also tried to restrict K between 5 and 15 (20 iterations/K) to increase resolution and decrease variance where K plateaus and thus approaches the real K (e.g. Pritchard & Wen 2003; Evanno *et al*. 2005). For the simulations in BAPS 3.2, we used different options, but we finally settled on clustering of nonpartitioned individuals followed by a population admixture analysis based on the resulting mixture clustering. Upper limit of number of partitionings was set at 25. In the admixture analysis, populations consisting of fewer than five individuals were removed. We used 100 as the input number of iterations, 200 simulated reference individuals from each population and 20 iterations for the reference individuals, following the advice in the software manual (Corander & Marttinen 2005).

Because of problems with low-quality template DNA, we could not obtain complete genotypes for seven specimens: *Herpestes auropunctatus* from Jamaica (4), Mauritius (1), Pakistan (1), and one grey mongoose (*H. edwardsii*). However, because these specimens failed at only two loci at most, we included them in all analyses.

Results

Each microsatellite locus produced between one and 15 alleles. Locus Hj5 was monomorphic across all populations and both species investigated. The total number of alleles observed within populations ranged from 14 to 53 and the mean number of alleles per locus per population from 1.6 to 5.9 (Table 2). We detected the most alleles in Bangladesh and Jamaica (53 in each), while the *Herpestes auropunctatus* sample from Fajou had only 18 and the sample from Pakistan only 20. The dearth of alleles in the Pakistan sample is noteworthy because this is a native region for this species. Notable also is that the population on Fiji, which according to the documented history stems from a

Table 2 Basic genetic data for populations. N_A is total number of alleles and N_{PA} number of private alleles for each population while N_{MA} is the mean number of alleles per locus. Estimates of F_{IS} along with observed (H_O) and expected (H_E) heterozygosities for each population. Allelic richness (R) as estimated for all populations except for the two specimens from Guyana is given in the final column

Population	N_A	N_{PA}	N_{MA}	F_{IS}	H_E	H_O	R
Bangladesh (35)	53	7	5.9	0.107	0.539	0.595	4.126
Pakistan (22)	20	2	2.2	–0.117	0.386	0.338	2.074
Jamaica (52)	53	6	5.9	0.009	0.578	0.577	3.794
Puerto Rico (9)	29	0	3.2	–0.074	0.593	0.523	3.099
Guadeloupe (9)	35	0	3.9	0.087	0.543	0.559	3.670
Fajou (59)	18	0	2	0.035	0.354	0.364	1.940
Hawaii (48)	30	2	3.3	0.028	0.507	0.516	2.753
Fiji (36)	46	2	5.1	0.093	0.494	0.537	3.530
Mauritius (35)	41	1	4.6	0.041	0.597	0.613	3.717
Amami-Oshima (44)	31	1	3.4	0.028	0.502	0.510	2.790
Okinawa (94)	32	1	3.6	0.102	0.470	0.521	2.898
Guyana (2)	14	0	1.6	0.077	0.333	0.264	NA
Herpestes edwardsii (8)	28	13	3.1	0.032	0.417	0.403	2.995

single pair introduced in 1883 (M. Gorman, personal communication), has 14 alleles more than theoretically possible assuming no supplemental introductions and no *in situ* mutations (two individuals heterozygote for different alleles at eight loci give 32 alleles maximum, but 46 alleles were detected; Table 2). The Bangladesh and Jamaica samples of small Indian mongoose had the highest number of private alleles, seven and six, respectively, while all other populations had two at most (Table 2). Our lowest estimate of allelic richness ($R = 1.940$) stems from the introduced population on Fajou, which has experienced at least three independent founder events (Table 2 and Fig. 1). The highest estimate ($R = 4.126$) stems from the specimens from the native range in Bangladesh (Table 2). From the foetuses from the Jamaica female, we could infer a single paternal genotype, which reveals the Mendelian inheritance of our markers.

