Journal of Herpetology, Vol. 54, No. 3, 317–324, 2020

Evidence for Nest-Site Fidelity but Not Natal Homing in Bog Turtles (*Glyptemys muhlenbergii*)

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Abstract.—**To mitigate habitat loss and increase the reproductive success of threatened Bog Turtles (*Glyptemys muhlenbergii*), managers often improve or restore open-canopy nesting habitats within or adjacent to occupied habitat. Restoring nesting habitat, however, does not guarantee that Bog Turtles will use these restored habitats; inertial mechanisms such as nest-site fidelity and natal homing may prevent female Bog Turtles from discovering and using restored habitats for many years or even generations. The objective of this study was to improve understanding of the role behavioral inertia may play in female Bog Turtle nest-site selection. From 2008 to 2012, at nine fens in New York and Massachusetts, we compared the average distance between previous and subsequent nests with null distributions assuming no nest-site fidelity. We also assessed whether pairwise genetic relatedness of nesting females was positively associated with geographic distance between nests. We found evidence for strong but incomplete fidelity to nesting-habitat patches within a wetland, likely driven by behavioral inertia. Individuals nesting closer together were not more closely related, and first-degree female relatives did not consistently nest within the same nesting-habitat patch, suggesting that Bog Turtles do not exhibit natal homing. Our results suggest that Bog Turtle populations may be slow to respond to newly restored nesting areas because of behavior inertia. However, testing this hypothesis will require long-term monitoring of habitat-restoration efforts coupled with further investigations of Bog Turtle nest-site selection.**

For nesting species such as turtles, females may repeatedly return to the same location to nest—a behavior known as nest-site fidelity (Loncke and Obbard, 1977; Freedberg et al., 2005). An extreme form of nest-site fidelity is natal homing, where females return to the same nesting areas from which they hatched (Carr and Carr, 1972; Meylan et al., 1990; Allard et al., 1994). Nest-site fidelity has been demonstrated in several freshwater turtles (e.g., *Chelydra serpentina*, Loncke and Obbard, 1977; *Chrysemys picta*, Valenzuela and Janzen, 2001; *Emydoidea blandingii*, Congdon et al., 1983; *Glyptemys insculpta*, Walde et al., 2007; *Trachemys scripta elegans* Tucker, 2001). However, as Freedberg et al. (2005) note, rarely do these studies include an examination of whether the fidelity is associated with natal homing. Although natal homing is frequently observed in sea turtles (reviewed in Bowen and Karl, 1997), researchers have also found that species of both migratory (Valenzuela, 2001) and nonmigratory freshwater turtles also tend to return to their natal nest site (Freedberg et al., 2005).

Bog Turtles (*Glyptemys muhlenbergii*) remain within their primary wetland habitat (fens and wet meadows) to nest, unlike many other freshwater turtle species that move from their aquatic primary habitats to nest in nearby uplands (U.S. Fish and Wildlife Service [USFWS], 2001; Whitlock, 2002; Ernst and Lovich, 2009). Bog Turtles often nest on hummocks in open-canopy sections of their heterogeneous fen habitats (Whitlock, 2002; Myers, 2011; Byer, 2015; Zappalorti et al., 2015). However, no published study has examined whether female Bog Turtles (1) return to the same nest locations year after year, (2) return to their natal nest sites, or (3) primarily use external cues (e.g., microhabitat characteristics) for nest-site selection.

Bog Turtles are listed as Threatened by the U.S. Fish and Wildlife Service (USFWS, 2001) and Critically Endangered by the International Union for the Conservation of Nature (Van Dijk, 2013). Because the loss of the Bog Turtle's specialized wetland/fen habitat is the primary threat for this species' survival, the U.S. Fish and Wildlife Service considers the preservation and restoration of Bog Turtle habitats as the most important recovery tool for this species (USFWS, 2001). Within these wetland/fen habitats, the amount of suitable nesting areas may limit the persistence or growth of existing populations (USFWS, 2001). Therefore, increasing the amount of nesting habitat could be an appropriate approach to improve Bog Turtle reproductive success (Travis et al., 2018). However, the effectiveness of efforts to improve or restore nesting habitat depends largely on whether nest-site fidelity or natal homing is a major determinant of nest-site selection in a focal population. If managers ignore nest-site fidelity or natal homing, they may falsely assume that the habitat conditions at known nesting areas are optimal for nesting, when, in fact, females are returning to their natal nesting areas regardless of the suitability of the habitat. Even more importantly, female turtles may not use restored, high-quality nesting habitat if they are likely to favor previously used or natal nest sites.

