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Male-biased dispersal in a tropical Australian snake (*Stegonotus cucullatus*, Colubridae)

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Abstract

Sex-based differences in dispersal distances can affect critical population parameters such as inbreeding rates and the spatial scale of local adaptation. Males tend to disperse further than females in mammals, whereas the reverse is true for birds; too few reptiles have been studied to reveal generalities for that group. Although reptiles are most diverse and abundant in the tropics, few tropical reptiles have been studied in this respect. We combine data from a long-term (10-year) mark–recapture study with genetic information (based on nine microsatellite markers) on slatey-grey snakes (*Stegonotus cucullatus*, Colubridae) in the Australian wet-dry tropics. Males attain larger body sizes than females, and both genetic and mark–recapture data show that males also disperse further than females. Recapture records show that hatchling males dispersed away from their release points whereas hatchling females did not, and adult males moved further than adult females. In the genetic analysis, males contributed less to overall F_{ST} and relatedness than did females ($F_{STm} = 0.0025$, $F_{STf} = 0.0275$, $P < 0.001$; $r_m = 0.0053$; $r_f = 0.0550$; $P < 0.001$). Spatial autocorrelation analyses within the largest population revealed a similar pattern, with spatial structuring stronger for females than males. Overall, our genetic analyses not only supported the mark–recapture data, but also extended our insights by revealing occasional long-distance dispersal not detected by the mark–recapture study.

Keywords: mating system, microsatellites, movement patterns, reptiles, sex-biased dispersal, spatial structure

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Introduction

The spatial distribution of gene frequencies within a population has important consequences (e.g. for inbreeding rates and opportunities for local adaptation) and can also provide significant insights into mating systems and movement patterns (e.g. DeWoody & Avise 2001; Dalerum *et al*. 2007; Keogh *et al*. 2007). If individuals are highly sedentary, or return to natal sites to breed, we expect to see strong spatial structure in gene frequencies; that is, local populations will diverge and inbreeding will be common. In contrast, we expect little spatial genetic structure in highly mobile, widely dispersing animals. Extensive studies suggest that the sexes often differ in their degree of dispersal and, hence, in their contribution to spatial genetic structure both within and among populations (Handley & Perrin 2007). The proximate and adaptive reasons for such sex differences have attracted considerable speculation, with inbreeding avoidance frequently suggested as an ultimate benefit of sex-specific dispersal (Packer & Pusey 1987; Wolff 1992), as it may lower the costs of inbreeding depression by increasing rates of genetic exchange between populations (Cockburn *et al*. 1985; Motro 1991; Gandon 1999; Perrin & Mazalov 1999). If members of one sex disperse to avoid inbreeding, there is no dispersal benefit to the other sex; theory predicts that one sex will remain philopatric while the other disperses (Gandon 1999; Perrin & Mazalov 1999). Short-distance dispersal may avoid inbreeding or kin competition, whereas long-distance dispersal may facilitate colonization of new territory (Clobert *et al*. 2001).

Perhaps reflecting inbreeding avoidance, sex differences in dispersal are common in vertebrates. Males usually disperse further than females in mammals, whereas the

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reverse is typical in birds (Greenwood 1980). Reptiles are less well-known in this respect, although recent studies report sex-biased dispersal in three major lineages: crocodiles (*Crocodylus johnstoni*: Tucker *et al*. 1998), turtles (*Caretta caretta*: Casale *et al*. 2002; Chaloupka & Limpus 2002; *Chelonia mydas*: FitzSimmons *et al*. 1997) and squamates (*Niveoscincus microlepidotus*: Olsson & Shine 2003; *Boa constrictor*: Rivera *et al*. 2006; *Cryptophis nigrescens*: Keogh *et al*. 2007). However, the proximate mechanisms underlying sex-biased dispersal may differ among species, involving phenomena such as displacement of subadult males by larger rivals in some cases (Tucker *et al*. 1998) and extensive mate-searching movements by adult males in others (Rivera *et al*. 2006).