After performing Bonferroni corrections for multiple comparisons (Rice 1989), we found observed heterozygote deficiencies for the three population-locus comparisons Bangladesh-Hj35 ($P < 0.01$), Bangladesh-Hj40 ($P < 0.05$) and Okinawa-Hj34 ($P < 0.05$). These deviations may stem from a Wahlund effect (Wahlund 1928), because structuring is expected within at least the Bangladesh sample, drawn from two or more different populations. Potentially, this explanation may hold for Okinawa too, but the deviation for this population was observed for only one locus. The F_{IS} estimates for the isolated non-native populations do not indicate inbreeding (Table 2). Rather, the highest estimated F_{IS} value was found in Bangladesh (0.107) within the native distribution. Finally, all nine primer pairs (including the monomorphic Hj5) proved suitable for amplification of the microsatellite primers for the eight individuals of grey mongoose (*Herpestes edwardsii*). In accord with our expectations, we found the largest number of private alleles (13) within this small sample of grey mongooses.

The differentiation between the mongoose on Jamaica and the Hawaiian population founded directly from Jamaica was among the lowest, with F_{ST} of 0.114 (Table 3). The F_{ST} between the small Indian mongoose populations and the grey mongoose sample from Bangladesh varied between 0.353 and 0.557 (Table 3). The F_{ST} estimate of 0.357 between Bangladesh *H. auropunctatus* and *H. edwardsii*, where these two species live in sympatry, is among the smallest. It is exceeded by eight intraspecific F_{ST} estimates, i.e. Pakistan–Fajou (0.516), Pakistan–Hawaii (0.443), Pakistan–Mauritius (0.373), Pakistan–Amami-Oshima (0.381), Pakistan–Okinawa (0.371), Fajou–Hawaii (0.424), Fajou–Amami-Oshima (0.372), Fajou–Okinawa (0.361). The estimates of Nei's D are typically larger than F_{ST}, and they are more in accord with our expectations (Table 3). For example, the interspecific genetic distance always exceeds the intraspecific distance.

Table 3 Estimates of pairwise genetic differentiation between populations. F_{ST} are given in the lower matrix and estimates of Nei's D (Nei 1978) in the upper matrix. All estimates are significant at $P < 0.05$ based on 10 000 permutations with Bonferroni corrections (Rice 1989)

	Bangl	Pakis	Jamai	Fajou	Hawai	Fiji	Mauri	Amami	Okina	Hedwa
Bangladesh (35)	0	0.679	0.234	0.408	0.503	0.223	0.440	0.444	0.389	1.105
Pakistan (22)	0.344	0	0.712	0.915	1.065	0.608	0.879	0.683	0.717	1.402
Jamaica (52)	0.125	0.351	0	0.503	0.172	0.233	0.461	0.407	0.362	1.128
Fajou (59)	0.286	0.516	0.314	0	0.852	0.437	0.620	0.600	0.631	1.315
Hawaii (48)	0.238	0.443	0.114	0.424	0	0.489	0.623	0.437	0.385	1.325
Fiji (36)	0.129	0.352	0.138	0.313	0.253	0	0.319	0.368	0.353	1.081
Mauritius (35)	0.183	0.373	0.197	0.345	0.262	0.164	0	0.563	0.515	1.150
Amami-Oshima (44)	0.223	0.381	0.215	0.372	0.247	0.215	0.250	0	0.071	1.404
Okinawa (94)	0.206	0.371	0.200	0.361	0.225	0.207	0.240	0.059	0	1.330
Herpestes edwardsii (8)	0.357	0.557	0.370	0.541	0.428	0.389	0.353	0.439	0.424	0

Table 4 Population assignments performed in GENECLASS2 (Piry *et al*. 2004) using the standard criterion of Rannala & Mountain (1997), the simulation algorithm of Paetkau *et al*. (2004) and 10 000 simulated individuals. A threshold *P* value of 0.05 was applied for assignments. Thus, some specimens may assign to more than one population, and some not at all

	Bangl	Pakis	Jamai	Fajou	Hawai	Fiji	Mauri	Amami	Okina	Hedwa
Bangladesh (35)	33	0	2	0	0	0	0	0	0	0
Pakistan (22)	0	22	0	0	0	0	0	0	0	0
Jamaica (52)	5	0	52	0	2	2	0	0	0	0
Fajou (59)	0	0	0	56	0	2	0	0	0	0
Hawaii (48)	0	0	33	0	46	0	0	0	0	0
Fiji (36)	2	0	3	0	0	36	0	0	0	0
Mauritius (35)	0	0	0	0	0	0	34	0	0	0
Amami-Oshima (44)	0	0	0	0	0	0	0	44	37	0
Okinawa (94)	0	0	0	0	0	1	0	52	90	0
Herpestes edwardsii (8)	0	0	0	0	0	0	0	0	0	8