Because nest-site fidelity (and possibly natal homing) is common in freshwater turtles and because knowledge of nest-site fidelity is critical for planning nest-site restoration for threatened species like the Bog Turtle, we designed this study to address two questions: (1) do Bog Turtles exhibit strong nest-site fidelity? and (2) if so, is this pattern a result of natal homing? We predicted that the distances between subsequent nest locations for an individual female Bog Turtle would be substantially shorter than random internest distances (based on all known nest sites). Furthermore, we predicted that, if Bog Turtles exhibit natal homing, then closely related females should generally nest

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DOI: 10.1670/19-073

closer to each other than random pairs of females sampled from the population.

MATERIALS AND METHODS

Study Species.—Bog Turtles are the smallest freshwater turtle in North America (Ernst and Lovich, 2009) and one of the most imperiled turtle species globally (Van Dijk, 2013). Bog Turtles are often found in small (<50 individuals), isolated populations throughout their range (New York to Georgia; USFWS, 2001; Shoemaker and Gibbs, 2013; Dresser et al., 2018). In the northern part of their range, populations occur primarily in mineral-rich open-canopy wetlands (hereafter referred to as fens) with soft, saturated soil and dominated by sedges and low, shrubby vegetation (Carter et al., 1999; Morrow et al., 2001; Ernst and Lovich, 2009; Rosenbaum and Nelson, 2010).

Site Description.—We performed nesting surveys for Bog Turtles at 10 fens in southeastern New York state. In addition, we incorporated nesting data from Bog Turtle surveys at two Massachusetts sites (Sirois et al., 2014, in 2008–2009 and Whitlock, 2002, in 1997). Fens and habitat complexes are identified by unique three-letter identification codes (see Shoemaker and Gibbs, 2013), and general site descriptions are provided in Appendix 1. Fen names, locations, and/or maps are not provided because of concerns about illegal collection of bog turtles. Only those fens with ≥4 nests of known maternity were included for genetic analyses of natal homing (seven sites in New York: CFP, EFP, SHR, WFP, SHA, SHF, SMF; two sites in Massachusetts: JEF, SCH; Appendix 1). For our site-fidelity analysis, only those sites for which we recorded ≥2 interannual nest locations for at least one female were included for analysis (four sites in New York: CFP, SHR, SMF, WFP and one site in Massachusetts: SCH; Appendix 1). We define a habitat complex as an association of fens separated by upland habitat types but located in close proximity (<2 km, based on evidence from Shoemaker and Gibbs, 2013, documenting apparent gene flow between Bog Turtle populations at this spatial scale).

Data Collection.—During each spring from 2008 to 2012 (although we did not perform nesting studies at each fen each year), we surveyed for Bog Turtles at our study sites in southeastern New York State using visual scanning and “muddling” (hand-probing in loose soil in rivulets and beneath hummocks and shrubs; Table 1). For each turtle captured, we recorded sex and approximate age (Sexton, 1959). We drew a small blood sample (<0.2 mL) from the caudal artery (following Shoemaker and Gibbs, 2013) and immediately placed the sample in a preservative lysis buffer (prepared following Longmire et al., 1997) or on FTA® cards (Whatman). Prior to the nesting season (mid-May to mid-June; Whitlock, 2002), we attached radio transmitters (4.5 g; L. L. Electronics) to a rear pleural scute of approximately 20 adult (>9 yr; Whitlock, 2002) females each year using waterproof PC-11 epoxy (PC-Products). We tracked adult females (n = 105; 2008–2012; Table 1) using a hand-held R-1000 telemetry receiver (Communications Specialists) approximately once per day at dusk, when Bog Turtles often exhibit nesting behaviors (Ernst and Lovich, 2009) until nesting occurred (approximately mid-June). When we observed a female exhibiting nesting behaviors (e.g., excavating soil with hind legs), we marked the location and returned the next day to determine whether eggs were laid. We recorded locations of the verified nests with eggs (n = 46) using a GeoXM GPS unit (Trimble). Geographic coordinates were postprocessed using Pathfinder software (Trimble) to <3-m accuracy. We additionally received GPS coordinates of eight nests at the Massachusetts site SCH from A. Sirois in 2008, leading to a total of 54 nests with corresponding maternity and GPS data (Table 1). Because of concerns about transmitter battery life, we did not keep transmitters on turtles over winter, and, therefore, had to recapture the same turtle opportunistically during the spring to track any one individual over multiple years. When we were able to record ≥2 nesting locations for an individual female (nine individuals at five sites), we measured the distance between her previous and subsequent nests using ArcGIS 10.1 (ESRI), and we assessed whether or not subsequent nest locations were located within the same nesting-habitat patch (see below).