The phenomenon of sex-biased dispersal in reptiles offers an excellent opportunity to test the generality of explanations advanced to account for this pattern in endothermic vertebrates. In particular, the diversity of life-history traits (e.g. reproductive mode, sex-determining systems, sexual size dimorphism, mating systems) within reptiles should enable tests of predictions from general theory (e.g. on intrasexual and intersexual competition, kin competition, and inbreeding avoidance: Handley & Perrin 2007). Frustratingly, however, we have data on this topic for so few reptile species that patterns in sex-biased dispersal cannot yet be identified with any confidence. Thus, detailed analyses of spatial genetic structure in reptiles, ideally combined with direct measurements of dispersal behaviour, can contribute substantially to our understanding of which sex disperses, and why it does so.

Materials and methods

Study species

Slatey-grey snakes (*Stegonotus cucullatus*, Colubridae) are large (to 1.5 m snout-vent length) slender nonvenomous snakes with a broad distribution through tropical Australia and New Guinea (O'Shea 1996; Cogger 2000). Abundant in many riparian habitats throughout their range, slatey-grey snakes are primarily terrestrial but with frequent arboreal activity (Shine 1991). Diets are diverse, including anurans, fishes, reptiles, mammals and reptile eggs (Shine 1991). Radio-tracking of slatey-grey snakes in the Australian wet-dry tropics revealed relatively small home ranges, averaging larger in males than females (11.4 vs. 4.6 hectares; G. P. Brown, unpublished) and showed that rates and distances of movement are highest during the wet season (Brown *et al*. 2005). Unlike most sympatric snake species, slatey-grey snakes do not shift their home ranges with the onset of seasonal flooding (Brown *et al*. 2005). Females produce one to two clutches of eggs between September and May (G. P. Brown, unpublished data). Unusually among snakes, adult males attain larger mean body sizes than do adult females (Shine 1991). We have seen mating between May and October (G. P. Brown, unpublished) but the mating system is poorly known. Male–male combat is likely given the correlation of this behaviour with male-larger dimorphism (Shine 1994) and observations of captive males attacking each other savagely if multiple animals are housed in a single cage (T. Madsen, personal observation).

Field data on movements and dispersal

From May 1998 to September 2007, we conducted a long-term year-round study on slatey-grey snakes on the Adelaide River floodplain 60 km east of Darwin in the Northern Territory. Snakes were captured by hand during nocturnal surveys on most nights of the year (total = 3099 nights). Our main study site (Fogg Dam) consisted of a 1300-m earthen embankment topped with a sealed road. The length of the wall was marked at 50-m increments and the location of each snake capture was recorded to the nearest 50 m. Captured snakes were brought back to the field laboratory for measuring and marking (scale-clipping). Sex was determined by eversion of hemipenes. Gravid females were retained in the laboratory until oviposition, whereupon the eggs were incubated (see Brown & Shine 2006 for detailed methods) and the females released at their site of capture. All other animals were released within a few days of capture (at initial capture sites) and neonates were released as soon as they had been measured and marked after hatching. Recaptures of these animals provided data on dispersal distances as a function of sex and age or body size.

Any sex differences in mean distance from the natal site at recapture might be due not to 'genuine' dispersal, but to a tendency for one sex to move around more than the other and, thus, on average be found further from the natal site. To test this possibility, we examined recapture locations in detail (see Field data on movement patterns and Field data on dispersal of hatchling snakes in the Results section). Lastly, we investigated the size and sex composition of animals that were first captured as juveniles or adults rather than released as hatchlings. Any sex or age bias in dispersal propensity should be reflected in the composition of that sample. For example, if dispersal is primarily by adult males, we should find large numbers of unmarked adult males in the study area.

Tissue sampling and DNA extraction

Tissue samples of 194 adult *Stegonotus cucullatus* (70 females and 124 males) were collected from 2002 to 2007 from three adjacent populations 3–6 km apart: Fogg Dam (FD, 125 samples: 12°34′13′S, 131°17′53′E), Harrison Dam (HD; 21 samples: 12°34′39′S, 131°20′16′E) and Beatrice Hill Farm (BHF, 48 samples: 12°36′25′S, 131°18′13″E). Total cellular

DNA was isolated from scales. Tissues were placed in 200 µL of 5% Chelex containing 0.2 mg/mL of proteinase K, incubated overnight at 56 °C, boiled at 100 °C for 10 min, and centrifuged at 13 200 *g* for 10 min. Then, the supernatant, containing purified DNA, was preleved and stored at –20 °C.

