

High mate and site fidelity in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat

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Abstract

While habitat alteration has considerable potential to disrupt important within-population processes, such as mating and kin structure, via changed patterns of dispersal, this has rarely been tested. We are investigating the impact of anthropogenic habitat alteration on the population biology of the rock-dwelling Australian lizard *Egernia cunninghami* on the Central Tablelands of New South Wales, Australia, by comparing deforested and adjacent naturally vegetated areas. The novel analyses in this paper, and its companion, build on previous work by adding a new replicate site, more loci and more individuals. The additional microsatellite loci yield sufficient power for parentage analysis and the sociobiological inferences that flow from it. Genetic and capture–mark–recapture techniques were used to investigate mate and site fidelity and associated kin structure. Analyses of the mating system and philopatry using 10 microsatellite loci showed high levels of site fidelity by parents and their offspring in natural and deforested habitats. Parentage assignment revealed few individuals with multiple breeding partners within seasons and fidelity of pairs across two or more breeding seasons was typical. Despite reduced dispersal, increased group sizes and significant, dramatic increases in relatedness among individuals within rock outcrops in deforested areas, no significant differences between deforested and natural areas were evident in the degree of multiple mating or philopatry of breeding partners within and across seasons. With the exception that there was a significantly higher proportion of unmated males in the deforested area, the social and mating structure of this species has so far been surprisingly robust to substantial perturbation of dispersal and relatedness structure. Nonetheless, approximately 10-fold elevation of mean pairwise relatedness in the deforested areas has great potential to increase inbred matings, which is investigated in the companion paper.

Keywords: family, habitat fragmentation, lizard, mate fidelity, microsatellite, site fidelity

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Introduction

Habitat fragmentation and the rationale of the Egernia cunninghami programme

Habitat fragmentation has been identified as a distinct threatening process in conservation biology (e.g. WCMC 1992; Bjørnstad *et al.* 1998; Boudjemadi *et al.* 1999). Considerable research has shown that restricted gene flow owing to fragmentation may elevate genetic differentiation

among populations, and can increase the rate of population extinction through genetic factors (Sarre 1995, 1996; Cunningham & Moritz 1998; Saccheri *et al.* 1998) as well as demographic factors (e.g. Boudjemadi *et al.* 1999). Nonetheless, the influences of habitat fragmentation on within-group processes (such as mating opportunities and kin structure) are much less well understood (Peacock & Smith 1997; Amos & Balmford 2001; Luck 2003). If within-group processes are affected by habitat alteration, the nature of residual populations may be changed deleteriously in ways that are potentially cryptic and cumulative. For example, human-induced habitat fragmentation could lead to postponed or impeded dispersal. This could lead to increased

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competition, unnaturally elevated philopatry and the disruption of potentially adaptive kin and social structures and breeding opportunities (Peacock & Smith 1997; Bjørnstad *et al.* 1998; Boudjemadi *et al.* 1999; Stow *et al.* 2001; Templeton *et al.* 2001). Certainly reduced juvenile dispersal can elevate genetic differentiation among local groups (Baker *et al.* 2000).

As with other phenomena in this context, several impacts of reduced dispersal on the mating system are possible. In the case of *E. cunninghami*, the subject of the present study, constrained dispersal (Stow *et al.* 2001) could potentially promote the formation of long-term pair bonds by holding individuals together, or could hold together individuals that would not otherwise be likely mating partners. Second, it has been suggested that females may facultatively use polyandry to counter increased risk of inbreeding, which may occur under increased natal philopatry (Petrie & Kempenaers 1998; Hosken & Blanckenhorn 1999; Tregenza & Wedell 2000). Third, increased aggregation of females may promote harem defence polygyny (Clutton-Brock 1989). Thus disruption to the mating system of *E. cunninghami* following dramatic decreases in dispersal and increases in density of close relatives (Stow *et al.* 2001) are plausible and even likely.

To enhance our understanding of the functions and evolutionary processes involved with long-term pair bonds, family group structure, and the potential impacts of habitat alteration, it is necessary to study such behaviours in phylogenetically and ecologically diverse taxa (Petrie & Kempenaers 1998; Bull 2000). We have been investigating the effects of habitat alteration on *E. cunninghami*, a large, secretive scincid lizard that utilizes mainly rocky retreat sites throughout its distribution in southeastern Australia. This convenient model lizard species was chosen because reptiles are under-represented in this type of research, yet they may be expected to show responses quite different from those of birds and mammals for which more data are available (Stow *et al.* 2001). Initial data on *E. cunninghami* from one site (Tarana) suggested that deforestation around rock outcrops dramatically reduced dispersal, particularly of females, and led to elevated local relatedness (Stow *et al.* 2001). However, the full implications of those findings could not be further investigated at that time because (i) a replicate site had not yet been identified and sampled, (ii) some loci in the microsatellite array were problematic and after their removal the genetic assay lacked sufficient resolution, e.g. for confident parentage estimation, and (iii) sample sizes of lizards were small for some analyses. A replicate site (Bathurst) has now been sampled and more samples and field data have been collected at Tarana. In addition, more microsatellite loci have been isolated (Stow 2002) and all sampled individuals have been typed at these. We can now report much more fully on the social structure and mating system of *E. cunninghami* and any impacts of deforestation. We present the data in a pair of

papers: this one focuses on mate and site fidelity and their impact on spatial organization of genetic relatives, while the other focuses on whether the coexistence of highly related individuals leads to inbred matings (Stow and Sunnucks 2002). In each paper, natural and adjacent deforested habitat is compared at two sites (Tarana and the recently added Bathurst).

