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Fine-scale genetic structure of a long-lived reptile reflects recent habitat modification

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Abstract

Anthropogenic habitat fragmentation — ubiquitous in modern ecosystems — has strong impacts on gene flow and genetic population structure. Reptiles may be particularly susceptible to the effects of fragmentation because of their extreme sensitivity to environmental conditions and limited dispersal. We investigate fine-scale spatial genetic structure, individual relatedness, and sex-biased dispersal in a large population of a long-lived reptile (tuatara, *Sphenodon punctatus*) on a recently fragmented island. We genotyped individuals from remnant forest, regenerating forest, and grassland pasture sites at seven microsatellite loci and found significant genetic structuring (R_{ST} = 0.012) across small distances (< 500 m). Isolation by distance was not evident, but rather, genetic distance was weakly correlated with habitat similarity. Only individuals in forest fragments were correctly assignable to their site of origin, and individual pairwise relatedness in one fragment was significantly higher than expected. We did not detect sex-biased dispersal, but natural dispersal patterns may be confounded by fragmentation. Assignment tests showed that reforestation appears to have provided refuges for tuatara from disturbed areas. Our results suggest that fine-scale genetic structuring is driven by recent habitat modification and compounded by the sedentary lifestyle of these long-lived reptiles. Extreme longevity, large population size, simple social structure and random dispersal are not strong enough to counteract the genetic structure caused by a sedentary lifestyle. We suspect that fine-scale spatial genetic structuring could occur in any sedentary species with limited dispersal, making them more susceptible to the effects of fragmentation.

Keywords: habitat fragmentation, microsatellites, sex-biased dispersal, spatial genetic structure, *Sphenodon punctatus*, tuatara

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Introduction

Habitat fragmentation is ubiquitous in modern ecosystems (Saunders *et al*. 1991). In addition to natural forces like dispersal, demography, and the mating system (Wright 1931, 1978; Crow & Kimura 1970; Bohonak 1999), anthropogenic habitat fragmentation and disturbance have strong effects on gene flow and genetic population structure (Manel *et al*. 2003; Lawton-Rauh 2008; Walker *et al*. 2008). Animals living in fragmented habitats often show decreased dispersal because of unwillingness or inability to move between fragments (Saunders *et al*. 1991; Debinski & Holt 2000; Couvet 2002). Fragmentation increases the likelihood of inbreeding by causing an accumulation of related individuals within fragments, and increases the rate of population differentiation due to genetic drift (Saunders *et al*. 1991; Couvet 2002; Allendorf & Luikart 2007). Thus, genetic studies of dispersal, relatedness and population structure can inform conservation management by elucidating the potential impact of habitat fragmentation and disturbance. Because the two primary effects of habitat fragmentation are isolation and alteration of the microclimate (Saunders *et al*. 1991), reptiles, with their naturally limited dispersal (Gibbons *et al*. 2000) and extreme sensitivity to environmental conditions (Janzen 1994a,b; Gibbons *et al*. 2000), may be more susceptible to the effects of fragmentation than other taxa.

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Genetic analyses based on individual genotypes have enabled detection of fine-scale genetic structuring, individual migration, and cryptic behaviour (Knutsen *et al*. 2003; Vignieri 2007; Clark *et al*. 2008). For instance, assignment tests can recognize probable migrants by identifying the population to which an individual's genotype has the highest likelihood of belonging. Likewise, patterns of dispersal and mating between relatives can be inferred by examining pairwise coefficients of relatedness among subpopulations or sexes. Molecular methods can be applied to systems where behavioural and dispersal patterns are difficult to observe or too low to detect by traditional ecological methods (e.g. radio-telemetry, capture–mark–recapture), and often enhance or provide results contrary to those obtained using traditional methods (Hughes 1998 and references therein).

Many fine-scale genetic techniques now incorporate detailed spatial information that goes beyond simple linear distances between populations. Incorporating advanced spatial analyses has enabled quantification of the effects of habitat and/or geographic barriers to explain genetic patterns (Manel *et al*. 2003; Spear *et al*. 2005; Storfer *et al*. 2007; Telles *et al*. 2007). For example, interpolation, a procedure that is commonly used in spatial analyses to calculate new data points to fit the range of known data points, is now being used to present novel graphical representations of spatial genetic structure (Miller 2005; Vignieri 2007). Interpolation enables population geneticists to model continuous genetic distances across a patchily sampled landscape without a priori grouping samples into subpopulations. When applied in the context of landscape-scale processes like habitat fragmentation, novel molecular methods are a powerful way of revealing genetic impacts to populations.

In this study, we use a large island population of tuatara (*Sphenodon punctatus*) to investigate the potential effects of recent habitat fragmentation on spatial genetic structure, dispersal, and relatedness. Endemic to New Zealand, tuatara are medium-sized (approximately 200 mm snout-vent length), territorial reptiles that are extremely long-lived (80+ years, Dawbin 1982), and have a long generation time (40–50 years, Allendorf & Luikart 2007). Although once distributed throughout the main and outlying islands of New Zealand, tuatara populations are now restricted to approximately 35 small offshore islands. Over half of extant tuatara (estimated at 30–50 000 individuals, Newman 1987) inhabit Stephens Island, a 150-ha island in the Marlborough Sounds (40°40′S, 174°00′E).

