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Original Article

Linking Genetic Kinship and Demographic Analyses to Characterize Dispersal: Methods and Application to Blanding's Turtle

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Abstract

Characterizing how frequently, and at what life stages and spatial scales, dispersal occurs can be difficult, especially for species with cryptic juvenile periods and long reproductive life spans. Using a combination of mark–recapture information, microsatellite genetic data, and demographic simulations, we characterize natal and breeding dispersal patterns in the long-lived, slow-maturing, and endangered Blanding's turtle (*Emydoidea blandingii*), focusing on nesting females. We captured and genotyped 310 individual Blanding's turtles (including 220 nesting females) in a central Wisconsin population from 2010 to 2013, with additional information on movements among 3 focal nesting areas within this population available from carapace-marking conducted from 2001 to 2009. Mark–recapture analyses indicated that dispersal among the 3 focal nesting areas was infrequent (<0.03 annual probability). Dyads of females with inferred first-order relationships were more likely to be found within the same nesting area than split between areas, and the proportion of related dyads declined with increasing distance among nesting areas. The observed distribution of related dyads for nesting females was consistent with a probability of natal dispersal at first breeding between nearby nesting areas of approximately 0.1 based on demographic simulations. Our simulation-based estimates of infrequent female dispersal were corroborated by significant spatial genetic autocorrelation among nesting females at scales of <500 m. Nevertheless, a lack of spatial genetic autocorrelation among non-nesting turtles (males and females) suggested extensive local connectivity, possibly mediated by male movements or long-distance movements made by females between terrestrial nesting areas and aquatic habitats. We show here that coupling genetic and demographic information with simulations of individual-based population models can be an effective approach for untangling the contributions of natal and breeding dispersal to spatial ecology.

Subject areas: Conservation genetics and biodiversity, Reproductive strategies and kinship analysis

Key words: breeding dispersal, *Emydoidea blandingii*, mark–recapture, natal dispersal, population simulation, spatial genetic autocorrelation

The study of animal movements among populations and habitats is a fundamental endeavor in ecology. Such movements can broadly be classified as natal dispersal, which reflects movements from natal to breeding sites, and breeding dispersal, which represents movements by adults among breeding sites. These 2 processes are shaped by fitness trade-offs associated with moving to new areas that differ by life stage and sex (Greenwood 1980). Moreover, age- and sex-based dispersal behaviors serve as the basis for emergent, population-level processes in heterogeneous landscapes such as source-sink (Pulliam 1988) and meta-population dynamics (Hansson 1991). Characterizing the relative influence of natal and breeding dispersal processes on population connectivity is thus key to understanding the dynamics of spatially structured populations (Fahrig and Merriam 1994), especially when adults display long-term fidelity to breeding sites (Berven and Grudzien 1990; Gamble et al. 2007).

Despite the importance of characterizing dispersal processes in ecology, quantifying and distinguishing between natal and breeding dispersal remains challenging for many species. Mark-recapture and telemetry techniques provide direct means for characterizing dispersal (MacDonald and Johnson 2001; Bullock et al. 2002), but estimating natal dispersal rates in species with long maturation times or cryptic life histories with these methods can be challenging (Godley et al. 2010). Indirect genetic methods provide an attractive and oft-used alternative for characterizing dispersal patterns in spatially structured populations when movements are difficult to track directly (Broquet and Petit 2009). However, indirect genetic approaches that rely upon distributions of genetic variation under equilibrium conditions, such as *F*-statistics, often misestimate migration rates when these assumptions of equilibrium are violated and cannot distinguish recent versus historic dispersal (Whitlock and McCauley 1999). Moreover, the performance of nonequilibrium approaches developed to detect recent dispersal events can be sensitive to genetic population structure. For example, population assignment methods (Rannala and Mountain 1997; Pritchard et al. 2000) have limited power to detect dispersers when genetic differentiation among populations is low (Berry et al. 2004; Paetkau et al. 2004, although see Hall et al. 2009), as can result from even infrequent dispersal (e.g., ≥ 1 migrant per generation; Wright 1951). Methods that rely on high genetic differentiation will thus have limited utility in the study of demographically linked populations such as those functioning as source-sink systems (Lowe and Allendorf 2010). Finally, while the rapidly expanding field of landscape genetics has provided novel insights into the environmental factors that impede or facilitate dispersal movements, distinguishing between historic and recent processes, as well as estimating per-capita dispersal rates, remains challenging (Epps and Keyghobadi 2015).

Genetic kinship methods have recently emerged as a promising alternative for characterizing dispersal patterns and estimating dispersal rates when “conventional” genetic approaches do not yield strong inference (Palsbøll et al. 2010). Kinship methods have proven useful in identifying fine-scale patterns of juvenile dispersal (Schunter et al. 2014) as well as long-term fidelity to natal sites (Feldheim et al. 2014) and social groups (Pilot et al. 2010). In the simplest sense, a recent dispersal event can be inferred when the 2 members of a dyad of close relatives (determined with genetic methods) occur in different populations. Inference applies to a time scale equivalent to the number of overlapping generations and is not hindered by genetic homogeneity resulting from frequent dispersal (Palsbøll 1999; Palsbøll et al. 2010). Indeed, genetic kinship methods are well suited for species with high dispersal rates (and thus low genetic divergence) given that frequent movements facilitate the

sampling of dyads split between 2 different populations. Moreover, these approaches only require that individuals be sampled on a single occasion (as opposed to mark-recapture methods), making them an appealing option for long-lived organisms with long maturation times. Finally, unlike many other genetic approaches, kinship methods can provide absolute estimates of per-capita dispersal rates by comparing observed spatial patterns of relatedness to expectations under different dispersal scenarios derived from individual-based population genetic models (Peery et al. 2008). Despite their potential utility, however, studies combining kinship inference with demographic information remain rare, and none to our knowledge have estimated natal dispersal rates among multiple populations from the distribution of closely related dyads while taking into account known breeding dispersal rates.

In this study, we integrate genetic kinship methods with demographic information and simulations of an individual-based population genetic model to estimate natal and breeding dispersal rates and patterns in Blanding’s turtle (*Emydoidea blandingii*), an IUCN-endangered species (van Dijk and Rhodin 2016). Blanding’s turtle is a semiaquatic species notable for its long-range overland movement capabilities (Beaudry et al. 2008, 2010a, 2010b). Breeding females display fidelity to particular terrestrial nesting areas, typically open areas spatially removed from resident marshes (Congdon et al. 1983; Standing et al. 1999). However, as in other turtle species, characterizing intergenerational patterns of dispersal, as well as movements over an individual’s lifetime, is difficult in Blanding’s turtle because cryptic behavior of juveniles, long maturation periods (≥ 14 years; Congdon et al. 1993), and long life spans (≥ 77 years; Brecke and Moriarty 1989).

Because dispersal is traditionally defined as the movement of organisms from their place of origin to their place of reproduction (Howard 1960) or among breeding sites, we focus primarily on movements among terrestrial nesting habitat patches by females rather than movements among aquatic habitats. We use a long-term mark-recapture dataset to estimate breeding dispersal in nesting females and integrate genetic kinship methods with demographic simulations to estimate natal dispersal rates. As a complement to these simulation-based analyses, we compare distributions of close kin over a broader spatial scale with more traditional spatial autocorrelation analyses (Smouse and Peakall 1999). While the manner in which genetic kinship methods are applied to questions of recent dispersal will vary by system, our work highlights the benefits of integrating genetic kinship methods with demographic approaches to study dispersal patterns in species with cryptic life history stages and long life spans.

