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Spatial genetic analysis and long-term mark–recapture data demonstrate male-biased dispersal in a snake

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Dispersal is an important life-history trait, but it is notoriously difficult to study. The most powerful approach is to attack the problem with multiple independent sources of data. We integrated information from a 14-year demographic study with molecular data from five polymorphic microsatellite loci to test the prediction of male-biased dispersal in a common elapid species from eastern Australia, the small-eyed snake *Rhinoplocephalus nigrescens*. These snakes have a polygynous mating system in which males fight for access to females. Our demographic data demonstrate that males move farther than females (about twice as far on average, and about three times for maximum distances). This sex bias in adult dispersal was evident also in the genetic data, which showed a strong and significant genetic signature of male-biased dispersal. Together, the genetic and demographic data suggest that gene flow is largely mediated by males in this species.

Keywords: sex-biased dispersal; mating system; movement patterns; spatial autocorrelation

1. INTRODUCTION

Dispersal is an important life-history trait with profound effects on population dynamics and the genetics of species. One widespread pattern in vertebrates is sex-biased dispersal whereby one sex is philopatric and the other sex disperses. Generally, mammals display male-biased dispersal, whereas most of the birds display the reverse pattern. Three hypotheses have been proposed to explain these sex differences in dispersal (reviewed in Clobert *et al.* 2001): (i) resource competition; (ii) local mate competition; and (iii) inbreeding avoidance. All three hypotheses predict male-biased dispersal in polygynous mating systems, but empirical tests of this prediction have been based almost entirely on the studies of birds and mammals (Clobert *et al.* 2001). Detecting dispersal is notoriously difficult, and there are little data on dispersal in groups, such as lizards and snakes (Gibbs & Weatherhead 2001; Olsson & Shine 2003). Several recent studies have studied the

dispersal of lizards between habitat fragments, but no clear patterns have emerged (reviewed in Chapple & Keogh 2005). No studies have combined long-term mark–recapture data with genetic information to infer dispersal in any species of snake.

In this study, we use molecular and long-term demographic data on a polygynous elapid snake to test the prediction that dispersal will be male biased. The small-eyed snake (*Rhinoplocephalus nigrescens*) is a habitat generalist that occupies a diversity of habitats in eastern Australia. The mating system is polygynous; mating occurs during autumn and spring, and males engage in vigorous ritualized combat to win access to females (Shine 1984). Previous demographic studies showed that females have higher recapture probabilities than males, consistent with male-biased dispersal (Webb *et al.* 2002*a*,*b*, 2003). Given the mating system of this habitat generalist, we made two specific predictions about local fine-scale genetic structure. First, we predicted that the small-eyed snakes should show little or no fine-scale genetic structure across the woodland matrix that separates our sandstone plateaux study sites. Second, we predicted that the small-eyed snakes should show a genetic signature of male-biased dispersal. Here, we test these two predictions with a detailed analysis of long-term mark–recapture data combined with analyses of fine-scale genetic structure using spatial autocorrelation and dispersal bias with assignment tests.

2. MATERIAL AND METHODS

(a) *Movements of snakes*

One of us (J.K.W.) carried out a 14-year (1992–2005) mark–recapture study on small-eyed snakes at three sites located on the western escarpment of a sandstone plateau (YN) in Morton National Park, New South Wales, Australia. Study sites were 1.1, 1.2 and 0.7 km long, 50 m wide, and were spaced 1.9 and 0.7 km apart. From August 1992 to February 1995, study sites were intensively sampled each month by turning rocks and cover objects (bark and logs). Thereafter, sites were sampled each year during the mating season. All snakes encountered were hand captured, permanently marked with PIT tags, a tissue sample collected and the snakes' exact location recorded with a GPS. Full details of field methods are described elsewhere (Webb *et al.* 2002*a*,*b*, 2003). Here, we analyse demographic data from 1992 to 2002, the time period that corresponds to the collection of samples for our genetic analyses. For all recaptured snakes, we calculated the straight-line distance (range distance) between the original capture site and the furthest location from that site.

