

# Limited effect of anthropogenic habitat fragmentation on molecular diversity in a rain forest skink, *Gnypetoscincus queenslandiae*

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## Abstract

To examine the effects of recent habitat fragmentation, we assayed genetic diversity in a rain forest endemic lizard, the prickly forest skink (*Gnypetoscincus queenslandiae*), from seven forest fragments and five sites in continuous forest on the Atherton tableland of northeastern Queensland, Australia. The rain forest in this region was fragmented by logging and clearing for dairy farms in the early 1900s and most forest fragments studied have been isolated for 50–80 years or nine to 12 skink generations. We genotyped 411 individuals at nine microsatellite DNA loci and found fewer alleles per locus in prickly forest skinks from small rain forest fragments and a lower ratio of allele number to allele size range in forest fragments than in continuous forest, indicative of a decrease in effective population size. In contrast, and as expected for populations with small neighbourhood sizes, neither heterozygosity nor variance in allele size differed between fragments and sites in continuous forests. Considering measures of among population differentiation, there was no increase in  $F_{ST}$  among fragments and a significant isolation by distance pattern was identified across all 12 sites. However, the relationship between genetic ( $F_{ST}$ ) and geographical distance was significantly stronger for continuous forest sites than for fragments, consistent with disruption of gene flow among the latter. The observed changes in genetic diversity within and among populations are small, but in the direction predicted by the theory of genetic erosion in recently fragmented populations. The results also illustrate the inherent difficulty in detecting genetic consequences of recent habitat fragmentation, even in genetically variable species, and especially when effective population size and dispersal rates are low.

**Keywords:** genetic diversity, *Gnypetoscincus queenslandiae*, habitat fragmentation, lizard, microsatellites, rain forest

Received 25 July 2003; revision received 15 October 2003; accepted 15 October 2003

## Introduction

Habitat fragmentation is predicted to have considerable long-term effects on the genetic and demographic viability of populations due to the combined effects of reduced population size and increased isolation (Frankel & Soule 1981; Young & Clarke 2000). Assuming that habitat fragmentation reduces local effective population size ( $N_e$ ) or migration

rates ( $m$ ), or both, increased genetic drift should redistribute genetic diversity such that variation within populations is decreased and variation among populations is increased (Wright 1931). In a closed population the average heterozygosity decreases at a constant rate of  $1/(2N_e)$  per generation, so that small populations have a higher rate of loss of heterozygosity, and are expected to have lower levels of genetic variation, than large continuously distributed populations (Wright 1978). Furthermore, fitness within isolates is expected to decrease because of accumulation of mildly deleterious alleles (Lande 1995), a process that is ameliorated by immigration (Schultz &

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Lynch 1997). The genetic structure of fragmented populations may therefore shift from a state of high connectivity towards isolated and genetically discrete demes with, on average, lower levels of variability and higher levels of genetic load (Falconer & Mackay 1996). However, paradoxically, total  $N_e$  and gene diversity may be higher in a subdivided system, as long as individual subpopulations remain viable (Gilpin 1991; Whitlock & Barton 1997).

The extent to which a species is affected by habitat fragmentation is determined by its degree of habitat specialization, dispersal potential and behavioural response to habitat fragmentation (Weins 1997). At one extreme, individuals (or their propagules) may disperse actively from one habitat isolate to another, increasing local diversity and decreasing differentiation among populations (Porter 1999). At the other extreme, low vagility habitat specialists may be trapped effectively within fragments, leading to reduced long-term fitness and population viability (Keller *et al.* 1994; Madsen *et al.* 1996; Saccheri *et al.* 1996). Despite the inherent difficulties (see below), surveys of genetic diversity in fragmented vs. continuous populations have an important place in understanding the response of a species to habitat modification.

Two complementary strategies have been used to examine the genetic effects of fragmentation; comparison of a particular set of populations through time under more or less fragmented states (e.g. Srikwan & Woodruff 2000) or comparison at a single point in time of different sets of populations inhabiting more or less fragmented landscapes (e.g. Leberg 1991; Sarre 1995a,b; Paetkau *et al.* 1998; Stow *et al.* 2001). The former is constrained typically by sampling and the latter sometimes by confounding effects of habitat differences or spatially varying population histories (Cunningham & Moritz 1998). In both cases, investigators face the general problem of distinguishing between historical connectivity and current migration as a cause of observed genetic similarity among populations, which is especially acute when using a small number of loci and traditional (e.g.  $F$  statistic) approaches (Slatkin 1987; Nielsen & Slatkin 2000). In the case of populations distributed across recently fragmented habitats, the problem is compounded by stochastic 'noise' in the approach to a new equilibrium value (Varvio *et al.* 1986; Steinberg & Jordan 1998). Given these and other limitations, it is unwise to seek to compare estimates of migration rates (Whitlock & McCauley 1999), although trends in within-population diversity (especially allele diversity, Nei *et al.* 1975) and qualitative differences in structure among populations (Slatkin 1993; Hutchison & Templeton 1999) can be informative. Increasingly researchers are producing work using a combined approach comparing current variation among populations with variation among those populations at a single time in the recent past (e.g. Mundy *et al.* 1997; Bouzat *et al.* 1998; Westemeier *et al.* 1998; Srikwan *et al.* 2002).