Lizard social group structures and mating systems

The most commonly reported mating systems in lizards involve multiple mating partners, with polygyny being most prevalent (Bull 2000). Although several studies show social monogamy in lizard species within a season (Toxopeus *et al.* 1988; Olsson & Shine 1998), research demonstrating the persistence of pair bonds across seasons is rare, being known in *Tiliqua rugosa*, *E. stokesii* and *E. saxatilis* (Bull *et al.* 1998; Gardner *et al.* 2002; O'Connor & Shine 2003). Presently, genetic evidence for family group structure in lizards has been published for only three lizard species, all in the genus *Egernia*: *E. cunninghami*, *E. stokesii* and *E. saxatilis* (Gardner *et al.* 2001; Stow *et al.* 2001; O'Connor & Shine 2003).

For offspring to delay dispersal and remain with parents may reflect a selective advantage (Emlen 1994). Potential benefits include extended parental care, access to resources, and altruistic benefits of social behaviours (e.g. Clutton-Brock 1991; Emlen 1994; Jones & Parker 2002; Schneider 2002). Social group organization has fitness consequences and is subject to evolutionary optimization (Stacey & Ligon 1991; Roberts 1996; Brown & Brown 2000). Thus it is important to understand the extent of the impact of habitat alteration/fragmentation on the social group structure and mating systems. In this study we investigate mate and site fidelity and kin structure in *E. cunninghami*, and the impact of deforestation (habitat fragmentation) on these aspects of the species' biology. We are not aware of another study that does this for a reptile.

Egernia cunninghami forms aggregations of up to 27 individuals or more, comprising adults of each sex and immatures of several age-cohorts (Barwick 1965; Stow *et al.* 2001). The species reaches sexual maturity at approximately 190 mm snout-vent length (SVL), is viviparous and usually has four to eight young per litter (Barwick 1965). Captive specimens can survive in excess of 35 years (P Harlow personal communication). Previous genetic work suggested that *E. cunninghami* lives in groups consisting of close kin, with elevated levels of relatedness among adults within groups, and lower levels of inferred dispersal associated with deforestation (Stow *et al.* 2001). Nonetheless these inferences were yet to be demonstrated by direct parentage analysis. In addition, more than one adult male or female are often captured together, therefore it was unknown whether the mating system involves long-term

pair-bond fidelity or multiple breeding partners. Here we attempt to fill these gaps in knowledge and estimate the effects of habitat fragmentation on social structuring and mating system in *E. cunninghami*.

Materials and methods

Study sites and sampling

Individuals of *Egernia cunninghami* were sampled from two study locations 'Tarana' (33°33' S, 149°55' E; the site reported on in Stow *et al.* 2001) and 'Bathurst' (33°27' S, 149°24' E) on the Central Tablelands of New South Wales, Australia. The two sites are separated by approximately 45 km and are similar in climate, geology and biotic factors. Each site contains some naturally vegetated and some deforested grazed habitat, with discrete rock outcrops. The minimum time since deforestation at each site is 70 years. The overstorey of the naturally vegetated habitat is largely *Eucalyptus*, with a shrub understorey. The range of distances between rock outcrops from which lizards were sampled was 27 m to 3096 m at Bathurst and 22 m to 1375 m at Tarana (Fig. 1). The *a priori* groups for *E. cunninghami* comprised individuals caught at the same rock outcrop. *Egernia cunninghami* typically uses deep crevices within rock outcrops as retreat sites, with aggregations of lizards often observed within one crevice. All lizards captured at a rock outcrop were captured within 15 m of one another. Strenuous efforts were made to sample all lizards observed, and capture-mark-recapture (CMR) findings indicate that this was largely successful (ca. 90% of adult male and female lizards captured). At

Bathurst, 198 individuals were sampled from 31 rock outcrops between September 2000 and January 2002, and at Tarana, 167 lizards were sampled from 23 rock outcrops between March 1998 and December 2001. (For the Tarana site, 143 of the lizards comprised the sample base for Stow *et al.* 2001.) The average number of sampled individuals within each group (rock outcrops with two or more individuals sampled) was eight lizards at each site (range 2–26 at Bathurst and 2–16 at Tarana). Group sizes were not significantly different between habitats, although groups were on average larger in deforested habitats (mean \pm SD, 9.08 ± 5.78) than the naturally vegetated habitats (7.06 ± 4.64 , Mann-Whitney *U*-test; $Z = -1.11$, $P = 0.254$) (Stow 2003). Individuals were captured, sexed, marked uniquely, measured and sampled for tissue as described in Stow *et al.* (2001).

Genotyping

DNA extraction and genotyping methods were essentially as described previously (Stow *et al.* 2001; Stow 2002). However, locus Tr5.20 exhibited high-frequency putative null allele(s) at Bathurst and was excluded from these analyses. Mistyping and recording errors were minimized through multiple polymerase chain reaction and electrophoretic runs of samples and repeated checks for input errors. Characteristics of the 10 microsatellite loci used are given in Table 1. Expected heterozygosity, exclusion probabilities and null allele estimates were calculated using the program CERVUS (Marshall *et al.* 1998). The conservative estimate for the probability of genotypic identity (P_{ID}) amongst full siblings (Waits *et al.* 2001) was calculated using the software GEMINI (Valière *et al.* 2002).