Materials and Methods

Study Area and Field Methods

This study was conducted in 2 adjacent wildlife reserves (Sandhill Wildlife Area [SWA] and Wood County Wildlife Area [WCWA]; Figure 1) covering approximately 75 km² in central Wisconsin. The study landscape is characterized by low human population density and dominated by extensive wetlands interspersed with deciduous and coniferous forests and several maintained openings used by Blanding’s turtles for nesting. Blanding’s turtles have been captured using a variety of methods (including trapping in aquatic habitats) and marked within SWA since 1990 (Reid et al. 2016). Formal nesting surveys within SWA began in 2001 and continued until 2013. These surveys consisted of visual surveys in the evening during the nesting season (late May and June) of terrestrial areas in which Blanding’s turtle were

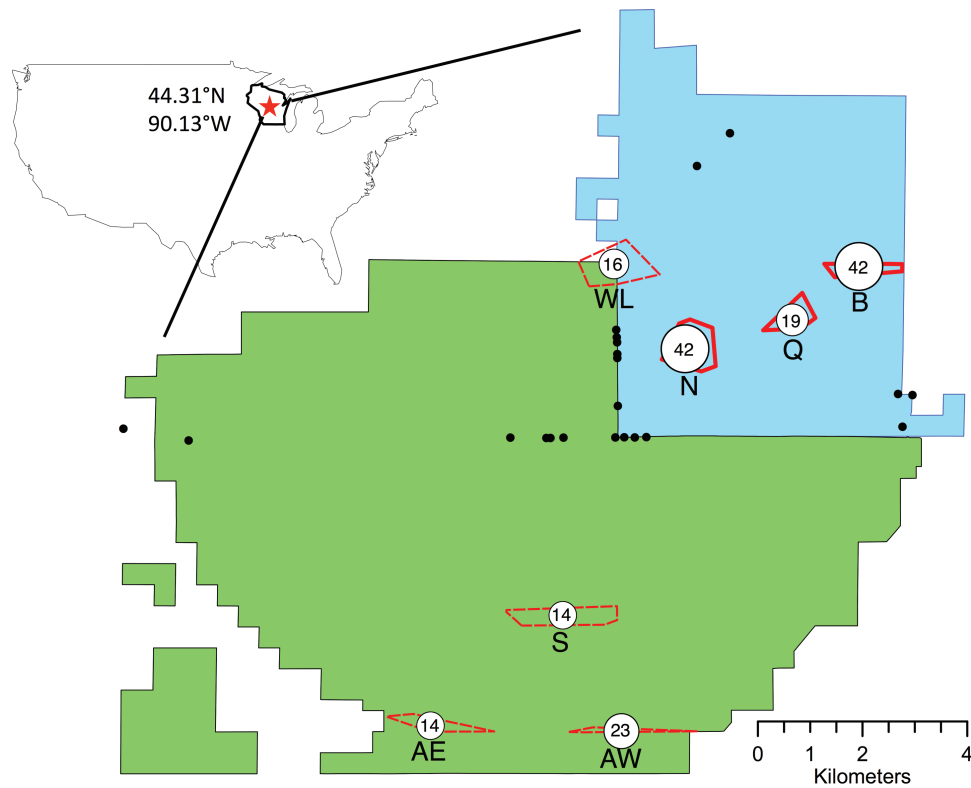


Figure 1. Map of study area. Inset indicates location of Wisconsin and the study area within the United States. Latitude and longitude are given for SWA headquarters. Red polygons encompass female nesting locations within designated nesting areas. Nesting areas within the blue polygon (SWA, in which nest surveys were conducted from 2000 to 2013 and genetic samples were collected from 2010 to 2013) are indicated by solid red lines, while nesting areas in the green polygon (WCWA or overlapping the 2 areas, in which nest surveys and genetic sampling were conducted in 2012–2013 only) are indicated with dotted red lines. Size of white bubbles is proportional to the number of individual genetic samples collected within each nesting area, and the number of genetic samples is shown inside the bubble. Black dots indicate the locations of females captured outside of nesting areas.

known or suspected to nest, focusing initially on SWA but expanded in 2012 to WCWA. Field protocols were approved by the University of Wisconsin-Madison Animal Care and Use Committee (ACUC assurance number A3368-01) and conducted under Wisconsin Department of Natural Resources Endangered Resource permit ER681. All turtles encountered, except those in the act of oviposition, were individually marked by notching marginal scutes with a triangular file for demographic analyses (Cagle 1939). A small blood sample (approximately 100 μ L) was collected via the dorsal coccygeal vein from all turtles captured from 2010 to 2013 for genetic analyses. Capture locations were either marked on a map (1990–2009) or recorded using a handheld GPS unit (2010–2013). Map locations were later georeferenced using Google Earth. In the course of nesting surveys, we identified 3 areas with concentrations of nesting activity within SWA (≥ 37 individuals in each area over 13 years; areas N [North Bluff], B [Bison Prairie], and Q [Quarry]; Figure 1) and 4 occurring partially or completely within WCWA (≥ 14 individuals in each area over 2 years; areas AE [Amundson East], AW [Amundson West], WL [West/Long Island], and S [South Bluff]). Over 85% of the turtles encountered during nesting surveys were found within these 7 areas, with the remainder encountered on access roads during nesting surveys (Figure 1).

Genotyping and Genetic Data Analysis

We genotyped all individuals for which samples were collected at 14 microsatellite loci developed for use in Blanding's turtle or closely related species (Table 1). DNA extraction and PCR procedures were conducted as described in Reid and Peery (2014).

Table 1. Locus characteristics for microsatellites used in this study

Locus	Alleles	H_o	H_e	Source
BTCA9	14	0.77	0.82	Libants et al. (2004)
Cp2	8	0.84	0.82	Pearse et al. (2001)
Eb09	8	0.61	0.6	Osentoski et al. (2002)
Eb17	6	0.68	0.64	Osentoski et al. (2002)
Eb19	4	0.66	0.64	Osentoski et al. (2002)
GmuD16	9	0.68	0.67	King and Julian (2004)
GmuD55	5	0.66	0.65	King and Julian (2004)
GmuD62	5	0.35	0.39	King and Julian (2004)
GmuD70	12	0.81	0.84	King and Julian (2004)
GmuD87	14	0.75	0.78	King and Julian (2004)
GmuD88	14	0.77	0.8	King and Julian (2004)
GmuD90	2	0.42	0.36	King and Julian (2004)
GmuD93	2	0.22	0.22	King and Julian (2004)
GmuD121	6	0.5	0.52	King and Julian (2004)

H_e , expected heterozygosity; H_o , observed heterozygosity.

