Microsatellite Analyses Provide Evidence of Male-Biased Dispersal in the Radiated Tortoise Astrochelys radiata (Chelonia: Testudinidae)

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Abstract

Dispersal is a major force in shaping the genetic structure and dynamics of species; thus, its understanding is critical in formulating appropriate conservation strategies. In many species, sexes do not face the same evolutionary pressures, and consequently dispersal is often asymmetrical between males and females. This is well documented in birds and mammals but has seldom been investigated in other taxa, including reptiles and, more specifically, nonmarine chelonians. In these species, nest-site fidelity observations are frequent but still remain to be associated with natal homing. Here, we tested for sex-biased dispersal in the radiated tortoise (Astrochelys radiata) from southern Madagascar. Using data from 13 microsatellite markers, we investigated patterns of relatedness between sexes in 2 populations. All Mantel tests indicated significant isolation by distance at the individual level in females but not in males. Furthermore, spatial autocorrelation analyses and 2 analytical approaches designed to assess general trends in sex-specific dispersal also supported male-biased dispersal. On the other hand, comparisons of overall genetic structure among sampling sites did not provide conclusive support for greater philopatry in females, but these tests may have low statistical power because of methodological and biological constraints. Radiated tortoises appear to be both polyandrous and polygynous, and evolutionary processes that may lead to a sex bias in dispersal are discussed with respect to tortoise breeding biology. Female natal homing is hypothesized as a key trait explaining greater female philopatry in A. radiata. These findings highlight the necessity of additional research on natal homing in tortoises, a behavioral trait with direct implications for conservation.

Key words: genetic distance, microsatellites, radiated tortoise, relatedness, sex-biased dispersal

Introduction

The differential propensity of males and females to disperse, or sex-biased dispersal, is a life-history trait closely related to the mating system of species (Greenwood 1980). Mutually nonexclusive hypotheses proposed to explain the direction of sex-biased dispersal across species can be classified into 3 main categories: 1) resource competition (Greenwood 1980), 2) local mate competition (Dobson 1982), and 3) inbreeding avoidance (Pusey 1987). These hypotheses allow predictions to be made depending on the mating system of species (Perrin and Mazalov 1999, 2000; Perrin and Goudet 2001). In polygynous species, all hypotheses predict male-biased dispersal because 1) females benefit the most from acquaintance with territory (i.e., philopatry) for better use of resources, which may lead to greater fecundity and better parental care; 2) the inclusive fitness of males is increased by dispersing to prevent competition with related males for mates; 3) females tend to select unrelated mates because inbreeding is more costly to them, and thus, fitness is increased for male dispersers. On the other hand, only the resource competition hypothesis predicts a bias in dispersal for monogamous species (female bias, reviewed in Favre et al. 1997). Based on these premises, the main focus of dispersal studies has traditionally been the comparison of dispersal patterns between mammal species, characterized by polygynous systems and birds, which are mostly

intense.
et al. 1998). For instance, there are comparatively few documented studies of sex-biased dispersal in reptiles, including lizards (Doughty et al. 1994; Stow et al. 2001; Massot et al. 2003; Olsson and Shine 2003; Chapple and Keogh 2005; Johansson et al. 2008), iguanas (Rassman et al. 1997), snakes (Rivera et al. 2006), and crocodilians (Tucker et al. 1998).

In chelonians, data are also sparse and come almost exclusively from sea turtles (reviewed in Bowen and Karl 2007). Marine turtles are characterized by a complex structure: Females exhibit strong homing to their natal beach but perform tremendous migrations between their feeding and nesting areas. Genetic analyses have revealed that gene flow among nesting beaches is male mediated, and opportunities for gene flow probably occur when adult populations overlap in feeding areas and migratory corridors (Bowen and Karl 2007). This original structure emphasizes the distinction to be made between ecological and genetic dispersal as defined in Johnson and Gaines (1990): Ecological dispersal is the movement of individuals from one place to another (including migrations), whereas genetic dispersal refers to individuals moving from their natal population to another population where they successfully breed. This distinction is similar to that emphasized by Clobert et al. (2001), who defined dispersal as “movement between the natal area and the area where breeding first occurs (natal dispersal) or between two breeding areas (breeding dispersal).” Population genetic approaches, such as the one employed in the present study, are powerful tools to assess sex-biased dispersal (Goudet et al. 2002; Prugnolle and de Meeus 2002), and they are especially useful to detect genetic dispersal because ecological dispersal does not affect the genetic structure of breeding populations. Henceforth, here we use the term dispersal synonymously with genetic dispersal.