Coastal forest covered Stephens Island until the early 1900s when over 80% had been cleared or severely degraded for livestock grazing (Dieffenbach 1843; Brown 2000; see Fig. 1A). Tuatara appear to have altered their behaviour and movement patterns significantly in only two or three generations since fragmentation. In both habitats, tuatara exhibit high territory fidelity and the adult spatial structure is relatively static over time (Moore 2008). Tuatara are nocturnal in the pastures, but they are active throughout the day and night in the forest (Gillingham *et al*. 1995). Further, population density is currently 10 times higher in the forest than in the pastures (~2700 tuatara/ha in the forest vs. ~250 tuatara/ha in the pasture; Moore 2008). Pastures do not appear to pose significant barriers to tuatara movement (e.g. many females traverse this habitat for nesting), and because tuatara are so long-lived, this population was assumed to be panmictic. However, although habitat fragmentation has affected the demography and behaviour of Stephens Island tuatara, any unrecognized genetic effects have yet to be revealed.

Genetic structuring and gene flow are well understood for long-lived plants (see Vekemans & Hardy 2004 for a review), but few studies have investigated fine-scale genetic structuring and dispersal of long-lived animals. Further, most studies of fine-scale genetic structuring and individual dispersal and relatedness patterns are conducted in taxa with well-developed social systems (e.g. insects — Ross 2001; Schrey *et al*. 2008; mammals — Coltman *et al*. 2003; Nussey *et al*. 2005; Frantz *et al*. 2008). Few reptiles have complex social systems (but see Bull & Cooper 1999; Stow *et al*. 2001; Chapple & Keogh 2005), and because of ecological and thermal constraints, many reptiles have extremely limited dispersal capabilities. Specifically, we ask:

1 Can dispersal or migration be sex-biased in a species with a simple territorial social structure?
2 Could fine-scale spatial genetic structuring (i.e. between subpopulations < 750 m apart) occur in a long-lived reptile, and what is the effect of recent habitat fragmentation?

Materials and methods

Sample collection

We collected blood samples from 272 tuatara from eight sampling sites distributed around Stephens Island (Fig. 1). In a remnant forest patch on Stephens Island (Keeper's Bush, F1 from now) three study plots (F1a, F1b, and F1c) were established and all individuals within the study plots (n = 142) were captured by hand on six separate sampling trips between November 2004 and March 2007. Four other sites (one regenerating forest, R1; three pastures, P1, P2 and P3) were sampled around the island and were chosen based on accessibility and permission from the New Zealand Department of Conservation (as researcher access is restricted to certain parts of the island). Individuals were located by opportunistic encounters, at night, between 28 February and 16 March 2006. Thirty tuatara were sampled in March 2003 from a forest remnant (the Frog Bank, F2) that is normally inaccessible to tuatara