Lizard age

Groups of *E. cunninghami* often contain individuals belonging to discrete size classes. *Egernia cunninghami* breeds maximally once a year, with litters produced usually during late January and February (Barwick 1965). The timing of fieldwork allowed direct capture of newborns and thus yielded their year of birth. For individuals not caught as newborns, growth curves estimated from SVL measurements obtained through CMR were used to estimate age (Stow 2003).

Lizards in their first and second years of growth were allocated an age-cohort and year of birth. However, to accommodate the possibility of accumulated individual differences in growth rates and incorrect age determination, lizards in their third or more year of growth but prior to maturity were placed in the category 'subadults'. Therefore only lizards captured in their first two years of growth are used to examine breeding-pair fidelity within and across two consecutive seasons. The subadult category

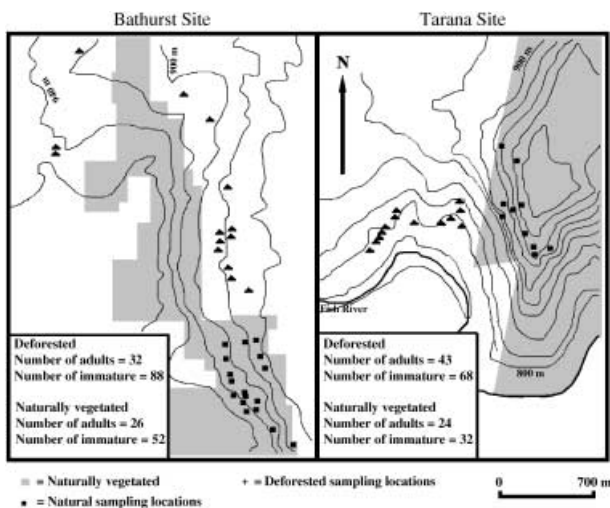


Fig. 1 Sampling locations of *Egernia cunninghami* at Bathurst and Tarana, in two different habitats, naturally vegetated and deforested. Individuals were sampled from a total of 54 rock outcrops, from which groups of lizards were sampled in 41 of these (single lizards were sampled from 13 rock outcrops).

Table 1 Number of alleles (N_a), heterozygosity estimates (H_E), exclusion probabilities (Ex), cumulative genotypic identity for first degree relatives ($P_{ID(Sib)}$) and null allele estimates (Null) of 10 microsatellite loci used for parental assignment

Bathurst site						Tarana site					
Locus	N_a	H_E	Ex	$P_{ID(Sib)}$	Null	Locus	N_a	H_E	Ex	$P_{ID(Sib)}$	Null
Est 13	22	0.906	0.674	3.035×10^{-1}	0.000	Est 13	19	0.925	0.728	2.914×10^{-1}	0.000
Est 1	23	0.898	0.660	8.998×10^{-2}	0.000	Est 1	26	0.927	0.734	8.472×10^{-2}	0.001
Tr5.21	20	0.935	0.761	2.573×10^{-2}	0.000	Tr5.21	18	0.871	0.587	2.737×10^{-2}	0.000
Est 9	9	0.630	0.239	1.230×10^{-2}	0.000	Est 9	15	0.516	0.160	1.525×10^{-2}	0.000
Tr3.2	25	0.916	0.705	3.765×10^{-3}	0.000	Tr3.2	27	0.915	0.703	4.533×10^{-3}	0.000
Ecu 3	11	0.821	0.467	1.344×10^{-3}	0.044	Ecu 3	12	0.867	0.574	1.483×10^{-3}	0.001
Ecu 5	17	0.895	0.646	4.157×10^{-4}	0.000	Ecu 5	13	0.808	0.466	5.325×10^{-4}	0.000
Ecu 4	31	0.924	0.733	1.218×10^{-4}	0.054	Ecu 4	38	0.955	0.825	1.463×10^{-4}	0.087
Ecu 2	13	0.843	0.521	4.141×10^{-5}	0.000	Ecu 2	13	0.861	0.553	4.828×10^{-5}	0.000
Ecu 1	17	0.906	0.676	1.252×10^{-5}	0.028	Ecu 1	20	0.921	0.717	1.420×10^{-5}	0.018

Total exclusionary power for first parent Tarana site = 0.999962.

Total exclusionary power for first parent Bathurst site = 0.999954.

was included to determine the number of cases wherein an individual has the same breeding partner in more than two seasons.

Parentage analysis

Parentage was estimated to provide an indicator of mate fidelity within and across breeding seasons and to assess the mobility of parents and offspring. Parentage analysis was conducted separately for each site, using 10 microsatellite loci (Table 1), with parentage assigned only for immature lizards (< 190 mm SVL). Lizards that could possibly have matured during the study were tested as potential parents and offspring.