Microsatellite analysis

Nine microsatellite loci isolated and characterized from *Stegonotus cucullatus* (Dubey *et al*. 2008; Steg_A4, Steg_A5, Steg_A105, Steg_B104, Steg_B105, Steg_C109, Steg_D1, Steg_D2, Steg_D11) were amplified and scored. Amplified products were genotyped with an ABI PRISM 377 DNA Sequencer using GENESCAN ANALYSIS 2.1 software (Applied Biosystems). Polymerase chain reaction amplifications were performed in a 9800 Fast thermal cycler (Applied Biosystems) as 5-µL reactions containing 0.075 U *Taq Ti* DNA polymerase (Biotech), 0.1 mM dNTPs, 0.4 mM of each primer, 20 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.25 $MgCl_2$ (2.0 mM for Steg_D2), and 15 ng of DNA. Cycling conditions included a hot start denaturation of 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 57 °C (58.5 °C for Steg_A5) annealing temperature for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 30 min. Amplified products were genotyped with an 3130 xl genetic analyser (Applied Biosystems) using GENEMAPPER software version 3.7 (Applied Biosystems).

Statistical analyses of genetic data

Gene diversities comprising observed (H_O), expected within-subpopulation (H_E) and expected overall heterozygosities (H_T) were estimated following (Nei & Chesser 1983). Genotypic disequilibria between loci in each sample and deviations from Hardy–Weinberg equilibrium within samples were tested based on 2880 permutations and 10 000 randomizations, respectively. Wright's fixation indices for within-subpopulation deviation from random mating (F_{IS}), as well as pairwise subpopulation differentiation (F_{ST}), were estimated following Weir & Cockerham (1984). We computed the deviation from random mating within populations (F_{IS}) per locus and sample with a bootstrap procedure (720 randomizations). Statistical support for pairwise population differentiation was obtained through exact *G*-tests on allelic frequencies as described by Goudet *et al*. (1996) with 10 000 randomizations. To compute these summary statistics and tests, we used FSTAT version 2.9.3.2 (Goudet 2002; FSTAT, a program to estimate and test gene diversities and fixation indices, http://www.unil.ch/izea/softwares/fstat.html). Significance values were corrected for multiple tests using the sequential Bonferroni procedure (Rice 1989). We tested for the detection of null alleles according to Chakraborty & Jin (1992).

We used two methods to quantify migration between the populations. First, we estimated the migration rate (*m*) using BAYESASS 1.1 (Wilson & Rannala 2003) to specifically consider short-term gene flow (i.e. during the past one to three generations). This Bayesian method relies on the tendency for immigrants to show temporary disequilibrium in their genotypes relative to the focal population, allowing their identification as immigrants or offspring of immigrants. BAYESASS yields unidirectional estimates of *m* for each population pair, which we averaged for comparisons with other methods. Unlike the other approaches, BAYESASS does not assume migration–drift equilibrium, an assumption frequently violated in natural populations (Whitlock & McCauley 1999). Initial runs showed that convergence was reached using $3 * 10^6$ Markov chain Monte Carlo (MCMC) iterations; for the final analysis, we used iterations $3 * 10^7$ MCMC of which $1 * 10^7$ were for the burn-in.

Second, we used the software MIGRATE 2.0.6 (Beerli & Felsenstein 2001; Beerli 2004) to estimate the scaled migration rate (*M*). This software is based on a coalescence model with mutation and migration, and estimates a measure of effective population size, θ, defined as $4Ne\mu$, where µ denotes mutation rate, and migration *M*, defined as m/μ, where *m* denotes migration rate. We assumed a stepwise mutation model and based estimates on 15 short (10^4MCMC steps) and five long (10^5MCMC steps) chains. To ensure convergence, we used the 'adaptive heating' option with one 'cold' and three 'hot' chains.

We performed four tests for sex-biased dispersal using FSTAT 2.9.3.2 (Goudet 2002) based on the entire dataset and on 10 000 randomizations: (i) We examined the possibility of sex-biased dispersal by comparing among-population F_{ST} (Weir & Cockerham 1984) in male and female cohorts. Under male-biased dispersal, we predict that F_{ST} will be greater among the female than the male cohorts of each sample, because allelic frequencies for individuals of the sex dispersing most should be more homogeneous than those for individuals of the more philopatric sex. (ii) We compared relatedness (*r*) between sexes for all individuals pooled, which should be lower for males under male-biased dispersal for the same reason as for the F_{ST}. (iii) We compared the mean of the corrected assignment index (*mAIc*; Favre *et al*. 1997), with immigrants tending to have lower *AIc* values than residents. Under sex-biased dispersal, the average index for the sex that disperses most is expected to be lower than that for the more philopatric sex. A *t*-statistic is used for the test. (iv) We also compared the difference of variance of *AIc* (*vAIc*). Because members of the dispersing sex will include both residents (with common genotypes) and immigrants (with rare genotypes), *vAIc* should be larger for the sex dispersing most.