Here we report a genetic analysis of fragmented vs. continuous populations of the prickly forest skink, *Gnypetoscincus queenslandiae*, a species restricted to rain forest habitats of the Wet Tropics region of northeast Australia. These rain forests have undergone both long-term natural fragmentation and recent human-induced fragmentation (Laurance 1990, 1994; Schneider *et al.* 1998; Hugall *et al.* 2002). The Atherton Tableland in the Wet Tropics has been subjected to substantial clearing for dairy-farming between 1920 and 1940, leaving numerous rain forest fragments that are surrounded by pastures (Winter *et al.* 1987). Large tracts (> 3000 ha) of continuous forest survive only on steeper hillsides encircling the Tableland, the majority of which have been logged selectively (Laurance 1990). Pahl *et al.* (1988) and Laurance (1990, 1991a,b, 1994) studied the responses of mammals to fragmentation on the Atherton Tableland and found that the ability to traverse the matrix between fragments was the most important factor determining a species' ability to survive in rain forest fragments. Laurance (1994) also suggested that small mammal communities were structured more intensively in fragments with strong competitive interactions occurring between ecologically similar species. Warburton (1997) found that bird species remaining in small rain forest fragments on the Atherton Tableland were matrix-tolerant species and that the avifauna became dominated by generalists.

*G. queenslandiae* is a habitat specialist, living under and within rotting logs on the rain forest floor (Naylor 1980; Cogger 1992). Prickly skinks are viviparous, giving birth to one to five young in the late wet season (January–March) and are fairly long-lived, with an estimated generation time of 6.5 years and a maximum age of approximately 10 years (Cunningham 1993; Sumner *et al.* 2001). Recapture and fine-scale genetic studies of the species in a continuous forest block (Sumner *et al.* 2001) indicate high density (a time scaled estimate of 0.006–0.014 individuals  $\times$  generation/m<sup>2</sup>) but low dispersal rates (404–843 m<sup>2</sup>/generation), resulting in a small neighbourhood area (1.05 ha/generation) and local effective population size (48–184 individuals). Many recaptured individuals are caught under the same log in consecutive years (Sumner 2002a). Prickly skinks are locally common, they are not currently threatened and their habitat is almost entirely contained within the Wet Tropics World Heritage Area. The skinks have been found in rain forest fragments as small as 0.25 ha on the Atherton Tableland (Sumner *et al.* 1999). Their specific habitat requirements and generally low vagility makes it very unlikely that they would traverse the open pastures that now separate fragments of rain forest. A mark-recapture study found no evidence of immigration to, or emigration from, fragments although the chance of detecting such an event is small (Sumner unpubl. data). The sampled skink populations in the forest fragments have been isolated for approximately nine to 12 generations,

using an estimated generation time for prickly forest skinks of 6.5 years (Sumner *et al.* 2001).

Cunningham & Moritz (1998) studied the genetic effects of recent anthropogenic forest fragmentation on the prickly forest skink on the Atherton Tableland using allozymes and mtDNA. They found significant inbreeding coefficients ( $F_{IS}$ ) in fragments but not continuous forest sites and significant population subdivision ( $F_{ST}$ ) among south Atherton fragments but not among the continuous forest sites. However, their results were potentially confounded by the differences in genetic structure between the sampled fragments and continuous habitat sites due to their location relative to Pleistocene rain forest refugia on the Atherton Tableland; the majority of the fragmented forest sites were located in one refugium, while most continuous forest sites were situated in another. Our research extends this work through more extensive sampling of sites within a single refugial area and by using microsatellite DNA markers which, because of their high mutation rate, may be less prone to confounding effects of population history and the higher number of alleles may make them more sensitive for fine-scale analysis of populations (Estoup *et al.* 1998).

We aimed to determine if there were changes in the genetic diversity and population structure of prickly forest skinks in the habitat islands remaining on the Atherton Tableland as a result of recent forest fragmentation. Specifically, we compared within-population diversity and among-population structure across fragments and continuous habitats than spanned a similar geographical distance. We investigated whether differences were a result of fragmentation *per se*, or whether the extent of change was related to the size of rain forest fragments.

## Methods

### Fieldwork

We sampled from seven rain forest fragments and from five sites within the continuous forest that surrounded the area of fragmented forest on the Atherton Tableland in northeastern Queensland (Fig. 1). The sites were located between latitude 145°34'–145°39' and longitude 17°30'–17°44'. The rain forest fragments were spread over a similar, but slightly longer and narrower, geographical area relative to the sites in the continuous forest. To avoid confounding the effects of historical fragmentation processes, sites were chosen to be within the same historical area, as defined by the skinks' mtDNA haplotype group (Cunningham & Moritz 1998; Schneider *et al.* 1998). The clearing that generated these fragments occurred through this area in the period 1920–40 (Winter *et al.* 1987) and, for the purpose of analysis, we assume similar isolation times for the fragments as isolation times are not known (see Table 1).