TABLE 1. Fen-specific summaries of radio-tracked Bog Turtle females and nests in New York and Massachusetts by year (2008–2012). Site codes with boldface type were the fens (and corresponding year) used in nest-site fidelity analyses. Complex name codes are listed in italics above sites belonging to that complex. Because nest location data from 1997 by Whitlock at JEF did not have specific GPS coordinates available, we did not include those females in this table or in the fidelity analysis (n = 7).

Complex/site code	Tracked females	Nests located
2008		
SCH	12	2
2009		
SCH	13	6
TMR complex		
CFP	3	2
EFP	2	2
WFP	9	5
SHR	9	6
2010		
TMR complex		
SHR	4	1
WFP	9	4
2011		
SMF	5	4
TMR complex		
CFP	1	1
EFP	2	1
WFP	8	4
SHR	5	4
2012		
SMF	7	
AMF complex		
SHA	5	3
SHF	11	1
Total	*105*	*54*

Nest-Site Fidelity Analyses.—Because we were unable to distinguish nesting habitat easily without observing nesting females, we defined nesting-habitat patches by applying a 10-m buffer around all observed nesting locations and dissolving (aggregating) all buffered nesting locations exhibiting overlap with neighboring (buffered) locations. We chose a 10-m buffer because Bog Turtles tend to exhibit <=5 m total displacement on a given day (Chase et al., 1989; Lovich et al., 1992; Morrow et al., 2001); however, Bog Turtles may move up to 20 m or more per day (Carter et al., 2000). Here, we examined nest-site fidelity in a similar manner as Rowe et al. (2005) and Freedberg et al. (2005) by determining fidelity (1) to a nesting-habitat patch within a fen (coarse-scale nest-site fidelity) and (2) to a specific nest site within a nesting-habitat patch (fine-scale nest-site fidelity).

To test for coarse-scale nest-site fidelity, we used a nonparametric bootstrap method to test whether the mean distance between subsequent nests was smaller than expected under the null hypothesis (no nest-site fidelity). Specifically, for each observed pair of subsequent nests at a particular fen, we randomly selected 10,000 pairs drawn from the set of all known nest sites at that fen. We then sampled from these bootstrapped distances to compute the expected internest distance under the null hypothesis. We computed the *P*-value for this test as the proportion of bootstrapped internest distances that were less than or equal to the observed mean internest distance.

We used a similar bootstrap analysis to test whether the proportion of females using the same nesting-habitat patch in subsequent nesting attempts was greater than expected under the null hypothesis (no fidelity to nesting-habitat patches). Specifically, for each observed nest-site pair, we randomly sampled 10,000 pairs drawn from the set of all known nest sites at that fen and determined the proportion of pairs that occurred within the same nesting-habitat patch. We then sampled from these bootstrapped proportions to compute the expected proportion renesting in the same patch under the null hypothesis. We computed the *P*-value for this test as the proportion of bootstrapped estimates that were greater than or equal to the observed proportion renesting in the same patch.

To determine whether female Bog Turtles that reused nest-site patches exhibited nest-site fidelity within their chosen patches (fine-scale nest-site fidelity; e.g., Najbar and Szuszkiewicz, 2007; Walde et al., 2007), we performed a final bootstrap analysis; this time, we repeated the internest distance analysis described above (within a fen), but we only selected random pairs of known nest sites within each female's selected nesting-habitat patch. We computed the *P*-value for this test as the proportion of bootstrapped estimates that were less than or equal to the observed internest distance. All bootstrap analyses were performed using R (Canty and Ripley, 2015; R Core Team, 2018).