Natal homing, such as that exhibited by sea turtles, is expected if female fitness is affected by nest-site quality, and daughters use the same nesting site as their mothers (Reinhold 1998). Dispersal in species with this behavioral trait is thus predicted to be male-biased, with females expected to be more philopatric. In freshwater and terrestrial chelonians, several nest-site fidelity observations have been made, but it remains unknown whether they are associated with natal homing (Freedberg et al. 2005), that is, whether females are nesting to their natal site or, alternatively, if they dispersed while being juveniles or subadults and instead exhibit fidelity to a nonnatal nesting site. Here, we investigate sex-biased dispersal in the Malagasy radiated tortoise (*Astrochelys radiata*), an endemic species of the spiny forest of southern Madagascar, with microsatellite data. We hypothesize that dispersal in *A. radiata* is male biased, a prediction that would be concordant with natal homing and the polygynous system generally observed in tortoises (e.g., Douglass 1986; Boglioli et al. 2003; Lagarde et al. 2003). Although many tortoise species (family Testudinidae) have been the target of population genetics studies (e.g., Ciofi et al. 2002; Cunningham et al. 2002; Beheregaray et al. 2003; Edwards et al. 2004; Schwartz and Karl 2005), the topic of sex-biased dispersal has rarely been specifically addressed in these animals. Ciofi et al. (2006) suggested male-biased dispersal as one of several possible explanations to the discrepancy between differentiation estimates from mitochondrial and nuclear markers in giant tortoises from Isabela Island, but no pattern was detected from microsatellite data. Knowledge about sex-biased dispersal patterns in tortoises is needed to improve our understanding of their evolutionary history and its ramifications for conservation. A strong signal for male-biased dispersal in the radiated tortoise, a rapidly declining, critically endangered species (Leuteritz and Rioux Paquette 2008), would emphasize the necessity to further study natal homing and nest-site fidelity in this species.

**Materials and Methods**

**Sampling and DNA Markers**

Blood or tissue samples from 180 wild adult radiated tortoises were used in this work (87 males and 93 females). Other samples from juveniles or individuals for which the identification of sex was uncertain were ignored. Sampling was mainly performed around 11 sites visited during the nesting season in 2000 and 2004. The approximate location of these sites is indicated in Figure 1, and geographic coordinates of each tortoise included in the analyses can be obtained from the authors. DNA extraction and genotyping at 13 highly polymorphic microsatellite markers were performed as described in Rioux Paquette et al. (2007). Summary details about these markers are provided in Supplementary Data online. The overall mean heterozygosity for this microsatellite panel was 0.76, and the estimated probability of identity (*P*ID) was 2 × 10^-15_. No linkage disequilibrium was detected among pairs of loci, but 5 out of 26 locus/population tests revealed significant deviations from Hardy–Weinberg equilibrium (HWE). However, these significant homozygote excesses were found in 4 markers known to harbor a significant frequency of null alleles (estimated between 0.09 and 0.20; Rioux Paquette et al. 2007).

**Data Analyses**

We analyzed isolation by distance (IBD) at the individual level for both sexes. Bayesian clustering analyses (Pritchard et al. 2000) have revealed that there were 2 genetically distinct populations in *A. radiata*, separated by a genetic barrier, the Menarandra River (Figure 1; see Supplementary Data online). Comparing IBD patterns between sexes requires that individuals can disperse within studied populations (Goudet et al. 2002; Prugnolle and de Meeus 2002). Therefore, we divided the data in 2 groups, called the Western (61 males and 58 females) and Eastern (26 males and 35 females) populations based on their geographic location with respect to the Menarandra River (Figure 1) and
performed the analyses described below separately in both populations. The absence of consistent deviations from HWE aside from those due to null alleles further supports our assertion that these 2 populations can be considered as panmictic groups.

