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Population Genetic Structure of Aldabra Giant Tortoises

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Abstract

Evolution of population structure on islands is the result of physical processes linked to volcanism, orogenic events, changes in sea level, as well as habitat variation. We assessed patterns of genetic structure in the giant tortoise of the Aldabra atoll, where previous ecological studies suggested population subdivisions as a result of landscape discontinuity due to unsuitable habitat and island separation. Analysis of mitochondrial DNA (mtDNA) control region sequences and allelic variation at 8 microsatellite loci were conducted on tortoises sampled in 3 locations on the 2 major islands of Aldabra. We found no variation in mtDNA sequences. This pattern corroborated earlier work supporting the occurrence of a founding event during the last interglacial period and a further reduction in genetic variability during historical time. On the other hand, significant population structure recorded at nuclear loci suggested allopatric divergence possibly due to geographical barriers among islands and ecological partitions hindering tortoise movements within islands. This is the first attempt to study the population genetics of Aldabra tortoises, which are now at carrying capacity in an isolated terrestrial ecosystem where ecological factors appear to have a strong influence on population dynamics.

Key words: Aldabrachelys, *islands, microsatellites, mitochondrial DNA, population structure*

Islands represent ideal model systems to describe the relative role of gene flow and genetic drift on the evolution of population diversity across discrete geographical areas. Although geological events and sea level changes may account for major patterns of island phylogeography, landscape ecology can often explain genetic signatures of population demography and biogeographical differentiation (Cowie and Holland 2008; Gillespie et al. 2008; Parent et al. 2008). The giant tortoise of the Aldabra atoll, *Aldabrachelys gigantea*, is the only extant species of giant tortoises in the Indian Ocean and has a relatively complicated evolutionary history. Stochastic processes such as orogenic, eustatic and ecological changes as well as deterministic factors (i.e., human disturbance) that occurred from the Pleistocene to present most probably contributed to the observed patterns of genetic diversity of extant populations (Austin et al. 2003; Palkovacs et al. 2003). Previous phylogenetic analysis suggest that colonization of Aldabra (as for the other islands of the western Indian Ocean) initiated from Madagascar (Palkovacs et al. 2002), and it is conceivable that changes in sea levels during the Pleistocene significantly affected patterns of dispersal. On Aldabra, abundant tortoise fossil records found at different sedimentary strata advocate repeated colonization events, with the latest occurrence during the second part of the last interglacial period (Taylor et al. 1979). Lack of variation found in the mitochondrial DNA (mtDNA) control region in a small sample set of *A. gigantea* suggested a relatively recent colonization event, probably about 80 000 years ago (Palkovacs et al. 2003). Since then, Aldabra was subject to significant geomorphological and topographical changes, the most recent being the breaching of the inner lagoon, between 4000 and 5000 years ago (Braithwaite et al. 1973). This probably resulted in the formation of the 4 islands