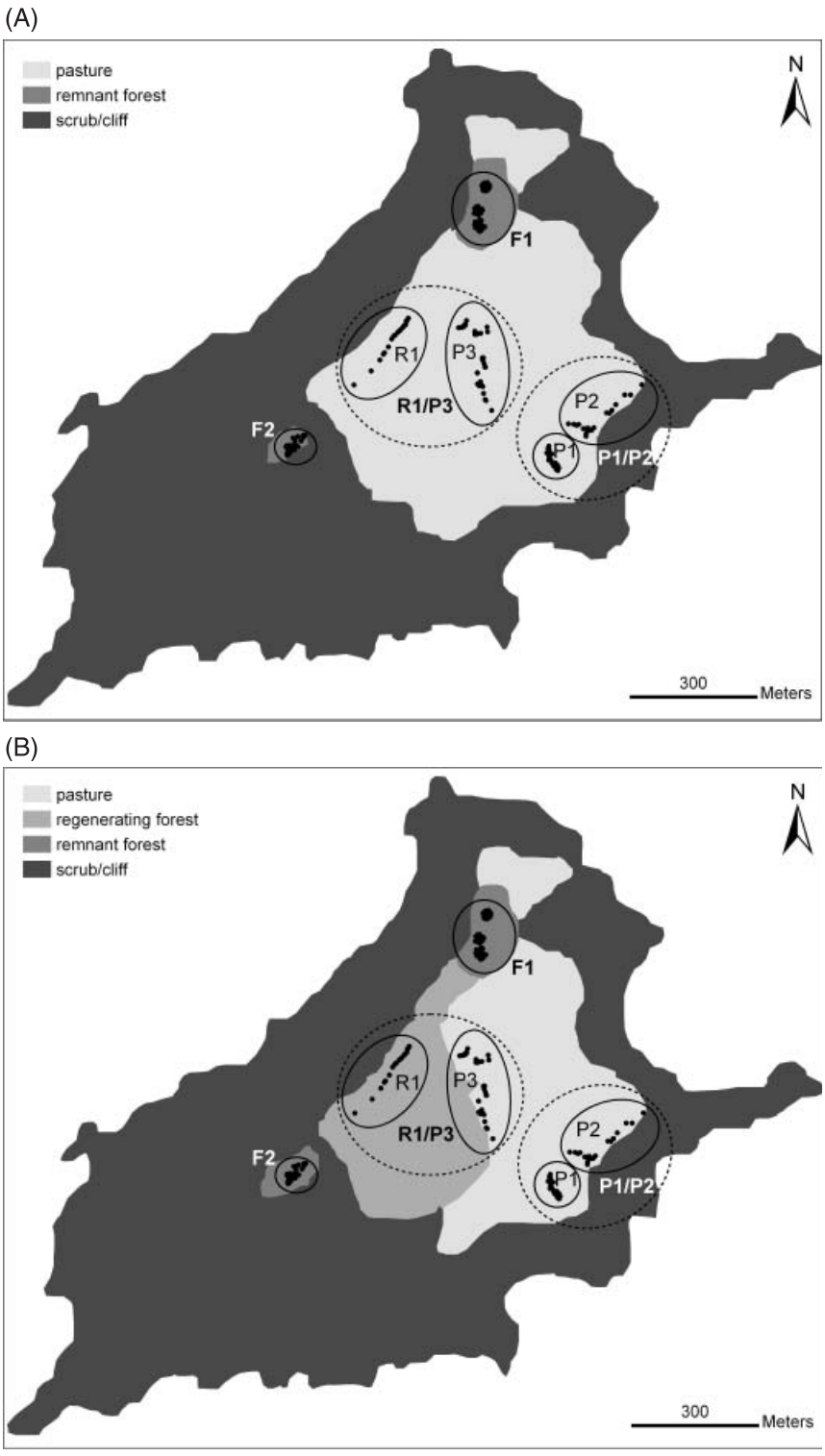

Fig. 1 Aerial view of Stephens Island with eight tuatara sampling locations (black dots are individual locations) by habitat type in (A) 1943 and (B) 1994. Data were grouped for some analyses into four subpopulations (indicated by dashed lines and bold lettering). Significant topography exists, with the island rising to near 300 m above sea level at the summit (near R1). Land cover layers were digitized based on high resolution aerial photographs and ground-truthed for accuracy.

researchers. Aside from the F1 site which was repeatedly sampled, no samples were collected during nesting season (November–December) when females would have been away from their home burrows. All individual capture locations were marked with a handheld GPS (Trimble GeoXT) and locations were post-processed to increase accuracy using Pathfinder Office software (Trimble Navigation Ltd.) and base files from Wellington, New Zealand. Data were then entered as a point coverage in a geographic information system (GIS).

Upon capture, all tuatara were weighed, measured, sexed, and 0.2–1.0 mL of blood was drawn from the caudal vein/artery. Sex of adults was determined by sexually dimorphic characters (e.g. larger head and spines of males vs. smaller spines and pear shaped abdomen of females; Cree *et al*. 1991) and individuals that were too young to be sexed were classified as juveniles. Blood samples were stored in cryotubes in either 70% ethanol at room temperature or snap- frozen in liquid nitrogen until they could be placed in long-term storage at –80 °C. Individuals in the F1 plots were marked with a subcutaneous passive integrated transponder (PIT) tag (AVID Identification Systems, Inc.), and all others were marked by writing a unique number code on the side of the animal with a non-toxic marker that was visible throughout the duration of the sampling trip, to ensure individuals were not resampled.

Genotyping and genetic analyses

Genomic DNA was extracted from 5–10 μL of whole blood using a proteinase K phenol-chloroform protocol (Sambrook *et al*. 1989) or with a DNeasy tissue kit (QIAGEN) following the manufacturer's protocol. We genotyped all individuals using seven reliable and polymorphic loci (*C2F*, *C11P*, *E11N*, *H5H*, *A12N*, *C12F* and *H4H*) in 15 μL reaction volumes, following polymerase chain reactions conditions outlined in Hay & Lambert (2008). Amplified products were multiplexed for genotyping and were run on an ABI 3730 Genetic Analyzer (Applied Biosystems) with the internal size standard GeneScan 500 LIZ (Applied Biosystems). Fragments were analyzed and visualized using GeneMapper software (version 3.0, Applied Biosystems) and sizes were manually scored by the same observer.

We calculated observed and expected heterozygosities and number of alleles per locus in GenAlEx 6 (Peakall & Smouse 2006), and tested for significant deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium at each locus for each sampling locality in GenePop 4.0 (Raymond & Rousset 1995). We used a Monte Carlo chain method (1000 dememorizations, 100 batches, 1000 iterations) following the algorithm of Guo & Thompson (1992) and applied a Bonferroni correction for a table-wide significance level of 0.05 (adjusted P value = 0.0008). We calculated allelic richness per locus for each sampling site using FSTAT 2.9 (Goudet 1995), and used Micro-Checker 2.0 (Van Oosterhout *et al*. 2004) to estimate the frequency of null alleles.

Population patterns of genetic diversity

We first examined subpopulation structuring in the Stephens Island tuatara using an analysis of molecular variance (AMOVA) framework in GenAlEx 6 following methods of Excoffier *et al*. (1992). AMOVA provides estimates of traditional *F*-statistics (Weir & Cockerham 1984), as well as their analogues (R_{ST} and Φ_{PT}). We also calculated pairwise F_{ST} and R_{ST}. A limitation to the AMOVA framework is that it requires a priori clustering of samples into subpopulations. Thus, we first explored pairwise F_{ST} and R_{ST} values of our sites as they were sampled (with eight separate subpopulations), and based on genetic similarities and differences, we grouped them according to spatial proximity (within 200 m of one another) and habitat and disturbance history. We then ran a second AMOVA with data grouped as four subpopulations: F1 (a random subsample of 50 individuals from the combined data from the three F1 plots), R1/P3 (all R1 samples combined with the P3 samples), P1/P2 (all samples combined from P1 and P2) and F2 (all of the F2 samples) (see Fig. 1). Because we only sampled a low number of juveniles in three of the eight sampling sites, we removed juveniles from these analyses. Significance testing was achieved by 9999 random permutations. Because F_{ST} has many limitations, we also used an assignment-based method that may be a more powerful alternative (Pearse & Crandall 2004; Clark *et al*. 2008). We calculated D_{LR}, the genotype likelihood-ratio distance (Paetkau *et al*. 1995), using Doh (Brzustowski 2002) with data grouped by sampling location. D_{LR} is the likelihood that a given genotype originated in the population where it was sampled relative to other populations. This measure appears to perform well at fine scales where populations show little differentiation (Paetkau *et al*. 1997). Significance of assignments was determined by creating random genotypes from pooled populations (in Doh) and recalculating population assignments for 1000 randomized datasets. The randomized datasets are then compared to the actual data sets to determine whether the level of self assignment in actual subpopulations is greater than in randomly constructed populations.