Mantel tests (Mantel 1967) were used to compare matrices of pairwise geographic distances (computed from geographic coordinates) and matrices of genetic distances for both sexes. Two different estimators of interindividual genetic distances were derived from microsatellite data for these comparisons: first, Rousset’s distance measure \( a \), which is the equivalent of the \( F_{ST}/(1 - F_{ST}) \) ratio but for pairs of individuals instead of pairs of populations, and it was conceived to study IBD at the individual level (Rousset 2000) and secondly, Queller and Goodnight’s relatedness coefficient \( R \); it is based on the proportion of identical alleles between 2 individuals (Queller and Goodnight 1989). Matrices of \( a \) and \( R \) values were computed with the software SPAGEDi (Hardy and Vekemans 2002) for each population, using their respective allele frequencies. Because Mantel tests should be computed with 2 distance (or “dissimilarity”) matrices (see Legendre P and Legendre L 1998), matrices of \( R \) were converted to distance matrices \( D \) with the simple operation \( D = 1 - R \).

It is worth mentioning that the occurrence of null alleles in microsatellite data increases inaccuracy in the estimation of relatedness coefficients (e.g. Wagner et al. 2006). However, there is no reason to believe that the introduced error is not equivalent across sexes. Furthermore, we calculated maximum-likelihood estimators of \( R \) corrected for null alleles following the methodology of Wagner et al. (2006), using the software ML-RELATE (Kalinowski et al. 2006) and found very strong congruence between the original \( R \) values and these corrected \( R \) values: Mantel correlation coefficients were 0.82 in males and 0.79 in females, with \( P < 0.0001 \) for both sexes. Thus, all analyses described below were performed with uncorrected \( R \) values, but the results of the different tests were the same with corrected \( R \) values (data not shown).

Mantel tests were performed with the VEGAN library (Oksanen et al. 2008) in R language (R Foundation for Statistical Computing, Vienna, Austria), and significance of Mantel correlation coefficients \( r_m \) was assessed with 10 000 permutations. Comparing sex-specific values of \( r_m \) cannot be achieved with traditional statistical methods because of pseudoreplication issues: The number of pairwise comparisons in each matrix \( n(n - 1)/2 \) used to calculate \( r_m \) is higher than the actual number of observations \( n \), which artificially inflates the number of degrees of freedom (see Prugnolle and de Meeus 2002). To circumvent this problem, 2 kinds of approaches developed by Knight et al. (1999) were employed to compare results from males and females. Both methods ensure that the number of compared values corresponds to the number of
observations, and the necessary calculations and data manipulation operations were performed with functions written in R language and available from the authors on request.

In the first approach, for each individual, a regression of genetic distance $a$ on geographic distance $d$ was calculated using all pairwise comparisons involving that individual and all other individuals of the same sex in the population. For instance, the number of calculated regressions was 61 for males and 58 for females in the Western population. The slope values ($b$) of these regressions were then compared between the sexes with a Mann–Whitney $U$ test computed in STATISTICA (Statsoft Inc). This analysis was repeated with $R$. For regressions of $a$, high slope values indicate strong structuring with respect to geographic distance. On the other hand, for regressions of $R$, highly negative values indicate that related individuals are likely to be found in close proximity (Knight et al. 1999).

The second approach was based on the comparison of the means of ranked $a$ values and their corresponding $d$ values for all individuals of the same sex in each population (Knight et al. 1999). For each individual, $a$ values were ranked in descending order and corresponding $d$ values were reordered accordingly. Then, for each sex and population, the first data point was calculated as the mean of the highest $a$ values of all individuals of the same sex and the mean of corresponding $d$ values. The second point was the mean of the second highest $a$ values and corresponding $d$, and so on. The regression of mean $a$ on mean $d$ was calculated in each population, and slopes were compared with Student’s $t$-tests for slope comparison (Zar 1996). A stronger, positive slope of $a$ on $d$ for females would indicate stronger genetic structuring, that is, related females are more likely to be found in close proximity than related males, thus suggesting male-biased dispersal. These analyses were repeated with $R$, and in that case, the opposite result was expected, that is, stronger negative slopes of mean $R$ on mean $d$ in females. Correlation coefficients were similarly compared using Fisher’s $\xi$ transformation.

To complement these analyses, a spatial genetic autocorrelation analysis (Smouse and Peakall 1999; Peakall et al. 2003) was performed. Computations were carried out in GENALEX 6.2 (Peakall and Smouse 2006), using the “combined” approach in order to compute an overall autocorrelation analysis from the data of both populations. Distance classes were not equal, and were instead selected to make the number of pairwise comparisons in each bin as even as possible. This was necessary because our sampling scheme was not homogeneous in space, but rather concentrated around sampling locations; thus, several distance classes would have no observations otherwise. Statistical significance of the genetic autocorrelation coefficient ($r_C$) was tested by permuting genotypes 1000 times to generate confidence limits around the null hypothesis ($r_C = 0$), with values outside this range considered as significant. Male and female values of $r_C$ were considered to be significantly different if their 95% confidence intervals (calculated from 1000 bootstrap replicates) did not overlap (Peakall et al. 2003).