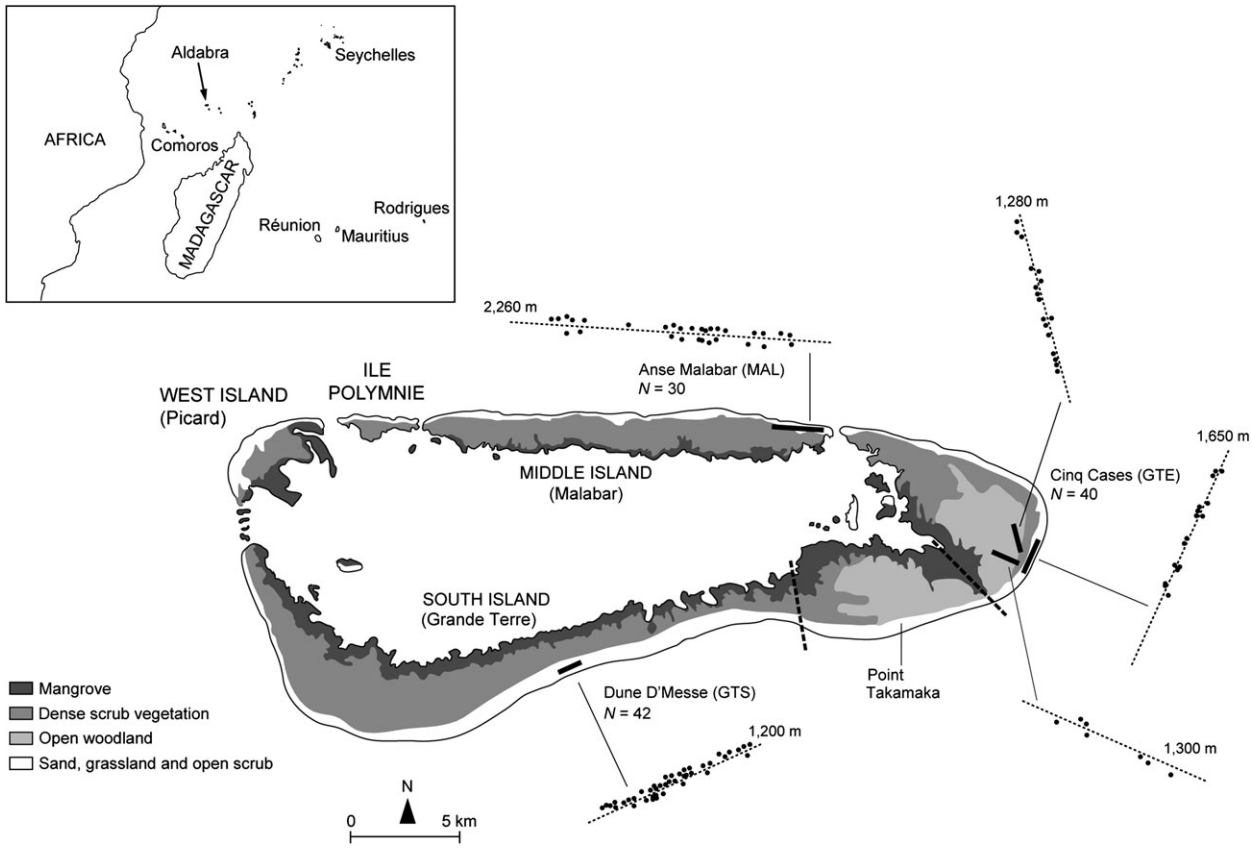

Figure 1. Map of Aldabra atoll and main habitat types of *Aldabrachelys gigantea*. Broken lines on South Island indicate partial habitat barriers described in Gibson and Hamilton (1984). Thick solid lines are approximate positions of line transects on South Island and Middle Island. Dotted lines show transect lengths. Sample size and approximate position of tortoises (closed circles) are reported for each transect.

currently making the land rim of the atoll (Figure 1). Giant tortoises are found on 3 of these formations (West Island, Middle Island, and South Island), which frame a shallow central lagoon linked to the ocean by 3 major channels and by several reef passages. Such partitions, although minor geographical barriers, may represent a significant vicariant factor affecting movements, demography, and genetic structure of tortoises. Moreover, the rugged, strongly weathered limestone has been eroded over the years to form a terrain of sharp rocks and numerous fresh and brackish water pits, which may represent major obstacles for this reptile. A patchy environment and narrow bands of open vegetation among unsuitable habitat types can also significantly affect movements across the atoll (Gibson and Hamilton 1983).

Several models of population structure have been suggested for Aldabra tortoises. Beside population subdivisions defined by major geographical barriers (i.e., sea water), Swingland et al. (1989) identified 3 major subpopulations in South Island based on movement records and intraspecific morphological differences (Figure 1). Gibson and Hamilton (1984) suggested an identical partition in 3 distinct subpopulations separated by habitat barriers (i.e., scrubs, open coastal areas of highly dissected rocks, and stretches of coastline devoid of shade) and described a fourth, putative subpopulation on the western edge of South Island. Occasional dispersal of animals among subpopulations was observed, but movements consisted mainly of migration events between alternative feeding grounds located within a limited range (Bourn and Coe 1978; Swingland and Lessells 1979). These preliminary descriptions of a putative population subdivision in Aldabra tortoises were based mainly on ecological survey techniques. No attempt has been made so far to describe patterns of gene diversity of Aldabra tortoises and compare genetic information to the available demographic data (e.g., Grubb 1971; Bourn and Coe 1978; Swingland and Lessells 1979) to further investigate levels of population subdivisions.

Aldabra tortoises are indeed unevenly distributed across the atoll, and their density appears to depend on favorable habitat, feeding grounds, and shade (Bourn and Coe 1978). The population size of Middle Island is just 4% of the total Aldabra population, with an average tortoise density of 12 individuals per hectare. Conversely, approximately 38% of the Aldabra tortoise population lives on eastern South Island with an average density of 18 tortoises per hectare

and 24% on the south coast of South Island with an average density of 13 tortoises per hectare (Bourn et al. 1999). The land rim of South Island has areas with open mixed scrubs and low-canopy trees suitable to giant tortoises, as well as sections of dense thickets, which hinder tortoise movements (Gibson and Phillipson 1983). The latter includes intervening zones formed by highly dissected rocks and thick *Pemphis* scrub with no shade for tortoises, described to the east and west of Takamaka point (Figure 1). These partial landscape barriers correspond to tortoise population partitions suggested by Gibson and Hamilton (1984) and Swingland et al. (1989).