We used GenePop version 4.2 (Rousset 2008) to assess deviations in observed heterozygosity from the expectations under Hardy-Weinberg equilibrium (HWE). We used the locus-specific test for heterozygote excess as well as the global test. We performed both tests using the default Markov chain parameters (1000 dememorization steps, 100 batches, 1000 iterations per batch). We then calculated genetic diversity and standard genetic divergence statistics (F_{ST} ; Weir and Cockerham 1984) for each pair of nesting areas, as

well as overall allele frequencies for each locus, using Genodive (Meirmans and Van Tienderen 2004). We used permutation tests (999 permutations) to determine whether F_{ST} values were significantly different from zero. Sequential Bonferroni adjustments (Holm 1979) were used to determine threshold significance values for F_{ST} and HWE tests. We estimated pairwise relatedness (r) for all possible dyads of individuals using maximum likelihood techniques (Milligan 2003) implemented in program ML-RELATE version 2 (Kalinowski et al. 2006).

Estimating Breeding Dispersal Rates With Mark-Recapture

We used multistate mark-recapture models (White et al. 2006) implemented in Program MARK version 8 (White and Burnham 1999) and our 13-year data set on marked turtles to estimate annual adult female dispersal rates among the 3 nesting areas (i.e., breeding dispersal) occurring within SWA. Each female capture was assigned a state corresponding to the nesting area in which it was captured in a given year. If a female was not captured in one of the 3 areas in a given year, the female was scored as uncaptured for that year. As the goal of this analysis was to estimate annual dispersal among nest areas, represented by state transition (Ψ) probabilities, we considered both recapture (p) and apparent survival (Φ) rates to be “nuisance parameters.” We first tested several models (holding Ψ constant) for p and Φ : 1) constant across years and nesting areas (p , or Φ); 2) annual variation (p_t or Φ_t); 3) nesting area-specific differences (p_A or Φ_A); or 4) both yearly and nesting area differences (p_{t+A} or Φ_{t+A}). Using the best-fit model for p and Φ , we then examined models incorporating yearly or area-specific Ψ . All transition probabilities were also modeled as symmetric (Ψ_{\pm} , meaning $\Psi_{i-j} = \Psi_{j-i}$) or asymmetric (Ψ_{\neq} , meaning $\Psi_{i-j} \neq \Psi_{j-i}$). The best-fit model in each case was identified using Akaike’s Information Criterion corrected for sample size (AIC_c) and relative model support was evaluated using differences in model weights (w) based on the difference in AIC_c between a given model and the best-fit model (ΔAIC_c). This best-fit model was tested for overdispersion using the bootstrap goodness-of-fit test and by estimating median \hat{c} using logistic regression in MARK.

Estimating Natal Dispersal Rates With Genetic Relatedness and Population Simulations

Our approach for estimating annual natal dispersal rates in Blanding’s turtles involved comparing observed patterns of relatedness within and among populations (i.e., genetic summary statistics) to expectations generated using an individual-based population genetic model parameterized with a range of possible values for dispersal rates. The value for natal dispersal that yielded genetic summary statistics most similar to observations from sampled individuals was assumed to reflect the most likely natal dispersal rate in Blanding’s turtles, similar in principle to approximate Bayesian computation (ABC) approaches (Beaumont 2010; Bertorelle et al. 2010; Csilléry et al. 2010). As both natal and breeding dispersal are expected to influence within- and among-population relatedness, the prior estimation of breeding dispersal rates using mark-recapture methods was key to our approach. Nevertheless, before estimating natal dispersal rates in Blanding’s turtles, we evaluated the robustness of our approach by exploring the sensitivity of spatial patterns of relatedness (i.e., relatedness summary statistics) to annual breeding (Ψ_B) and natal dispersal rates (Ψ_N) as well as sampling intensity (see Evaluating Model Performance section below).

The Population Model

The individual-based population genetic model generated expected joint distributions for 2 kinship-based summary statistics (see below) under different assumed values for Ψ_B and Ψ_N in a 2-population system. The 2 populations were projected forward in time based on expected stage-specific birth, survival, and dispersal rates according to an annual time step using the program *spim_m*, a multipopulation implementation of *spim* (Anderson and Dunham 2005). Individuals reaching the age-of-first-breeding had a one-time probability of either nesting in their natal area ($1 - \Psi_N$) or switching areas (i.e., natal dispersal; Ψ_N). Each year after the age of first breeding, individuals could either nest in the same area as they did in the previous year ($1 - \Psi_B$) or switch areas (i.e., breeding dispersal; Ψ_B). We assumed random mating within populations and no migration of males among populations, as adult male Blanding’s turtles tend to remain in a particular residence wetland for most of their lives (Congdon et al. 2011). After migration, simulated individuals survived and reproduced stochastically in each time step based on specified age- and sex-specific probabilities. Probability of survival, reproduction, and age-specific fecundity was obtained from a published life table for Blanding’s turtle (Congdon et al. 1993). Additional information regarding model structure and a complete list of model parameters can be found in the [Supplementary Material 1](#) online.

Multilocus genetic data were generated for each simulated individual according to the rules of Mendelian inheritance (i.e., offspring inherited one allele from their mother and one allele from their father) under the assumption of no mutation. The number of loci simulated and initial allele frequencies for these loci were set to the overall values observed for Blanding’s turtles in our study area. Pairwise relatedness was estimated in the final year of the model projection for all possible dyads as described above for observed microsatellite data. Pairs of individuals in dyads with $r \geq 0.5$, which corresponds to the expected r for all first-order kin (parent-offspring or full-sibling dyads), were considered to be “close kin.” Although r may fail to identify the “true” relationship for some simulated dyads due to the stochastic nature of allele-sharing among individuals in populations (Milligan 2003), rates of erroneous identification should in this case be similar between simulated and empirical datasets as both are generated from genetic data with the same number of loci and level of polymorphism.

We calculated 2 summary statistics based on the number of close kin dyads observed within each population (K_1 and K_2), the number of close kin dyads split between populations (K_{12}), and the number of total possible dyads within each population or between populations based on ending population sizes (N_1 and N_2). The number of possible dyads within a population of size N is equal to $\frac{1}{2}N(N - 1)$, and the number of possible dyads between 2 populations of size N_1 and N_2 is simply N_1N_2 . We note that summary statistics based on the $r \geq 0.5$ threshold yielded less error in estimates of natal dispersal rates than the most likely inferred relationship or population mean relatedness ([Supplementary Material 3](#) online). The first summary statistic (proportion related within areas, or PRw) was calculated as the proportion of close kin dyads within both populations relative to the total number of possible split dyads within both populations:

$$PRw = \frac{2(K_1 + K_2)}{N_1(N_1 - 1) + N_2(N_2 - 1)}$$

The second summary statistic (proportion related between areas, or PRb) quantified kinship patterns between populations and was calculated as the proportion of “split” dyads relative to the total number of possible split dyads between the 2 areas, or:

$$PRb = \frac{K_{12}}{N_1 N_2}$$

Evaluating Model Performance

We evaluated model and summary statistic performance by simulating scenarios in which either Ψ_N or Ψ_B were varied while the other parameter was held constant. A full list of parameters used for simulations is given in [Supplementary Material 1](#) online. All simulations were repeated 30 times in order to obtain a joint distribution for the 2 summary statistics, PRw and PRb , for each set of parameters considered. The number of parameter sets and the number of simulations that could be run for each parameter set was limited in part by the need to convert simulation output files into the format required by ML-Relate and the lack of a batch processing mode in ML-Relate. A standard workflow and custom scripts were used to streamline the process of conducting simulations and calculating relatedness statistics where possible ([Supplementary Material 2](#) online). We used “leave one out” cross-validation methods within an ABC framework using the local linear regression method to quantify accuracy and bias of dispersal rate estimation with summary statistics, as well as error associated with different tolerance levels ([Supplementary Material 3](#) online) and then plotted joint distributions of summary statistics for each dispersal scenario to evaluate the sensitivity of the joint distribution to changes in Ψ_N or Ψ_B . For a subset of simulations, we also randomly subsampled either 90% or 60% of simulated dyads and re-plotted the resulting joint distributions of PRw and PRb to determine the potential effect of incomplete sampling on dispersal estimation.