We tested whether there was an overall pattern of isolation by distance across subpopulations. We grouped data as six subpopulations F1, F2, R1, P1, P2, and P3 because this analysis is reliant upon geographic distances. We calculated the geometric centres of individual geographic locations for each of the six subpopulations using the 'mean centre' tool in ArcGIS 9.1 (ESRI). Pairwise distances between subpopulation centres were determined by performing a surface length analysis in ArcGIS 9.1 based on a 25-m digital elevation model (DEM) for Stephens Island. Surface length distances are three-dimensional lengths that take into account the topography of a landscape, thus providing more accurate estimates of distance when significant topographical features exist. Surface lengths between subpopulation centres were 1–32 m longer than planimetric (i.e. flat) distances. We performed a Mantel test for matrix correspondence in GenAlEx 6 comparing pairwise R_{ST} values to pairwise surface length distances for the six subpopulations. We used R_{ST} because it is more appropriate for markers with high levels of variation, and F_{ST} can be

biased downwards when variation within subpopulations is high (Allendorf & Luikart 2007). To examine whether dispersal and movement may be limited to within a habitat type, we used a Mantel test to compare pairwise habitat difference/similarity to genetic distance (pairwise R_{ST}) in GenAlEx6. We generated a pairwise habitat matrix for the six sites by designating like pairs (i.e. forest–forest) with a value of 1 and unlike pairs (i.e. forest–pasture) with a value of 2. Significance of matrix correspondence was tested by 9999 random permutations.

Because of the fragmented nature of our study site, we tested the hypothesis that individuals within historic forest fragments (F1 and F2) are more related than individuals in pasture sites (P1/P2 and R1/P3). We calculated mean pairwise relatedness (*R*) using the formula of Queller & Goodnight (1989) in GenAlEx 6 (Peakall & Smouse 2006) for each of the four subpopulations. We tested for significant differences in subpopulation means by performing 999 random permutations of our data, and 95% confidence intervals around each mean *R* were estimated by 999 bootstraps.

Detailed patterns of spatial genetic structure across Stephens Island were visualized using the 'genetic landscape shape' (GLS) interpolation procedure in Alleles in Space (AIS, Miller 2005). This method is designed to facilitate visualization of patterns of diversity across a landscape by creating a three-dimensional surface plot where the *x*- and *y*-axes correspond to geographic coordinates and the *z*-axis corresponds to genetic distance. The procedure thus creates peaks in areas where genetic distances between individuals are high, and valleys or troughs where genetic distances between individuals are low, and is particularly effective at identifying geographic barriers. This method has recently been used to investigate spatial genetic structure at very fine scales (e.g. Vignieri 2007) and is a powerful approach for estimating genetic structure across sampled and unsampled individuals. In AIS, we first created a pairwise location-based connectivity network for all individual locations. Pairwise genetic distances were then calculated following equation 3 in Miller (2005). Residual genetic distances (derived from the linear regression of all pairwise genetic distances on geographical distance) were then assigned to the midpoints of each connection in the network. Genetic structure across the landscape was inferred from measured genetic distances using an inverse distance weighted interpolation across a uniform grid laid over the entire sampling area. A grid size of 50×50 was selected (we also tested a 100×100, and 25×25 grid), with a distance weighting parameter (*a*) of 0.5 (we also tested $a = 0.6$–1.5).

Individual dispersal and migration

In addition to testing for population-wide patterns of diversity, we investigated whether there were differences at the individual level in the form of sex-biased dispersal or migration. Although male-biased dispersal is predicted in polygynous systems (Mossman & Waser 1999; Prugnolle & de Meeus 2002), nesting by forest tuatara occurs outside of home ranges and no direct evidence for natal philopatry exists. Thus, we hypothesized sex-biased dispersal would not be evident in pasture or forest tuatara. We conducted four separate indirect tests for sex-biased dispersal and migration of males and females across sites: (i) mean pairwise relatedness, (ii) mean corrected assignment index (*mAIc*), (iii) variance of assignment index (*vAIc*), and (iv) spatial autocorrelation. Assignment indices follow the methodology of Favre *et al.* (1997) and Mossman & Waser (1999). We included all data from the F1 plots and analyzed each as separate sites (F1a, F1b, and F1c), in addition to the F2, R1/P3 and P1/P2 subpopulations. If sex-biased dispersal exists, the dispersing sex is expected to have a lower average relatedness than the nondispersing sex (Prugnolle & de Meeus 2002). Likewise, *mAIc* should be lower for the dispersing sex because immigrants have lower *AIc* values than residents, and *vAIc* should be higher for the dispersing sex because members of the dispersing sex will include both immigrants (low *AIc*) and residents (high *AIc*) (Mossman & Waser 1999). We calculated mean relatedness between male–male and female–female pairs, *mAIc*, and *vAIc* in FSTAT 2.9. Significance testing was achieved by comparing actual values to randomized values for 10 000 permutations. Multi-locus spatial autocorrelation analyses, following the methods of Smouse & Peakall (1999), were performed in GenAlEx 6. This technique calculates an autocorrelation coefficient (*r*) for predefined distance classes. Under a model of restricted dispersal, the expectation is that genetic and geographic distance will be positively autocorrelated at short distances. Significance tests are performed using 1000 random permutations and 95% confidence intervals for estimates of *r* are determined by 1000 bootstraps.