Natal Homing Analysis.—Ideally, to measure natal homing, we would have marked hatchlings at their emergence from a nest and recaptured the marked females when they were nesting themselves to determine whether females returned to the same nest site (Almany et al., 2007). However, mark–recapture studies on small turtles, such as Bog Turtles, are difficult because researchers have no well-established and permanent way to mark hatchlings, and females exhibit delayed sexual maturity (ca. 10 yr; Whitlock, 2002). An alternate method is to measure the relatedness of nesting females, because, if natal homing is occurring, then closely related females should nest closer together than random pairs of females sampled from the population (e.g., Freedberg et al., 2005). Because natal homing for nesting (as opposed to spawning/mating) is an exclusively female behavior, often mitochondrial DNA (mtDNA) markers (i.e., genetic material passed on through maternal lineages) are used to evaluate natal homing in turtles (Meylan et al., 1990; Allard et al., 1994; Freedberg et al., 2005). However, Amato et al. (1997) and Rosenbaum et al. (2007) revealed remarkably low levels of mtDNA variability within Bog Turtles considering the small population sizes and low dispersal capacity of this species. Therefore, mtDNA may not provide sufficient variation to determine relatedness within a Bog Turtle population. Based on the higher genetic diversity of microsatellite regions of DNA, Freedberg et al. (2005) suggested that microsatellite data are better than mtDNA markers at determining genetic structure within nesting Bog Turtle females. Therefore, for this study, we used microsatellite data to investigate natal homing.

Building on the genetic data from Shoemaker and Gibbs (2013), we extracted genomic DNA from blood samples using DNeasy 96-well plate extraction kits (Qiagen, Valencia, California) if the sample was preserved in lysis buffer or using FTA® extraction solution (Whatman) if the sample was preserved on FTA® cards. We prepared the extraction products for polymerase chain reaction (PCR) with the use of 15 previously tested and described microsatellite loci for Bog Turtles (King and Julian, 2004; Shoemaker and Gibbs, 2013). We analyzed PCR products using fluorescently labeled microsatellite primers using an ABI 3730 DNA Analyzer (Applied Biosystems) at the Sackler Institute of Comparative Genomics at the American Museum of Natural History (New York, New York). We genotyped the ABI3730 data by visualizing electropherograms and determined allele sizes via GENEMAPPER v.4.0 (Applied Biosystems). We verified results across laboratories by re-extracting the DNA and reanalyzing the PCR products from 10% of the blood samples in the Shoemaker and Gibbs (2013) study. We found no differences in allele calling between institutions and researchers, and therefore we included all data, regardless of institution or researcher, resulting in a total of 202 genetic samples for the following analyses.

We tested for potential genotyping errors using MICRO-CHECKER (Van Oosterhout et al., 2004). We tested for deviations from Hardy–Weinberg expectations with GENEPOP v.4.0 (Raymond and Rousset, 1995) and linkage disequilibrium using Markov-chain exact tests. We determined maximum-likelihood estimation of pairwise relatedness (*r*; reviewed in Blouin, 2003) using ML-RELATE (Kalinowski et al., 2006). Because ML-RELATE estimates of relatedness assume a closed population (i.e., no immigration or emigration), and because a previous study determined gene flow between fens within 2 km of each other (Shoemaker and Gibbs, 2013), we ran ML-RELATE analyses on all genotyped individuals (i.e., males, females, juveniles) at the habitat complex level (grouping all sites within 2 km of each other) to incorporate the allelic diversity and null allele information of the entire complex population.

Because we were interested in nesting females to address the question of natal homing, we created a subset of the ML-RELATE pairwise relatedness to include only females with observed nests at each fen ($n = 42$). With this subset of relatedness data, we performed a Mantel test (9,999 permutations) using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012) to compare the pairwise difference between the relatedness coefficients between each nesting female within a population and the distance between their respective nests within a fen. When females nested multiple years, we used the most recently recorded nest location in the pairwise geographic distance matrix for the Mantel test. We did not perform a Mantel test if a fen (1) had fewer than four nests with known maternity or GPS coordinates, and (2) could not be grouped with nearby fens (<1 km away). Consequently, fens SHA and JEF were not included in this analysis.