In this study, we analyze patterns of genetic variation and distinctiveness among Aldabra giant tortoises from the 3 presumed subpopulations of South Island and Middle Island identified by Swingland et al. (1989), separated by either water barriers (Middle Island vs. South Island) or landscape and ecological elements that may hinder tortoise dispersal (southern vs. eastern South Island). We characterize mtDNA control region sequences using a larger data set than that examined by Palkovacs et al. (2003), describe levels of population distinctiveness and gene flow across the atoll using microsatellite markers, and test whether population genetic divergence corroborates patterns of population structure suggested by previous ecological work.

Materials and Methods

Study Area, Sampling, and DNA Extraction

Aldabra is a volcanic atoll with a land area of 155 km^2 made of coral limestone raised to about 8 m above sea level. The lagoon shores are fringed with dense mangroves, and the seaside rim has several habitat types. On West Island, Middle Island, and the southwest coast of South Island, tortoises are found along a relatively narrow coastal belt of grassland and open mixed scrub vegetation. Sand dunes also cover part of the south coast and support grass swards grazed by tortoises during the wet season. The east of South Island is characterized by grassland and sedge swards and a much more open mixed scrub and woodland community. The presence of shade, freshwater pools, and tortoise turf (a distinctive plant community which constitutes the main food of *Aldabrachelys*) yield a better environment for tortoises than the other locations. Inland, most of the atoll is made of uplifted mushroom-shaped coral formations (champignon) covered by very dense scrub vegetation unsuitable for tortoises (Gibson and Hamilton 1983).

Blood samples were collected from a total of 112 tortoises (21 males, 55 females, and 36 subadults) from the 3 subpopulations identified on South Island and Middle Island by Swingland et al. (1989). Thirty individuals were sampled on the north coast of Middle Island (MAL), 40 on the east coast of South Island (GTE), and 42 tortoises on the south coast of South Island (GTS) along 5 linear transects walked in mixed open scrub, woodland, and grassland habitat (Figure 1). Approximate position of tortoises was recorded with reference to stakeout poles regularly spaced along each transect and reported on a 1:25 000 topographic map. Tortoises were temporarily marked with paint to avoid resampling. Blood samples were stored in a lysis buffer containing 0.1 M Tris buffer, 0.1 M ethylenediaminetetraacetic acid (EDTA), 0.2 M NaCl, and 1% sodium dodecyl sulfate, pH 8.0. DNA was extracted using the DNeasy extraction kit (Qiagen) following the manufacturer's protocol, resuspended in TE buffer (10 mM Tris–HCl; 1 mM EDTA; pH 7.2), and stored at −80 °C.

Table 1 Characteristics of 8 microsatellite loci in *Aldabrachelys gigantea* (for PCR primer sequences, see Ciofi et al. 2002 and Palkovacs et al. 2003)

Locus	Cloned repeat	Number of alleles	Allele size (bp)	T_a (°C)
GAL50	CA_{24}	3	116–148	60
GAL85	CA_{22}	3	81–91	60
GAL94	CA_{18}	6	95–111	51
GAL100	CA_{26}	3	86–100	55
GAL127	CA_{21}	6	85–143	55
GAL136	CA_{20}	5	85–109	49
GAL247	CA_{39}	2	69–93	56
GAL263	CA_{17}	7	94–120	59

Genetic Analysis

We amplified a 915-bp fragment of the mtDNA control region using primers ALD-DLAFor and ALD-DLBRev (Palkovacs et al. 2003). Samples were screened for sequence diversity using the single-stranded conformation polymorphism (SSCP) technique as described in Beheregaray, Ciofi, Caccone, et al. (2003). SSCP is a simple and precise method for detecting mtDNA sequence identities (for a review, see Sunnucks et al. 2000), and it has been demonstrated to provide reliable results for sequences of over 700 bp (e.g., Beheregaray, Ciofi, Geist, et al. 2003; Beheregaray et al. 2004). All our samples presented the same SSCP phenotype. Sequence identity was confirmed by polymerase chain reaction (PCR) amplification and sequencing (described in Palkovacs et al. 2003) of 42 random samples with the same SSCP gel band and by comparison of our data set with 10 Aldabra tortoise sequences (Palkovacs et al. 2003) used as controls in all gels. Individuals with the same SSCP phenotype as the control samples had identical sequences, whereas the reverse was observed for individuals with different sequences. Microsatellite allele variation was assessed using PCR primers developed for the Galápagos giant tortoise (Ciofi et al. 2002). We used 8 polymorphic loci (Table 1), all but one (GAL127) tested in *Aldabrachelys* by Palkovacs et al. (2003). Amplification was carried out as in Ciofi et al. (2002). Thermal profiles consisted of an initial denaturation step at 94 °C for 4 min, 35 cycles of 45 s at 94 °C, 30 s at annealing temperature, and 45 s at 72 °C, with a final extension step of 7 min at 72 °C. PCR products were analyzed by gel electrophoresis using an ABI 373 DNA sequencer (Applied Biosystems).

