

'Neighbourhood' size, dispersal and density estimates in the prickly forest skink (*Gnypetoscincus queenslandiae*) using individual genetic and demographic methods

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Abstract

Dispersal, or the amount of dispersion between an individual's birthplace and that of its offspring, is of great importance in population biology, behavioural ecology and conservation, however, obtaining direct estimates from field data on natural populations can be problematic. The prickly forest skink, *Gnypetoscincus queenslandiae*, is a rainforest endemic skink from the wet tropics of Australia. Because of its log-dwelling habits and lack of definite nesting sites, a demographic estimate of dispersal distance is difficult to obtain. Neighbourhood size, defined as $4\pi D\sigma^2$ (where D is the population density and σ^2 the mean axial squared parent–offspring dispersal rate), dispersal and density were estimated directly and indirectly for this species using mark–recapture and microsatellite data, respectively, on lizards captured at a local geographical scale of 3 ha. Mark–recapture data gave a dispersal rate of 843 m²/generation (assuming a generation time of 6.5 years), a time-scaled density of 13 635 individuals * generation/km² and, hence, a neighbourhood size of 144 individuals. A genetic method based on the multilocus (10 loci) microsatellite genotypes of individuals and their geographical location indicated that there is a significant isolation by distance pattern, and gave a neighbourhood size of 69 individuals, with a 95% confidence interval between 48 and 184. This translates into a dispersal rate of 404 m²/generation when using the mark–recapture density estimation, or an estimate of time-scaled population density of 6520 individuals * generation/km² when using the mark–recapture dispersal rate estimate. The relationship between the two categories of neighbourhood size, dispersal and density estimates and reasons for any disparities are discussed.

Keywords: dispersal, *Gnypetoscincus queenslandiae*, isolation by distance, microsatellites, neighbourhood size, skinks

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Introduction

Dispersal, or the amount of dispersion between an individual's birthplace and that of its offspring, is of great importance in population biology, behavioural ecology and conservation (Koenig *et al.* 1996). This life-history trait has profound effects on the dynamics and persistence of populations and the distribution and abundance of species. From an evolutionary perspective, dispersal determines the level of gene flow between populations, the size of local

genetic populations (Wright 1943) and affects processes such as genetic drift, local adaptation and speciation (Endler 1977; Dieckmann *et al.* 1999). For conservation dispersal can be vital in maintaining viable populations in increasingly fragmented ecosystems by reducing the effects of inbreeding and allowing recolonization of areas in which populations have gone extinct (Brown & Kodric-Brown 1977; Hanski 1998).

Obtaining direct estimates of dispersal from field data on natural populations has long been plagued with biases, typically attributed to systematic underestimation of dispersal as a result of the finite size of the majority of study areas but also resulting from a number of other factors

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(Rousset 2001a,b). Previous studies have tried to overcome this problem using Barrowclough's correction (Barrowclough 1978) or extremely large study areas (Van Vuren & Armitage 1994).

Models of isolation by distance describe the accumulation of local genetic differences under geographically restricted dispersal. Following Wright, it has often been considered that the basic unit of population structure is the 'neighbourhood' (e.g. Wright 1943; Slatkin & Barton 1989). Neighbourhood size (NS) is here defined as $4\pi D\sigma^2$, where D is the population density and σ^2 the mean axial square of parent-offspring dispersal rate. This parameter determines the increase of genetic differentiation with distance (Rousset 1997, 2000). NS estimates allow the evaluation of either dispersal given that density is known, or alternatively of density if dispersal is known.

Indirect genetic estimates of dispersal distance address the actual genetic input that dispersers have in the population, which is rarely attainable using demographic methods. The degree of population differentiation, measured as F_{ST} , is usually used to determine the rates of gene flow between population subunits, from which estimates of the number of migrants per generation can be made (Slatkin 1987; Waser & Elliott 1991; Koenig *et al.* 1996; and see Rousset 2001a,b for review). Assignment methods are also available which identify individual migrants and their likely origin and from which it would then be possible to estimate dispersal rates (Paetkau *et al.* 1995; Rannala & Mountain 1997). Unfortunately, the results of direct demographic and indirect genetic methods are often quite disparate and may depend on both the validity of the various assumptions (e.g. Eastale 1985) and the method of analysis (reviewed in Rousset 2001a,b).

Rousset (2000) derived a method of determining genetic differentiation between individuals from an earlier population-based method (Rousset 1997). The new method considers a unit of analysis as an individual in a population, rather than a subpopulation as part of the total population. It is based on the analysis of a continuous population according to the closest possible 'lattice' model and makes use of randomly distributed samples taken from the continuously distributed population. A regression of the genetic differentiation between pairs of individuals and their geographical distance allows estimation of the NS (i.e. the product $4\pi D\sigma^2$). A direct estimate of population density, such as from a mark-recapture study, can be substituted to calculate dispersal distance and conversely, population density can be estimated if a direct estimate of dispersal distance is available.