Parentage was assigned using the software CERVUS (Marshall *et al.* 1998) which determines the logarithm of the odds (LOD) score for all candidate parents and carries out simulations to estimate the critical difference in LOD score between the most likely and second most likely candidate parent, at 80% and 95% confidence levels. Simulations for both sites were run with the following parameters: 10 000 cycles, 60% of candidate parents sampled, 100% of loci typed and a genotyping error rate of 1% (approximated from multiple assays). It was assumed that 60% of candidate parents had been sampled, an extremely conservative estimate, far lower than the likely real value (Results). Parental assignments were accepted if the candidate was not genetically incompatible at more than one locus and could be the parent with 80% or 95% confidence. In addition, the level of genetic compatibility in parent-offspring assignments made at 80% confidence had to be higher than that obtained between the offspring and any other sampled candidate parent. Parentage assignment at less than 80% confidence was accepted for 12 adults that

presented no genetic mismatches with offspring, and had the highest positive LOD score. This was considered reasonable because no other sampled candidate parents were genetically compatible with these offspring, and the majority of parents (10) were assigned with at least 80% confidence to other offspring that most probably belonged to their clutch (based on genotypes, age and capture location). According to these criteria, parentage was assigned to each sex in a stepwise manner (Marshall *et al.* 1998). Offspring paternity was determined first, then maternity was assigned with some offspring already having an identified father.

The estimates for null alleles segregating at each locus (Table 1), suggests that nonamplifying alleles may be present at some loci. These could result in homozygote mismatches between parent and offspring. Null alleles tend to underestimate LOD scores and result in a more conservative parental assignment (Marshall *et al.* 1998). However, it was important to ensure that estimates of breeding-pair fidelity were not biased by the false exclusion of one parent because of null alleles. Using the assignment criteria above, genotypes of all offspring that were assigned to only one parent were compared with the genotypes of the most likely candidate as second parent. If offspring were found to be incompatible with this candidate parent solely because of homozygous mismatches at loci that potentially have null alleles, the single parent-offspring assignment was excluded from analysis.

Breeding-pair fidelity and single parent assignments

The procedures outlined in this section were applied within and among seasons to test for fidelity on those two time scales. Estimation of mate fidelity was most straightforward

when two parents per individual could be identified for putative offspring arrays (i.e. groups of offspring belonging to one or several age-cohorts that had at least one parent in common). However, groups of siblings for which all or some individuals had only a single parent assigned were still useful, in the following ways. First, polygamy was inferred where a pair of parents was identified as contributing to a progeny array but one was excluded as a parent for a subset of the array. Second, an assigned parent's genotype could be used to deduce allelic combinations contributed by unsampled parents and the minimum number of unsampled parents contributing (using GERUD, & Jones 2001). In a progeny array, the presence of three or more alleles (at any locus) not attributable to the identified parent points to multiple unknown parents. This assumes no mutation or genotyping errors and requires three or more offspring assigned to a single parent (Dewoody *et al.* 2000). Where many offspring can be explained by a single unsampled genotype, that genotype can be reconstructed (Jones 2001), and such 'reconstructed genotypes' can be tested as parents of offspring or arrays. To be sufficiently confident in this, a reconstructed genotype was accepted only when it could account for a whole litter or for all offspring assigned to a parent. We note the high probability of genotypic uniqueness and power of exclusion (Table 1).

Movement among rock outcrop groups

Levels of mobility for *E. cunninghami* were inferred by the location of parents and offspring and by direct observation through CMR. To allow for any disturbance to movement patterns resulting from capture, repeat location data from CMR was included only for time periods of 7 days or more between capture and recapture. For every lizard captured, the date and rock outcrop at which it was located was recorded. Distances were measured between all rock outcrops at which lizards were captured. The geographical extent of a rock outcrop site was defined to include 11 m from the edge of the rock outcrop, calculated as half the minimum distance between rock outcrops at the two sites. Dispersal amongst rock outcrops was detected using records of individual capture–recapture locations.

Habitat comparisons

The sites (Bathurst and Tarana) were matched as closely as possible for climate, structure of rock outcrops and time since deforestation. To examine the impacts of habitat alteration on breeding-pair fidelity and philopatry, data were first examined at each site to ensure consistency, then pooled across study sites to increase sample size and statistical power. Unless noted otherwise, comparisons were made using Fisher's Exact Test as it is robust to small

Table 2 Total number of sampled immatures and adults of each sex for each habitat and study site

Site/habitat	Offspring	Adult Males	Adult Females
Bathurst/deforested	88 (75)	12 (5)	20 (13)
Bathurst/natural	52 (49)	13 (7)	13 (8)
Tarana/deforested	68 (60)	22 (8)	21 (9)
Tarana/natural	32 (17)	13 (2)	11 (4)

The number of immature lizards assigned to at least one parent and the number of male and female lizards assigned as parents are given in parentheses.

expected frequencies (Siegel & Castellan 1988). All probability values reported are two-tailed.

Results

Parentage assignment

From both sites combined, 201 of 240 offspring sampled were assigned to at least one sampled parent (Table 2). In total, 94% of parenthoods were assigned with 80% or 95% confidence, most (78%) at the 95% level. Three offspring were assigned a parent but excluded from further analysis because the second most likely parent was not incompatible if a null allele was invoked. Because the two most likely parents for these three offspring were each assigned as parents for other immature lizards of the same age-cohort, their exclusion will not influence breeding-pair fidelity estimates across seasons. There were 24 sampled adult pairs identified as parents for a total of 129 offspring. The mean, standard deviation and range of offspring numbers per sampled pair were 5.38 ± 4.17 (1–17). For the remaining 72 offspring, only a single parent could be assigned. A pool of 21 parents was identified, each with 1–12 offspring (mean \pm SD, 3.43 ± 3.25). These 21 'single parents' include six individuals also identified as members of the 24 breeding pairs. There was no significant habitat difference observed in the proportion of progeny arrays for which one or both parents could be assigned (deforested $n = 30$, natural $n = 14$; $P = 0.510$).