Measures of Genetic Diversity

We assessed microsatellite allelic diversity, observed and unbiased expected heterozygosity, and tested for departure from Hardy–Weinberg (HW) equilibrium and genotypic linkage disequilibrium using GENEPOP 4.0 (Rousset 2008). Probability of type I error was calculated using a sequential Bonferroni procedure. Deviation from random mating was tested by estimating statistical significance of the inbreeding coefficient f using the randomization approach implemented in GENETIX 4.05 (Belkhir et al. 2004). Errors due to large allele dropout or stutter bands and evidence for the presence of null alleles at each locus were evaluated using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Null allele frequencies were then estimated using the expectation maximization algorithm as described in Chapuis and Estoup (2007).

Genotypic differentiation was estimated using the log likelihood ratio (G) test implemented in GENEPOP 4.0. Differences in allele frequency between sampling sites were assessed by the F_{ST} estimator θ using GENETIX 4.05. R_{ST} values were obtained by calculating standard deviations from the global mean of microsatellite repeat unit numbers using RSTCALC 2.2 (Goodman 1997). We also estimated θ values from a data set corrected for null alleles by limiting the computation to visible allele states using FREENA (Chapuis and Estoup 2007). Patterns of relative similarities among sampling sites were also resolved by principal component analysis (PCA) implemented in GENALEX (Peakall and Smouse 2006).

Population Structure and Gene Flow

Evidence of population structure was assessed using the Bayesian clustering analysis implemented in STRUCTURE 2.2 (Pritchard et al. 2000). We ran the program without prior population information with a burn-in period of 100 000 iterations, and we estimated the probability of the observed genotypes given a number of populations K ranging from 1 to 4 (the number of sampling sites plus one) by Markov Chain Monte Carlo (MCMC) methods using 10 000 repetitions. We calculated the mean likelihood $L(K)$ over 20 runs for each K. We then assessed the mean difference between successive likelihood values of K, $L'(K) = L(K) - L(K-1)$ and the absolute value of the difference between successive values of $L'(K)$, $|L''(K)| = |L'(K+1) - L'(K)|$. Finally, we estimated ΔK and the most likely number of populations K as described in Evanno et al. (2005). The K value was then used as prior information to estimate the probability that an individual belongs to a given population.

A test for past population contraction was performed by estimating the relative reduction in the number of alleles as compared with gene diversity. Because the number of alleles is reduced relatively quicker than gene diversity by a bottleneck event, a reduction in effective population would produce an excess of heterozigosity compared to the expected heterozigosity calculated from the observed number of alleles under the assumption of a constant size (equilibrium) population (Cornuet and Luikart 1996). A Wilcoxon sign-rank test was performed to assess whether any of the 3 Aldabra populations exhibited a significant number of loci with heterozigosity excess under either an infinite allele model or a 2-phased model of microsatellite mutation.

We inferred the genealogical history of alleles between the 3 sampling sites from South Island and Middle Island using a coalescent approach and MCMC simulation, and calculated the relative likelihoods of a genetic drift population model and an immigration-drift model given the observed microsatellite frequency counts using 2MOD (Ciofi et al. 1999). Convergence of parameters from individual runs was confirmed by running a total of 4 independent runs of 1.0×10^6 iterations after a burn-in of 10^4 iterations. Estimates of recent migration rates and 95% confidence intervals between populations were obtained using the MCMC procedure implemented in BAYEASS 1.3 (Wilson and Rannala 2003). The MCMC was ran for 3.0×10^6 iterations, with the first 10^6 iterations discarded as burn-in to allow the chain to reach stationarity. Samples were collected every 2000 iterations to infer posterior probability distributions of parameters of interest.