Estimating Natal Dispersal Rates in Blanding’s Turtles

To estimate natal dispersal rates in Blanding’s turtles in SWA, we compared simulated joint distributions of the 2 relatedness summary statistics with observed values for each pairwise combination of the 3 nesting areas within SWA. When estimating natal dispersal rates, we simulated a range of 9 discrete values for Ψ_N (0–0.8) while fixing Ψ_B at the rate estimated using mark-recapture methods described above. Initial population sizes corresponded to the estimated population sizes of nesting areas within SWA based on the number of females captured in each area and the cumulative probability of capturing and sampling an individual in these areas ([Supplementary Material 1](#) online). As a simple visual heuristic for evaluating natal dispersal hypotheses, we plotted observed and simulated values for the summary statistics PRw and PRb and considered all simulated dispersal scenarios for which the observed values for PRw and PRb fell within the simulated joint distribution (defined by a convex hull containing all summary statistics estimated from simulations conducted under a specific dispersal scenario) to be scenarios potentially supported by the data. To more formally evaluate the support for different dispersal scenarios, we used the package ABC version 2.1 ([Csillery et al. 2012](#)) in program R version 3.1 ([R Core Development Team 2013](#)) to calculate the posterior model probability for each dispersal scenario as well as a point estimate of natal dispersal rate. Posterior model probability was evaluated using simple rejection, while point estimates were calculated using the local linear regression method. We used a tolerance level of 0.3, which resulted in relatively low error compared to other tolerance levels based on our cross-validation analyses ([Supplementary Material 3](#) online).

Characterizing Spatial Patterns of Relatedness

We complemented our simulation-based estimation of natal dispersal rates among nesting areas in SWA with 2 analyses incorporating genetic data and spatial patterns of relatedness from the wider survey area including both SWA and WCWA.

Spatial Distribution of Close Kin

We calculated PRw for all nesting areas and PRb for all pairs of nesting areas, including the 4 more recently surveyed areas located in WCWA for which breeding dispersal estimates were unavailable, to examine how kinship changed over larger spatial scales. Specifically, we performed a linear regression with distance as the independent variable and arcsine-square root transformed summary statistics PRw and PRb as the dependent variable to determine whether there was a statistically significant correlation between these 2 variables. Distances associated with PRw were set to zero, and distances associated with PRb for a given pair of areas were calculated as the Euclidean distance between the average of all coordinates for capture locations of individuals in each area.

Genetic Autocorrelation Analyses

To examine whether patterns of relatedness for Blanding’s turtles of other sexes, age classes, and habitats were similar to those for nesting females, we performed spatial genetic autocorrelation analyses using the Excel add-in GenAlEx ([Peakall and Smouse 2012](#)). This approach involves examining the extent to which individuals found within a given distance of one another are more genetically similar than would be expected under the assumption of no relatedness. Since the power to detect spatial differences among groups in dispersal pattern using genetic autocorrelation is low when sample sizes are <100 ([Banks and Peakall 2012](#)), we conducted 2 separate analyses; one including only females caught during nesting surveys ($n = 204$) and another where we pooled all other individuals (males, juveniles, and females caught during aquatic trapping; $n = 123$). In cases where individuals were captured multiple times during the course of the study, we used the centroid or average of x and y coordinates across all captures (incorporating only nesting survey or non-nesting survey captures for females) as the spatial location of the individual. Distance bins were delineated by 7 breakpoints, 2 corresponding to distances within nesting areas (500 and 1000 m), one corresponding to distances both within and among nesting areas (2000 m), and the remainder corresponding to distances among nesting areas (5000, 8000, 12000, and 16000 m). We performed 999 permutations to estimate confidence levels for expected autocorrelation under the assumption of no relatedness in each bin and 999 bootstraps to determine the variance associated with observed autocorrelation within each bin.

Results

Genetic Data Analysis

We obtained genetic samples from 220 adult females, 56 adult males, and 34 juveniles from 2010 to 2013 ([Table 2](#)). Of the adult females sampled, 204 were captured during nesting surveys, and 170 of these were captured within one of the 7 nesting areas ([Figure 1](#)). No loci exhibited statistically significant deviation from HWE after sequential Bonferroni correction for either the local or global tests, and expected heterozygosity was similar to observed heterozygosity for all loci ([Table 1](#)). Mean observed heterozygosity was 0.62 (range: 0.22–0.84), with an average of 7.8 alleles per locus (range: 2–14;

Table 2. Number of captures and genetic samples collected each year for mark–recapture and genotypic datasets

	Number captured (number genotyped)												
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
SWA—overall	21	38	32	41	29	52	31	52	26	59 (57)	73 (54)	87 (49)	81 (29)
Area N	14	22	15	19	18	23	18	22	11	20 (18)	29 (17)	26 (6)	23 (1)
Area B	1	0	4	0	3	8	2	3	2	6 (6)	12 (10)	20 (14)	26 (12)
Area Q	1	5	5	9	3	5	3	13	8	7 (7)	6 (4)	14 (6)	17 (2)
WCWA—overall	—	—	—	—	—	—	—	—	—	10 (10)	11 (11)	51 (43)	82 (57)
Area WL	—	—	—	—	—	—	—	—	—	1 (1)	2 (2)	6 (4)	10 (9)
Area S	—	—	—	—	—	—	—	—	—	—	—	7 (7)	10 (7)
Area AE	—	—	—	—	—	—	—	—	—	—	—	12 (12)	22 (11)
Area AW	—	—	—	—	—	—	—	—	—	—	—	4 (4)	12 (10)

For all years (2001–2013), the total number of captures (including recaptures) in a given area for the carapace-marking dataset is indicated, and from 2010 to 2013, the number of individuals sampled and genotyped for the genetic dataset (excluding recaptures) is indicated in bold. “Overall” values indicate the total number of individuals (males, females, and juveniles) captured in a given year in either SWA or WCWA, and the “Area” values indicate the number of nesting females captured in each of the 7 nesting areas in each year.

Table 1. F_{ST} values were generally low between nesting areas (average = 0.009, range 0.001–0.028), and all F_{ST} values for pairs of populations within SWA were <0.01 (**Table 3**). Among the 170 nesting females captured in one of the 7 nesting areas, we identified 44 pairs with $r \geq 0.5$, where both members occurred within the same nesting area and 55 pairs with $r \geq 0.5$, where the members were split between 2 nesting areas.