(b) *Microsatellite genotyping and genetic analyses*

A total of 93 snakes were genotyped for five polymorphic microsatellite loci that we described elsewhere (Stapley *et al.* 2005). We excluded 13 snakes from outlying populations with small sample sizes and restricted our spatial genetic analyses to 80 snakes from the three study sites, 64 from the main site (YN north), 7 snakes from the same plateau (YN south) and 9 snakes from a second plateau (MG, see map in electronic supplementary material). Our sex-biased dispersal analyses were based on 77 of these snakes (44 females and 33 males) because three animals could not be sexed.

We used four complementary approaches to examine fine-scale genetic patterns among the three populations using GENALEX v. 6.0 (Peakall & Smouse 2006). We used Principal Coordinates Analysis to explore multivariate patterns of molecular diversity relative to populations. We used Analysis of Molecular Variance (AMOVA) to perform the analysis of genetic differentiation following the procedures in Peakall *et al.* (2003). We used Mantel tests of matrix correspondence following the methods of Smouse *et al.* (1986) to test for isolation by distance. Finally, we used a powerful new microspatial autocorrelation technique developed by Smouse & Peakall (1999) for multiallelic codominant loci that uses pairwise geographical and genetic distance matrices to dissect out positive genetic correlation at various geographical size classes (see Peakall *et al.* 2003 for review; Chapple & Keogh 2005; Double *et al.* 2005). A significant departure from the null hypothesis of no structure
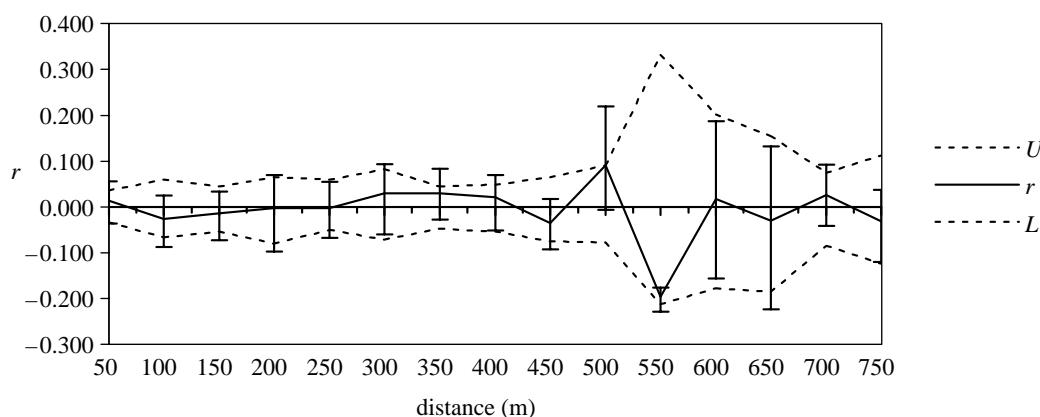
Figure 1. Correlogram showing the results of the fine-scale spatial autocorrelation analysis where genetic correlation values (r) are plotted as a function of distance. U and L represent 95% CIs around the null hypotheses of no structure. The plot shows that there is no significant positive genetic structure at any distance class.

($r=0$) is obtained when a positive r-value falls outside of the 95% CI. Based on information from the detailed mark–recapture data, we chose a distance size class of 50 m and evaluated 15 size classes, giving an evaluation over 750 m of space. One potential criticism is that the uneven sample sizes across our three sites could bias our results, so all of these analyses were repeated for snakes just from the site with the largest sample size (YN North, 61 snakes). The same patterns emerged from the smaller dataset (results not shown), therefore, we report the results based on analyses of all the data to give the most complete picture possible of the spatial genetic information.

We used a modified population assignment test to test for sex-biased dispersal using the software GENALEX v. 6.0 (Peakall & Smouse 2006). This method produces an Assignment Index correction (*AIc*) value for each sex following the method of Mossman & Waser (1999). Negative *AIc* values characterize individuals with a higher probability of being immigrants, whereas positive values characterize individuals with a lower probability of being migrants. Mean *AIc* values for each sex were then compared with a non-parametric Mann–Whitney *U*-test.