The prickly forest skink (*Gnypetoscincus queenslandiae*) represents a monotypic genus endemic to the rainforest in the wet tropics on the north-eastern coast of Australia (Cogger 1992). This small, viviparous lizard is common locally and is found under and within rotting logs on the

rainforest floor (Cogger 1992). Nothing is known about dispersal in the prickly skink because of the cryptic habits of the species and the lack of definite nesting sites from which to measure natal dispersal. Hence, an 'indirect' estimate of dispersal distance would be particularly useful.

In this study, we simultaneously utilized a direct demographic method using mark-recapture data, and an indirect genetic method using microsatellite markers to estimate NS, density and dispersal rate. More specifically, we addressed whether the estimates of NS, dispersal rate and density gained using demographic and genetic methods were consistent, and how any deviations were related to the methods used and their respective assumptions.

Materials and methods

Field work

Field work was carried out at Massey Creek Research Station (145°34' E, 17°37' N), an area of continuous forest on the Atherton Tableland in the wet tropics of Australia. This site consists of complex notophyll vine-forest (Tracey 1982). Seven trips were made to the site, once in the late dry season (November), and once in the late wet season (February–April) from November 1995 to 1998. During each trip a complete search was made of an 'L'-shaped 3-ha area that was divided into a grid consisting of 48 25 × 25 m squares. Animals were hand-captured, and each animal caught was marked using an individual combination of toe clips, a tail tip was taken for DNA and the specific location within the site was recorded to the nearest 5 m using the grid markings for reference. Morphological measurements were taken (Sumner *et al.* 1999), the sex and reproductive condition were recorded if possible, and the animal was replaced under the log where it was found.

Demographic analysis

We based a large part of the treatment of our demographic data on the work of Rousset (1999a) in order to minimize discrepancies between the specific parameters that are estimated in the demographic and genetic analyses. Only the major steps are presented here, the detailed computations applied to our biological model are presented below.

Age, size and survival rate. In order to estimate the age of individuals, we first calculated growth rate (GR) for the lizards as the change in snout-vent length ($dSVL$) per month (dT) between the first and last capture such that $GR = dSVL/dT$. A linear regression was used to determine the relationship between GR and SVL, which was then integrated to generate an equation relating age and SVL (Van Devender 1978; Kaufman 1981). The size at which individuals reach maturity was estimated from this

equation, and was used to calculate survival rate(s) and, subsequently, an estimate of dispersal distance.

Density

Individuals were identified as either adult or juvenile, with animals classified as adults at 65 mm SVL and above; this is the smallest size for which a gravid female was captured in this population. Males and females mature at approximately the same size (Cunningham 1993) so males were assumed to also be mature at 65 mm SVL. The population size, for adults only, was estimated using the program JOLLY (Pollock *et al.* 1990). The 'inbreeding' effective population size was computed according to eqns 48 and 56 in Rousset (1999a) using available information on adult sex ratio and age structure. The 'effective' population density (D_e ; for adults only) was deduced by dividing the population size estimate by the studied area.

Dispersal rate estimate

We were not able to estimate dispersal rate per se (i.e. distance from an individual's birthplace to that of its offspring) using our field data. However, we obtained a dispersal rate comparable with the genetic one by recording the distance moved between captures at the site from the seven sampling periods, for those individuals recaptured on more than one occasion. A dispersal rate (σ^2) for individuals within identified age classes was then calculated such that the σ^2 per month is equal to:

$$\frac{1}{2} \frac{\sum (dX)^2}{\sum dT};$$

where dX is the distance moved by an individual from its release point dT months ago. Note that our demographic estimation of individual movements is not axial because it is based on the movements of animals in two dimensions: the noncentral second moment of parent-offspring Euclidean distance is $2\sigma^2$ and we wish to calculate σ^2 so the factor '1/2' is necessary in the equation above (see Appendix I). An estimate of effective dispersal rate (σ_e^2) was then calculated according to equation 22 in Rousset (1999a). For comparison with genetic estimates, the neighbourhood size was computed as $NS = 4\pi D_e \sigma_e^2$.

Genetic analysis

Cloning and genotyping of microsatellites. DNA was extracted for cloning from the fresh liver of a single *Gnypetoscincus queenslandiae* and from a single *Eulamprus amplus*, a closely related species (Greer 1989), using a high molecular mass extraction protocol (Sambrook *et al.* 1989). DNA fragments were cut using either *Sau3AI* restriction enzyme or a