Finally, population genetics approaches comparing overall structure among sites were also carried out. Instead of relying on data from individual tortoises in 2 populations, the data were reorganized by sampling sites (see Figure 1 for sample sizes for each site) and 4 different tests were computed to detect sex-biased dispersal among sites: comparison of sex-specific $F_{ST}$ values, comparison of mean pairwise relatedness values ($R$), comparison of the mean of the corrected assignment index (mAIC), and comparison of the variance of the corrected assignment index (vmAIC) (Goudet et al. 2002). These 1-tailed tests (alternate hypothesis = male-biased dispersal) were done in FSTAT 2.9 (Goudet 1995) with 1000 randomizations.

**Results**

In both populations, the values of Mantel correlation coefficients ($r_a$) were higher in females than in males for either Rousset’s genetic distance ($d$) or $1-R$ (Table 1). All 4 $r_a$ values were highly significant in females, whereas none was significant in males (Table 1).

The comparison of individual regressions of genetic distances on geographic distance also supported this difference between sexes. Results of Mann–Whitney $U$ tests are provided in Table 2; females had significantly higher slope values ($b$) in both populations for $a$. Conversely, for $R$, $b$ values were significantly more negative in females, as expected.

In addition, the method based on the comparison of means of ranked $a$ and $R$ values generated very similar results. In the 2 populations, the regression slope of mean $a$ on mean $d$ (geographic distance) was greater in females and the regression slope of mean $R$ on mean $d$ were negatively greater in both cases also (Figure 2). Statistical tests to assess the significance of the differences in regression slopes between sexes revealed that for mean $R$, the difference was significant in both populations (Western population: $t = -2.41$, $P = 0.017$, df = 113; Eastern population: $t = -2.87$, $P = 0.006$, df = 55), whereas it was only significant in the Eastern population for $a$ (Western population: $t = 1.55$, $P = 0.125$; Eastern population: $t = 2.68$, $P = 0.009$). Correlation coefficients ($r_C$ indicated in Figure 2) were significantly greater for females in all 4 cases.

Results of the spatial genetic autocorrelation analysis are reported in Figure 3. Females that were geographically close to each other were more genetically similar than were males, as evidenced by significantly higher spatial autocorrelation coefficients ($r_C$) at the first 2 distance classes (Figure 3C). At these distances, both males and females showed structuring, with significant positive $r_C$ values, followed by a significant negative $r_C$ for the fourth (23 km) distance class (Figure 3A, B). No noticeable pattern was observed in greater distance classes (from 69 to 150 km), and these are consequently not reported in Figure 3.
On the other hand, none of the tests based on pairwise comparisons among sampling sites yielded significant results. Mean \( F_{ST} \) and \( R \) values appeared higher in females (\( F_{ST} = 0.055, R = 0.099 \)) than in males (\( F_{ST} = 0.039, R = 0.070 \)), but these differences were not significant (\( P = 0.14 \) and 0.15, respectively). Likewise, tests based on the assignment index were not significant (\( m_{AIc} = -0.082 \) in males and 0.077 in females, \( P = 0.39 \); \( v_{AIc} = 13.9 \) in males and 15.1 in females, \( P = 0.64 \)).

### Discussion

Overall, results support our initial hypothesis of male-biased dispersal in radiated tortoises. Mantel test results indicate that IBD at the individual level is only significant in females, and other analyses also reveal a greater tendency of females to be genetically related to other geographically close females. The fact that similar trends were consistently found with both the regression slope comparison approach and the means of ranked values approach in both populations with two genetic estimators appears to make a strong case for male-biased dispersal and greater female philopatry.