We used GENALEX version 6.0 (Peakall & Smouse 2006) to perform a microspatial autocorrelation technique (as developed by Smouse & Peakall 1999) for multi-allelic

codominant loci. This method uses pairwise geographical and genetic distance matrices to dissect out positive genetic correlation at various geographical size classes (see Peakall *et al*. 2003; Chapple & Keogh 2005; Double *et al*. 2005; Keogh *et al*. 2007). When several locations were reported for an individual in 1 year or more than 1 year, we used the mean location, based on all the different locations, for that animal. The analyses were performed for both sexes within the largest population (Fogg Dam). The results of the autocorrelation analyses are presented graphically in correlograms depicting changes in genetic correlation values (r) over distance. The correlogram presented here represents the average r-value within each class (15 sections of 100 m each in the present study), for the nine microsatellite loci.

Results

Field data on movement patterns

A total of 935 slatey-grey snakes were marked and released over the course of this study. Recapture rates were high (total number of captures = 2043; range = 1 to 24 captures per individual), especially for adult snakes living at our main field site (Fogg Dam). Mark–recapture analysis of Fogg Dam snakes indicates that survival rates of females and males are similar, both in juveniles and in adults (juveniles 0.48 vs. 0.46; adults 0.50 vs. 0.55, G. P. Brown, unpublished). Recapture rates, however, are higher among females than males (juveniles 0.46 vs. 0.28; adults 0.56 vs. 0.46, G. P. Brown, unpublished).

For each individual at Fogg Dam that was recaptured after a minimum of 1 month (N = 215), we calculated the maximum distance between captures. As a covariate, we used the number of days between capture events. The interaction term between sex and this covariate was not significant ($F_{1,211}$ = 0.025 P = 0.88) and was removed from the model. After controlling for recapture interval (number of days), the maximum distance between recaptures averaged significantly greater in males than in females (LSMeans = 285 m vs. 207 m, ANCOVA, $F_{1,212}$ = 6.82; P = 0.01). This result suggests that both male and female slatey-grey snakes tended to move further from their initial capture site over time, but that males moved further than females. We repeated this analysis after restricting the data set to adult animals only (> 80 cm snout-vent length), and found that males moved further on average than did females (295 m vs. 219 m), but the difference was no longer statistically significant ($F_{1,123}$ = 2.84, P = 0.10).

Field data on dispersal of hatchling snakes

We also examined dispersal of 91 neonatal snakes (hatched in the laboratory, and released a few days later in the field at their mother's site of capture). The distance between the site a hatchling snake was released, and the place that it was recaptured, tended to increase with time (age) for males but not for females (Fig. 1). To evaluate this apparent difference statistically, we used ANCOVA to compare the relationship between maximum dispersal distance and age between the sexes. The ANCOVA produced a significant interaction term (time*sex $F_{1,86}$ = 8.61, P = 0.0043); that is, males and females dispersed at different rates. Thus, we carried out separate regressions of maximum distance vs. time (age) for each sex. For male hatchlings, the maximum distance from the release point increased through time ($F_{1,24}$ = 5.87, P = 0.023). In contrast, a female's distance from her release point did not increase with age ($F_{1,62}$ = 0.03, P = 0.98). In other words, males gradually moved away from their natal release sites whereas females did not.