mixture of *AluI*, *HaeIII* and *RsaI* enzymes. The library was screened using ^{32}P -radiolabelled dinucleotide probes for 300–700-bp DNA fragments inserted into puc18 plasmids. Sixty positive clones were detected and sequenced for *G. queenslandiae*, 28 from *E. amplus* and from these primers were designed and fluorescent-labelled for 17 loci. Ten of those loci were polymorphic and gave unambiguous amplification patterns (nine from *G. queenslandiae* and one from *E. amplus* primers; Table 1). Individual DNA was extracted using a standard phenol-chloroform extraction procedure (Sambrook *et al.* 1989) on lizard tails, fresh frozen, or stored at room temperature in a solution of 10% dimethylsulfoxide saturated with NaCl. Final concentrations in the polymerase chain reaction (PCR) mixture consisted of: 1 μL DNA, 1 \times PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1% Triton X-100, pH 8.5), 2 mM MgCl_2 , 40 mM for each dNTP, 0.75 U *Taq* DNA polymerase (Perkin-Elmer), 100 nM labelled primer and 250 nM unlabelled primer, mQH_2O –15 μL . Microsatellites were amplified on a GeneAmp PCR system 9700 thermocycler. Conditions consisted of an initial denature at 94 °C for 105 s, followed by 35 cycles of 94 °C for 15 s, annealing temperature of 52 or 56 °C for 20 s, 72 °C for 10 s, then 120 s at 72 °C, or a touchdown PCR (Taylor *et al.* 1994) with an initial denaturation at 94 °C for 105 s, six cycles of 94 °C for 15 s, 60 °C for 20 s decreasing 1 °C each cycle to 55 °C, 72 °C for 10 s, then 30 cycles of 94 °C for 15 s, 54 °C for 20 s, 72 °C for 1 s, then 72 °C for 120 s. PCR products were resolved on a model 373 DNA sequencer and analysed using GENESCAN and GENOTYPER software (Applied Biosystems). Primer sequence and PCR conditions are given for each locus in Table 1.

In a continuous population isolation-by-distance patterns occur when genetic differentiation among individuals increases with their geographical distance (Wright 1943). We described isolation-by-distance data using the statistic a_r , a multilocus estimator of an $F_{ST}/(1 - F_{ST})$ analogue between pairs of individuals. When applied on a small geographical scale, and when the population is continuous, a_r is approximately linearly related to the logarithm of distance, $a_r \approx \ln(d)/4\pi D\sigma^2 + \text{constant}$ (Rousset 2000), where d is the geographical distance between two individuals, D is a time-scaled density of individuals in the population, σ is the mean axial dispersal rate per unit time and the constant is the intercept with the y -axis at unit distance. Here, we interpret D and σ^2 as 'effective' parameters as in Rousset (1999a). Note that the equivalent parameters for the demographic estimates are denoted D_e and σ_e^2 . Values of a_r were regressed against the log of geographical distance between the paired individuals to determine whether an isolation-by-distance pattern existed. Ninety-five per cent confidence intervals around the regression slope value were computed using the ABC bootstrap procedure described in DiCiccio & Efron (1996). The relationship between a_r and geographical distance is not linear at very

Table 1 Primer information including sequence, repeat array, annealing temperature, size range of polymerase chain reaction (PCR) product and allele number for the 10 microsatellite loci amplified for *Gnypetoscincus queenslandiae*. Loci were named according to the species from which they were cloned (*G. queenslandiae*; GQ or *Eulamprus amplus*; EA). The fluorescently labelled primer has the suffix 'F'. TD refers to touchdown style PCR. Gene diversity and proportion of heterozygous individuals (Nei 1987) for each locus, and significance levels for any deviations from Hardy–Weinberg proportions; NS: nonsignificant, N: number of individuals genotyped

Locus and primer sequence	Repeat array	Annealing temp. °C	Size range of PCR product (bp)	No. of alleles	Gene diversity	Proportion of heterozygous individuals	HWE	N
GQ10: AATCTGCTGTTCTATTGTACCTG GQ11F: AGGTTTAAGTTCAAGGTGACC	(TG) ₂₀	52	235–256	13	0.66	0.66	NS	137
GQ16: GCCATAGTTTGTACTCCCC GQ17F2: TTCCTCCTTGGGCTTTGCTACC	(GT) ₂₀	60–55 TD	252–283	9	0.61	0.58	NS	119
GQ18: GGGAAATAAAGAGAAAATCATC GQ19F: TTCATGGAATCTATTGCTAGGT	(TC) ₁₄ (AC) ₁₈	60–55 TD	155–201	11	0.43	0.49	NS	132
GQ20: GCAAAGATGTTTTCCAAAAGTGGG GQ21F: GGCTACACAAATCCAGACCTAAG	(CT) ₂₁ (CA) ₁₉	56	161–202	9	0.74	0.77	NS	138
GQ24F: GGAGGATGGGAAGAACTGTTG GQ25: GCGAATGGCAGGCACACTAATG	(TG) ₃₃	52	182–222	18	0.81	0.81	NS	134
GQ30B: GTTCTGGCTCTGTCAACACTGGACC GQ31F: TGGGGAAACAGAGGCTGAT	(AC) ₂₂	60–55 TD	122–144	6	0.39	0.37	NS	135
GQ36: GGAAGGTTTGGGACTTTAGTCAG GQ37F: GGGCTGCCACTCAATTAACAC	(AC) ₂₇	56/60–55 TD	157–210	14	0.78	0.73	$P < 0.05$	133
GQ38B: GTGTCTGTGTGACAACTGTATAACC GQ39F: GTCATTCTCCCTCTCCACAAG	(GT) ₁₉	60–55 TD	227–233	5	0.15	0.14	NS	127
GQ42B: GTTTCTATTTGCTTGCAACCACAGT GQ43F: ATCGGAAGCTCTTTGGGGAC	(CA) ₁₂	56	155–169	6	0.53	0.47	$P < 0.0001$	137
EA1F: GTTTCCTTATGCCAGATGATTCGT EA2B: GTTTGACCATCAGACCCCTCTTGC	(TC) ₁₄ (AC) ₁₈	60–55 TD	186–244	16	0.83	0.82	NS	136