Mark–Recapture Estimation of Breeding Dispersal

We captured 159 individual females during nesting surveys within the 3 SWA nesting areas, with a total of 446 capture records from 2001 to 2013 for these individuals (**Table 2**). Ten captures of these females occurred outside of the 3 designated nesting areas within SWA, resulting in 436 capture records for the multistate mark–recapture analysis. The best-supported multistate mark–recapture model $\{\Phi, \Psi_{\rightarrow}, p_{t+A}\}$ incorporated annual variation in recapture probabilities, constant survival rates among nesting areas, and symmetric transition probabilities that differed among pairs of nesting areas (**Table 4**). This model was approximately 9 times more likely than the next best model $\{\Phi, \Psi_{\rightarrow}, p_{t+A}\}$, which included asymmetric transition probabilities. There was no significant evidence of overdispersion (bootstrap $P = 0.098$) and the estimated overdispersion parameter was close to 1 (median $\hat{c} = 1.07$). Estimated apparent survival rates for this model were high ($\Phi = 0.935$, 95% confidence interval [CI] = 0.906–0.956), in line with previous estimates for SWA ($\Phi = 0.939$; Reid et al. 2016) and only slightly lower than the “true” survival estimate of 0.96 used in the published life table for Blanding’s turtle (Congdon et al. 1993). The estimated transition rate was highest between nesting areas B and Q (0.0275, 95% CI: 0.0111–0.0665), followed by N to Q (0.0057, 95% CI: 0.0018–0.0178). Negligible transition rates (<0.0001) were calculated between areas N and B, and no individuals were observed nesting in both areas.

Summary Statistics and Model Performance

Increasing breeding and natal dispersal in our population model led to increases in PRb and decreases in PRw , and thus had the expected effect on the joint distribution of these 2 statistics (**Figure 2a, b**). Indeed, small increases in breeding dispersal had large effects on the 2 kinship statistics; for example, a 1% increase in breeding dispersal caused a shift in the joint distribution of PRb and PRw roughly similar to the shift resulting from a 20% increase in natal dispersal. PRb and PRw stabilized rapidly with increasing breeding dispersal

Table 3. Pairwise genetic differentiation for *Emydoidea blandingii* nesting areas sampled (below diagonal) and permutation P values (above diagonal)

	AW	AE	S	WL	N	B	Q
AW	—	0.376	0.414	0.398	0.07	0.361	0.032
AE	0.001	—	0.032	0.008	0.007	0.003	0.001
S	0.001	0.013	—	0.169	0.041	0.057	0.075
WL	0.001	0.015	0.006	—	0.131	0.287	0.005
N	0.009	0.013	0.01	0.005	—	0.027	0.042
B	0.001	0.014	0.009	0.002	0.005	—	0.155
Q	0.013	0.028	0.011	0.019	0.008	0.004	—

Bold indicates values were significantly different from zero after sequential Bonferroni adjustment.

Table 4. Model characteristics for multistate mark–recapture models

Model	K	AIC_c	ΔAIC_c	w
$\Phi, \Psi_{\rightarrow}, p_{t+A}$	18	1202	0	0.87
$\Phi, \Psi_{\rightarrow}, p_{t+A}$	21	1206.3	4.3	0.1
$\Phi, \Psi_{\rightarrow}, p_{t+A}$	16	1209.5	7.4	0.02

Ψ represents transition probability between areas, Φ represents survival probability, and p represents capture probability. For all parameters, (.) indicates a single parameter was used for all areas and years, and (t) indicates year-specific parameters were used. For recapture and survival, (A) indicates area-specific capture parameters were used. For transition, (=) indicates a single parameter was used for each pair of areas (i.e., symmetric transition rates), while (≠) indicates that different parameters were used for each possible directional transition (i.e., asymmetric transition rates). K indicates the total number of parameters in a given model. ΔAIC_c indicates the difference in AIC_c between a given model and the best-supported model. w indicates the model weight. Only models with $w > 0.01$ are shown below.

rates and joint distributions of PRb and PRw were similar when $\Psi_B = 0.03$ and $\Psi_B = 0.04$. On the other hand, PRb and PRw increased almost monotonically as natal dispersal increased from $\Psi_N = 0.0$ to $\Psi_N = 0.80$, indicating that these kinship statistics were sensitive to changes in natal dispersal over a broad range of values for Ψ_N .

Joint distributions for PRb and PRw were very similar for 90% and 100% sampling across all values of Ψ_N considered, indicating that the presence of a small percentage of unsampled

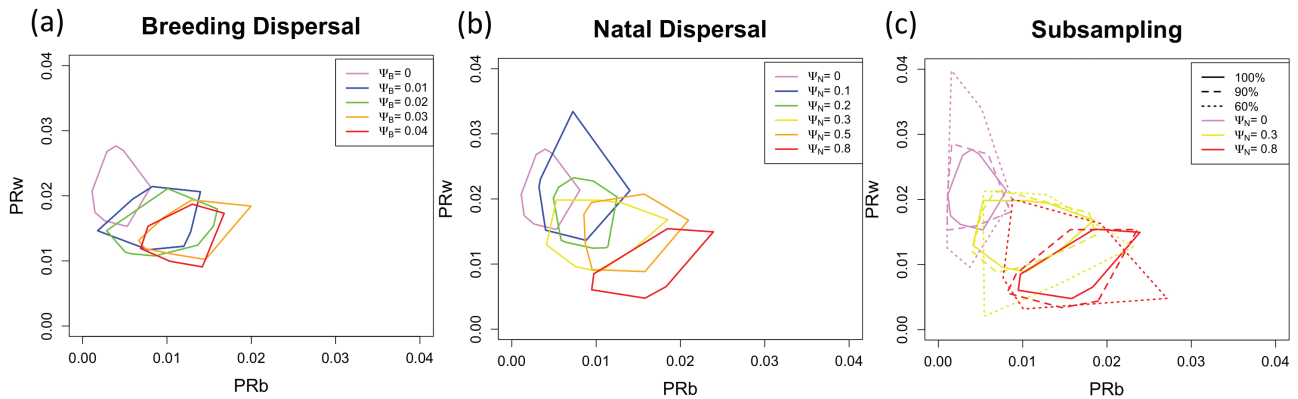


Figure 2. Polygons encompassing simulated distributions of PRb and PRw under (a) variable breeding dispersal (holding natal dispersal probability at 0); (b) varying natal dispersal (holding annual breeding dispersal rate at 0); and (c) subsampling (3 different natal dispersal scenarios subsampled; percent of each population subsampled indicated by line type). Ψ_N = annual breeding dispersal rate; Ψ_B = natal dispersal probability.

individuals did not impact the ability to estimate natal dispersal rates (Figure 2c). The breadth of the joint distribution increased with 60% sampling, but expected distributions still overlapped very little for $\Psi_N = 0, 0.30,$ and $0.80,$ suggesting that it was possible to discriminate among no, moderate, and high natal dispersal with high confidence even when sampling was substantially incomplete (Figure 2c).