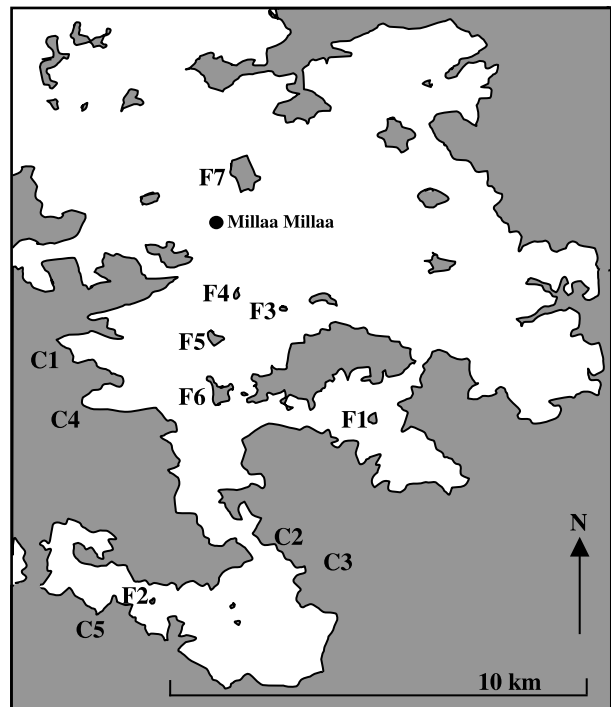


Fig. 1 Locations of sites sampled on the Atherton Tableland in the Wet Tropics of southeastern Australia. Fragments (F1–F7) are labelled according to increasing fragment size and continuous forest sites (C1–C5) are labelled alphabetically according to the site name (see Table 1). Note that fragment F1 is divided by a river and only the 2-ha area to the south was sampled. Grey areas indicate primary forest and white areas indicate cleared land.

Our fragments covered a range of sizes from 2 to 65 ha. Four were small (< 6 ha) and three were moderate (24–65 ha)-sized fragments. Fragment size was mapped using a combination of 1991 Landsat TM digitization and 1:50 000 topographical maps. Corrections for minor boundary alterations subsequent to 1991 were made after ground truthing. All the fragments were at least 200 m from neighbouring forest, more than twice the greatest recorded distance moved by an individual during a 3-year mark-recapture study (Sumner *et al.* 2001). This minimum distance is also substantially greater than the dispersal rate found in that study (a time-scaled estimate of  $20.1 \text{ m/generation}^{1/2}$ ). From this information we assume that immigration into fragments is negligible and that most or all emigrants perish. The vegetation at all sites consisted of original complex notophyll vine forest (Tracey 1982), although most sites have undergone some degree of logging in the past 100 years.

DNA samples were collected during a single trip in January 2000, except for Massey Creek, for which samples were collected in November 1998 (Sumner *et al.* 2001). Skinks were captured by hand, measured for snout to vent and total length (Sumner *et al.* 1999), and the sex and

**Table 1** Sites sampled and the number of individuals genotyped (No.), the site type, the fragment area as generated by remotely sensed mapping and the approximate time of isolation prior to 2000 (years) from Pahl *et al.* (1988) and from topographical map (Maalan Road only)

Site name	Code	No.	Site type	Frag. area (ha)	Frag. age (years)
Souita Falls	F1	25	Fragment	2.00	> 49
Maalan Road	F2	42	Fragment	2.46	> 22
Waltham	F3	27	Fragment	2.64	49
Pat Daley Park	F4	18	Fragment	5.96	> 49
Nose Ring	F5	29	Fragment	24.19	> 49
Whiteing Road	F6	30	Fragment	36.31	< 49
Millaa Millaa Falls	F7	27	Fragment	65.06	< 50
Brotherton	C1	28	Continuous	—	
Cross-eye	C2	30	Continuous	—	
Mt Father Clancy	C3	32	Continuous	—	
Reynolds	C4	28	Continuous	—	
Massey Creek	C5	94	Continuous	—	

reproductive condition was recorded if possible. A tail tip was taken and fresh-frozen on dry ice (CO<sub>2</sub>) for later extraction of DNA. The animal was then replaced under the log where it was discovered. Sites had also been sampled during previous trips (Sumner *et al.* 1999) and the tissue stored at room temperature in 20% dimethylsulphoxide saturated with sodium chloride. DNA could not be amplified consistently from these samples, however, due possibly to tissue degradation.

#### Genetic data

DNA was extracted using a standard phenol-chloroform extraction procedure (Sambrook *et al.* 1989), and nine microsatellite loci with dinucleotide repeat motifs were amplified according to the polymerase chain reaction (PCR) conditions given in Sumner *et al.* (2001). Primer pairs that were used are: GQ10/11F GQ16/17F2, GQ18/19F, GQ20/21F, GQ24F/25, GQ36/37F, GQ38B/39F, GQ42B/43F and EA1F/2B. PCR products were resolved on an Applied Biosystems model 373 DNA sequencer and analysed using the manufacturer's proprietary GENESCAN and GENOTYPER software.

Homogeneity of microsatellite allele distributions for all pairs of populations was tested using the probability test in GENEPOP version 3.2 (Raymond & Rousset 1995) or, where possible, exact tests (Haldane 1954). Tests for each population pair were combined across all nine loci (Fisher 1970).

Loci were tested for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium using GENEPOP version 3.2 (Raymond & Rousset 1995). An exact test based on a Markov chain algorithm was conducted on each population to test for deviations of loci from HWE. The alternate hypothesis was designated as a deviation from HWE due to a heterozygote deficit. Global tests were

performed for each site across all loci and for each locus across all sites. Each pair of loci was tested for linkage disequilibrium using probability or exact tests and then tests were combined across all populations. Bonferroni corrections were applied to multiple tests to reduce Type I error.

We examined two estimates of within-population diversity; allelic diversity ( $A$ ) and expected heterozygosity ( $H_E$ ). Of these, allele diversity is expected to be most sensitive to recent reductions in population size (Nei *et al.* 1975; Garza & Williamson 2001). As the number of alleles identified at each site is effected by sample size, the average allele number ( $A$ ) was adjusted to a common sample size of  $n = 42$  (or slightly less, depending on the highest number of individuals typed at each locus), using a program based on the formula of Ewens (1972; A. Estoup pers. comm.). The formula of Ewens (1972) assumes an infinite alleles model of mutation; however, there is no analytical correction for sample size available under any other mutation model, and the difference in allele number after correction is very small even under extreme models such as the stepwise mutation model (A. Estoup pers. comm.). We chose to standardize the results to the size of the second largest sample of individuals genotyped at one site, as the largest sample ( $n = 94$  at Massey Creek) is more than twice that of the closest sample, and more than five times that of the smallest sample (Table 1) and we wanted to minimize errors from overextrapolation (Leberg 2002). The average allele number for Massey Creek was calculated in two ways, first by scaling the sample down to  $n = 42$  using the formula of Ewens (1972) as above, and second by taking the average of the allele number of 100 iterations of a random subsample of 42 individuals. All analyses were calculated using both estimates; however, the difference between the results was negligible so only the results of calculations using the first estimate are given.