Genetic Boundaries

We inferred population boundaries by looking for zones of sharp changes in genetic data using the wombling approach implemented in the R package WOMBSOFT (Crida and Manel 2007). This method interpolates multilocus allele frequencies recorded at different locations into values of a continuous function defined over the study area. The function, or surface, is generally represented by a matrix of values at the nodes of a geographic grid (Barbujani et al. 1989). We used a grid with a resolution of 500 × 500 m. Bandwidth was set at 45 km, which relates to the dispersal distance of tortoises in this study, that is, the distance over land from the southwestern location to the sampling site on Middle Island. The partial derivative of each node was then computed recording both the magnitude and direction of the surface slope. Genetic boundaries were identified by searching for regions where the absolute value of the surface slope is larger than 5% of a set of simulated slopes (Womble 1951; Barbujani and Sokal 1990). This was achieved by implementing a binomial test with percentile of 0.1.

Results

Genetic Diversity

No variation was found in the mtDNA control region sequence among the Aldabra tortoises sampled in this study. All 8 microsatellite loci were polymorphic with a number of alleles per locus ranging from 2 to 7 alleles (Table 1). Tortoises from the southern coast of South Island showed the highest level of allelic diversity and heterozygosity. No significant linkage disequilibrium was detected for any of the 28 pairwise locus combinations after Bonferroni correction.

Table 2 Genetic diversity of Aldabra tortoises from 3 sampling locations

Location	N	A	H_E	H_O	HWE	f
GTE	40	4.3 ± 0.6	0.50 ± 0.08	0.40 ± 0.09	<0.01	0.213*
GTS	42	4.4 ± 0.7	0.58 ± 0.06	0.49 ± 0.07	<0.01	0.156*
MAL	30	3.5 ± 0.5	0.51 ± 0.07	0.42 ± 0.08	<0.01	0.158*

N, number of individuals; A, mean number of alleles per locus; H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; HWE, statistical significance of deviation from HW equilibrium; f, F_{IS} estimator after Robertson and Hill (1984). Errors are 1 standard error.

*$P < 0.01$.

Table 3 Pairwise comparison matrix of θ (above diagonal) and R_{ST} (below diagonal) values among sampling sites on the Aldabra atoll

Location	GTE	GTS	MAL
GTE	—	0.109 (0.096)	0.059 (0.056)
GTS	0.122	—	0.062 (0.063)
MAL	0.094	0.067	—

Values of θ corrected for null alleles are shown in parenthesis. All values are significant with $P < 0.01$.

There was no evidence for scoring errors due to stuttering or large allele dropout. Deviation from HW proportions and significantly high values of inbreeding coefficient were recorded for all sampling locations (Table 2). The HW test computed for each locus per sampling site showed deviation from HW expectation at 2 loci for GTE, 6 loci for GTS, and 2 loci for MAL after adjustment for multiple tests. Excess of homozygotes for most allele size classes ($P < 0.01$) suggested presence of null alleles at 3 loci for each sampling site. In particular, GAL50 showed evidence of null alleles in all tortoise populations. Allele frequencies were therefore adjusted with respect to the estimated cumulative frequency of null alleles calculated from the observed proportional heterozygote deficiencies following Brookfield (1996).

Genetic Differentiation of Populations

No differences in allelic diversity (analysis of variance [ANOVA]; $F = 0.634$, $P = 0.540$) and mean expected and observed heterozygosity (ANOVA; $F = 0.287$, $P = 0.753$ and $F = 0.265$, $P = 0.770$, respectively) were recorded among sampling sites. On the other hand, there was significant genotypic differentiation among sampling sites ($P < 0.01$). Similarly, pairwise θ and R_{ST} values showed significant divergence ($P < 0.01$) with the strongest differentiation between GTE and GTS (Table 3). Significant differences ($P < 0.05$) among sampling sites were also found with male and female genotypes analyzed separately. F_{ST} analysis of genetic differentiation using allele frequencies adjusted for the presence of null alleles resulted in slightly different θ values than those obtained with uncorrected frequencies; however, significance levels of genetic diversity did not change. In the PCA, the first 2 principal components explained 72.44% and 27.56% of total inertia, respectively. The first component clearly differentiated GTE from GTS and the second component distinguished the tortoise population of South Island from the population of Middle Island.

Population Structure and Gene Flow

Population structure analysis revealed that the mean value of the log likelihood of the data increased for K values from 1 to 4. On the other hand, we found a modal value of $\Delta K = 18.4$ for $K = 3$, whereas ΔK values of 13.6 and 13.8 were recorded for $K = 2$ and $K = 4$, respectively. We used $K = 3$ as prior population information for calculating the posterior probability of individual assignment. A very high proportion of tortoise genotypes was assigned to each of the 3 clusters identified by the analysis (range: 0.918–0.977), advocating a strong pattern of population structure. Three tortoises from GTE showed a significant posterior probability of being assigned to or having a recent ancestry in either MAL or GTS. All other tortoises from the east and south coast of South Island and from Middle Island had a high posterior probability of belonging to their sampling locations (Figure 2). Between the 2 models of population structure considered in this study, the likelihood of the drift model was much higher (P(drift model) = 0.98) than that of the immigration-drift model, advocating a strong influence of genetic drift on population divergence and the absence of a significant level of gene flow. Moreover, according to the mean posterior probabilities and 95% confidence intervals for migration rates reported in Table 4, each location had a very high percentage of resident individuals (0.87–0.95).