Ten of the 21 'single parents' were assigned three or more offspring (5.90 ± 3.21), and from these 10 arrays it was possible to assess the number of unidentified parents. For eight of the 10, a single unsampled parental genotype could generate all offspring. In the two cases where more than one unsampled parent was deduced, three or more alleles unattributable to the known parent were found at two and five loci, indicating multiple unsampled parents rather than mutation. One case indicated polygyny within a breeding season, and the other suggested a female changing partner between seasons. In the latter case, there were

Table 3 Summary data describing mating systems in the deforested and naturally vegetated habitats

Habitat	N	Multiple partners	No. of partners (mean \pm VAR)	No. of partners	
				1.	2.
Deforested					
male	23	3	1.13 \pm 0.119	16 (2)	11 (1)
female	22	1	1.05 \pm 0.045	15 (2)	11 (1)
Natural					
male	11	1	1.10 \pm 0.100	7 (0)	4 (0)
female	16	0	1.00 \pm 0.000	7 (0)	4 (0)

Number (*N*) of breeding pairs from which polygyny could be detected and litters for which multiple paternity could be assessed. The number of cases for each sex where multiple breeding partners were detected within breeding seasons and the mean number (\pm VAR) of within-season breeding partners for individuals of each sex detected as breeding. Number of cases for which male and female individuals are detected breeding in: 1. consecutive seasons, 2. three or more seasons. In parentheses, the number of individuals not keeping at least one partner in common in each of the breeding seasons assessed.

sufficient offspring to reconstruct the paternal genotype. This reconstructed genotype was included alongside the eight unsampled genotypes reconstructed from whole progeny arrays, and these nine reconstructed genotypes were examined for uniqueness and for their possible reproductive success. One case of polygyny was inferred where alleles consistent for one male were assigned to a total of 21 offspring in progeny arrays of two females. The other reconstructed genotypes mismatched with each other at four or more heterozygous loci, and were thus taken as separate individuals. With 24 breeding pairs assigned and nine confidently reconstructed partnerships, data were available for a total of 33 breeding pairs.

Breeding-pair fidelity within and across breeding seasons

Within breeding seasons, low numbers of individuals were detected with multiple breeding partners. Out of a total of 33 breeding pairs estimated from genetic data only four males (14%) were detected as breeding with more than one female in a season. For male lizards that were detected breeding, the mean number, variance and range of breeding partners per male in a season were 1.12 \pm 0.11, 1–2 (Table 3).

There were 38 litters that could be assessed for potential multiple paternity (Fig. 2). Of these there was only one possible but inconclusive case of multiple paternity. The female was assigned four first year offspring. One sampled male was assigned to two of these, but all candidate sires mismatched the other two offspring at several loci. A single unsampled father could explain the genotypes of the

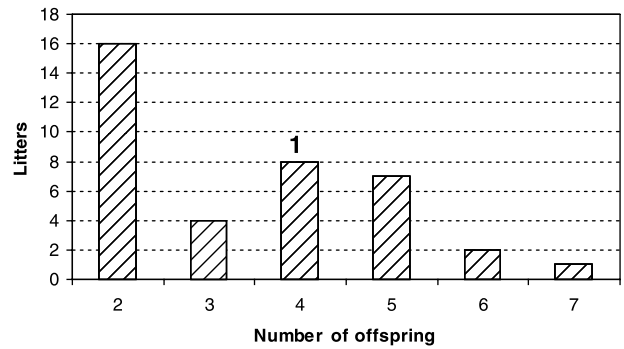


Fig. 2 The frequency of litter sizes assessed for potential multiple paternity in *Egernia cunninghami*. One possible case of multiple paternity (marked by 1) was observed in a litter of four offspring.

four offspring if mutation resulted in three nonmaternal alleles found at one locus. Including this tentative case of multiple paternity, the mean number, variance and range of breeding partners per female in a season was 1.026 \pm 0.026 (Table 3).

High levels of breeding-pair fidelity were observed over breeding seasons. Twenty-six of 30 individuals assigned parentage over two seasons kept at least one partner in common. The remainder (two of each sex) changed partners over seasons (Table 3). In the four cases of polygyny, three males each had a consistent partner but took an additional female mate in one season. The remaining male showed stable polygyny with a pair of females over several seasons. Finally, of the 15 males and 15 females for which offspring were sampled that constituted three or more age-cohorts, only two individuals (a male and female) were not detected with the same breeding partner in at least three breeding seasons. That is, 14 pairs showed mate fidelity in at least three breeding seasons (Table 3).

Habitat differences in the level of mate fidelity were not apparent (values of *P* range from 0.289 to 1.0 depending on sex and season categories). However, given the very high overall level of breeding-pair fidelity, the frequency of multiple breeding partners limits statistical power (Table 3).