The ratio of the total number of alleles to the overall range in allele size (defined as sequence or repeat length) can be used to detect a population bottleneck (Garza & Williamson 2001). The average  $M$  ratio ( $M = k/r$ ) was calculated for each site, where  $k$  is the number of alleles and  $r$  is the range in allele size +1 at each locus (Garza & Williamson 2001). Rather than testing for significant deviations from mutation-drift equilibrium, which requires an a priori estimate of  $4N_e\mu$  (Garza & Williamson 2001), we tested for greater deviations (lower  $M$ ) in fragments vs. continuous forests, assuming that equilibrium  $4N_e\mu$  was the same across the sampled sites prior to habitat fragmentation.

$T$ -tests were used to test for differences in allele numbers and  $M$  ratios between fragments and continuous forest, analysis of variance (ANOVA) to test for differences between small fragments (< 6 ha), large fragments (> 24 ha) and continuous forest sites and a linear regression to determine if there was a relationship between fragment size and allele number or  $M$  ratio. Significance of differences in  $H_E$  was tested using paired  $t$ -tests of arcsine transformed  $H_E$  calculated for individual loci and repeated for each pair of sites in turn (Archie 1985; Nei 1987).

#### Population structure

Sewall Wright's  $F$ -statistics, calculated following Weir & Cockerham (1984), were used to measure population structure between sites using FSTAT 2.9.1 (Goudet 2000) and GENEPOP 3.2a software. ARLEQUIN 2.000 (Schneider *et al.* 2000) was used to test significance of differences between pairwise  $F_{ST}$  values.  $F_{ST}$  was used to calculate genetic distance as opposed to other distance metrics such as Slatkin's  $R_{ST}$ , as it has been found to be the most conservative approach when the number of loci analysed is less than 20 and the number of samples less than 50 (which is the case for the majority of sites in this study; Gaggiotti *et al.* 1999).

Isolation by distance (IBD) patterns were determined by comparing genetic distance between sites, as measured by pairwise  $F_{ST}$ , to geographical distance (Hutchison & Templeton 1999) using GENEPOP 3.2 (Raymond & Rousset 1995) and the significance tested using a Mantel test. We looked first for an overall pattern of isolation by distance by comparing all sites then looked at IBD among sites separated into fragments and continuous forest sites. We predict that IBD patterns would start to break down in fragments due to suppressed migration and, thus, the enhanced effects of drift (Hutchison & Templeton 1999). To test for this we compared the slopes of the regressions of geographical distance vs.  $F_{ST}$  for the continuous and the fragmented forest sites.

$T$ -tests, ANOVA, analysis of covariance (ANCOVA) and factor analyses were carried out using STATISTICA 4.1 (Statsoft 1994). The effect of fragment size was assessed using the ln of fragment size (ha) in all cases to normalize data.

#### Results

A total of 411 individuals from 12 sites (seven of which were fragments and five of which were continuous forest sites;  $n = 18-94$ ; Table 1) were genotyped at nine microsatellite loci. Complete genotypes were obtained for 362 individuals (88%). The number of missing genotypes at each locus ranged from one (0.24%) to 20 (4.87%) individuals, with 2.14% of genotypes missing across the entire data set. The total number of alleles per locus ranged from five to 28, with an average of 19.6 alleles per locus across all sites. A large number of alleles unique to a site (5.8 unique alleles averaged across all loci) contribute to this high allelic diversity across sites. The average allele number per locus for all loci within sites is much lower (8.3 alleles; see below).

Of the 66 pairs of populations in our study, all had allele distributions that differed at the 5% level after correction for multiple tests. In fact, all but one pair (F5 and C1) had allele distributions that differed at the 0.01% level. We were confident from this result that all sites could be treated as independent units for the purpose of statistical analysis.

Our samples and the apparently unlinked markers (see below) suggest that mating is close to random in this species. Departure from Hardy-Weinberg equilibrium for each population and for each locus was no higher than that expected due to Type I error. We tested for null alleles using global tests across all sites, and it was found that locus GQ10/11 differed significantly from Hardy-Weinberg proportions ( $P = 0.007$ ). This result was not significant when corrected for the number of tests, and appears to be driven by a single highly significant result at Whiteing Road (F6). We did not obtain complete genotypes for this locus, with seven individuals untyped, three of which were from Whiteing Road and the remaining four spread across three other sites. This may be suggestive of null, or nonamplifying, alleles at this locus, perhaps at a higher frequency at Whiteing Road, or may be due to chance alone. A small proportion of alleles at this locus have repeatable one base-pair differences between alleles, which may also be responsible, as there is an increased chance of miss-scoring. This locus is included in all analyses, however, as the result was not significant when corrected for the number of tests and there was no evidence of null alleles in other populations.