The bottleneck test showed heterozygosity excess consistent with past reduction in population size in tortoises from Malabar and the south coast of South Island for both the infinite allele model ($P < 0.01$) and the 2-phased model ($P < 0.05$) of microsatellite mutation. No deviation from expected gene diversity values was recorded for the eastern South Island population.

The wombling procedure identified a region of genetic discontinuity in South Island (significance of binomial test: $P < 0.05$) as a putative boundary to gene flow to and from Middle Island and between the southern and the eastern region of South Island. This area stretches from GTS to the north of GTE and includes areas of ecological transition (Figure 3). Such boundaries were maintained when various parameters were changed in the wombling algorithm (data not shown). No polygons were resolved close to the East channel between Middle Island and South Island.

Discussion

Patterns of Genetic Variation

Giant tortoises of Aldabra have endured reiterated events of population extinction and colonization as a consequences of orogenic displacements and changes in sea level which altered the topography of the atoll during the Pleistocene

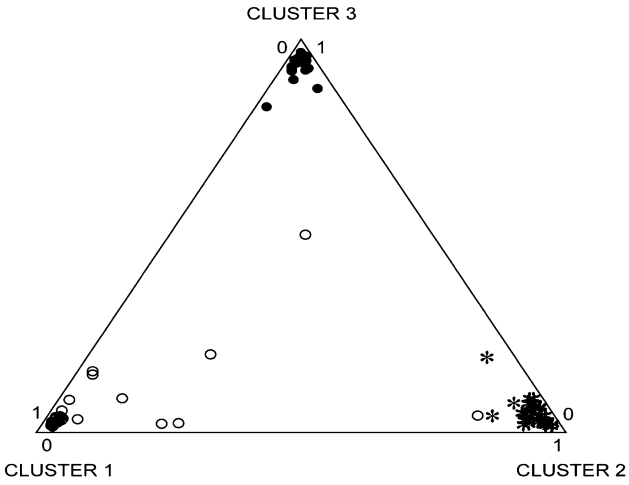

Figure 2. Triangle plot showing the proportion of each tortoise's genome that originated from each cluster inferred by population structure analysis. Admixture proportions are shown for individuals sampled in GTE (circles), GTS (stars), and MAL (bullets).

(Taylor et al. 1979). For many island species, formation of new water barriers and landscape features leave clear signatures in the genetic components of populations (Ciofi and Bruford 1999; Beheregaray et al. 2004; Bloor et al. 2008; Jordan and Snell 2008), and the Aldabra atoll provides a good example of how a mosaic of land and ecological discontinuities may affect population subdivision. In this study, we assessed the amount of genetic variability among Aldabra giant tortoises and substantiate patterns of population structure inferred by earlier ecological surveys.

We found no evidence of mtDNA variation among tortoises from the 2 main land rim formations of South Island and Middle Island. This result corroborated previous findings based on a much smaller sample set from Aldabra and the Seychelles (Palkovacs et al. 2003). A similar pattern was also found by Austin et al. (2003) for different non-Madagascan *Aldabrachelys* species. Low levels of mtDNA sequence divergence have been reported for terrestrial reptiles with limited vagility following isolation, postcolonization expansion, or population decline (Walker et al. 1998; Hay et al. 2003; Bloor et al. 2008). Moreover, records of single or very few mtDNA sequence haplotypes were described for species that recovered, naturally or via in situ recruitment programs, to large population sizes after a bottleneck or have been reduced to island populations after rising of sea levels at the end of the last ice age (e.g., Maldonado et al. 1995; Hinten et al. 2003). It is possible that a founding event of few individuals (Taylor et al. 1979) resulted in an initial low level of genetic variation, which could have been maintained by a combination of factors including the lack of recurrent immigration, lower rates of mtDNA evolution in turtles relative to other vertebrates (Avise et al. 1992), and low effective population size of mtDNA versus nuclear genes. New mutations would have been additionally limited by severe population depletion during the 19th century caused by human exploitation, which reduced this species to less than 1000 tortoises (Bourn et al. 1999). The hypothesis of a past contraction in effective population size was corroborated by the bottleneck test, where a significant deviation from heterozygosity values expected under the assumption of a constant-size population was recorded for the Malabar population and the tortoise population from the southern coast of South Island.