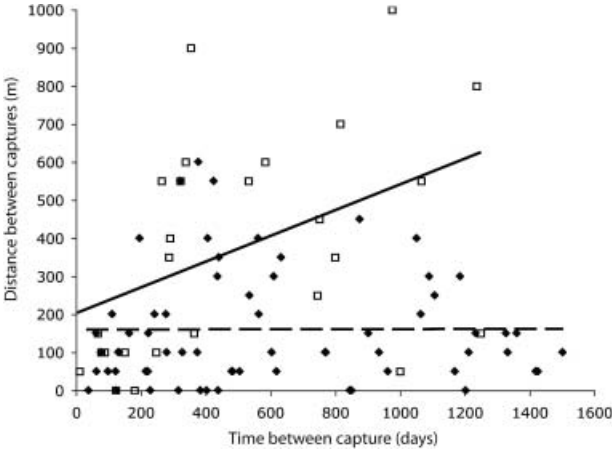

Fig. 1 Dispersal of male (open squares and solid line) and female (black diamonds and dotted line) slatey-grey snakes in the field. Dispersal distances are expressed as distance in metres between natal release point and subsequent recapture, as a function of the snakes' age at recapture (in days).

To check that this sex difference reflected genuine dispersal away from the natal site, not simply greater movement by males, we extracted data for all individuals that were recaptured at least three times after being released as hatchlings (n = 32 females, 10 males). We calculated the minimum distance each individual was captured from their release site, and examined the degree of overlap between the natal site and the animal's subsequent home range (based on all capture locations). There was no significant sex difference in the mean number of captures (5.7 vs. 5.6, t = 0.07, P = 0.94), nor in the mean ages of males vs. females when they were captured closest to their release point (729 days vs. 825 days, t = 0.55, P = 0.59). However, the minimum distance that females were captured from their release site was smaller than for males (122 m vs. 535 m, t = 4.65, P < 0.0001). Of the 32 females, 75% were recaptured within 200 m of their natal site, whereas this

was true for only 2 of the 10 males. Based on recaptures, the initial release site was included within the subsequent home ranges for 69% of the females but only 30% of the males ($\chi^2 = 4.75$, $P = 0.029$).

Field data on the age and sex of unmarked snakes arriving in the study area

Males comprised most of the 274 'new arrivals' (adult males comprised 38% of the new snakes, and juvenile males comprised an additional 28%). Juvenile and adult females comprised 21% and 13% of new arrivals, respectively. Although a numerical minority among new arrivals, these juvenile snakes outnumbered the resident juveniles (i.e. the newcomers comprised the majority of total numbers of both male and female juveniles in the study area: 79% and 58% for males and females, respectively). New arrivals made up 34% of captures of adult male snakes and 21% of captures of adult females. New arrivals thus accounted for a larger proportion of captures among males than among females (juveniles: 58% vs. 79%, d.f. = 1, $\chi^2 = 9.4$, $P = 0.002$; adults: 21% vs. 34%, d.f. = 1, $\chi^2 = 9.9$, $P = 0.0016$).

Another way to examine the sex ratio of unmarked snakes arriving in the study area is to compare it to the sex ratio of the resident snakes. The sex ratio of new arrivals was 66% male (93 F : 181 M), compared to 57% males among the resident snakes (92 F : 124 M). This difference in sex ratios is statistically significant (d.f. = 1, $\chi^2 = 8.81$, $P = 0.003$), with males over-represented among the new arrivals.

Genetic analyses

No linkage disequilibria or null alleles were detected, and all loci were in Hardy–Weinberg equilibrium. Consequently, all loci were included in the following analyses. For the nine microsatellite loci, the number of alleles per locus ranged from 3 to 11 (Table 1; average = 8.33), with a total of 75 alleles across 9 loci. The allelic richness within-population ranges were 5.80 for FD, 6.10 for HD, and 5.31 for BHF, with an average of 5.94 (Table 1). Observed heterozygosities within populations (H_O) were 0.77 for FD, 0.79 for HD, and 0.76 for BHF, with an average of 0.77. Expected heterozygosity (H_E) values were 0.73 for FD, 0.72 for HD, and 0.74 for BHF, with an average of 0.73 (Table 2). There was a significant deviation from random mating in the analysed populations with a negative global F_{IS} of –0.048 ($P < 0.001$). The genetic subdivisions between populations (pairwise F_{ST}) were low, ranging from 0.0108 to 0.0128, but all the values were significant ($P < 0.05$), as was the overall F_{ST} ($F_{ST} = 0.012$; $P < 0.001$).