Site fidelity of parents and offspring

An examination of the mating system shows that *Egernia cunninghami* parents and offspring display a high degree of site fidelity. Of the 201 offspring assigned parent(s), 51 of 56 assigned parents were captured at the same rock outcrop as all of their sampled offspring (Table 4). A total of 25 groups of full siblings were identified (mean number per group 7.36 \pm 3.80). In 24 of these groups, all siblings were captured together at the same rock outcrops. This is indicative of low levels of movement for both offspring and parents. The generality of low levels of dispersal for *E.*

Table 4 Inferred movement from parentage data and direct observation from the capture–recapture locations

	Same location	Movement > 11 m	Median distance (range) in m
Parentage*			
sib	184	1	48
father	17	3	70 (22–170)
mother	33	0	–
Recapture			
immature	85	2	20 (18–22)
male	16	7	43 (15–200)
female	17	1	40

From parentage data, the number of full siblings (Sib) located together at one rock outcrop and the number of individuals captured at a different location to a group of its siblings. The number of adults of each sex that were captured only at the same location as all their offspring and the number of parents either captured at a different rock outcrop to one containing a group of their offspring or a breeding partner–offspring assignment. For recapture data, the number of adult male, adult female and immature individuals recaptured only at the one location and the number of captured beyond 11 m from the edge of the rock outcrop at which they were first captured. For inferred and observed movements the median and range of the distance (m) moved.

*Each of the four movements inferred had parent–offspring assignments at 95% confidence.

cunninghami was also shown by recapture data. There were 192 records of recapture obtained from 128 individuals consisting of 41 adult and 87 immature lizards. Of these, 176 recaptures (92%) were within 11 m of the rock outcrop from which the individual was originally captured. Movement beyond this distance was observed for eight male and two female lizards (Table 4). There was no significant relationship between time and distance lizards moved from their site of original capture (Spearman Rank, $b = -0.047$, $P = 0.521$). Individuals were recaptured at the same location for time intervals up to 1358 days (mean 302.54 ± 314.70), suggesting long-term site tenure for some *E. cunninghami*. These data provide no indication of kin-biased dispersal amongst discrete rock outcrops for immature or adult lizards. Correspondingly, groups of siblings are considered to be located at their natal rock outcrop. The high proportion (42%) of breeding pairs sampled at the same location together with their offspring consisting of three or more age-cohorts further indicates long-term site tenure in *E. cunninghami*.

At the temporal scale assessed by CMR and parentage data, differences were only minor between deforested and intact habitat in site fidelity of parents and offspring. The number of parent(s) sampled at the same location as all

their offspring was not significantly different between natural and deforested habitats ($P = 0.397$). In addition, no significant habitat differences were evident for the duration of site tenure. The proportion of breeding pairs with offspring constituting three or more age-cohorts that were detected together only at the same rock outcrop was 40% and 43% in the deforested and naturally vegetated sites, respectively (deforested $n = 23$; naturally vegetated $n = 10$, $P = 1.00$). However, habitat comparisons for levels of site fidelity for all categories of *E. cunninghami* are less clear from the current data. While the proportion of individuals that were recaptured only at their initial capture location did not differ significantly between deforested and natural habitats (deforested $n = 103$, natural $n = 25$, $P = 1.00$), the proportion of individuals recaptured in the naturally vegetated habitats was significantly lower than that in the deforested habitats ($P = 0.0003$).

Spatial structure of family groups

The 201 offspring with parents assigned were located in 32 separate rock outcrops (deforested $n = 19$, natural $n = 13$). In 23 rock outcrops, two or more offspring were assigned parents (mean 8.35 ± 4.80 SD) allowing within-group characteristics of parental allocation to be assessed (Table 2). In 16 rock outcrops, parentage was assigned to all offspring sampled, while in the remaining seven groups parentage was assigned to the majority of offspring sampled (average over this second assemblage = 72%; Table 2). In 13 groups, all offspring for which parents were assigned were full siblings. In the 10 groups in which more than two parents contributed to offspring sampled, four groups each contained a single father siring offspring of two females, and in one group there was a possible case of multiple paternity (see Results on breeding-pair fidelity). In the remaining five groups there were either partner changes in different breeding seasons or rock outcrops contained multiple breeding pairs. Despite the tendency towards higher numbers of adults within groups in the deforested ($N = 16$, mean 3.63 ± 1.31 SD) than naturally vegetated habitats ($N = 7$, 2.71 ± 0.951 , Mann–Whitney U -test; $Z = -1.72$, $P = 0.115$), there was no significant habitat difference in the number of adults detected breeding per group (deforested, mean 2.65 ± 0.957 ; natural 2.29 ± 0.487 ; $Z = -0.633$, $P = 0.624$). Correspondingly, there were higher numbers of individuals not detected as breeding in the deforested habitats. Significantly higher numbers of nonbreeding adult male lizards were present within groups in the deforested habitats (0.813 ± 0.655) compared with the natural habitats (0.143 ± 0.377 , Mann–Whitney U -test; $Z = -2.34$, $P = 0.033$). Numbers of nonbreeding adult females within groups were on average higher in the deforested than natural habitats, although this difference was not significant (deforested, 0.625 ± 0.719 ; natural, 0.428 ± 0.786 ; $Z = -0.751$, $P = 0.535$).