Following a Bonferroni correction for multiple tests we found genotypic disequilibrium for two pairs of loci: GQ24/25 and GQ18/19, and GQ16/17 and GQ36/37, each in a single population. This can indicate linkage on the same chromosome, similar selection pressures on these genotypes or stochastic processes in the population. However, if either of the first two explanations were the case we would expect to see disequilibrium more consistently across the 12 sites we sampled. As this was not the case we assumed that loci were independent.

**Table 2** Average number of alleles for each locus and each site adjusted to 42 (the sample size of F2). Results of random subsampling of 42 individuals from C5 are bracketed. Site codes follow Table 1

Site	GQ20/21	GQ10/11	GQ24/25	GQ38/39	GQ18/19	GQ16/17	GQ36/37	EA1/2	GQ42/43	Average
F1	6.8	9.23	10.47	3.28	6.87	10.94	13.0	8.0	4.23	8.09
F2	8	11	10	3	6	10	10	11	4	8.11
F3	5.5	11.72	12.09	4.41	12.48	3.2	10.09	14.9	3.22	8.62
F4	6.12	8.89	17.61	2.22	8.89	6.34	13.35	7.49	4.93	8.43
F5	9.98	11.28	16.63	4.36	12.48	8.82	16.13	8.82	5.41	10.43
F6	8.74	8.74	11.42	5.34	11.15	8.91	15.91	13.35	4.26	9.76
F7	5.5	9.01	15.34	3.21	6.87	7.14	13.87	12.64	5.5	8.79
Average	7.23	9.98	13.37	3.69	9.25	7.91	13.19	10.89	4.51	8.89
C1	8.91	9.01	10.09	4.34	10.09	6.66	17.64	11.28	4.36	9.15
C2	8.74	5.37	12.19	3.17	8.74	8.74	14.52	13.35	4.26	8.79
C3	11.93	9.69	10.81	5.27	7.54	10.91	11.93	13.06	4.23	9.49
C4	7.75	8.91	12.64	4.3	18.63	10.09	12.48	12.48	4.32	10.18
C5	7.56	9.91	13.6	3.53	8.4	7.66	11.43	12.15	3.53	8.64
	(7.59)	(9.89)	(13.47)	(3.3)	(7.33)	(8.2)	(11.73)	(12.14)	(4.0)	(8.63)
Average	9.33	8.25	11.43	4.27	11.25	9.1	14.14	12.54	4.29	9.40

### Comparisons of within-site diversity

Average adjusted allelic diversity ( $A$ ) per site ranged from 8.09 to 10.43 alleles per locus (Table 2; raw scores can be calculated from Table 3). There was no significant difference in average allele numbers between fragmented and continuous forest sites overall (average = 8.89 and 9.40, respectively;  $t = 0.78$ ;  $P = 0.45$ ; d.f. = 10). A regression of the average allele number and  $\ln$  (size) of fragments approached significance ( $F = 4.532$ ;  $P = 0.087$ ; d.f. = 1,5;  $r^2 = 0.475$ ) and when fragments were divided into small (< 6 ha) or moderate (> 24 ha) categories there was a significant difference in average allele number among the categories ( $F = 5.11$ ,  $P = 0.033$ , d.f. = 2; continuous forest sites = 9.40; small fragments = 8.31; and moderate fragments = 9.66 alleles/locus). A Tukey's test of pairwise differences found significant differences between small and moderate fragments ( $P = 0.035$ ), a near significant result between small fragments and continuous forest sites ( $P = 0.093$ ) and no difference between moderate fragments and continuous forest sites ( $P = 0.618$ ).

The expected heterozygosity ( $H_E$ ) per locus across all sites ranged from 0.24 to 0.83 (average  $H_E$ : 0.67; Table 3). There was no significant difference in average heterozygosity across loci between fragments (average  $H_E$ : 0.67; range: 0.07–0.89 per locus) and continuous forest sites (average  $H_E$ : 0.67; range 0.17–0.90 per locus;  $t = -0.13$ ,  $P = 0.90$ , d.f. = 10); nor was there any association between  $H_E$  and fragment size ( $F = 0.030$ ;  $P = 0.870$ ; d.f. = 1,5;  $r^2 = 0.006$ ). Following correction for multiple tests, there were no significant differences between  $H_E$  for any pair of sites.

$M$  ratios averaged across loci ranged from 0.295 to 0.386 (Table 4). Fragments had significantly lower  $M$  ratios on average than the continuous forest sites (average = 0.316

and 0.344, respectively;  $t = -2.33$ ;  $P = 0.042$ ; d.f. = 10). There was no significant linear relationship between fragment size and  $M$  ratio ( $F = 0.1177$ ;  $P = 0.328$ ; d.f. = 1,5;  $r^2 = 0.191$ ).

### Comparisons of population structure

Values for pairwise  $F_{ST}$  calculated between sites were generally small, ranging from 0.004 to 0.125 (Table 5). Most sites exhibited a fairly similar range of values, although Mt Father Clancy (C3) and Millaa Millaa Falls (F7), the most easterly and northerly sites, respectively, had larger values on average than the other sites. Global  $F_{IS}$  scores for fragments and continuous forest sites calculated separately provided no indication of nonrandom mating within fragmented forest sites ( $F_{IS} = 0.013$ ; 95% confidence interval (CI):  $-0.016$ – $0.04$ ) or continuous forest ( $F_{IS} = -0.01$ ; 95% CI  $-0.031$ – $0.011$ ). Similarly, there was negligible difference in overall population subdivision: fragments had  $F_{ST} = 0.046$ ; 95% CI  $0.036$ – $0.058$ ; continuous forest sites had  $F_{ST} = 0.057$ ; 95% CI  $0.044$ – $0.072$ .