Table 5 Average allocation percentages for mongoose samples from simulations in BAPS 3.2 (Corander *et al*. in press), using clustering of nonpartitioned individuals followed by a population admixture analysis based on the resulting mixture clustering. In the admixture analysis populations with fewer than five individuals were removed, which resulted in two populations removed, consisting of three specimens from Bangladesh (thus, 32 individuals remained). The partitioning resulted in 10 different clusters (C1-C10), mimicking the actual number of 10 sampling localities (left column). For the simulations, we used 100 input numbers of iterations, 200 simulated reference individuals from each population and 20 iterations for the reference individuals (e.g. Corander & Marttinen 2005). The rightmost column show the number of individuals for each population that significantly appeared as having an admixed origin ($P < 0.05$)

Origin/allocation	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	$N_{ADMIXED}$
Bangladesh (32)	0.88	0.01	0.01	0.03	0.01	0.03	0	0.01	0	0	1
Pakistan (22)	0	1	0	0	0	0	0	0	0	0	0
Jamaica (52)	0.03	0	0.75	0.02	0.10	0.07	0.01	0.01	0.01	0	2
Fajou (59)	0	0	0	1	0	0	0	0	0	0	1
Hawaii (48)	0	0	0.02	0.01	0.95	0.01	0	0	0.01	0	1
Fiji (36)	0.02	0.01	0.02	0.02	0.01	0.86	0.03	0.02	0.01	0	0
Mauritius (35)	0	0.01	0.01	0.03	0.01	0.02	0.89	0	0.02	0	1
Amami-Oshima (44)	0	0	0	0	0	0.01	0	0.80	0.18	0	0
Okinawa (94)	0	0	0	0	0.01	0.01	0	0.61	0.37	0	3
Herpestes edwardsii (8)	0	0	0	0	0	0	0	0	0.01	0.99	0

The simulation in GENECLASS2 indicates that the two Japanese populations (Amami-Oshima and Okinawa) are very similar, because several individuals assign to both when the threshold *P* value is 0.05 (Table 4). Similarly, the specimens from Hawaii often assign to Jamaica, which is the proposed origin of the Hawaiian mongoose (Bryan 1938; Laycock 1966; Lever 1983). A few specimens from several populations also assign to the Bangladesh sample. The results from the simulations in STRUCTURE 2.1 show that the probabilities for different *K* plateau at the actual number of 10 populations for the admixture model with correlated allele frequencies. The estimates vary considerably however, and the nonadmixture models with uncorrelated allele frequencies failed to recognize, or even get close to, 10 populations. The estimates of *K* did not improve when *K* was restricted between 5 and 15 and number of iterations was increased to 20. Thus, the STRUCTURE software detects the signal provided in our genetic data, but it is not as prominent as we expected from the seemingly high degree of differentiation (F_{ST} between 0.059 and 0.557). The results from the analyses with BAPS 3.2 are more unambiguous and settle on 10 populations (Table 5) after removal of two populations that consists of one and two individuals, respectively (see below). As with GENECLASS2, BAPS 3.2 verifies the recent common history of the Japanese populations with near equal allocation to Amami-Oshima and Okinawa, respectively.

In the admixture analysis based on individual-based clusters in BAPS 3.2, three small Indian mongoose specimens from Bangladesh (B1, B3 and B24) fall out as two separate 'populations'. Two of these specimens, B1 and B3, were collected in southwest Bangladesh (Borodal), while all other Bangladesh specimens except one (B2) are from the vicinity of Dacca. The third specimen, B24, is from Dacca. This specimen is homozygous for one allele at locus Hj35 (length 274 bp) that was not present in any other investigated

small Indian mongoose individual. However, a grey mongoose individual from southwest Bangladesh (BHe7) was also homozygous for this allele, and two additional grey mongooses were heterozygous (BHe5 and BHe11). This small Indian mongoose was also heterozygous for two alleles common to both species at locus Hj14 and heterozygous for one allele common to both species at locus Hj51. At all other loci, this individual displays alleles not detected among the eight investigated grey mongoose individuals. The results for this individual may indicate that hybridization between the small Indian mongoose and the grey mongoose occurs among native sympatric populations.