Discussion

This study demonstrates breeding-pair fidelity beyond one year in *Egernia cunninghami*, and that both parents and offspring show high levels of site fidelity. The majority of rock outcrops have only a single breeding pair over several seasons. These structures generate groups of lizards consisting of parents and offspring of several age-cohorts. The finding of such strong family group structure in this species accounts for previous research results demonstrating high levels of within-group relatedness in *E. cunninghami* (Stow *et al.* 2001). Long-term breeding-pair fidelity and family group structure was found in both deforested and naturally vegetated habitats, indicating that it was not a consequence of the anthropogenic disturbance investigated. Results obtained for *E. cunninghami* are very similar to those reported for the congeneric *E. stokesii*, for which strong longitudinal family group structure and long-term monogamy were characteristic (Gardner *et al.* 2001, 2002). This provides further evidence for the suggestion that complex sociality may be widespread in the lizard genus *Egernia* (Chapple 2003).

High mate and site fidelity

Few *E. cunninghami* were detected having multiple breeding partners within a breeding season. Although actual levels may be higher than those detected owing to incomplete sampling of offspring or parents, the lack of convincing evidence for multiple paternity from 38 litters and only four males showing polygyny in 33 breeding pairs strongly suggest that the mating system employed by *E. cunninghami* does not generally involve multiple breeding partners. The high degree of breeding-pair fidelity across breeding seasons and low levels of multiple breeding partners within seasons are suggestive of long-term genetic monogamy in *E. cunninghami*. More accurate determination of the degree of multiple breeding partners may be obtained by paternity testing litters from gravid females (cf. Morrison *et al.* 2002). Furthermore, it may be of interest to investigate the degree of multiple mating, sperm competition and sperm selection by females (Birkhead & Møller 1993; Olsson *et al.* 1996; Olsson & Madsen 2001).

Two main hypotheses have been proposed to explain social monogamy in species with no direct paternal care: males adopt monogamy when unable to maintain reproductive access to multiple females, and females adopt monogamy if conditions make them vulnerable to 'helpful coercion' from males (Bull & Cooper 1999). *Tiliqua rugosa*, a close relative of *Egernia* skinks, is approximately as monogamous as the present data suggest for *E. cunninghami*: about 15% of offspring result from extra-pair copulations. In *T. rugosa*, males who spend less time with their

partner are more likely to be cuckolded, so mate guarding may be a driver for faithfulness in males. Females seem to derive benefits from strong association with a given male; they will seek them out if separated, and are likely to 'divorce' inattentive males the next season. Benefits could be nongenetic (e.g. vigilance) as well as genetic (e.g. inbreeding avoidance) (Bull & Cooper 1999; Gardner *et al.* 2002).

We have shown that *E. cunninghami* lives in large groups of close relatives with data from deforested areas showing sharply reduced dispersal (especially in females) and greatly elevated relatedness of individuals living in close proximity (Stow *et al.* 2001 and present study). Thus, it was surprising that habitat fragmentation (deforestation) had so little measurable impact on levels of multiple mating, particularly as we detected a significant increase in the proportion of unmated males (and nonsignificant increase for females). This may reflect very strong inbreeding avoidance, a phenomenon that is tested for in the companion paper (Stow & Sunnucks 2004).

Sex and age of dispersers

Based on genetic data, deforestation was associated with reduced dispersal in both sexes, disproportionately so for females to the point of creating significantly stronger male-biased dispersal (Stow *et al.* 2001; Stow 2003). Direct observations of dispersal reported in this study support that inference of male-biased dispersal, although sample sizes are low for statistically meaningful comparisons between natural and altered habitats. A higher proportion, approaching significance, of male than female adult lizards were observed by CMR to move from their original capture location ($P = 0.059$; Table 4). In addition, only fathers were captured at sites away from their female partner and offspring ($P = 0.049$; Table 4).

The capture locations of parents and offspring indicate that most dispersal is by sexually mature individuals. A significantly higher proportion of adult than immature lizards were recaptured at distances beyond 11 m from the rock outcrop at which they were first captured ($P = 0.003$; Table 4). In addition, low rates of dispersal in immature lizards are suggested by groups of full siblings where nearly all members were captured together at the same rock outcrops (Table 4). This is consistent with previous research wherein dispersal rates of immature lizards inferred by relatedness structure were low and not significantly different for comparisons between natural and modified habitats even though significant differences were seen in adults (Stow *et al.* 2001). It is also apparent that levels of migration for breeding pairs are low in deforested and naturally vegetated habitats. The overall proportion of parent-offspring groups extending over three or more age-cohorts did not differ significantly between habitats. This indicates little

difference according to habitat type in the level of dispersal between rock outcrops for parents at the time-scale investigated here. Levels of dispersal amongst age-cohorts of immature lizards have not been addressed because other factors such as habitat differences in survival may also influence age structure and rates of recapture (Wheeler *et al.* 2003).

Large aggregations of close kin

The spatial structuring of family groups shows that offspring grouped within a rock outcrop largely have one or both parents in common. This is unlikely to be an artefact of incomplete sampling of parents because the majority of offspring sampled within these rock outcrops (94%) were assigned parentage with high confidence. Aggregations of offspring numbered up to 23 at one rock outcrop, with a median size of eight in family groups. In all of these groups offspring were composed of at least two age-cohorts, all had at least one parent captured at the same location with 92% of all most likely parents sampled within the same rock outcrop as their offspring.