Results

Genetic analyses

Expected heterozygosity ranged from 0.73 to 0.78 and was highest for the F1b and P3 sites ($H_E = 0.78$) and lowest for the F2 site ($H_E = 0.73$) (Table 1). Allelic richness ranged from 8.1 to 9.5 alleles per locus. Individuals in the R1 subpopulation showed the highest allelic richness (9.5 alleles) while the F2 individuals showed the lowest allelic richness (8.1 alleles) (Table 1). Following the Bonferroni correction, only one locus (*H5V*) in one subpopulation (P3) showed a significant deviation from HWE. We did not find any overall linkage disequilibrium between any of the loci. Although null alleles were detected at low frequencies in one locus for three subpopulations, and two loci for one

Table 1 Sample sizes (N), habitat type, mean number of alleles, expected (H_E) heterozygosity by locus, and allelic richness (number of alleles corrected for sample size) for each tuatara sampling locality on Stephens Island

	N	Habitat type	No. of alleles	H_E	Allelic richness
F1a	42	Remnant forest	11.29	0.76	8.9
F1b	47	Remnant forest	11.71	0.78	9.2
F1c	53	Remnant forest	11.57	0.77	9.0
F2	30	Remnant forest	8.86	0.73	8.1
R1	20	Regenerating forest	9.71	0.77	9.5
P1	20	Pasture	8.86	0.75	8.7
P2	20	Pasture	9.00	0.76	8.9
P3	40	Pasture	11.14	0.78	9.0

Table 2 Pairwise population R_{ST} (above diagonal) and, for comparison, F_{ST} (below diagonal) estimates for tuatara from four subpopulations from two habitat types (forest, F2 and F1; pasture, P1/P2 and R1/P3) on Stephens Island. Asterisks indicate significant differences from zero ($P < 0.05$)

	F2	F1	P1/P2	R1/P3
F2	—	0.009	0.053*	0.030*
F1	0.003	—	0.004	0.000
P1/P2	0.007*	0.000	—	0.004
R1/P3	0.011*	0.000	0.001	—

subpopulation, null alleles were not detected at the same loci across subpopulations. Therefore, we retained all seven loci for analyses.

Population patterns of genetic diversity

The AMOVA with data grouped as four subpopulations based on discreet sampling locations showed generally low but significant levels of differentiation (overall $R_{ST} = 0.012$, $P = 0.025$), with only 1.2% of total genetic variation due to differences among subpopulations. Pairwise estimates of R_{ST} between the F2 and P1/P2 and R1/P3 sites were low, but significant (Table 2). We found no evidence for an isolation-by-distance pattern. The F2 and R1/P3 sites (located ~400 m from one another) were significantly genetically differentiated, while sites (F1 and F2) that were a greater distance apart (~750 m) were not. Pairwise D_{LR} values ranged from 0.0 to 1.46, indicating a low to moderate average likelihood of observing individual genotypes in the subpopulation from where they were sampled to that of other subpopulations (Clark *et al*. 2008). Furthermore, 29% of individuals were correctly assigned to the subpopulation from which they were sampled and the randomization procedure showed significant self-assignment in the two forest remnant sites (F1 and F2), but not in the other sites (Table 3). No individuals from the R1 site assigned to the R1 site, but rather they assigned evenly across all other sites. The Mantel test comparing pairwise surface lengths to pairwise genetic distance showed no significant patterns of isolation by distance ($R = 0.47$, $P = 0.078$). However, the Mantel test comparing pairwise habitat difference/similarity to genetic distance was weakly significant ($R = 0.47$, $P = 0.048$). Mean pairwise relatedness was generally low (values ranging from –0.14 to 0.062). Only the individuals within the F2 subpopulation were significantly more related than the other three subpopulations (mean = 0.062, $P = 0.002$; Fig. 2) as determined by permutation testing.

The genetic landscape shape suggests increased genetic distance between individuals in the middle of the island, with lower genetic distance between individuals around the edges, and extremely low distances (represented as a trough) in the region of the F2 site (Fig. 3). The peaks

Table 3 Proportion of sampled tuatara assigned from column site to row site from six sampling sites around Stephens Island (based on genotype likelihood ratio distances). Only individuals from the two remnant forest sites (F1 and F2) showed significant self-assignment to their subpopulation of origin, as indicated by the asterisks ($P < 0.05$)

	F2	F1	P3	P2	P1	R1
F2	0.40*	0.23	0.10	0.13	0.00	0.13
F1	0.18	0.36*	0.18	0.10	0.09	0.09
P3	0.05	0.30	0.20	0.08	0.15	0.23
P2	0.15	0.15	0.20	0.20	0.15	0.15
P1	0.05	0.20	0.25	0.20	0.15	0.15
R1	0.15	0.25	0.30	0.20	0.10	0.00