Table 1 Genetic diversities of slatey-grey snakes at nine microsatellite loci, H_O, observed heterozygosity; H_E, expected heterozygosity; N_a, number of alleles

Locus	H_O	H_S	H_t	N_a
Steg_A4	0.62	0.55	0.55	3
Steg_A5	0.84	0.83	0.83	11
Steg_A105	0.77	0.73	0.74	8
Steg_B104	0.77	0.73	0.73	8
Steg_B105	0.75	0.69	0.69	10
Steg_C109	0.80	0.80	0.82	10
Steg_D1	0.76	0.76	0.78	8
Steg_D2	0.82	0.77	0.79	11
Steg_D114	0.80	0.74	0.74	6

Concerning the unidirectional migration rates (M or m) estimated within population pairs, four of the six in total were asymmetric [i.e. where 95% confidence interval (CI) and standard deviation did not overlap; Table 3]. The MIGRATE analysis clearly showed that migrations occurred between populations. In addition, the migrations were asymmetric for the pairs FD-BHF and FD-HD, with FD identified as the most important contributor to gene flow. Higher M (scaled migration rate) values were obtained from FD to BHF (4.47) and to HD (4.42). The BAYESASS analysis yielded results qualitatively similar to those obtained using MIGRATE. Migration rates (m) were asymmetric for two pairs out of the three (FD-BHF and FD-HD), and FD was suggested to be the most important contributor to gene flow. In addition, inferred migration rates (m) were high from FD to BHF (0.31) and from FD to HD (0.30).

Table 2 Genetic diversities within four populations of slatey-grey snakes based on 9 microsatellite loci

Population	n	H_O	H_S	F_{IS}	Ar
Fogg Dam	125	0.77	0.73	–0.052 NS	5.80
Harrison Dam	48	0.79	0.72	–0.091 NS	5.31
Beatrice Hill Farm	21	0.76	0.74	–0.019 NS	6.10
Overall	194	0.77	0.73	–0.048***	5.94

n, sample size; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, fixation indices for within-population deviation from random mating; Ar, allelic richness. NS, not significant. ***, $P < 0.001$.

Table 3 Unidirectional geneflow and migration-rate estimates within pairs of slatey-grey snake populations. The 95% confidence intervals are given in parentheses for MIGRATE (Beerli 2004), while for BAYESASS (Wilson & Rannala 2003) estimates contain the standard deviation in parentheses. The direction of gene flow/migration is as follows: the first population listed into the second population listed

	FD and BHF		FD and HD		BHF and HD	
Method of asymmetric gene flow and migration	FD → BHF	BHF → FD	FD → HD	HD → FD	BHF → HD	HD → BHF
M (= m/μ; Beerli 2004)	4.47 (4.14–4.82)	2.81 (2.63–3.02)	4.42 (4.05–4.82)	1.18 (1.06–1.33)	0.93 (0.76–1.12)	1.15 (1.01–1.32)
m (Wilson & Rannala 2003)	0.312 (0.015)	0.005 (0.006)	0.302 (0.021)	0.004 (0.004)	0.017 (0.016)	0.008 (0.008)

BHF, Beatrice Hill Farm; FD, Fogg Dam; HD, Harrison Dam.