small geographical distances (see Figs 2 and 3 in Rousset 1997, 1999b, respectively) so a second analysis was processed selecting individuals separated by a distance higher than the demographic estimate of σ (Rousset 1997). The inverse of the regression slope was used to estimate the 'neighbourhood size' ($NS = 4\pi D\sigma^2$). Analyses were performed using GENEPOP 3.2a (Raymond & Rousset 1995).

The mean axial parent-offspring distance (σ) was determined from the product $4\pi D\sigma^2$ (estimated as described above) using the demographic estimate of population density (D_e). The population density (D) was estimated from the same product, $4\pi D\sigma^2$, using the estimate of σ_e^2 obtained from the demographic study.

Note that the product $D\sigma^2$ does not depend on the arbitrary choice of time interval (generation time here). One could simply not consider any generation time in the computation, or choose any definition of generation time and scale dispersal and 'density' accordingly. Here, care was taken to use the year as the basic time interval as it corresponds to the time interval of the demographic matrix used.

Results

Demographic data analysis

A total of 513 individuals were caught, including 156 recaptures across the seven trips, with an average of 96 individuals caught per trip. The percentage of recaptured individuals per trip ranged from 1.1% in the second trip to 41.7% in the sixth, with an average of recaptured individuals across all trips of 24.4%. Of the 128 individuals recaptured, the majority were recaptured once (41 adults and 64 subadults; animals were classified as adult at 65 mm SVL or over) or twice (9 adults and 10 subadults). Three adults were captured four times in all, and one subadult was captured five times.

Age, size and survival rate

The growth model used to calculate generation time predicted that growth rate in SVL is a linear function of body length:

$$GR = a + bSVL,$$

where $a = 2.0021232$ and $b = -0.0231229$.

Integration of this equation generated an equation relating age and SVL;

$$\text{Age } (k) = (1/b) \ln(a + bSVL) + c, \quad (1)$$

where c is a constant of integration that can be calculated if age at any specific size is known. Size at time zero (birth) is known approximately as the smallest captured individual

after parturition (32 mm SVL). Calculation using this value gives $c = 10.07$.

The inferred size at which prickly forest skinks reach maturity is $SVL = 65$ cm (Sumner *et al.* 1999), which gives an age at maturity of 40.12 months, or 3.34 years. We can assume from this that individuals start to breed in their fourth year. Using eqn 1, we determined generation length by calculating the average size of gravid females (Stearns 1992) at Massey Creek (77.66 mm SVL, $N = 47$), which gives an approximate generation length of 78.31 months, or 6.53 years.

The estimate of size at maturity (ϕ) determined from mark-recapture data can be used to calculate an estimate of survival rate(s) per year and subsequently an estimate of dispersal rate. Given that the survival rate per year is s , the frequency of females in successive age classes is in proportion to 1, s , s^2 , ... s^k , summing to 1, for k age classes. Because $\sum_{k=0}^{\infty} s^k = 1/(1-s)$, the frequencies of successive age classes are $(1-s)$, $s(1-s)$, $s^2(1-s)$, ... $s^k(1-s)$. Size at maturity is then the sum of size, given age per frequency of age class, that is:

$$\phi = (1-s) \sum_{k=0}^{\infty} s^k \frac{b(40.12 + 12k - c) - a}{b} \quad (2)$$

Size at maturity (ϕ) was estimated as the mean SVL (75.27 mm) of all mature individuals, male and female, collected during the fieldwork, and by solving for s , the survival rate per year was calculated as 0.86. This survival rate was used to estimate density as described below.

Density

The analysis of population size was found to fit best ($P = 0.27$) a model which assumes constant survival rate and capture probability in the system and allows for overlapping generations. However, there was little difference between the three models tested (Table 2). The adult

Table 2 Goodness-of-fit tests for the adult population size estimate under various models using the program JOLLY. Model A allows capture and survival probabilities to vary over sampling periods, model B assumes constant survival probabilities over the whole capture period, and Model D assumes constant survival rate and constant capture probability over the whole capture period

Model	Chi-square	d.f.	Probability	Mean N	SE
A	8.328	6	0.215	459.35	135.85
B	11.558	9	0.239	518.64	352.51
D	16.776	14	0.268	484.45	227.04

d.f., degrees of freedom; N , population size estimate; SE, standard error.

population size was estimated at 484 (SE \pm 227.04) individuals across the 3-ha site (Table 3).