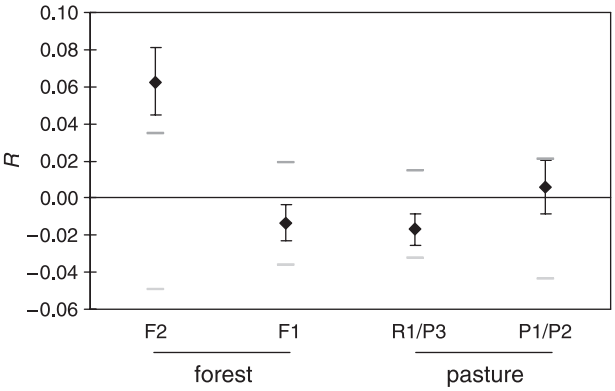

Fig. 2 Mean pairwise estimates of tuatara relatedness (bound by 95% confidence intervals) for forest (F1 and F2) and pasture subpopulations (P1/P2 and R1/P3). Individuals in the F2 subpopulation are significantly more related than expected ($P = 0.002$). Grey bars are upper and lower 95% confidence limits across subpopulations.

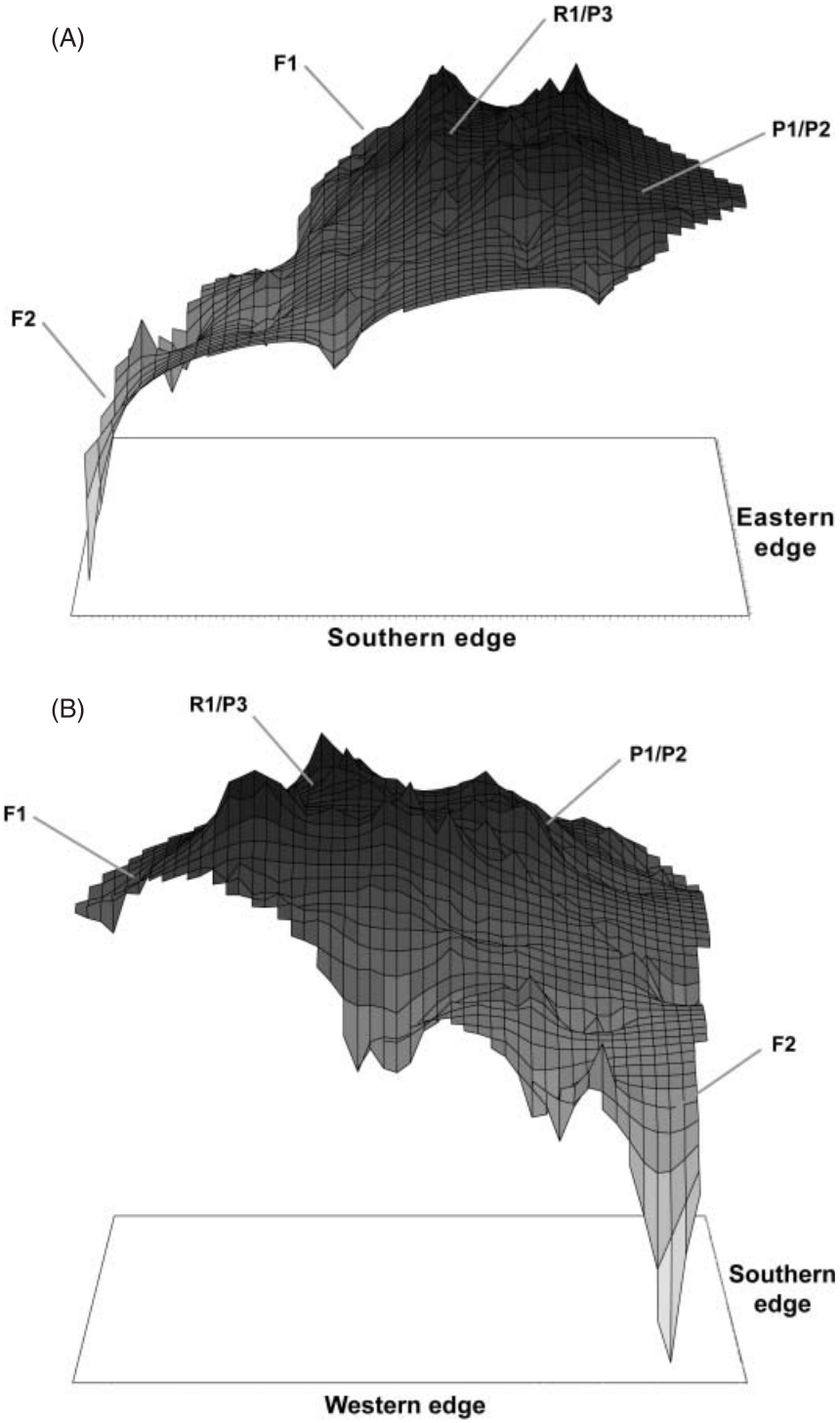

Fig. 3 Genetic landscape shape showing patterns of spatial genetic distance for tuatara across Stephens Island. *X*- and *y*-axes correspond to geographic coordinates and the *z*-axis (height) corresponds to genetic distance between individuals. Peaks are indicative of areas with high pairwise genetic distance and valleys or lighter colours are indicative of areas of low pairwise genetic distance. Approximate centres of sampling sites are indicated for clarity.

appear to approximately correspond to regions that historically would have been most disturbed (pastures), while troughs correspond to least-disturbed areas (forest fragments). The interpolation is bounded by the outside of the sampling area, and thus does not include anything beyond the outermost edges of the sampling areas (e.g. cliffs).