Discussion

Genetic differentiation and population assignments

Our investigation of genetic differentiation among small Indian mongoose populations with a well-documented history generally verifies expectations from population genetic theory. In general, F_{ST} values below 0.05 are expected with current gene flow, values between 0.05 and 0.1 indicate that populations are semi-isolated, and values above 0.1 suggest that populations are isolated (Wilson *et al*. 2003). As none of our F_{ST} estimates is lower than 0.1, our results confirm the present isolation of the investigated populations. The large differentiation between populations most likely results from rapid alteration of the genetic variation present during the founder event and subsequent enhancement of genetic differentiation during the population expansion phase. In this way, founder events may actually constitute an initial step towards reproductive isolation and, ultimately, speciation (Templeton 1980; Carson & Templeton 1984; Gavrilets & Boake 1998). Moreover, intraspecific estimates of F_{ST} often exceed interspecific estimates between the small Indian mongoose and the grey mongoose, while Nei's D for interspecific distance is typically twice the value of intraspecific distances (Table 3). Thus, Nei's D seems to be a more accurate tool for assessing the relationship between these species.

The different population assignment tests mostly accord with the documented history of the populations. For example, the mongoose on Amami-Oshima stems from a proposed introduction of 30 specimens from Okinawa in 1979 (Abe *et al*. 1991; Sekiguchi *et al*. 2001). Subsequently, these two populations appear mixed in the resulting simulations (Tables 4 and 5). Nevertheless, while specimens from Hawaii are almost as likely to assign to Jamaica as to Hawaii (Table 4), the F_{ST} between these populations is estimated at 0.114 (Table 3). Thus, we expected that there would be few cross-assignments. Bogdanowitcz *et al*. (1997) observed this problem with introduced populations of gypsy moths. When they applied assignment methods, certain Chinese specimens were assigned to North American populations, leading Bogdanowitcz *et al*. (1997) to urge caution in conclusions on moth origin based on these tests. Although the resulting simulations in BAPS 3.2 successfully allocated our samples into 10 different populations in agreement with their actual origins, there were a few individuals that sorted out as significantly admixed, although they could not possibly have a mixed origin (Table 5). We therefore suggest that assignments of population origin of individuals should be viewed as tentative and that before results are applied, they should be confirmed through use of different algorithms for the calculations.

Bottleneck effects

An investigation of genetic variation and differentiation between introduced West Indian and Hawaiian populations of the small Indian mongoose using allozyme markers shows limited loss of variation from population bottlenecks, presumably because ancestral alleles carried by the founder specimens were retained (Hoagland & Kilpatrick 1999). Allozymes typically have a lower level of resolution than microsatellites, and results from parallel analyses with the two classes of alleles sometimes conflict (e.g. Estoup *et al*. 2001). Our results show that the Jamaican population investigated for allozymes by Hoagland & Kilpatrick (1999) has also retained microsatellite variation, but during subsequent introductions from Jamaica to Puerto Rico, Guadeloupe, Fajou and Hawaii microsatellite alleles have been lost (Table 2). This depletion is particularly apparent for the sequential introduction of mongoose from Jamaica (53 alleles) to Guadeloupe (35 alleles) and then from Guadeloupe to Fajou (18 alleles). The pattern is also seen in the estimates of allelic richness, which for example decrease from 3.794 on Jamaica, to 3.670 on Guadeloupe and 1.940 on Fajou (Table 2). The observed reduction of genetic variation is in accordance with what we would expect from a linear stepping-stone model of sequential founder events (Le Corre & Kremer 1998).

The genetic variation of introduced populations could exceed that of native populations because of repeated introductions and mixture of specimens with different geographical origins (Petit *et al*. 1997; Thulin & Tegelström 2001). On Fiji, the proposed introduction of a single pair in 1883 (M. Gorman, personal communication) does not satisfactorily explain the current observation of 46 alleles over eight loci and the relatively high estimate of allelic richness (Table 2). This increase in number of alleles from an initial maximum of 32 may be explained by *in situ* mutations and subsequent accumulation of novel alleles during the population expansion (e.g. founder flush; Carson 1990). However, it is more likely that there have been repeated, undocumented introductions in addition to the

first successful introduction in 1883. By contrast, the native Pakistan mongoose population displays one of the lowest measures of expected heterozygosity and allelic richness in our study (Table 2). The reason for this low allelic variation is unclear, but our observation encourages extended investigations of genetic differentiation within the native range of the small Indian mongoose.