For some species, extended parental care has been suggested as a benefit to family group structure (Clutton-Brock 1991). However, parental care has rarely been reported in lizards (Shine 1988). Perhaps more likely benefits of aggregations involve predation, with reduced risk through predator vigilance, 'dilution' and 'confusion' effects, or the 'selfish herd' effect (Hamilton 1971; Roberts 1996; Uetz *et al.* 2002). Kin-based access to resources such as food and refuge may also encourage delayed dispersal, particularly if constraints on dispersal are high, for example through high levels of predation or a lack of suitable habitat or mates (references in Introduction).

These data on *E. cunninghami* will contribute to future investigations into the evolution of sociality within this interesting lizard lineage. In addition, this study validates a model system in which to investigate the effects of anthropogenic habitat fragmentation on within-patch population biology. In summary, *E. cunninghami* skinks have been shown to be very faithful over time to their breeding partners and natal sites. The typical spatial arrangement is a breeding pair of at least a few years' tenure, accompanied by many offspring of different cohorts. This structure suggests considerable potential for favourable kin interactions. The long-term stability of the groups, long lifespans of individuals and naturally patchy nature of the *E. cunninghami* habitat may have predisposed these lizards to resist profound impacts on their social organization in deforested areas. On the other hand, given greatly elevated relatedness within rock outcrops, inbred matings could be common unless blocked by effective inbreeding avoidance. That is the subject of the companion paper (Stow & Sunnucks 2004).

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The study reported here, in the companion paper (Stow & Sunnucks 2004), and in Stow *et al.* (2001) and Stow (2002) is part of Adam Stow's PhD research programme. Adam has interests in behavioural ecology, especially of reptiles, and in the consequences of habitat fragmentation for population genetics and social interactions. Paul Sunnucks' research focuses on the application of molecular markers to mobility and gene flow of animals in natural and human-impacted habitats.

Appendix 1

Composition of *Egernia cunninghami* groups for which parentage data were obtained in deforested (D) and naturally vegetated habitats (N) at Bathurst and Tarana sites

Group	Within-group adults		Immature lizards	Male parents	Female parents	Offspring	Year of Birth
	males	females					
1D	1	1	10	M1	F1	2	2000
						3	1999
						5	≤ 1998
2D	1	2	17	M1	F1	5	2000
						2	1999
						10	≤ 1998
3D	2	2	8	M1†	F1	5	2000
						1	1999
4D	2	3	4	M1	F1	1	2000
				M1	F2	1	1999
				M1	F3	1	≤ 1998
5D*	1†	3	23	M1†	F1	6	2000
						5	1999
						1	≤ 1998
				M1†	F2	3	2000
						4	1999
						3	≤ 1998
6D	3	3	3	M1 ^a	F1	1	1999
					F1	1	≤ 1998
7D*	2	1	8	M1 ^a	F1	2	2001
					F1	2	2001
					F1	1	≤ 1998
8D	2	1	7	M1	F1	1	2001
	2	1	7	M1	F1	1	2001
9D	0	1	1		F1	5	2000
						1	≤ 1998
						1	2001
10D	4	2	11	M1	F1	2	1998
						2	1999
				M1	F1	2	1997
						1	≤ 1996
						1	≤ 1996
11D	2	2	9	M2 ^b	F3†	3	1997
						M1	F1†
				2	1999		
				3	1998		
				1	1997		
12D*	2	2	8	M1	F1†	3	1998
				M1	F2†	4	1998
						1	1997
13D*	2	2	8	M1	F1	1	1999
						1	1998
						3	≤ 1996
						3	1998
14D	1	1	10	M1	F2†	3	1998
						M1 ^c	F1
				4	1999		
				1	1998		
1	≤ 1996						

Appendix 1 *Continued*

Group	Within-group adults		Immature lizards	Male parents	Female parents	Offspring	Year of Birth
	males	females					
15D	1	1	6	M1+ M2+	F1 F1	1	2000
						1	1998
						2	1997
16D	1	1	5	M1	F1	1	2001
						1	2000
						3	1998
17D	4	3	2	M1 ^c	F1§	1	1997
18D	2	2	4	M1	F1	2	1998
						1	≤ 1996
19D	2	1	1	M1 ^b	F1	1	1999
1N	1	1	9	M1	F1	4	2001
						2	2000
						2	1999
						1	≤ 1998
2N	1	3	8	M1+	F1	2	2001
						2	2000
						2	1999
						2	≤ 1998
3N	1	1	1	M1	F1	1	2000
4N*	1	2	14	M1 M1	F1 F2	7	2001
						5	2001
						2	2000
5N	1	1	8	M1	F1	6	2001
						2	2000
6N	1	1	7	M1 M1‡	F1 F2+	4	2001
						3	≤ 1999
7N	1	1	1	M1	F1	1	2001
8N	1	1	1	M1		1	2001
9N	0	3	2		F1	1	1998
10N	1	1	12	M1	F1	4	2001
						5	1999
						2	1998
11N	0	1	2		F1	1	1999
12N	2	2	8	M1	F1	1	1999
						2	1997
						1	≤ 1996

*groups in which polygamy was detected.

†Reconstructed genotypes.

‡Parentage also assigned to a 'solitary' subadult male (see Table 4).

§A single parent-offspring assignment for which the mother was located in a separate group to her subadult male offspring (see Table 4).

^{a,b,c}The same letter identifies pairs of groups where offspring in each group share the same father.