Estimating Natal Dispersal Rates

When estimating natal dispersal probability among the 3 focal nesting areas in SWA, we assumed complete sampling because 1) the proportion of individuals sampled at these sites likely exceeded 90% based on cumulative capture probabilities over the 4 years over which genetic samples were obtained and 2) the joint distribution of the 2 summary statistics PRw and PRb was unaffected by not sampling 10% of the population (Figure 2c). For 2 pairs of areas (NB and NQ), the observed values of PRw and PRb fell within the distribution of simulated values only for low values of Ψ_N ($\Psi_N = 0$ or 0.1), while for the third pair of areas (BQ), the observed summary statistics fell outside of the expected joint distribution of PRw and PRb for all values of Ψ_N (Figure 3). Specifically, PRb was lower than expected for areas B and Q under any potential natal dispersal scenario, suggesting that breeding dispersal rates may have been overestimated for this pair of populations. For all 3 pairs of nesting areas, the highest posterior probabilities were for scenarios in which $\Psi_N = 0$ or $\Psi_N = 0.1$ (Figure 3). Point estimates of natal dispersal probability derived with ABC methods were similar for dispersal between areas N and B (NB $\Psi_N = 0.080$) and areas N and Q (NQ $\Psi_N = 0.078$) and higher between nesting areas B and Q (BQ $\Psi_N = 0.12$).

Characterizing Spatial Patterns of Relatedness

Spatial Distribution of Close Kin

Among all 7 nesting areas, mean PRw (0.0178) was approximately 4 times greater than mean pairwise PRb (0.0046), indicating that pairs of closely related females were more likely to be sampled in the same nesting area than in different nesting areas. PRw ranged from 0.0047 to 0.0204 across the 7 nesting areas and PRb ranged from 0 to 0.0186 among pairs of nesting areas (Figure 4). The proportion of closely related nesting females declined with increasing distance between nesting areas ($P < 0.001$; Figure 4).

Genetic Autocorrelation Analyses

Spatial genetic autocorrelation for nesting females was significantly greater than expected in the absence of structure ($r = 0.017$; $P = 0.001$) for the shortest distance bin (0–500 m). Autocorrelation values for nesting females tended to decrease with distance and a negative autocorrelation was observed at distances between 8000 and 12000 m ($r = -0.009$; $P = 0.001$), mirroring results from the spatial distribution of close kin (Figure 4). For non-nesting turtles, the autocorrelation differed significantly from expectation of the absence of structure only for the 5000–8000 m bin, where a negative autocorrelation value was observed ($r = -0.007$; $P = 0.012$).

Discussion

Coupling genetic kinship inference methods with demographic data has considerable potential for providing robust inference regarding dispersal patterns and processes, especially when dispersal occurs during cryptic life stages that are difficult to observe directly (Telfer et al. 2003; Jones et al. 2005; Waser et al. 2006; Nutt 2008). In our study system, for example, we observed infrequent breeding dispersal among nesting areas coupled with significant spatial genetic autocorrelation at short distances. At the same time, genetic differentiation among these nesting areas was low, suggesting potential gene flow mediated by cryptic juveniles. As none of the genetic methods used to characterize dispersal here provide inferences regarding rate or timing of dispersal, however, demographic simulations (informed by known rates of breeding dispersal) were necessary in order to distinguish the relative contributions of natal and breeding dispersal rates.

Simulation-based studies are particularly powerful in this regard as they allow for estimation of rates of unobserved dispersal in cryptic life stages given realistic population models that incorporate complex life history and observed rates of dispersal in less cryptic life stages. An alternate method for estimating dispersal rates using kinship inference has recently been proposed as well (Wang 2014). While this method provides an attractive likelihood-based framework for estimating dispersal, the estimation makes simplifying assumptions regarding dispersal behavior (only natal dispersal) and demography (discrete generations) that may introduce biases when these assumptions are violated. For long-lived organisms in particular, we have shown here that even infrequent breeding dispersal can significantly effect the distribution of kin, as annual rates are compounded over long reproductive life spans. With a simulation-based method, such as the one used here, the contributions of natal and

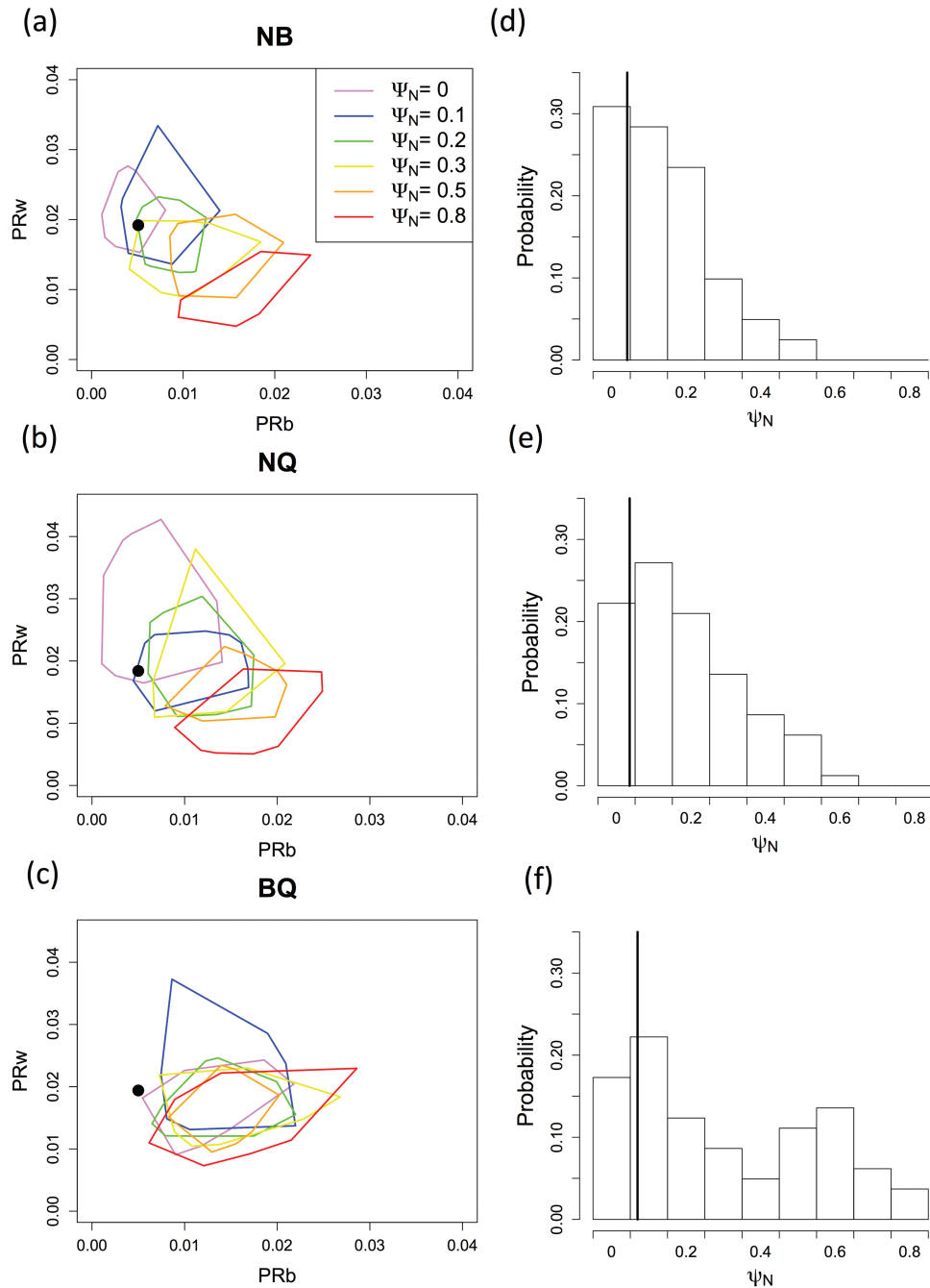


Figure 3. (a–c) Polygons encompassing simulated distributions of PRb and PRw under different rates of natal dispersal (Ψ_N) for the 3 focal nesting areas in SWA (NB, NQ, and BQ). Observed values for the corresponding pair of nesting areas are indicated by black circles. (d–f) Bar plots showing posterior distributions of model probabilities for natal dispersal scenarios derived using ABC for the 3 pairs of nesting areas. Location of weighted median estimates of natal dispersal rate is shown as solid vertical lines.

breeding dispersal can be estimated independently while accounting for the life history of the study organism.