We also measured the relatedness of nesting females by examining whether groups of mothers with nests clustered together within a fen (nesting group) had greater relatedness compared to overall relatedness of all nesting females within the fen. For this analysis, we clustered females into nesting groups by creating 20-m buffers around any known nest included in the natal homing study (i.e., any nest for which we had genotyped maternity information). We merged any buffers that overlapped with buffers from other females' nests and any females' nests found within these merged buffers were considered part of the same nesting group. For this analysis, we used a 20-m buffer because the 10-m buffer method as described in the fidelity analysis had too few genotyped females within nesting groups to compare to other groups within the fen. We tested whether nesting group explained a fraction of the total genetic variation

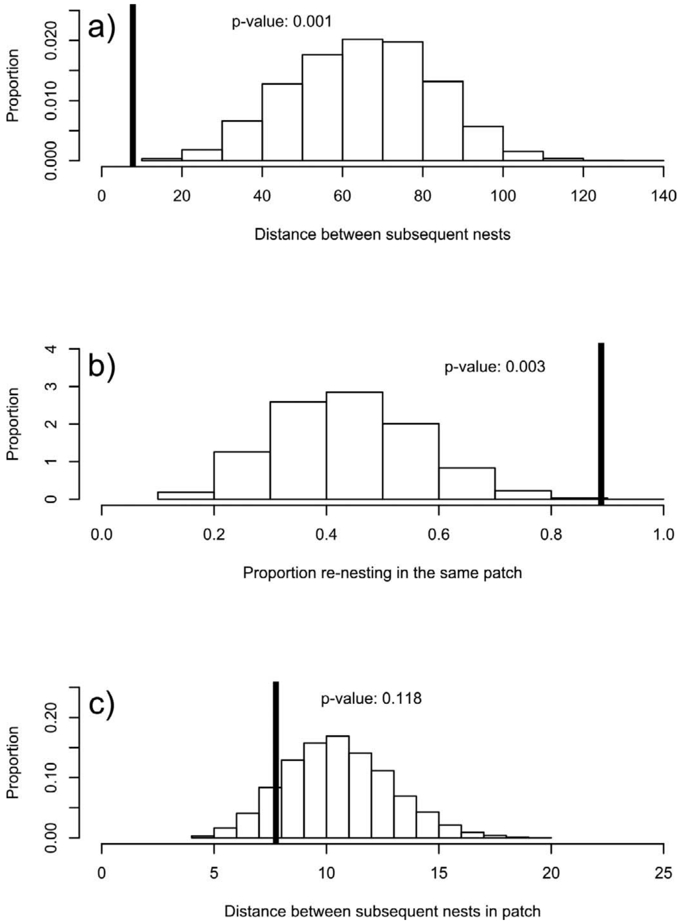

FIG. 1. Results from nonparametric bootstrap analyses of nest-site fidelity in female Bog Turtles. (a) Mean observed internest distance (black vertical line; $n = 9$, based on data from five sites over 8 yr) vs. the distribution of this test statistic generated by sampling random pairs of known nesting sites. (b) Mean observed fraction of females renesting in the same habitat patch (black vertical line; $n = 9$) vs. the distribution of this statistic generated by sampling random pairs of known nesting sites. (c) Mean observed internest distance for females observed to nest within the same nesting-habitat patch (black vertical line; $n = 8$) vs. the distribution of this test statistic generated by sampling random pairs of known nesting sites (within the same nesting-habitat patch). *P*-values were computed as the fraction of bootstrapped statistics as or more extreme than the observed mean internest distance.

among females using AMOVA ('Pops Mean' function in GenAlEx; 9,999 permutations).

In 1997, Whitlock (2002) recorded nest locations of known (notched) females at two Massachusetts sites (SCH and JEF). In 2011, we genotyped some of the nesting females ($n = 7$) Whitlock had observed nesting in 1997. Because the 1997 data set did not have specific GPS coordinates, but instead had anecdotal comparative distance between the nests (A. Whitlock, pers. comm.), we could not perform a Mantel test based on exact distances between the nests, but instead used the nesting-group relatedness comparison method.