To compute 'effective' population size, and hence 'effective' density (D_e) we used eqns 56 and 48 in Rousset (1999a):

$$\frac{1}{D_e} = \frac{\sum (\epsilon_i^2 - \epsilon_{i+1}^2)}{D_k}$$

where ϵ is the reproductive value of the different age classes. Reproductive values are computed as described in Appendix II.

The effective density was calculated for females only, initially, so corrections were made for sex ratio, and the density of gravid females. The sex ratio in *Gnypetoscincus queenslandiae* is female biased with a ratio of 1:1.5 (Cunningham 1993; Sumner *et al.* 1999). Only 44% of adult females ($N = 142$) captured were gravid during the breeding season (October to March), suggesting less than annual reproduction in the species. No females recaptured in consecutive years during the breeding season were gravid each year ($N = 8$). It is unlikely that we missed gravid females due to the infrequency of our trips as females appeared to give birth

Table 3 Estimate of adult population size under model D of the program JOLLY, assuming constant survival rate per unit time and constant capture probability

Period	N	Variance	SE
2	442.33	9919.71	99.60
3	409.59	7901.71	88.89
4	642.00	17130.66	130.88
5	422.40	8050.02	89.72
6	486.72	10401.72	101.99
7	503.67	12574.17	112.13
Mean	484.45	51547.26	227.04

N, population size estimate; SE, standard error.

Table 4 Age classes calculated for individuals caught at Massey Creek showing snout-vent length (SVL), dispersal rate and the number of individuals in each age class

Age (years)	SVL (mm)	Dispersal rate (m ² /per month)	No. of individuals
0	32.00	—	—
1	45.23	32.11	33
2	55.25	33.68	23
3	62.84	22.80	17
4	68.59	19.31	11
5	72.95	18.57	6
6	76.26	2.32	18
7	78.76	7.02	3
8	80.66	0.61	11
9	82.09	2.32	2
10	83.18	1.82	1

concurrently and neonates were found only late in the wet season. During the February–March trips either gravid females were captured, but no neonates, or no gravid females were captured and neonates were. There is no evidence for differences in age structure between males and females, and the percentage of effective breeding males is not known, so no corrections were made for these parameters in calculating effective density.

Total density ($D_t = 484$) and the estimated survival rate ($s = 0.86$) were used to calculate the effective density (D_e ; Appendix II). The effective density expressed in the same units as σ^2 is $D_{em} = D_e/30\,000 = 0.089$ individuals * year/m² (89 000 individuals * year/km²), and 1.36×10^{-2} individuals * generation/m² (13 600 individuals * generation/km²).

Dispersal

The expected size at different ages (birth, 1 year, 2 years ...) was calculated using eqn 1 (Table 4). These sizes were used to split the individual dispersal distances calculated from mark–recapture data into age classes. The dispersal rate,

$$\sigma_k^2 = \frac{1}{2} \frac{\sum (dX)^2}{\sum dT},$$

was then calculated for each age class, k ,

where dX is the distance moved by an individual from its release point dT months ago (Table 4).

An estimate of effective mean axial dispersal rate (σ_e^2) was calculated according to eqn 22 in Rousset (1999a) using the reproductive values (ϵ) calculated for the density estimate:

$$\sigma_e^2 = \epsilon_{4,s} \sum_{k=1}^4 \sigma_k^2 + \sum_{k=5}^{10} \epsilon_{k,5} \sigma_k^2$$

which gives $\sigma_e^2 = 10.76$ m per month.

There is a possibility that we have missed an older age class, but if this is the case, the change to the effective dispersal rate is negligible (an additional 0.7 m per month assuming a dispersal rate of 4 m/month in older classes). The 'effective' rate per year is therefore $10.76 \times 12 = 129.12$ m² which gives $\sigma_e^2 = 843.15$ m²/generation (Table 5).

For comparison with genetic estimates, the neighbourhood size using mark–recapture data was computed as $4\pi D_e \sigma_e^2$ and is thus 144.07 individuals (Table 5).

Genetic analysis

One hundred and thirty-nine individuals were genotyped and analysed at 10 microsatellite loci. The number of alleles per locus ranged from 5 to 18 (mean, 10.7), and the proportion of heterozygous individuals per locus ranged from 0.15 to 0.83 (mean, 0.59).