Significant IBD was identified among all sites ( $P < 0.0001$ ;  $F_{ST} = 0.0072 + 0.0040$  (distance);  $r^2 = 0.52$ ). When fragments and continuous forest sites were tested for IBD separately, significant patterns were detected for both fragments ( $P = 0.030$ ;  $F_{ST} = 0.0245 + 0.0022$  (distance);  $r^2 = 0.32$ ) and continuous forest sites ( $P = 0.017$ ;  $F_{ST} = -0.0022 + 0.0053$  (distance);  $r^2 = 0.68$ ; Fig. 2). An ANCOVA between fragments and continuous forest sites with  $F_{ST}$  as the dependent variable and geographical distance as the covariate found a significant difference between the slopes of the regressions ( $F = 4.372$ ;  $P = 0.046$ ; d.f. = 1,27). Further, there is a marked increase in the spread of  $F_{ST}$  values relative to distance in fragments ( $r^2 = 0.32$ ) relative to continuous sites ( $r^2 = 0.68$ ).

**Table 3** The number of alleles (No.), the proportion of heterozygous individuals ( $H_O$ ) and the gene diversity ( $H_E$ ) for each locus at each site, and the significance levels for any deviations from Hardy–Weinberg proportions (heterozygote deficit; \* $P < 0.05$ , \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Site codes follow Table 1

Locus	Site	F1	F2	F3	F4	F5	F6	F7	C1	C2	C3	C4	C5	Average
GQ20/21	No.	6	8	5	5	9	8	5	8	8	11	7	9	7.42
	$H_O$	0.72	0.86	0.63	0.67	0.76	0.70	0.74	0.71	0.67	0.72	0.71	0.75	0.72
	$H_E$	0.73	0.77	0.66	0.65	0.76	0.73	0.57	0.73	0.71	0.65	0.59	0.74	0.69
GQ10/11	No.	8	11	10	7	10	8	8	8	5	9	8	12	8.67
	$H_O$	0.72	0.76*	0.68	0.72	0.61**	0.40***	0.70*	0.67	0.67	0.88	0.64	0.65	0.68
	$H_E$	0.68	0.73	0.75	0.77	0.72	0.67	0.78	0.66	0.67	0.84	0.57	0.66	0.71
GQ24/25	No.	9	10	10	12	14	10	13	9	11	10	11	17	11.33
	$H_O$	0.68*	0.83	0.91	0.83	0.92	0.67	0.75*	0.79	0.80	0.66	0.85	0.82	0.79
	$H_E$	0.78	0.83	0.86	0.87	0.89	0.80	0.83	0.84	0.80	0.69	0.81	0.82	0.82
GQ38/39	No.	3	3	4	2	4	5	3	4	3	5	4	4	3.67
	$H_O$	0.25	0.12	0.56	0.11	0.30	0.33	0.07	0.15	0.23	0.28	0.21	0.15	0.23
	$H_E$	0.32	0.12	0.43	0.11	0.27	0.30	0.07	0.18	0.24	0.31	0.31	0.17	0.24
GQ18/19	No.	6	6	11	7	11	10	6	9	8	7	16	10	8.92
	$H_O$	0.63	0.48	0.64	0.61	0.71	0.79	0.58	0.82	0.70	0.65	0.89	0.53	0.67
	$H_E$	0.70	0.46	0.71	0.60	0.70	0.73	0.66	0.73	0.71	0.66	0.84	0.46	0.66
GQ16/17	No.	9	10	3	5	8	8	6	6	8	10	9	9	7.58
	$H_O$	0.82	0.52	0.36**	0.63	0.45*	0.75	0.81	0.59	0.80	0.77	0.68	0.56*	0.65
	$H_E$	0.83	0.50	0.47	0.53	0.58	0.68	0.71	0.61	0.78	0.81	0.58	0.60	0.64
GQ36/37	No.	11	10	9	10	14	14	12	15	13	11	11	14	12.00
	$H_O$	0.80	0.83	0.75	0.72	0.82	0.90	0.82	0.89	0.93	0.81	0.71	0.79	0.82
	$H_E$	0.87	0.82	0.78	0.77	0.85	0.89	0.84	0.90	0.87	0.84	0.73	0.80	0.83
EA1/2	No.	7	11	13	6	8	12	11	10	12	12	11	15	10.67
	$H_O$	0.72	0.86	0.86	0.78	0.79	0.97	0.78*	0.96	0.80	1.00	0.82	0.79	0.84
	$H_E$	0.77	0.83	0.83	0.74	0.80	0.86	0.87	0.86	0.82	0.84	0.83	0.83	0.82
GQ42/43	No.	4	4	3	4	5	4	5	4	4	4	4	4	4.08
	$H_O$	0.76	0.64	0.63	0.61	0.76	0.73	0.56	0.52*	0.67	0.68	0.82	0.48	0.65
	$H_E$	0.66	0.59	0.65	0.64	0.76	0.74	0.65	0.73	0.59	0.58	0.68	0.53	0.65
All loci	Average no.	7.00	8.11	7.56	6.44	9.22	8.78	7.67	8.11	8.00	8.78	9.00	10.44	
	Average $H_O$	0.68	0.66	0.67	0.63	0.68	0.69	0.65	0.68	0.70	0.72	0.71	0.62	
	Average $H_E$	0.70	0.63	0.68	0.63	0.70	0.71	0.66	0.69	0.69	0.69	0.66	0.62	