A clear pattern of genetic diversity was instead recovered by microsatellite analysis. Positive amplification of loci in *A. gigantea* using primers designed for *Chelonoidis elephantopus* is evidence of retention of microsatellite flanking sequences over long evolutionary time. The Galápagos lineage split from its closest living relative 6–12 Ma, whereas tortoise colonization of Madagascar and the Indian Ocean occurred not earlier than about 17.5 Ma (Palkovacs et al. 2002). Conservation of microsatellite flanking sequence between the Aldabra and Galápagos tortoise lineages could therefore date back to the Paleocene more than 20 Ma. Amplification of microsatellite loci and the persistence of polymorphism across taxa have been documented in reptiles and other poikilotherms for which homologous microsatellite loci can persist for more than 400 My (FitzSimmons et al. 1995; Rico et al. 1996; Zardoya et al. 1996). Allelic diversity and heterozygosity values recorded in Middle Island and South Island were nevertheless lower than those recorded in Galápagos tortoises (Ciofi et al. 2002). Different patterns of polymorphism have been described where dinucleotide repeats are most variable in their respective source species; however, these tests failed to show a significant loss of variability as time of divergence increases (e.g., Rico et al. 1996). Against the hypothesis of an ascertainment in the selection of loci analyzed, it has also been suggested that evolution of microsatellite loci could proceed at different rates in different species (Rubinsztein et al. 1999) and an increase in mutation rates may be found in heterozygotes where there is a large length difference between alleles (Amos 1999). This suggests an increase in the rate of microsatellite evolution in large populations, where a higher average level of heterozygosity is generally retained, an hypothesis that better applies to the Galápagos lineage with longer intraspecific evolutionary time and significantly different patterns of demographic changes among islands than the Aldabra tortoise. The presence of null alleles

Table 4 Means and 95% confidence intervals of the posterior probabilities for migration rates between Aldabra tortoise populations from South Island (GTE and GTS) and Middle Island (MAL)

	Rate from		
To	GTE	GTS	MAL
GTE	0.87 (0.796–0.942)	0.04 (0.008–0.100)	0.08 (0.016–0.159)
GTS	0.01 (0.000–0.043)	0.95 (0.856–0.998)	0.04 (0.000–0.119)
MAL	0.06 (0.000–0.164)	0.01 (0.000–0.058)	0.92 (0.817–0.997)

Migration rates >0.10 are underlined.

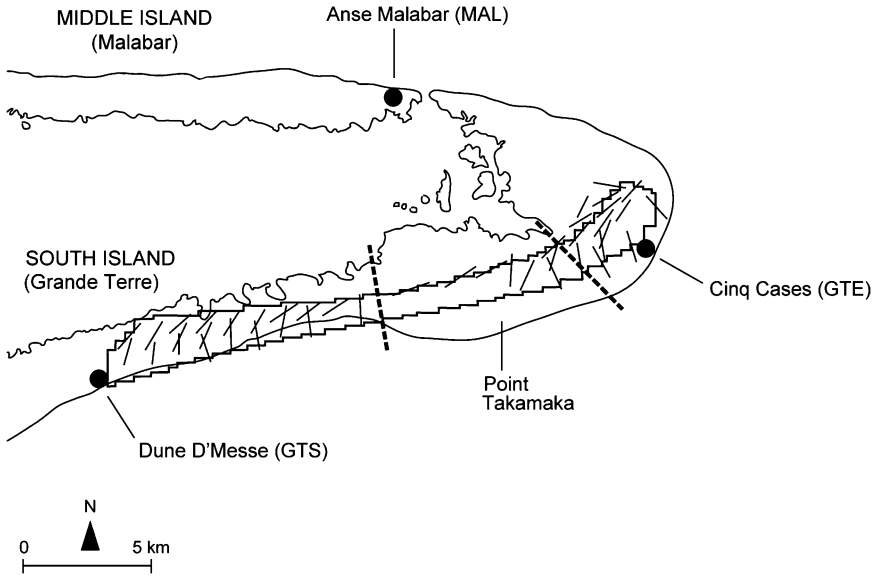

Figure 3. Map of the eastern portion of the Aldabra atoll showing approximate position of sampling sites and results of the wombling analysis. Broken lines on South Island indicate partial habitat barriers as in Figure 1. The area delimited by the serrated line on South Island represents genetic boundaries where the binomial function inferred by the wombling approach was significant at the 5% level. Rods show the directions of the gradient, and their length is proportional to the magnitude of the resulting surface slope. Main habitat types of *Aldabrachelys gigantea* as in Figure 1.