Simulation-based methods, of course, require sufficiently accurate estimates of demographic parameters included in the population model as well as a model that reasonably describes the life history of the species in question. The need for an estimate of breeding dispersal rates increases the data required for this approach compared to alternative genetic methods. An important consideration relative to the current study is that direct measurement of dispersal provides estimates of all dispersal events, while our simulation method

incorporates only effective dispersal (i.e., dispersal potentially leading to successful reproduction; Greenwood 1980; Broquet and Petit 2009). Dispersal events could occur in the absence of effective dispersal if reproductive success is lower in the new nesting area than in the turtle's natal area. If turtles disperse without the chance of successfully producing offspring, fewer split pairs of close kin (and thus a lower Prb) would be expected between populations. This may explain the fact that the observed distribution of split pairs between the 2 areas with the highest estimated breeding dispersal rates was lower than expected in any of the natal dispersal scenarios, implying

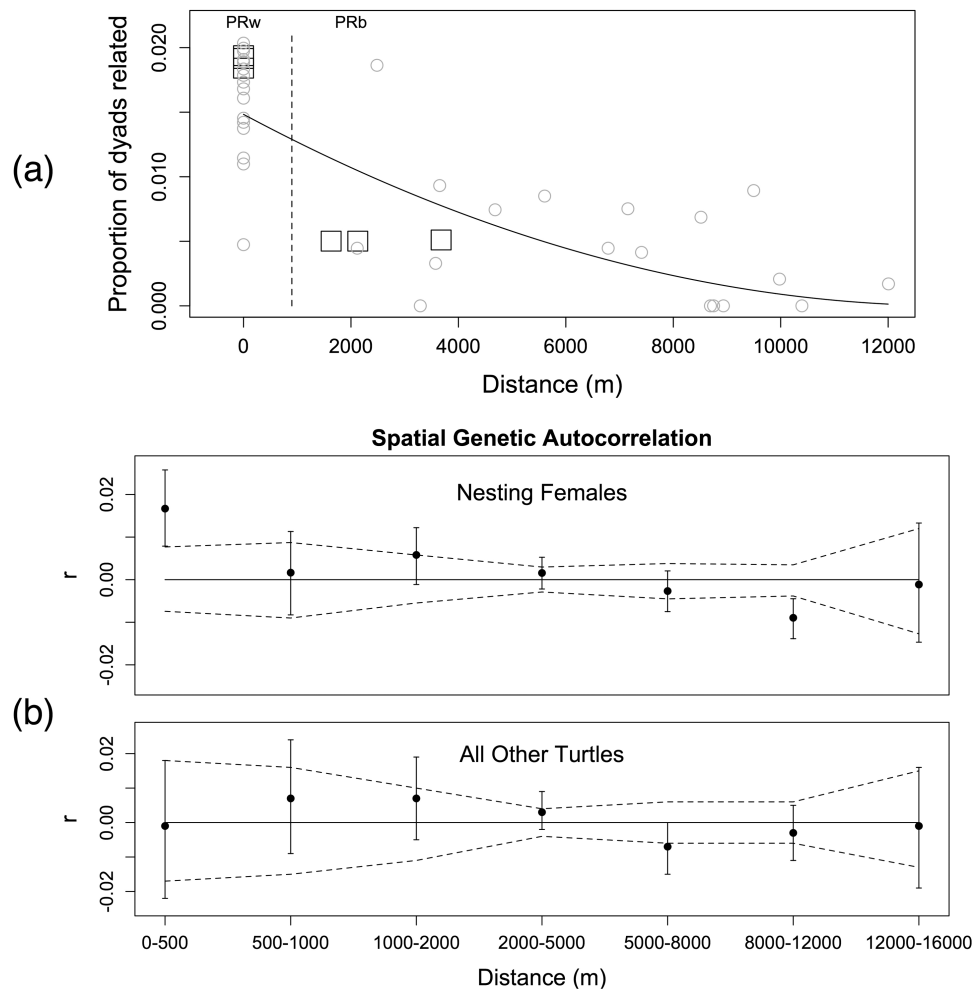


Figure 4. (a) Values for PRw and PRb for each nesting area pair are shown on the y axis. PRb is plotted against distance between centroids (the average of all coordinates for capture locations of individuals) of each area. PRw was assigned a distance of zero, and the fitted regression line between distance and proportion related is shown. Values for comparisons of nesting areas within SWA are shown as squares, while all other comparisons are shown as circles. (b) Spatial genetic autocorrelation analysis. Dotted lines represent 95% confidence levels for expected autocorrelation under the hypothesis of no autocorrelation given the number of individuals in each bin, while error bars represent 95% bootstrap CIs for the observed autocorrelation within each bin. Results are shown for (top) nesting females only and (bottom) all other turtles.

that effective dispersal rates could be lower than apparent dispersal rates between these 2 areas. Alternatively, true breeding dispersal rates for this pair of populations may simply be lower than the point estimate used for conducting simulations while remaining within the CIs estimated through mark–recapture analyses. Although computationally prohibitive here, an ABC approach that simultaneously estimates effective breeding dispersal rates using a probability distribution based on mark–recapture rather than a point estimate would be potentially useful for incorporating uncertainty in the true rate of effective breeding dispersal.

While we aimed here to use a realistic demographic model to construct expected distributions of relatedness within a population, no model can fully encompass the demographic complexities of a real population. Some of these complexities could potentially result in additional biases for model-based estimation of dispersal using kinship. For Blanding's turtle, several aspects of species biology in particular (sex determination and multiple paternity) could potentially affect the distribution of sibling pairs in the population. Blanding's turtles exhibit temperature-dependent sex determination (TSD; Gutzke and Packard 1987), and as such the sex ratio of clutches could vary from year to year or exhibit consistent biases toward one sex or the other.

A preponderance of unisexual clutches has been observed in the wild in some turtle species with TSD (Janzen 1994) and would result in more full-sibling female pairs; however, the frequency of unisexual clutches has not been examined for Blanding's turtle.