**Table 4**  $M$  and interlocus variance in  $M$  for each site. Site codes follow Table 1

Site	$M$	Variance
F1	0.295	0.021
F2	0.340	0.027
F3	0.354	0.045
F4	0.302	0.045
F5	0.326	0.022
F6	0.302	0.010
F7	0.296	0.021
C1	0.344	0.036
C2	0.328	0.019
C3	0.342	0.020
C4	0.368	0.034
C5	0.340	0.025

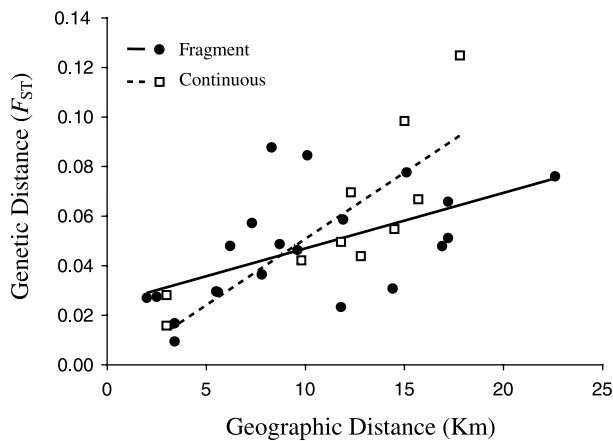
## Discussion

Our research indicates that the populations of prickly forest skinks in rain forest remnants on the Atherton Tableland have only undergone small genetic change as

a result of habitat fragmentation. None the less, each of the three changes detected is in the direction predicted by theory. First, we observed a slight but significant decrease in allelic diversity within the set of smallest fragments, presumably due to increased genetic drift. In addition, the relationship between allele number and fragment size had a high  $r$ -squared value indicating a strong relationship, despite approaching significance only at the 5% level. Second, a greater reduction in the number of alleles relative to allele size range was found in fragments consistent with changes predicted in simulations of a decrease in effective population size over a few generations (Garza & Williamson 2001). Third, the relationship between geographical and genetic distance was significantly weaker in fragments compared to continuous forest sites. The latter observation could be interpreted as increased gene flow among fragments (Porter 1999) and this is supported by evidence for increased dispersal in male prickly forest skinks in rain forest fragments (J. Sumner unpubl. data), but coupled with reduced allele numbers and our knowledge of the natural history of prickly skinks (see below) we suggest

**Table 5** Pairwise  $F_{ST}$  estimates for all loci on the lower matrix. The upper matrix indicates  $P$ -values for pairwise comparisons: NS indicates no significant difference, \* $P < 0.05$ , \*\* $P < 0.01$ ; \*\*\* $P > 0.001$ . Site codes follow Table 1

Site	F1	F2	F3	F4	F5	F6	F7	C1	C2	C3	C4	C5
F1	—	***	***	***	***	***	***	***	***	***	***	***
F2	0.0511	—	***	***	***	*	***	NS	***	***	***	NS
F3	0.0877	0.0658	—	***	***	***	***	***	***	***	***	***
F4	0.0845	0.0478	0.0270	—	***	***	***	***	***	***	**	***
F5	0.0463	0.0307	0.0167	0.0274	—	NS	***	NS	***	***	***	***
F6	0.0364	0.0233	0.0296	0.0293	0.0094	—	***	NS	***	***	**	***
F7	0.0776	0.0760	0.0571	0.0479	0.0487	0.0586	—	***	***	***	***	***
C1	0.0567	0.0250	0.0293	0.0313	0.0077	0.0045	0.0371	—	***	***	***	***
C2	0.0473	0.0473	0.0809	0.0939	0.0498	0.0337	0.0934	0.0439	—	NS	***	***
C3	0.0463	0.0591	0.1114	0.1177	0.0768	0.0514	0.1172	0.0668	0.0158	—	***	***
C4	0.0916	0.0557	0.0453	0.0365	0.0280	0.0253	0.0821	0.0281	0.0984	0.1248	—	***
C5	0.0562	0.0040	0.0840	0.0698	0.0487	0.0318	0.0917	0.0421	0.0496	0.0548	0.0696	—

**Fig. 2** Isolation by distance (IBD) pattern among fragments ( $F_{ST} = 0.0245 + 0.0022$  (distance);  $r^2 = 0.32$ ;  $P = 0.030$ ) and among continuous forest sites ( $F_{ST} = -0.0022 + 0.0053$  (distance);  $r^2 = 0.68$ ;  $P = 0.017$ ).

that increased isolation among fragments (Hutchison & Templeton 1999) may be more plausible.

Loss of rare alleles is predicted to occur in a population in the generations immediately following fragmentation due to a reduction in population size (Nei *et al.* 1975; Frankel & Soule 1981). A decrease in  $H_E$  has been documented in a large number of cases for long-isolated islands vs. continental populations (Frankham *et al.* 2002). Heterozygosity ( $H_E$ ) may not decrease substantially unless a decrease in population size is maintained for a number of generations, or the population size is greatly reduced (Nei *et al.* 1975; Frankel & Soule 1981). The slight decrease we found in  $A$  in small fragments in the absence of change in  $H_E$  may be as a result of a moderate reduction in the effective population size of skinks in the fragments. Within continuous forest, neighbourhood area estimates for *G. queenslandiae* are

approximately 1.05 ha (Sumner *et al.* 2001) and our smallest fragments, at 2–6 ha, are only slightly larger than this estimate. The reduced density of skinks in fragments found by Sumner *et al.* (1999) and the reduction in the proportion of adults in fragments compared to populations in continuous forest sites may decrease further the effective population size in small fragments in particular. However, overall we would expect only a slight decrease in effective size and thus  $A$  to have occurred in fragments since isolation.