### Detecting Sex-Biased Dispersal with Different Analytic Approaches

Difficulties may arise when studying dispersal in a small sample of a large population, especially if we consider that microsatellite data can lead to noisy relatedness estimates (Van de Casteele et al. 2001; Csilléři et al. 2006). Furthermore, tests that are based on the comparison of accurate pairwise values (like Mantel tests) are particularly prone to be affected by these problems (Knight et al. 1999). Mantel tests are known for lacking statistical power in some instances (e.g., Legendre 2000; Legendre et al. 2005; Bookstein 2007) and the inherent levels of variance associated with genetic estimators of relatedness could thus considerably affect the power of Mantel tests to detect individual IBD. However, these limitations did not prevent us from obtaining significant results in this study. In addition, 2 analytical methods more robust to this problem were also used, allowing a better evaluation of broad dispersal patterns based on general trends in genetic distance or relatedness values (Knight et al. 1999).

The choice of an appropriate sampling scale is important because mating eradicates the nuclear DNA signal for sex-biased dispersal in subsequent generations. Although the regression slope and means of ranked values methods are typically applied at very short geographic scales (e.g., Knight et al. 1999; Stow et al. 2001), they can be correctly used at larger scales when the life history and ecology of the studied species can reasonably allow individuals to disperse freely within the boundaries of the study area before being sampled. For instance, the regression slope comparison test was performed among Canada lynx (\( L. canadensis \)) individuals sampled as far as 850 km apart (Campbell and Strobeck 2006), knowing that lynx are characterized by long-distance movements (Slough and Mowat 1996). For \( A. radiata \), all individuals included in this study exhibited sexually dimorphic characters and had thus reached at least 16–20 years old (Pedrono 2008). Coupled with observations that radiated tortoises the largest extant vertebrates in southern Madagascar are capable climbers that move routinely between inland limestone plateaus and the coast and between populations along the coast (Leuteritz et al. 2005) and that there is no genetic differentiation between most surveyed sites (Rioux Paquette S, unpublished data), it appears that the potential for natal dispersal in \( A. radiata \) is substantial.

The absence of IBD (as revealed by Mantel tests) among males was nevertheless unexpected considering that significant genetic differentiation, albeit very small, has been

### Table 1  Results of Mantel correlation tests between pairwise genetic and geographic distances among individual radiated tortoises \( (A. radiata) \)

<table>
<thead>
<tr>
<th>Population</th>
<th>Genetic distance</th>
<th>Sex</th>
<th>( r_m )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western</td>
<td>( a )</td>
<td>Males</td>
<td>-0.0054</td>
<td>0.5349</td>
</tr>
<tr>
<td></td>
<td>( 1 - R )</td>
<td>Males</td>
<td>0.0339</td>
<td>0.1538</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>0.1291</td>
<td>0.0020</td>
</tr>
<tr>
<td>Eastern</td>
<td>( a )</td>
<td>Males</td>
<td>-0.0365</td>
<td>0.6446</td>
</tr>
<tr>
<td></td>
<td>( 1 - R )</td>
<td>Males</td>
<td>-0.0416</td>
<td>0.6634</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>0.1903</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

\( r_m \) Mantel correlation coefficient; \( a \), Rouset's genetic distance; \( R \), relatedness coefficient.

### Table 2  Results of Mann–Whitney \( U \) tests comparing the slopes of regressions of genetic on geographic distance for individual male and female radiated tortoises

<table>
<thead>
<tr>
<th>Population</th>
<th>Genetic distance</th>
<th>Sex</th>
<th>Mean rank</th>
<th>Sum of ranks</th>
<th>( U )</th>
<th>( z )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western</td>
<td>( a )</td>
<td>Males</td>
<td>47.61</td>
<td>2904</td>
<td>1013</td>
<td>4.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>73.03</td>
<td>4236</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( R )</td>
<td>Males</td>
<td>76.10</td>
<td>4642</td>
<td>835</td>
<td>-4.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>43.07</td>
<td>2498</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern</td>
<td>( a )</td>
<td>Males</td>
<td>19.96</td>
<td>519</td>
<td>168</td>
<td>4.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>39.20</td>
<td>1372</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( R )</td>
<td>Males</td>
<td>37.69</td>
<td>980</td>
<td>281</td>
<td>-2.54</td>
<td>0.0112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>26.03</td>
<td>911</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a \), Rouset's genetic distance; \( R \), relatedness coefficient.
noticed among peripheral sampling sites (Rioux Paquette et al. 2009). It appears that the low but significant differentiation values obtained between peripheral sites within both populations may be mainly imputable to female philopatry, counterbalancing considerable levels of male-mediated gene flow. The conformity to HWE in both populations (excluding markers with genotyping artifacts) also supports high gene flow. Yet, the “diffusion” of close relatives in the landscape should generate a pattern of IBD at the scale of the study, but it might simply be too weak for detection with matrix correlation tests. In fact, a significant pattern of genetic autocorrelation at the sampling site scale was detected in males with spatial autocorrelation analysis. Autocorrelation analyses are most insightful when samples are collected evenly throughout space, which was not possible in our case.