Clutches with varying levels of multiple paternity also occur in turtles (Uller and Olsson 2008), and multiple paternity would tend to reduce have the opposite effect of TSD with regard to expected patterns of within-clutch kinship (i.e., reducing the number of full siblings expected within a clutch). However, reported proportions of clutches with multiple paternity and the distribution of paternity within these clutches vary widely for Blanding's turtle (Refsnider 2009; McGuire et al. 2013; Anthonysamy et al. 2014). Additionally, sperm storage and repeat mating with the same male across years have both been reported for Blanding's turtle (McGuire et al. 2013; Anthonysamy et al. 2014), and these phenomena would tend to increase the number of full-sibling pairs across clutches produced in different years. Given the limitations of the simulation program used and the uncertainties regarding these potential biases in our study species, we assumed random mating, single paternity, and equal sex ratios. A more complex simulation framework could allow for better modeling of these factors and evaluation of their effects on the results.

Insights Into Turtle Dispersal From Genetics and Demography

Nest-site fidelity is well known in marine turtles (Bowen and Karl 2007), and females of several freshwater turtle species display fidelity to nesting areas based on mark–recapture studies (Lindeman 1992; Janzen and Morjan 2001; Rowe et al. 2005) and genetic data (Scribner et al. 1984; Freedberg et al. 2005; Sheridan et al. 2010), suggesting that nest-site fidelity is a common trait in turtle species. The spatial scale of nest-site fidelity, however, is more difficult to assess and may vary among species. Sheridan et al. (2010) detected significant spatial autocorrelation at short distances (<50 m) in nesting diamondback terrapins (*Malaclemys terrapin*). Nesting areas defined in our study were spatially extensive, including some large continuous tracts of nesting habitat in which nests were located in some cases over 1000 m from one another. As we observed significant spatial autocorrelation at distances less than 500 m, however, it is probable that Blanding's turtles display natal and breeding fidelity to subregions within these larger areas. Congdon et al. (2011) noted that most female Blanding's turtles monitored for more than 4 years often use multiple nesting areas (up to 6). However, nesting areas delimited in that study occurred in close proximity (as close as 200 m from one another) and our results are thus not inconsistent with Congdon et al. (2011). We have also observed Blanding's turtles moving hundreds of meters while searching for nesting sites (Reid et al. 2016). Given the extent of movements made by females between aquatic habitat and nesting areas (often greater than 1000 m; Beaudry et al. 2010b; Refsnider and Linck 2012) relative to the observed scale of spatial genetic autocorrelation, we conclude that migrations to nest sites in Blanding's turtle are motivated by natal fidelity to general nesting areas but not necessarily to precise nest sites.

While a number of studies have quantified the spatial scale of nest-site fidelity, few have attempted to quantify the probability that new or experienced breeders will disperse to different nest sites, and to the best of our knowledge, our study provides the first estimates of natal dispersal rates in a freshwater turtle species. Genetic kinship analyses indicate that natal and breeding dispersal among nesting areas is infrequent female Blanding's turtles, a result supported by spatial genetic autocorrelation analysis. At the same time, a lack of spatial genetic autocorrelation among non-nesting turtles and low levels of genetic differentiation among nesting areas suggest extensive local gene flow despite a general pattern of natal and breeding fidelity in females. To reconcile these 2 findings, we note that multiple potential mechanisms for local gene flow exist in this species that are consistent with the genetic patterns we observed. First, observed patterns of kinship for nesting areas within SWA were consistent with low but measurable rates of natal dispersal (0.08–0.12). Estimates of breeding dispersal rates were close to zero for 2 of 3 pairs of nesting areas and less than 0.03 for all pairs within SWA; however, as these rates represent annual probabilities of dispersal (and are thus compounded over long reproductive life spans of potentially 60 years or more), there could be a significant overall “lifetime” probability of a female using a nesting area different from its natal area.

Blanding's turtles are also highly mobile in general; females make relatively long movements from aquatic habitats to nesting habitats compared to other turtles (Steen et al. 2012) and use multiple wetland habitats throughout the active season (Refsnider and Linck 2012). Males may also move between and mate with females in multiple wetlands (Anthonysamy et al. 2014), and both males and females make long-distance movements overland to different aquatic habitats throughout the active season (Beaudry et al. 2010a) and have large multiyear home ranges (Schuler and Thiel 2008). As juveniles are in general more cryptic than adults in this species, their movements are

more difficult to rigorously quantify, although we have anecdotal evidence of juveniles of unknown sex within SWA that have moved in excess of 4 km over the course of several years (Reid BN, unpublished data). It is also possible, therefore, that extensive local gene flow occurs through mating in aquatic habitats or dispersal by males even as females exhibit low dispersal among nesting areas.

The complex interplay between local site fidelity and long-distance dispersal in Blanding's turtle may help to explain distributional and population genetic patterns described in this species, which include both striking genetic similarities between widely disjunct localities and genetic discontinuities between nearby populations (Mockford et al. 2007; Davy et al. 2013; Sethuraman et al. 2014). Gene flow may be spatially restricted by female site fidelity but punctuated by occasional (and somewhat random) instances of long-distance dispersal or founder effects resulting from colonization of novel habitats. The spatial genetic structure of Blanding's turtle in Wisconsin is partially related the historic distribution of suitable nesting habitat but also characterized by genetic similarity for some widely disparate sites (Reid 2016), supporting the view that nesting site fidelity as well as long-distance dispersal have both shaped landscape-scale patterns of genetic variability in this species.

Conservation Implications

Infrequent natal dispersal has a number of management implications for turtles. For species whose numbers are impacted by nest depredation and low juvenile survival, such as Blanding's turtle, head-starting is commonly used for augmenting declining populations (Heppell et al. 1996). Strategies for head-starting *E. blandingii* usually involve excavating a clutch of eggs immediately following oviposition, after which the eggs are incubated ex situ until hatching. Turtles are then released the following year, generally into wetlands near the original nest site (Glowacki and Kuhns 2010). While this strategy greatly reduces mortality due to nest and hatchling depredation and increases juvenile survivorship, it may disrupt natal homing behaviors, making it more difficult for adult females to find suitable nesting habitat once they mature. Alternative strategies such as in situ nest protection or removal of nest predators may be more appropriate given the spatial ecology of these species. A better understanding of female dispersal patterns among nesting habitats may also be useful in planning nesting habitat creation and restoration (Kiviat et al. 2000; Dowling et al. 2010). In turtles with TSD, nest-site fidelity may also retard adaptive responses to climatic warming, resulting in an increased potential for sex ratio biases and concomitant increased vulnerability to climate change (Morjan 2003).

The simulation-based methodology used here may be useful to conservation planning, as it allows for the estimation of unknown demographic parameters while also providing a framework for identifying and predicting the potential effects of management interventions on populations. Once plausible rates of natal dispersal are estimated from observed kinship distributions, these estimates (and the uncertainty around these estimates) can then be incorporated into further simulations in which other demographic parameters (such as juvenile or adult survival) or simulation conditions (such as the number of potential breeding areas) corresponding to proposed management actions can be manipulated to determine the potential effects of these actions on the population level. Continued monitoring of demographic and kinship patterns can then be used within an adaptive management framework (McCarthy and Possingham 2007; Schwartz et al. 2007) to both improve understanding of the system and to identify whether conservation actions have had the desired effect.

Supplementary Material

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

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Data Availability

All location data and microsatellite genotypes for individual turtles incorporated in this study have been archived online and are available at Dryad: <http://dx.doi.org/10.5061/dryad.p5c04>.

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