The matrix of pairwise multilocus a_r values estimated from all 139 individuals showed a significant correlation

Table 5 Summary for direct field-based demographic methods and indirect genetic methods of estimating 'neighbourhood size' ($NS = 4\pi D\sigma^2$), effective dispersal distance (σ) and effective population density (D) in the prickly forest skink, *Gnypetoscincus queenslandiae*. No methods are available to compute 95% confidence intervals on NS from the demographic data

	NS (individuals)	95% confidence on NS (individuals)	σ (m/generation ^{1/2})	D (individuals * generation/m ²)
Direct estimate	144.07	Not computed	29.04	1.36×10^{-2}
Indirect estimate	69.12	47.69–183.7	20.1	6.52×10^{-3}

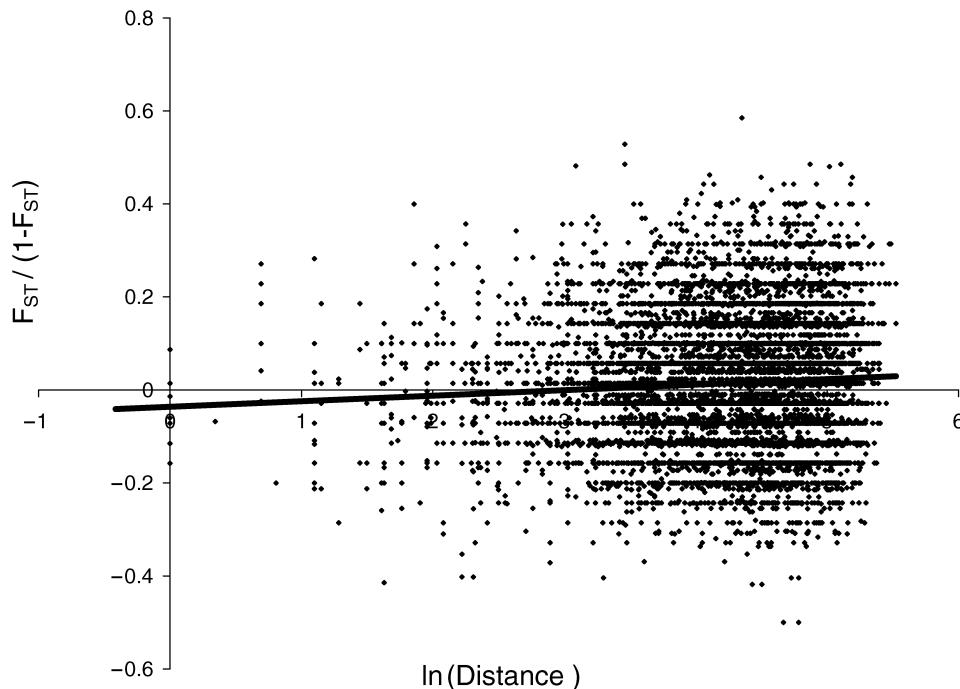


Fig. 1 Genetic differentiation in the prickly forest skink, *Gnypetoscincus queenslandiae*, shown as the regression of pairwise genetic differentiation between individuals against the logarithm of distance (m). $a_r = -0.036 + 0.0119 \ln(\text{distance})$.

with geographical distance, which indicates a significant isolation-by-distance pattern (Mantel's test; $P = 0.026$). The regression of genetic distance against the log of distance between all individuals gave a slope of 0.0119 (Fig. 1), corresponding to a NS of 84 individuals. Computation of a 95% confidence interval on the NS gave lower and upper values of 47.7 and 183.7 individuals (Table 5).

Removing all pairwise comparisons at distances less than the demographic estimate of $\sigma = 29.0$ m/generation^{1/2} (see Materials and methods), gave a NS of 69 individuals [$a_r = -0.028 + 0.0149 \ln(d)$]. The mean effective dispersal rate (σ^2) was deduced from this NS estimation as being equal to 20.1 m/generation^{1/2} (Table 5) using the demographic density estimate ($D_e = 0.0136$ individuals * generation/m² or 13 635 individuals * generation/km²). Conversely, population density was calculated using the estimate of dispersal rate obtained from the demographic study ($\sigma_e^2 = 843$ m²/generation), giving a value for D of 6.52×10^{-3}

individuals * generation/m² or 3466 individuals * generation/km² (Table 5).

Discussion

The major conclusions of this study are that a significant isolation-by-distance pattern was found between the individual a_r values and the geographical distance of individuals using the individual-based genetic method, and that the demographic and genetic methods are in good agreement as they both gave low estimates of neighbourhood size (mark-recapture estimate of 144 individuals vs. genetic estimate of 69 individuals). The demographic estimate of NS (144 individuals) falls within the confidence intervals for the genetic estimate of NS (48–184). The twofold difference between the estimates of neighbourhood size may be explained by imprecision of the estimators and/or by biases in some of the methods

used, in particular, the demographic estimate of density, as discussed below. Simulations in Rousset (2000) indicate that a twofold difference in the estimates is similar to the degree of accuracy to be expected from the genetic estimate. This level of precision is higher than found in many estimation methods in population genetics.

Although significant, the slope of the regression of individual a_r values against the geographical distance between individuals explains little of the variance in the sample, as indicated by the low r^2 value. This result is expected under the model, and is a general observation of mutation/drift models (Rousset 2000).