Individual dispersal and migration

We did not detect an overall pattern of sex-biased dispersal or migration. Mean pairwise relatedness between males ($R = 0.008$) and females ($R = 0.01$) was not significantly different ($P = 0.27$). Although the mean corrected assignment

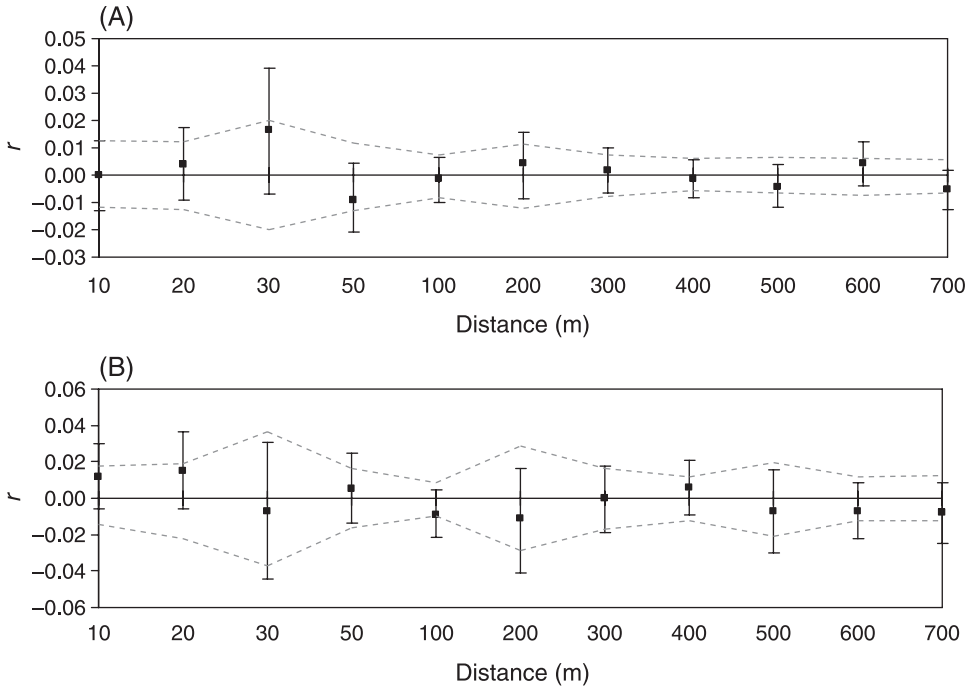

Fig. 4 No significant pattern of spatial autocorrelation exists for male (A) or female (B) tuatara on Stephens Island as indicated by the correlograms of correlation coefficients (r) of geographic and genetic distance at variable distance classes. Upper and lower error bars are bound by 95% confidence intervals around each r, and dashed lines indicate 95% confidence limits around the null hypothesis of a random spatial distribution of genotypes.

index was lower on average for males ($mAIc = -0.16$) than females ($mAIc = 0.30$), the difference was not significant ($P = 0.11$). Likewise, variance of assignment index was higher for males ($vAIc = 8.01$) than females ($vAIc = 7.63$), but the difference was not significant ($P = 0.35$). We found no obvious patterns from the spatial autocorrelation analyses. Neither sex showed a significant decline in genetic distance with geographic distance (Fig. 4), suggesting that neither sex is philopatric.

Discussion

We show that large populations of long-lived animals with high genetic variation can exhibit genetic structuring on a very small scale (< 500 m), even in the absence of sex-biased dispersal or complex social systems. Although the level of genetic differentiation in Stephens Island tuatara was low, the fact that any significant genetic structuring exists at this scale in such a long-lived species is surprising. We found no evidence for sex-biased dispersal, and assignment tests reflect individual movement patterns consistent with recent habitat modification.

The low but significant genetic structuring of Stephens Island tuatara is primarily driven by the differentiation of the southern forest remnant (F2) individuals. The most parsimonious explanation for this result is that the F2 site has simply diverged over time because it has been the least disturbed throughout the history of the island (Brown 2000), and it may be naturally more isolated due to the topography of the island. However, this does not explain why the two forest remnants were not genetically differentiated, although they were geographically more distant than sites that were. We envisage two possible explanations for this pattern. First, the scrubby cliffs along the western edge of the island may have functioned as a corridor between the two forest remnants during and after fragmentation. Although inaccessible to people and livestock, these cliffs pose no barrier to tuatara movement and probably provide greater cover than the pastures. Radiotelemetry has shown that some female tuatara from the northern forest remnant (F1) nest on the western cliffs, subsequently returning to their home ranges (J. Moore, unpublished data). Juveniles dispersing from western cliff nests to either of the forest remnants, avoiding the disturbed areas in between, may have homogenized these two sites.

Second, allele frequencies in the forest remnants may represent what was present across the island prior to habitat modification, and increased admixture in the pasture and regenerated sites has caused these sites to exhibit different genetic profiles from the F2 site. Deforestation of pasture sites and constant disturbance from livestock grazing has altered behaviour and movement patterns of

adult tuatara (Gillingham *et al*. 1995; Moore *et al*. 2007) and may have caused increased mortality. Further, conversion of forest to pasture has altered the thermal regime enabling nesting in pastures that were too cold to support egg development when forested (Thompson 1990). Therefore, genetic signatures of F1 individuals are regularly introduced to the pasture sites by F1 females that now nest in these sites (N. Nelson, unpublished data). The high genetic distances (calculated in AIS) between individuals in the pastures (see GLS in Fig. 3), the even assignment of pasture individuals across all other sites, and the lack of differentiation between the F1 and pasture sites support this supposition. In this respect, the forest fragments may have acted as refugia for established resident tuatara that were able to maintain their natural spatial structure and behavioural patterns throughout the period of disturbance, as well as nearby juveniles that may have immigrated during heavy disturbance. The significant self-assignment of only individuals from forest sites and findings from our habitat Mantel test provide evidence for this.