3. RESULTS

(a) *Distances moved by adult males and females*

We captured 252 snakes (165 individuals) during the 10-year study. Range distances differed significantly between males and females (ANOVA, $F_{1,40}=6.86$, $p=0.01$). On average, females remained closer to their original capture sites (mean distance $=35$ m, range $= 0$–140 m) than males (mean distance $=99$ m, range $= 0$–346 m). Overall, 55% of females were recaptured less than 20 m from their original capture sites, and six females were recaptured under the same rocks where they were first captured. For females, there was no significant correlation between the time between recaptures and range distance (log transformed data, $r^2=0.12$, $p=0.10$). By contrast, range distances of males were significantly correlated with time between captures (log transformed data, $r^2=0.30$, $p=0.01$). As time progressed, adult males tended to move farther away from their original capture sites.

(b) *Fine-scale genetic structure and sex-biased dispersal*

We did not detect significant genetic differentiation across the three sites (AMOVA, $F_{st}=0.017$, $p=0.090$). AMOVA analysis revealed that less than 2% of genetic variance was found between populations. This result was corroborated by the Mantel test, which showed no evidence of isolation by distance
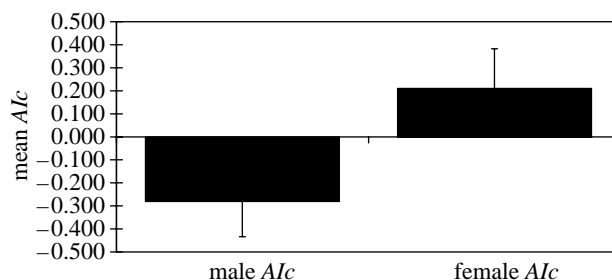
in the genetic data (Mantel, $R_{xy}=0.008$, $p=0.462$) and by PCA, which showed that while 70.68% of the variance was accounted for by the first three principal components, there was no clear pattern of hierarchical genetic structure among the three sites (results not shown). Individually and together, these three tests suggest that there is little restriction to gene flow among the three sites. This result was corroborated by the spatial autocorrelation analysis, which does not show a significantly positive r-value at any distance class (figure 1). Analysis of sex-biased dispersal showed that males have strongly negative *AIc* values, indicating that males are more likely to be immigrants or the more widely ranging sex (figure 2, Mann–Whitney *U*-test, $U=495$, $p=0.0174$).

Figure 2. Mean Assignment Index correction (*AIc*) values for male and female small-eyed snakes demonstrating that males are the wider ranging sex.

4. DISCUSSION

Our long-term demographic data showed that adult female small-eyed snakes were sedentary whereas adult males were highly mobile; on average, males moved twice as far as females. This sex bias in movements is consistent with a previous demographic analysis showing that females have significantly higher recapture probabilities ($p=0.50$) than males ($p=0.25$; Webb *et al.* 2002b). Thus, both the mark–recapture and the genetic analyses indicate that dispersal is male biased in this snake population.

Male-biased dispersal is predicted to occur in polygynous species, especially when the sexes differ in their ability to secure resources needed for reproduction (Bohonak 1999). Our results support this hypothesis. Male small-eyed snakes engage in

male–male combat, and because victory is determined by body size, larger males gain exclusive access to females. Large males also evict smaller males from 'hot' rocks that females use as diurnal thermoregulatory sites. By contrast, females often share hot rocks with other females (Webb & Shine, unpublished data). Consequently, males gain the most from dispersal in this system. From a male's perspective, the locations of females are also predictable in space and time; we found that most females did not move far from their original site of capture, and some females used the same rocks as diurnal shelter sites each year during the reproductive season. Since a high proportion of females in this population reproduce every year (Webb *et al.* 2002*a*,*b*), females represent a predictable resource that males can exploit during the reproductive season. Consequently, to maximize their reproductive success, males should move over large distances to search for receptive females.

The apparent absence of genetic structure among the three populations also conforms with *a priori* prediction, because small-eyed snakes are habitat generalists that use a broad range of habitat structures (bark, logs and rocks) as diurnal shelter sites. Our genetic analyses show that individuals of this species (especially males) readily disperse across the matrix of tall, open forest growing in the steeply dissected valleys that separate the study plateaux. The genetic data not only corroborate the demographic data, but also suggest that gene flow may even be greater than is evident from our mark–recapture records. We would expect such a pattern because extremely long-range dispersal is unlikely to be recorded in a (spatially focused) mark–recapture study, but will be detected by genetic analyses. Together, our genetic and demographic data strongly support the hypothesis that gene flow is largely mediated by males, and hence provide the first evidence of sex-biased dispersal in a snake species.

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