Comparison of demographic and genetic estimates

The individual-based genetic method of Rousset (2000) makes only weak assumptions about the distribution of dispersal distances and is robust for distributions of dispersal more leptokurtic than normal, a feature commonly found in natural populations. We can expect some bias in the genetic estimate as Malecot's model, on which the genetic estimate is based, describes a population evenly distributed on a lattice and this does not describe the distribution of individuals in our population exactly. Rousset (2000) found that the estimators of $D\sigma^2$ yield estimates that are closer to parameter values than usually recognized using other methods when σ is small (as ours is) and when most of the individuals present within an area of $10\sigma \times 10\sigma$ are sampled. We sampled an area of 3 ha, which is less than the suggested area (which would equal ≈ 4 ha). Our study site is 'L'-shaped, however, so we did sample a range of distances between individuals equivalent to those of a square, 4-ha site. Our mark-recapture data indicates that we were not capturing all the individuals within the area so again we may have lost some precision in our estimators due to that fact. The number of loci we used should increase our level of precision, however, as simulations indicate that an increase in loci, rather than individuals, is a better way to increase accuracy (Rousset 2000).

The method of Rousset (2000) allowed us to analyse genetic differentiation within a continuous population, without requiring the arbitrary definition of subpopulations or demes of previous methods. For example, Fleischer (1983) used the approximation $F_{ST} = (1 + 4Nm)$ in his comparison of theoretical and genetic estimates of F_{ST} . This method requires well-delineated subpopulations with low dispersal rates between them, and so is not applicable to a 'continuous' population. This individual-based genetic method also allows studies to be carried out at a local geographical scale, which may give more valuable estimates because heterogeneity of demographic parameters is reduced (Rousset 2000).

The method only requires the use of genetic and geographical data from a single sampling occasion, hence, this

genetic method appears easier to apply than demographic methods. It is worth stressing, however, that the genetic estimate of NS is a product of D and σ^2 , and in order to obtain an estimate of either D or σ^2 , a parallel demographic study is needed to estimate independently one or both of those parameters.

The demographic estimates of D_e and σ_e^2 are not straightforward, and require careful computations as described above in Materials and methods and Results. The ease and precision with which one can estimate those parameters depends greatly on the ecology of the individual species. Species that are easily captured and have discreet home ranges allow more accurate estimation, or even an exact calculation of dispersal or density (e.g. Waser & Elliott 1991). Prickly forest skinks are not easily seen or captured without disturbance of their log habitat, making it difficult to accurately estimate either parameter using demographic methods.

A significant source of error in the demographic estimate of density may occur when using the Jolly Seber method to estimate population size, then dividing by area to calculate density. First, the presence of 'marginal' individuals at the periphery of the study area may induce a strong heterogeneity of capture which may, in turn, have a strong effect on the robustness of population size estimation (Carothers 1973). Second, and more importantly, the Jolly Seber estimate of population size is valid only for a discrete randomly mixing population, so the estimate of density would be valid only for such a population; genetic data showed a significant isolation-by-distance pattern, indicating that we sampled within a nonrandomly mixing population in a continuous tract of forest. A distance sampling method (e.g. Buckland *et al.* 1993) that is not based on recapture of individuals would be more appropriate for some species, but as it relies on sighting individuals from a distance, could not be carried out on a log-dwelling species such as the prickly skink. For this species, in a continuous area of forest, there does not seem to be a method available to estimate density accurately.

The calculation of movement per generation made from the monthly movement distance assumes one-way movement throughout life, which is highly unlikely, and would result in an overestimation of the field-based dispersal distance. This may also introduce some disparity into the estimates found between the demographic and genetic estimates.

Finally, the estimate of σ_e^2 from mark-recapture data may under-sample long distance dispersers. However, Rousset's (2000) method may also fail to detect long-distance dispersal if the proportion of long-distance dispersion events in the population is low, despite being robust for distributions of dispersal more leptokurtic than normal. In this case, both estimates are likely to reflect local processes only (Rousset 2001a), and differences

between the estimates are unlikely to be due to the effects of under sampling of long distance dispersers.

Ecology of the prickly forest skink

In estimating the parameters discussed above, we have gained some insight into the ecology of the prickly forest skink. Our research indicates that prickly forest skinks are a long-lived species that exhibit high site fidelity. The age of the largest individuals caught, estimated as 10 years, is supported by the analysis of bone growth rings by Cunningham (1993), whose data indicated that this species lives for up to 9 years, assuming annual deposition of growth rings. Prickly forest skinks have a low reproductive rate; females have an average of three offspring (Cunningham 1993) every second year, offset by high adult survivorship as estimated here. Fewer older individuals were captured than expected from the high survival rates calculated (see Table 4). This suggests that there is some senescence in the population, despite our assumption of no female senescence in calculating the demographic estimate of density. Alternatively, individuals may be harder to catch as they age, however, there is no clear evidence for this. From our data, movement distances in prickly forest skinks range from 33 m² per month to 0.6 m² per month, and are greatest in sub-adults up to 2 years. Movement distances per month decrease after individuals reach maturity in their fourth year.