Our assignment tests show that individuals in the reforested R1 site are recent immigrants. The R1 site, and the entire region between the two forest remnants (Fig. 1), was once completely denuded by livestock and naval activity (Brown 2000). This area was replanted in 1989 by the New Zealand Department of Conservation to provide a corridor between the remaining forest patches, which now resembles original closed canopy forest remnants. Reforestation appears to have established a refuge for pasture animals by providing increased cover from avian predators, greater food resources (Walls 1981), and little competition from already established resident tuatara. Kanowski *et al*. (2006) advocate revegetating corridors between remnant habitat fragments for successful re-establishment of reptile populations. Our data suggest that corridor reforestation has been a successful approach for tuatara, and most likely for other lizard species as well (Stephens 2004).

We found no strong pattern of sex-biased dispersal or migration in tuatara. Although values from the individual tests point to males being the more mobile sex and females the more philopatric sex, no test was significant. Sex-biased dispersal may evolve as a mechanism to avoid inbreeding (Perrin & Mazalov 2000; Prugnolle & de Meeus 2002), but because female tuatara in the forest do not nest in their home ranges, the risk of inbreeding in large forest remnants is low. Sex-biased dispersal may be more prevalent in reptiles that live in family groups or have more complex social systems (e.g. some lizards in the *Egernia* genus, Bull & Cooper 1999; Stow *et al*. 2001). Our ability to detect natural dispersal patterns is probably confounded by the disturbance and habitat fragmentation in this population (e.g. Stow *et al*. 2001; Sumner 2005). A more appropriate test may be to examine only patterns of juvenile dispersal, or patterns of dispersal on a warmer northern island where local climatic conditions could allow females to nest in their home ranges.

Spatial genetic structuring has now been detected at fine scales (100 m–2 km) in a number of mobile animal species (e.g. Gibbs *et al*. 1997; Spruell *et al*. 1999; Brouat *et al*. 2003; Coltman *et al*. 2003; Peakall *et al*. 2003; Double *et al*. 2005; Clark *et al*. 2008). However, many of these species have a more complex social system, and shorter lifespan, than tuatara. For instance, fine-scale genetic structuring can be reinforced by a strong tendency for female philopatry, a pattern that is common in many mammals (e.g. red deer, *Cervus elaphus*, Nussey *et al*. 2005; Frantz *et al*. 2008). The relatively simple territorial spatial structure of tuatara is highly stable over years (Moore 2008), and possibly decades. The risk that juveniles will coincidentally disperse to the same forest fragment as their parents increases when preferred forest habitat is limited, thereby increasing relatedness within small fragments (e.g. for Cunningham's skink, *Egernia cunninghami*, Stow *et al*. 2001). Thus, the sedentary lifestyle and limited dispersal of adult tuatara, and many reptiles (Gibbs *et al*. 1997; Prosser *et al*. 1999), may be strong enough to result in fine-scale genetic structuring even in the absence of a more complex social system.

We found no overall pattern of isolation by distance for Stephens Island tuatara. At mutation–migration–drift equilibrium, and for species with limited dispersal, genetic differentiation should increase with geographic distance (Slatkin 1993). Habitat fragmentation and disturbance may have caused Stephens Island tuatara to diverge from this theoretical expectation. Driscoll & Hardy (2005) found that populations of agamid lizards (*Amphibolurus nobbi*) in uncleared forested habitat showed significant isolation by distance, whereas populations in linear farmed habitat did not. Further, small *A. nobbi* populations in farmed habitat had similar levels of genetic variation to large populations in nature reserves, which the authors attributed to a burst of movement during land clearing resulting in migrations of lizards from many sources finding refuge in remnant forest populations (Driscoll & Hardy 2005). Stephens Island tuatara appear to reflect a similar pattern, although it is perhaps equally plausible that the isolation-by-distance expectation would not hold true at this fine scale. The apparent alteration of genetic structuring and dispersal across the island has occurred on a very short timescale for long-lived tuatara (only two to three generations), and hence, the current patterns are more indicative of increased migration of long-lived individuals, rather than genetic drift.

Anthropogenic habitat modification and disturbance have had a profound effect on gene flow for many reptiles, because of their naturally low dispersal and extreme sensitivity to changes in the thermal environment (Cunningham & Moritz 1998; Stow *et al*. 2001; Sumner *et al*. 2004; Driscoll & Hardy 2005; Sumner 2005; Gardner *et al*. 2007). If weak

fine-scale genetic structuring due to limited dispersal and a sedentary lifestyle is not counteracted by extreme longevity, large population size, or a simple social structure or random dispersal pattern (e.g. for tuatara), we suspect that any species with limited dispersal or mobility could exhibit very fine-scale spatial genetic structuring. These species may thus be more susceptible to behavioural alteration from anthropogenic habitat fragmentation or disturbance, which would ultimately affect patterns of gene flow and genetic differentiation and potentially increase extinction risk.

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