recorded for all sampling sites on Aldabra may reflect mutations within the priming sites of the nonsource species (a taxon different from the one for which PCR primers are designed) and can explain in part the relatively lower heterozygosity found in the Aldabra population with respect to Galápagos tortoises. However, the 5 microsatellite loci with homozygote excess due to null alleles and the other loci for which null alleles were not detected showed similar values of heterozygosity. The level of genetic diversity observed in *Aldabrachelys* populations was lower or comparable with that recorded in other tortoise species separated by water barriers or habitat fragmentation (e.g., Ciofi et al. 2002; Cunningham et al. 2002; Paquette et al. 2007) and may be representative of the minimum amount of genetic diversity currently retained on the Aldabra atoll. South Island and Middle Island tortoises have recovered from repeated harvesting during the last 2 centuries and the variability we see today within and among populations is likely persistent from the prebottleneck period.

Population Structure of Aldabra Tortoises

A significant genetic structure and a high proportion of resident individuals recorded at each sampling site supported previous observations by which areas of topographical and vegetational discontinuity may significantly limit migration of tortoises between islands or to distant locations across unsuitable habitats in South Island (Bourn and Coe 1978). South Island and Middle Island are approximately 300 m apart at their closest point and the distance becomes even shorter as the water level of lagoon falls during low tide. Although the short distance between islands would facilitate occasional migration of tortoises carried by tidal currents (Grubb 1971; Bourn and Coe 1978), our study showed that movements between land formations are rare. On South Island, the strong pattern of genetic divergence recorded between sampling locations was corroborated by results of an individual-based wombling approach that delineated an area of sharp genetic changes between the southern and eastern coast of South Island. Moreover, assignment analysis of individual genotypes from the east coast of South Island recorded only a few individuals of mixed ancestry with tortoises from the south coast or from Middle Island. More intensive sampling could help in testing for a pattern of isolation by distance across South Island. However, the sharp genetic divergence and extremely low levels of migration estimated between the southern and eastern coastal areas and the clear absence of tortoise records east of the Takamaka point (Bourn and Coe 1978) advocate the hypothesis of a strong population substructure. Although preliminary analysis of tortoise recapture data showed that some seasonal migrations do occur, these were mainly local movements, and tortoises were rarely relocated more than 1000 m from their initial marking point (Bourn and Coe 1978; Swingland and Lessells 1979).

The high likelihood value of the drift model of population structure recorded for microsatellite loci suggests that current patterns of genetic diversity may be the result of drift-induced divergence associated with past biogeographical events of island formation and development of more recent landscape and ecological barriers, which developed

after the breaching of the Aldabra atoll land rim and formation of the present lagoon, about 4000 years ago (Braithwaite et al. 1973; Taylor et al. 1979). Indeed, this pattern is observed in tortoise populations with dispersal restricted by water barriers or limited to rare events of passive transportation by sea currents rather than in other reptiles with life-history traits that facilitate over water dispersal (Rassmann et al. 1997; Caccone et al. 2002; Calsbeek and Smith 2003; Paquette et al. 2007). Moreover, genetic differentiation recorded at microsatellite loci without an extreme reduction in genetic diversity is further indicated that divergence across the atoll may reflect repartitioning of ancestral variation (Knowles and Richards 2005; Carstens and Knowles 2007).

Our work provides a further contribution to the evolutionary ecology of islands with distinctive biogeography as the Aldabra atoll. In particular, genetic analysis supported patterns of population structure and analysis on tortoise movements suggested by previous ecological surveys by which population divides were identified between the eastern and southern coast of South Island (Swingland et al. 1989). We also reiterated the utility of microsatellite markers designed for *C. elephantopus* in *A. gigantea* and provided additional evidence of microsatellite flanking sequence conservation over relatively long evolutionary time. In the last 80 years, population size of Aldabra tortoises has grown to an estimated 100 000 (Bourn et al. 1999). The abundance of this large herbivorous reptile makes Aldabra a unique island complex and lends special attraction to study the ecology and genetics of a species basically regulated by resource availability and strongly influenced by habitat heterogeneity (Swingland and Lessells 1979). Further understanding of fine-scale demography and population structure of Aldabra giant tortoises would certainly benefit from more extensive surveys across the atoll, including West Island. With a population size at carrying capacity and a density much larger than its endangered relative from the Galápagos, the Aldabra giant tortoise remains the only large herbivore occupying such a dominant position in a terrestrial ecosystem and a natural model for the study of intraspecific demography and genetic structure evolution.

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