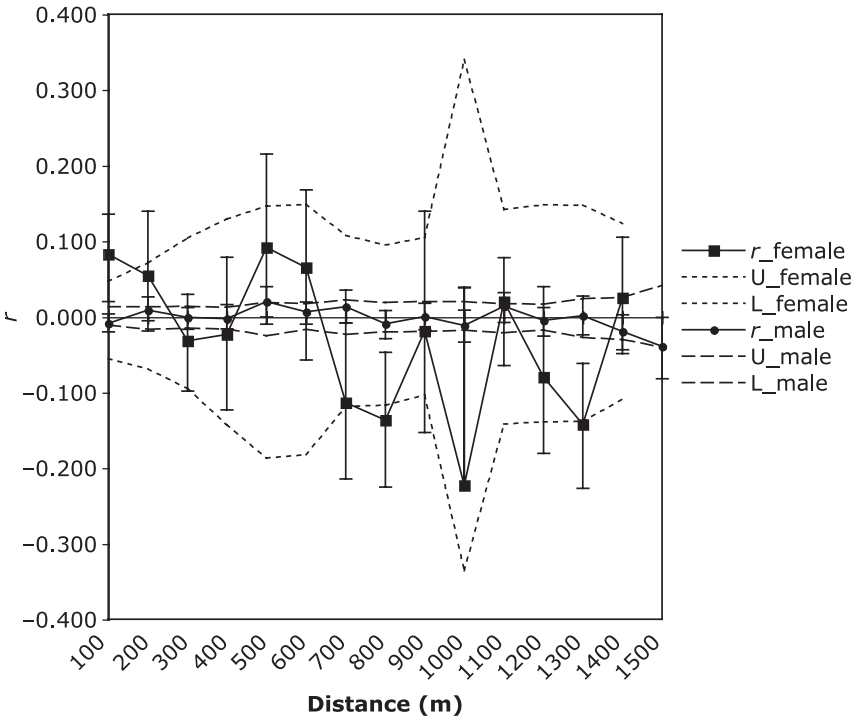

Fig. 2 Correlogram showing the results of the fine-scale spatial autocorrelation analyses where genetic correlation values (r) are plotted as a function of distance for males and females. U (upper) and L (lower) lines represent 95% confidence intervals around the null hypotheses of no structure. The plot shows that there is no significant positive genetic structure at any distance class for the males, whereas there is significant positive genetic structure for the females at the first distance class.

Male snakes contributed significantly less to overall F_{ST} (0.0025) than did females (F_{ST} = 0.0275, P = 0.0006). Similarly, the mean relatedness, $r = 2\ F_{ST}/(1 + F_{IT})$, of males ($r_m$ = 0.0053) was lower than that of females (r_f = 0.0550; P = 0.0007), revealing male-biased dispersal.

Mean assignment indices (*mAIc*) were similar between the sexes (P = 0.59; $mAIc_m$ = 0.03 and $mAIc_f$ = – 0.05). However, the proportions of correct assignments in this test were close to 0, and thus unlikely to provide any insights into gene flow. The variances of *AIc* (*vAIc*) did not differ significantly between the sexes (P = 0.29; $vAIc_m$ = 8.67 and $vAIc_f$ = 5.93).

Spatial autocorrelation analyses performed on data from the largest population (FD) did not show significantly positive r values at any distance class for male snakes (r value outside the 95% CI; Fig. 2), with the mean r value oscillating between –0.11 and –0.02. In contrast, autocorrelation analyses based on data from females revealed a significant and positive r value (0.08) for the class 0–100 m. This result indicates that females within that distance class were more similar genetically than were females drawn at random from the total sample. In addition, genetic similarity among females also was significantly negative at the distance class 700–800 m (–0.14) and the mean r value oscillated over a larger range (–0.22–0.09). Therefore, overall our analyses suggest that gene frequencies in female slatey-grey snakes are nonrandomly distributed in space, whereas those in males are more homogeneously distributed.

Discussion

Our basic result is very clear: male slatey-grey snakes disperse further than do conspecific females. That result is shown not only by direct measures of neonatal dispersal, but also by movement patterns of adult snakes. In keeping with radiotelemetric monitoring that showed larger home ranges in adult male than adult female slatey-grey snakes (Brown *et al*. 2005), our field studies demonstrate higher mobility in males than in females. However, the tendency for males to be recaptured further from their natal sites than females is not simply a reflection of larger home range sizes in males. Our detailed analyses of recaptured hatchlings confirmed that females do indeed remain close to the natal site whereas males do not. Additionally, males predominated among unmarked snakes arriving in the study area, and once marked, male snakes were less likely to be recaptured than were females (despite similar survival rates), as expected under male-biased dispersal. That conclusion is reinforced by our genetic analyses. Both within the single largest population, and in overall comparisons, relatedness among snakes living within the same area tended to be lower for males than for females.

Despite this broad consistency, however, not every measure detected significantly sex-biased dispersal. The failure of some methods to detect the phenomenon, despite unambiguous evidence for its presence from the entire data set, is of interest methodologically in suggesting that alternative methods differ in their power to detect sex-specific dispersal. In our own study, the tests based on F_{ST} and relatedness (r) clearly revealed significant male-biased dispersal, whereas the tests based on mean assignment index (*mAIc*) and its variance (*vAIc*) failed to detect this bias. This difference in conclusions from different methods may reflect a higher sensitivity of F_{ST} to detect a bias, especially (i) when compared to a *vAIc*-based test and (ii) when the dispersers constitute a large proportion of the sample (Goudet *et al*. 2002; Pope *et al*. 2005; Hammond *et al*. 2006; Handley & Perrin 2007). This nonsignificance compared to the significance of the other statistics (F_{ST} and r) therefore suggests a high overall rate of dispersal. This condition likely was satisfied in our study, as shown by (i) the unidirectional indices of migration (M and m) observed between our populations, with very high values from FD into BHF and HD (M = 4.47 and 4.42; m = 0.31 and 0.30, respectively), and (ii) the low global structure observed between the three populations (global F_{ST} = 0.012), as well as within both sexes (male: F_{ST} = 0.0025; female: F_{ST} = 0.0275), with pairwise F_{ST} ranging from 0.0108 to 0.0128.