To investigate relationships (i.e., unrelated, half siblings, full siblings, parent–offspring) between pairs of individuals, we used the relationship function of ML-RELATE. We considered pairs of females to have a strong kin relationship (i.e., first-degree relatives: full sibling or parent–offspring) if the log-likelihood of relatedness was at least two log units greater than the log-likelihood of the pair being unrelated (Kalinowski et al., 2006). We ran a Fisher's exact test to determine if the observed frequency of first-degree relative pairs nesting in the same nesting group (as their first-degree relative) varied from the expected frequency.

RESULTS

At the five fens for which multiple-year data were available, we recorded at least two nest locations for eight individuals. One of these individuals (identified as 2 WFP) had three recorded nest locations (2009, 2010, and 2011). Subsequent nests generally ranged from 3 to 14 m from the original observed nest location (mean distance = 8 m ± 5 SD). All but one observed pair of subsequent nesting attempts (1 out of 9 pairs) occurred in the same patch of nesting habitat; the one exception was one pair of subsequent nests for female 2 WFP who renested in a different habitat patch located 86 m from her original observed nest location. Based on the bootstrapping analysis for coarse-scale nest-site fidelity, Bog Turtles exhibited strong nest-site fidelity within a fen ($n = 9$; bootstrapped $P = 0.001$; Fig. 1a) and tended to reuse the same patch of nesting habitat more often than expected under the null hypothesis ($n = 9$; bootstrapped $P = 0.003$; Fig. 1b). However, we did not detect evidence of fine-scale nest-site fidelity—nest-site fidelity within a nesting-habitat patch ($n = 8$; bootstrapped $P = 0.12$; Fig. 1c).

When performing preliminary genetic analyses, we observed no evidence for allelic dropout (i.e., when one or more allelic copies of a loci fail to amplify) via the MICRO-CHECKER analyses. Allelic frequencies at both the site and complex level met Hardy–Weinberg expectations, and, across fens/complexes, no pairs of loci were designated as highly significant when testing for linkage disequilibrium using Markov-chain exact tests. Therefore, we included all 15 microsatellite loci in the remaining analyses.

Based on Mantel tests, we report low and nonsignificant R^2 values of the relatedness and distance between nests within each site (Table 2; Fig. 2), meaning that closely related individuals did not nest closer together (as compared to more distantly related individuals).

When examining mean relatedness of nesting groups (as compared to the relatedness across all females), we observed two separate nesting groups in New York fens WFP and SHR, two separate nesting groups in Massachusetts fens SCH and JEF (Sirois, 2014), and two separate nesting groups at SCH from Whitlock (2002). At WFP, mean relatedness ($r = 0.25$) within the northernmost nesting group (WFP 1) was greater than expected by chance ($P = 0.01$). The mean relatedness within the WFP 2 nesting group did not differ from relatedness across all nesting females ($r = 0.06$; $P = 0.76$). At SHR, the mean relatedness within the SHR 2 nesting group did not differ from random ($r = 0.19$; $P = 0.33$), but the mean relatedness within the SHR 1 nesting group was greater than expected by chance ($r = 0.23$; $P = 0.02$). When analyzing nesting data from Massachusetts fens, the females within the two nesting groups at SCH, from both 2008 [(SCH 1; $r = 0.05$; $P = 0.29$) (SCH 2; $r = 0.08$; $P = 0.29$)] and 1997 [(SCH 1; $r = 0.01$; $P = 0.95$) (SCH 2; $r = 0.08$; $P = 0.26$)] as well as the females within the two groups at JEF in 2008 [(JEF 1; $r = 0.00$; $P = 1.0$) (JEF 2; $r = 0.06$; $P = 0.40$)] were not more related than expected by chance.

Considering only first-degree relatives (Table 2), we observed 70% (7 of 10 pairs) of females nesting within the same nesting group as their first-degree relative. Within the TMR Complex fens of CFP, EFP, and WFP, we found two full-sibling relationship pairs with one pair nesting within the same nesting

TABLE 2. Fen-specific summaries of Bog Turtle natal homing via pairwise comparisons of maximum-likelihood relatedness and nest distances. Mantel tests performed with 9,999 permutations. CFP, EFP, and WFP were examined together because of the geographic proximity (<1 km) and because CFP and EFP had sample sizes too small to perform Mantel tests on at the fen level. Because WFP had adequate sample sizes, it was also examined on its own. Number of first-degree relative pairs listed as determined by ML-RELATE maximum-likelihood relationship determinations of parent–offspring or full-sibling pairs. Nests from Whitlock (2002) were not included in this analysis (see text for details). One first-degree pair found split between the sites SHF and SHA in the AMF Complex is not listed in this table because SHA was dropped from the Mantel test analysis (see text for details).