This study illustrates the importance of combining molecular and demographic methods in studies of individuals within populations. Genetic methods such as those used here may help to overcome the difficulties of mark-recapture programmes, especially in long-lived and cryptic species.

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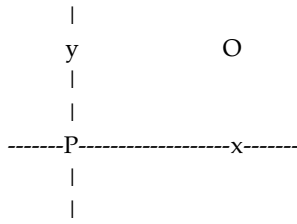
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Appendix I

Consider a plot of the position of an offspring 'O' relative to the position of its parent (i.e. origin of the axes 'P'), as well as projections 'x' and 'y' on the axes;



The square parent offspring distance is $dX^2 = x^2 + y^2$, with the axial square parent offspring distance being x^2 on one axis and y^2 on the other. The parameter σ^2 of our genetical model is the mean axial square parent offspring distance, $E[x^2]$ on one axis and $E[y^2]$ on the other, and the mean square parent offspring distance is $E[dX^2] = E[x^2] + E[y^2]$. Assuming $E[y^2] = E[x^2]$, we have $E[dX^2] = 2E[x^2] = 2\sigma^2$. In other words, the noncentral second moment of parent-offspring Euclidean distance is $2\sigma^2$ (see also Crawford 1984; Rousset 1997).

Appendix II

Assuming no senescence among females at maturity, reproductive values (ϵ) are deduced from a matrix of the form:

$$\begin{pmatrix} 0 & 0 & 0 & 1 - ss & (1 - ss)ss & (1 - ss)ss^2 & \dots \\ 1 & 0 & 0 & 0 & 0 & 0 & \dots \\ 0 & 1 & 0 & 0 & 0 & 0 & \dots \\ 0 & 0 & 1 & 0 & 0 & 0 & \dots \\ 0 & 0 & 0 & 1 & 0 & 0 & \dots \\ 0 & 0 & 0 & 0 & 1 & 0 & \dots \\ \dots & \dots & \dots & \dots & \dots & \dots & \dots \end{pmatrix}$$

giving the age class reproductive values; $\epsilon_{k,s} = \frac{1 - s}{1 + 3(1 - s)}$

for $k = 1 \dots 4$, and $\frac{(1 - s)s^{k-4}}{1 + 3(1 - s)}$ for $k \geq 4$, for k years.

The number of effective individuals, ' N_k ', per 3 ha [30 000 m²], corresponds to the effective density $D_k = D_g(1 - s)s^{k-4}$ for $k \geq 4$ where D_g is the density of gravid females:

$$\epsilon_{k,s} = \frac{(1 - s)s^{k-4}}{1 + 3(1 - s)};$$

$$D_{k,s} = D_g(1 - s)s^{k-4}.$$

Thus the inverse of the effective density of females

$$\frac{1}{D_{ef}} = \sum_{k=4}^{\infty} \frac{\epsilon_k^2 - \epsilon_{k+1}^2}{D_k} \text{ which simplifies to } \frac{1 - s^2}{(4 - 3s)^2 D_g} \text{ so that}$$

$$D_{ef} = \frac{(4 - 3s)^2 D_g}{1 - s}.$$

Note that D_{ef} relates to females only. Under the assumptions that: (i) adult males and adult females are related by a 1:1.5 ratio (Sumner *et al.* 1999), so that the total density $D_t = 5D_f/3$; (ii) the density of gravid females is $D_g = 0.44D_f$; (iii) the age structure is the same for males and females; and (iv) there is no extra variance in the number of offspring not accounted for by this model, one has the effective (male and female) density D_e :

$$\begin{aligned} \frac{1}{D_e} &= \frac{(1/2)^2}{D_{ef}} + \frac{(1/2)^2}{D_{em}} = \frac{1}{4} \left(\frac{1 - s^2}{(4 - 3s)^2 D_g} + \frac{1 - s^2}{(4 - 3s)^2 D_m} \right) \\ &= \frac{1 - s^2}{4(4 - 3s)^2} \left(\frac{5}{0.44 * 3D_t} + \frac{5}{2D_t} \right) = \frac{D_t(4 - 3s)^2}{1.57(1 - s^2)}. \end{aligned}$$

Because D_t and s were estimated to be 484 individuals in 3 ha and a rate of 0.86, respectively, it follows that the effective density, $D_e = 2670.99$ per year for 3 ha. This is more than D_t by a factor of 5.5, however, per unit time (1 year = one class) the effective parameter would be $D_e/6.53 = 409$. The effective density expressed in the same units as σ^2 is: $D_{em} = D_e/30\ 000 = 0.089$ individuals * year/m² (89 000 individuals * year/km²), and 1.36×10^{-2} individuals * generation/m² (13 600 individuals * generation/km²).