We found a higher average adjusted allele number in moderate fragments ( $A = 9.66$ ) than in continuous forest sites ( $A = 9.25$ ). This may be due to emigration of individuals from the large surrounding area at the time of habitat clearance (Porter 1999), resulting in an artificially high  $A$ . If this were also the case for the small fragments, the significantly lower  $A$  found in those fragments may perhaps reflect an even greater degree of genetic erosion (Woodruff 2002). However, the limited dispersal ability of this species (Sumner *et al.* 2001) is likely to have minimized the number of refuges able to relocate to remaining forested areas.

That there is no detectable effect on  $H_E$  is not surprising, as this parameter is less sensitive than  $A$  to increased rates of genetic drift over the short term (Nei *et al.* 1975). Average heterozygosity decreases at a rate of  $1/(2N_e)$  per generation in a closed population (Wright 1978) and the following crude estimation of  $N_e$  in small fragments suggests that the size of the expected effect is small and likely to be obscured by sampling error and stochastic variance. The density of effective individuals was estimated to be approximately 85% of the estimated total density of prickly forest skinks (see Appendix II, Sumner *et al.* 2001), and in the mark-recapture study in continuous forest the actual captures were less than 25% of the estimated population size (Sumner *et al.* 2001). We searched the four small fragments in their entirety so, assuming the same  $N_e/N$  ratio and the

same sampling rate, we can extrapolate from the number of individuals caught that effective population sizes in these fragments may be in the range of 61–143 individuals. The fragments have been isolated for 50–80 years (Winter *et al.* 1987; Pahl *et al.* 1988), and if we use the estimated generation time for prickly forest skinks of 6.5 years (Sumner *et al.* 2001), we can infer that these populations have been isolated for at least 10 generations. Using these estimates, heterozygosity in the sampled fragments would be expected to have decreased by just 3–8%, an effect likely to be obscured by the large stochastic variance (Steinberg & Jordan 1998).

The values of  $M$  found for populations in this study are all much lower than found across a range of taxa by Garza & Williamson (2001). This reflects, perhaps, the long-term history of successive bottlenecks in the species due to rain forest contraction and expansion (Joseph *et al.* 1995; Schneider *et al.* 1998). However, a simpler explanation is that the alleles do not fit the stepwise model; a number of the loci have large gaps between alleles resulting in a bimodal distribution. This would lead to an increase in  $r$  (the difference between largest and smallest alleles) and a resulting decrease in  $M$ .

The genetic diversity of *G. queenslandiae* in fragments is also expected to be affected by increased isolation. In the larger fragments and continuous forest sites exchange is expected to occur across overlapping genetic neighbourhoods, ameliorating the effect of local genetic drift (Wright 1943). However, in the smaller fragments genetic exchange is restricted to an area approximating a single neighbourhood. Although there was no difference between fragments and continuous forest sites in overall population subdivision ( $F_{ST}$ ), the effect of increased isolation in fragments was evident from disruption of the strong pattern of isolation by distance evident among populations in continuous forest (Templeton *et al.* 2001). The lower than expected values for genetic distances between fragments greater than 10 km apart is likely to be an artefact of a greater increase in variance in  $F_{ST}$  for larger  $F_{ST}$  values, which may have had the net effect of reducing the slope, or at least increasing the noise in the system (see Fig. 1 in Hutchison & Templeton 1999).

*G. queenslandiae* is not threatened currently with extinction; it is locally common and its habitat is almost entirely contained within the protected Wet Tropics World Heritage Area. It is a rain forest obligate, however, so may be a useful model for gauging the effect of fragmentation on the rarer rain forest species found in the area (Covacevich & McDonald 1993; Sumner 2002b). A significant decrease in effective population size was detected in fragments in this common species, which might indicate that rarer species will be equally or more strongly affected by the same degree of habitat fragmentation. Prickly forest skinks are maintaining substantial populations in fragments despite their apparent inability to utilize the matrix between

fragments, the most important factor found to determine extinction proneness in mammals (Laurance 1991a) and birds (Warburton 1997) on the Atherton Tableland. The naturally limited movement of this species and the smaller habitat area requirements perhaps render them less susceptible to extinction in fragments of the same size as for mammals and birds. Maintenance of moderate effective population size in the future may maintain local genetic variation in this species, despite its limited dispersal ability. However, given the early signs of isolation identified in this study and the potential for accumulation of slightly deleterious alleles in small populations, such as those in the fragments (Lande 1995; Schultz & Lynch 1997; Higgins & Lynch 2001), as well as the evidence of changes, albeit slight, in morphology observed between fragments and continuous forest sites (Sumner *et al.* 1999), it is possible that genetic erosion (Woodruff 2002) and loss of fitness will occur unless habitat linkages are restored.

### Acknowledgements

This research was carried out as part of the PhD research of the first author, with support from an Australian Postgraduate Award and the Rainforest Cooperative Research Centre. We would like to thank all the volunteers who gave their time and energy to this work, John Winter and Helen Myles at Massey Creek Research Station for access to their property and unlimited hospitality and Keith McDonald for his hospitality, knowledge and time. Thank you to Bill Sherwin and two anonymous reviewers for comments on the manuscript.

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Joanna Sumner is interested in molecular approaches to ecological and conservation questions, especially concerning reptiles. Tim Jessop's interests lie in life-history trade-offs, hormones and behaviour, but dabbles occasionally with molecular techniques. David Paetkau is interested in developing molecular methods that have practical utility in the study of population ecology. Craig Moritz has broad research interests that include the application of genetics to conservation problems, 'hybrid zones' in rain forests and speciation across ecotones.

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