Site code	No. of females	No. of pairwise comparisons	Relatedness range (*r*)	Nest distance range (m)	Mantel test (R^2)	*P*-value	No. of first-degree pairs
CFP	2	91	0.00–0.73	2–355	0.003	0.709	2
EFP	3						
WFP	9						
WFP alone	9	36	0.00–0.40	2–175	0.045	0.060	1
SHR	9	36	0.00–0.72	6–132	0.056	0.105	5
SHF	8	27	0.00–0.50	<1–127	0.005	0.392	2
SMF	4	6	0.00–0.22	6–38	0.136	0.665	0
SCH	7	21	0.00–0.26	1–300	0.029	0.258	0

group (WFP 1) and the other pair having one sister nesting in the WFP 2 nesting group and the other nesting at a different fen (EFP) within the habitat complex, located approximately 320 m from the WFP 2 nesting group.

At SHR, we found one full-sibling pair, and this pair nested in the same nesting group (SHR 1). Also in SHR, four mother–daughter pairs were found, three of which had both individuals of the pair found within the same nesting group (SHR 1); the remaining pair had one individual nesting in SHR 1 and one individual in SHR 2. When we examined SHR with CFP, EFP, and WFP at the habitat complex level (TMR Complex), no additional first-degree relatives were observed between these fens (i.e., no evidence of migration of nesting first-degree relatives between SHR and other TMR Complex fens).

In SHF, one full-sibling pair and one mother–daughter pair nested in the same nesting group. At the AMF Complex level (SHA and SHF together), we recorded one mother–daughter pair with one female nesting in a nesting group at SHA and the other nesting almost 1.5 km away in a nesting group at SHF. Of the sampled individuals at the remaining three fens (SMF, SCH, and JEF), we did not find the nests of any first-degree relative pairs.

Overall, 7 of 10 first-degree relative pairs had individuals nesting in the same nesting group as their first-degree relative (Fisher test; $P = 0.34$). Three first-degree relative pairs had individuals nesting in different nesting groups; for two of these pairs, individuals nested at a completely different fen within their habitat complex.

DISCUSSION

Bog Turtles in southeastern New York and western Massachusetts generally displayed fidelity to nesting-habitat patches (coarse-scale nest-site fidelity); with one notable exception (80-m distance between subsequent nests), females returned to their original nesting-habitat patch in subsequent nesting events. We found no evidence for site fidelity within nesting habitat patches (~10 to 20 m), although small sample size ($n = 8$ nest-site pairs) may have prevented detection of weak signals at this scale. Our results are congruent with Whitlock (2002), who reported four Bog Turtles nesting within 5 m of their first observed nest and