Morphological and genetic differentiation

Morphological variation in the small Indian mongoose, as evidenced by skull length and upper canine diameter, is not distributed in accord with introduction history (Simberloff *et al*. 2000). Individuals in the introduced populations are all similar and much larger than those from the native source region. This observation fit well with the modified island rule for mammals, which states that small mammals grow larger on islands (Lomolino 1985). Further, among the introduced populations, morphology does not track history. For example, individuals from the Oahu population, founded from the Hawaii population, have much longer skulls than the latter. The two most morphologically similar introduced populations are those from Mauritius and Hawaii, completely distinct historically. As opposed to the morphological differentiation, the microsatellite differentiation between Mauritius and Hawaii is relatively large (F_{ST} = 0.262) and confirms that these mongoose populations have different histories. Thus, as hypothesized, we found no apparent congruence between genetic differentiation of microsatellite data and this morphological differentiation.

The lower coefficients of variation observed among morphological characters on islands (Simberloff *et al*. 2000) may result from an introduction bottleneck and subsequent decrease in amount of available genetic variation. Although we believe bottlenecks have induced differentiation of microsatellite DNA between mongoose populations, there is no apparent effect of bottlenecks on degree of morphological differentiation. Allelic variation has obviously been depleted in several introduced populations because of the small number of founders, and this depletion is reflected in the lowered morphological coefficients of variation of the introduced populations, but these populations all appear to have evolved rapidly towards larger size (Simberloff *et al*. 2000).

Hybridization and its possible role in character release

Character release in the small Indian mongoose may be a response to differences in food use or resource limitations (Simberloff *et al*. 2000). However, a changed female preference for larger males would also explain the character release, especially given the increased sexual size-dimorphism. Niche expansion of males in allopatry may call for more aggressive territorial behaviour, encouraged by female preferences and resulting in larger body and canine size in males. Our discovery of a small Indian mongoose from Bangladesh with alleles found only in the sympatric grey mongoose (B24) bears on this hypothesis. This individual is unlikely to be a first-generation hybrid but may very well be a recent backcross. In any event, the genotype of this specimen suggests that interspecific gene transfer occurs between these two species in sympatry. A close relationship of the two parental species was also documented in a recently revised phylogeny of the genus *Herpestes* (Veron *et al*. 2004; G. Veron, personal communication) and the low estimates of F_{ST} between the two species may indicate that a small degree of introgression occurs.

The small Indian mongoose is sympatric with the grey mongoose in much of its native range. There are many ecological similarities between these species, for example diurnal activity, solitary behaviour, food choice and habitat use (Rood 1986; Corbet & Hill 1992). An explanation for character displacement could be natural selection against potential hybridization (Brown & Wilson 1956), leading to sexual selection for smaller body size in areas where the small Indian mongoose is sympatric with the grey mongoose. In sympatry, female preference for larger males could be disadvantageous if it led to interspecific mating and lowered reproductive success. In areas of allopatry, however, release from these constraints would be expected. Such released female preferences could rapidly alter male morphology and increase sexual size dimorphism.

Conclusions

In our study of genetic differentiation in the small Indian mongoose, many expectations based on introduction history, such as loss of alleles and relationships among populations, are confirmed. For populations on Jamaica, Guadeloupe and Fajou, which have experienced sequential founder events, we observe a reduction of genetic variation that was expected from a linear stepping-stone model (Le Corre & Kremer 1998). Moreover, the high number of alleles in the Fiji population of the small Indian mongoose indicates that there have been additional introductions to the documented origin of a single pair in 1883 (M. Gorman, personal communication). By contrast, the low allelic variation in the native Pakistan mongoose population calls for extended investigations of genetic differentiation within the native range of the small Indian mongoose.

With population assignment methods, we observe a few specimens that cross-assign and/or appear to have a mixed ancestry, despite large estimates of population differentiation (F_{ST} between 0.059 and 0.557). Therefore,

we suggest that population assignments of individuals should be confirmed by using different algorithms before results are applied in conservation or management. Further, we find no congruence between previously reported morphological differentiation and the sorting of microsatellite variation. Finally, we find alleles from the sympatric grey mongoose (*Herpestes edwardsii*) in one small Indian mongoose within the native range, suggesting an alternative explanation for morphological differentiation involving a shift in female preferences in allopatry.

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