Nevertheless, genetic autocorrelation was significantly greater in females for the same distance classes, supporting findings obtained with the other methods. Autocorrelation analyses are most insightful when samples are collected evenly throughout space, which was not possible in our case.

The lack of a decisive signal for male-biased dispersal in site comparison tests is not surprising when one considers conditions under which these tests achieve satisfactory power: Extensive sampling is required, and the tests have limited power unless the sex bias in dispersal is severe (Goudet et al. 2002). In addition, these tests rely on the assumption that differentiation among sites is sufficient to observe sex differences in allele frequencies. If gene flow is so high that no difference can be detected overall among sites, it would be vain to expect uncovering sex-biased patterns. This is likely to explain the nonsignificance of most

Figure 2. Relationship between the means of ranked a (Rousset’s genetic distance) values (A, B) or ranked R (relatedness) values (C, D) and corresponding mean geographic distances calculated separately in male and female radiated tortoises from the Western (A, C) and Eastern (B, D) populations. Filled circle symbols indicate data points from females, x symbols indicate data points from males, black lines represent female regressions, and dash lines represent male regressions. Regression equations and their corresponding correlation coefficients are indicated next to regression lines. Data points were obtained by ranking, for each individual, pairwise a or R values in descending order, and the first point was obtained by calculating the mean of first-ranked values across individuals and the mean of the corresponding geographic distances, then repeating for second-ranked values (second point), and so on. Axes should not be interpreted as actual geographic or genetic distances observed between pairs of individuals. (See text for further details).
of these tests in our radiated tortoise analyses, because the great majority of pairwise $F_{ST}$ values among sites within either the Western or Eastern populations are not significant (Rioux Paquette S, unpublished data). We believe that the other approaches used here (individual IBD patterns, spatial autocorrelation) provide interesting alternatives when constraints, both methodological (i.e., low sample sizes for one or both sexes at the different sites) and biological (i.e., no genetic differentiation among sites), prevent the more commonly used methods focusing on among-site comparisons of attaining reasonable power.

**Genetic and Ecological Dispersal in Females**

Female philopatry does not necessarily correlate with lower ecological dispersal in females. Although there are observations of larger home ranges and movements in males of some tortoise species (e.g., McRae et al. 1981; Diemer 1992; O’Connor et al. 1994; Eubanks et al. 2003; Tuberville et al. 2005), opposite observations have also been made (e.g., Longepierre et al. 2001; Lagarde et al. 2003), including in radiated tortoises, in which females may travel great distances outside their usual home range to lay eggs (Leuteritz 2002). Similarly to female sea turtles, these unusual movements may correspond to migration (on a sizably smaller scale) to the nesting site, suggesting nest-site fidelity in these tortoises. Furthermore, female body mass in tortoises is correlated with egg size, which is in turn correlated with larger hatchlings and ultimately, higher survival rates (Leuteritz and Ravolanaivo 2005; O’Brien et al. 2005). Thus, females may have to move more than males during the breeding season, while foraging to accumulate resources in order to produce larger eggs. Nonetheless, overall net dispersal might be minimal, as supported by data from 7 female radiated tortoises radio-tracked during a whole breeding season (October–March), among which the largest net movement recorded was 64 m (O’Brien 2002). Accordingly, our results suggest that even if adult female radiated tortoises perform substantial ecological dispersal, they tend, on average, to remain closer to their natal area than males. This apparent incongruity emphasizes the role of genetic markers in dispersal studies.