Because we have extensive field as well as genetic data, we can explore the ontogenetic and seasonal timing of sex divergences in dispersal rates. Such correlates can illuminate the selective forces and proximate mechanisms responsible for the existence of sex differences in movement patterns (as shown in Clobert *et al*. 2001; Keogh *et al*. 2007). In the case of slatey-grey snakes, the strongest result to emerge is the lack of any clear temporal association between reproductive activity and higher dispersal rates in males. Thus, males dispersed further than females even as neonates, long before they attained reproductive maturity; and the pattern for longer-distance movement by adult males (compared to adult females) was evident year-round both in our recapture data and in telemetry studies (Brown *et al*. 2005). Because reproduction by slatey-grey snakes is highly seasonal at our study site (G. P. Brown, unpublished, see Materials and methods), it is clear that mate-searching activities by adult males cannot be the proximate cause of male-biased dispersal in this species (unlike *Boa constrictor*: Rivera *et al*. 2006). Instead, a tendency to disperse is more marked in male slatey-grey snakes than in females from the time of hatching, and this difference appears to persist throughout their lives. Dispersal during the first few years of life, prior to reproduction, may be the most important.

The lack of any clear temporal correlation between male movements and reproductive activities makes it difficult to infer either the proximate causes or the adaptive significance of male-biased dispersal in slatey-grey snakes. For example, the proximate causes may lie either in intrinsic sex differences in behavioural attributes of the snakes, or in displacement of males by larger rivals even during juvenile life. The adaptive significance may lie in avoidance of inbreeding, or such avoidance may be a secondary consequence of sex divergences in dispersal patterns that have evolved for some other function. More detailed information on mating systems and mate choice in this system might clarify these issues.

In a more general context, male-biased dispersal and female philopatry appear to be the norm in mammals, whereas birds exhibit female-biased dispersal and male philopatry (Greenwood 1980). Of the few studies on reptiles, most show male-biased dispersal (but see Olsson & Shine 2003). Greenwood (1980) hypothesized that the direction of dispersal bias is driven by the mating system, with female-defence polygyny favouring male-biased dispersal. This explanation may well apply to slatey-grey snakes also. To test this hypothesis, we will need evidence come from paternity analyses aimed at identifying sires of clutches laid by wild-caught females. The slatey-grey snakes of Fogg Dam would provide an excellent system for such an analysis.

Conclusions

Our study demonstrates the utility of combining ecological and genetic data to document patterns of gene flow within a population of secretive and highly cryptic animals. Although snakes are abundant in many tropical habitats,

the animals may be virtually unobservable even if radio-transmitters are used and, thus, we need to develop novel approaches if we are to reveal aspects of their biology. Our study is also of interest in hinting that the male-biased sexual size dimorphism of our study species (unusually extreme among snakes: Shine 1994) is accompanied by, and hence may be functionally linked to, the marked sexual divergence in dispersal rates within our study population. In addition, these divergences occurred in juveniles as well as adults, and consequently before many sex-specific behaviours are expressed. Although we know too little about the magnitude of sex divergences in gene flow within other snake species for any comparative analysis of links between such traits, the opportunities for such work are exciting. The combination of ecological and genetic data likely will be far more informative than either approach in isolation. For example, our ecological data were pivotal in clarifying whether sex differences in genetic structure represent genuine dispersal effects rather than simply greater mobility in males; and our genetic data reveal significant levels of migration among subpopulations, despite the fact that we have never recaptured an adult snake in any population other than the one in which it was marked.

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The research interests of the authors concerns the interface between evolution and ecology in reptiles and amphibians, as well as their conservation.