**Figure 3.** Spatial genetic structure correlograms for male (A) and female (B) radiated tortoises from 2 combined populations. Observed autocorrelation coefficients ($r_C$) are indicated by the black lines, while the dash lines represent 95% confidence intervals for $r_C = 0$. The $P$ values of significant $r_C$ values are indicated on figure. (C) Comparison of male and female $r_C$ values with their 95% bootstrap confidence intervals. Nonoverlapping intervals indicate significant differences and are identified with asterisks.
Male-Biased Dispersal in Light of Tortoise Breeding Ecology

Several tortoise species exhibit a polygynous system of the “scramble competition” type (Thornhill and Alcock 1983), where males attempt to outrace other males to receptive females (Boglioli et al. 2003). Theoretical models (e.g., Perrin and Mazalov 2000) predict male-biased dispersal in such species as a mechanism to avoid either kin competition or inbreeding. Little is known about the mating system of radiated tortoises, but it might actually be described as both polygynous and polyandrous; both males and females court repeatedly over a breeding season, often with different partners (Leuteritz and Ravolanaivo 2005). Furthermore, multiple paternity is frequent in tortoises (e.g., Pearse and Avise 2001; Johnston et al. 2006; Moon et al. 2006), and females can store sperm and utilize it several years later to fertilize eggs (Kuchling 1999; Pearse and Avise 2001), creating a system where sperm competition and female choice might be as important as intramale competition in sexual selection. Even females that mate only once in a season could effectively be polyandrous because of long-term sperm storage (temporal polyandry). Additionally, vocalizations are a key feature of radiated tortoise courtship and mating (Auffenberg 1978), and recent evidence in other tortoise species (genus Testudo) suggest that females use male vocalizations as a cue to assess male quality, providing a mechanism allowing them to choose their mates (Sacchi et al. 2003; Galeotti, Sacchi, Fasola, et al. 2005; Galeotti, Sacchi, Pellitteri Rosa, and Fasola 2005).

With respect to this mating system, not all 3 hypotheses (local resource competition, local mate competition, inbreeding avoidance) are equally likely as the main force driving male-biased dispersal in A. radiata. The local mate competition scenario predicts a male bias in dispersal when males benefit the most from multiple matings (Dobson 1982; Perrin and Goudet 2001). However, in the system described above for radiated tortoises, females also benefit from a high number of mating opportunities. Similarly, the inbreeding avoidance hypothesis predicts a sex bias in dispersal only when inbreeding costs are unequal between sexes, but this disparity may be at least partially countered by female radiated tortoise mating strategies (sperm storage, multiple paternity, and recognition of quality mates). Although synergistic interactions are to be expected (Perrin and Goudet 2001), the local resource competition hypothesis may be the predominant factor in explaining the observed bias in dispersal. Acquaintance with territory might help females increase their fecundity (and fitness) during the breeding season, but this alone cannot explain why juvenile and subadult females would not disperse and get acquainted to new areas in the 16–20 years before they reach sexual maturity. On the other hand, high-quality nesting sites represent a key resource within the natal territory, and natal homing coupled with nest-site fidelity appears as a likely mechanism maintaining greater female philopatry. As explained in the Introduction, if female fitness is affected by the choice of nesting sites and the same sites are used by female offspring, natal homing is expected to occur (Reinhold 1998). Olfaction appears to be involved in the homing behavior of displaced tortoises (Chelazzi 1992), so olfactory imprinting could be the mechanism involved in natal homing and nest-site fidelity. In this scenario, males may have a higher propensity to disperse because dispersal costs are lower for them, but interactions with aforementioned factors are also possible. Further studies of natal homing in tortoises and freshwater turtles are needed not only to demonstrate the hypothesized relationship between male-biased dispersal and natal homing in these species but also because they provide important insights into sex allocation theory and the evolution of sex ratio and environmental sex determination (Reinhold 1998; Freedberg et al. 2005).

Conservation Implications

The existence of male-biased dispersal and the possibility of natal homing in radiated tortoises emphasize the importance of locating nesting sites and identifying nest-site preferences in order to implement effective conservation schemes for this species. Overexploitation has been identified as the main threat to the survival of this species (O’Brien et al. 2003; Randriamahazo et al. 2007), but habitat loss, which is currently worse in the spiny forest than elsewhere in Madagascar (Harper et al. 2007), may significantly impact the species if suitable nesting sites, to which females were homing, are destroyed. Furthermore, female philopatry may hinder the recovery of depleted populations, even if poaching is completely stopped. Sex ratios are male biased in exploited populations as a result of poacher preference for females because they sometimes contain eggs and their meat is fattier (Pedrono et al. 2000). Consequently, if very few female immigrants disperse from nearby populations, the recovery of impacted populations may take longer than anticipated.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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