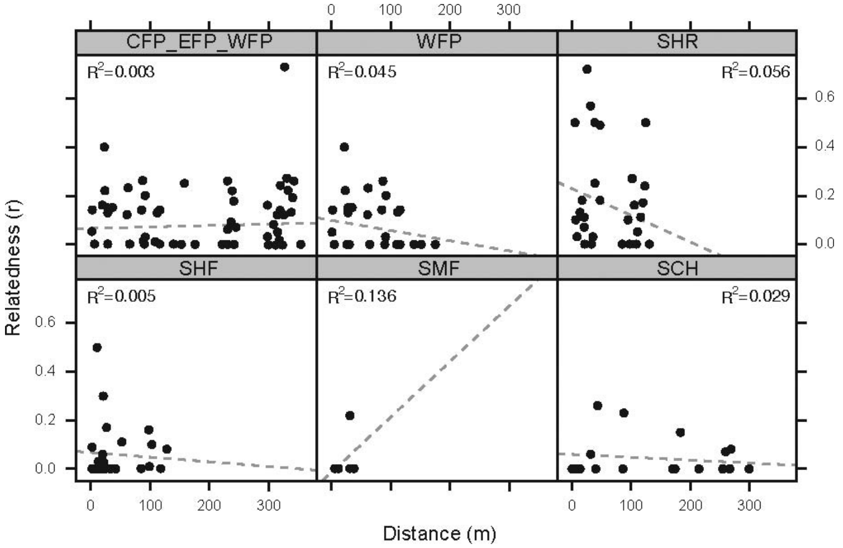

FIG. 2. Pairwise nest distances (m) and genetic relatedness (*r*) of nesting Bog Turtle females at natal homing study sites—calculated via a Mantel test with 9,999 permutations. For Mantel tests, if natal homing was expected, then plots would display high relatedness at low geographic distances (negative slope of regression line). No Mantel test has a significant R^2 value (see Table 2 for summaries), suggesting closely related females are not nesting close together (i.e., no evidence for natal homing). Dashed lines are included for visualization purposes only—lines do not convey significant trends.

another individual nesting nearly 300 m from its original nest location. Incomplete nest-site fidelity has been documented for several other species of freshwater turtle, whereby many individuals are observed to exhibit nest-site fidelity and others migrate to new nesting areas (Congdon et al., 1983; Walde, 1998; Joyal et al., 2001).

The observed fidelity to nesting-habitat patches did not appear to be attributed to natal homing, as more closely related female Bog Turtles did not lay their nests closer together within a fen. Additionally, when examining natal homing by measuring the relatedness of females with nests found clustered together (nesting group relatedness), 8 of 10 nesting groups were not more related than expected. Although 7 of 10 first-degree relative pairs were found nesting within the same nesting-habitat patch as their relative, this result was not statistically significant. Strikingly, two pairs of first-degree relatives not only nested in different patches, but the patches they selected were at different fens within their habitat complex. Because Bog Turtles generally do not perform nesting migrations (they tend to nest in the same fen where they spend the remainder of the year; Ernst and Lovich, 2009), these two examples of first-degree relatives found at different fens probably reflect dispersal rather than seasonal migration events. The timing or reason for these dispersal events is not known, but first-degree relative females occurring at different sites is especially interesting considering that, if natal homing were occurring, the homing behavior should be most obvious between mothers and daughters and full siblings (first-degree relatives).

Because of the difficulty of locating individual turtles over multiple years, we only once were able to identify >2 nest locations for a single female. However, the nest locations for this female—one of which was near to her first nest and the other of which was located a long distance from her first nest—raises the intriguing possibility that females may exhibit substantial variation in nest-site fidelity among years. Interannual variation in individual nest-site fidelity remains poorly understood for most turtles, and yet has important management implications; even if females exhibit nest-site fidelity in most years, interyear variation in nest-site fidelity could enable some fraction of the population to find and use restored or improved nesting habitats each year. To study the nesting habits of individual females over multiple years more effectively (and thereby study temporal patterns of nest-site fidelity), researchers might consider tracking females until hibernation, and then returning to known hibernacula to capture focal females. We caution against keeping radio transmitters on the turtles over the winter months, as Bog Turtles with transmitters have been observed becoming trapped within their hibernacula (J. Tesauro, pers. comm.).

Although our observation of strong nest-site fidelity in Bog Turtles may indicate behavioral inertia in this species (e.g., natural selection can favor nest-site fidelity where the reproductive outcome is unpredictable; Switzer, 1993), apparent nest-site fidelity in our study may also arise from among-individual variation in nesting-habitat preference. More research is needed to tease apart these alternative explanations for apparent nest-site fidelity in Bog Turtles, as the results may have implications for conservation (e.g., whether to prioritize microhabitat diversity within restored nesting sites). It is important to note that our finding of strong nest-site fidelity in Bog Turtles is unlikely to be an artifact of microhabitat selection at the population level, because our null model for statistical tests involved resampling from known nesting locations rather than resampling randomly within each wetland. Regardless, habitat and microhabitat characteristics almost certainly play a key role in nest-site selection at certain critical temporal and spatial scales (Congdon et al., 1983; Walde, 1998; Rasmussen and Litzgus, 2010; Foley et al., 2012). An evidence-based approach to nesting habitat restoration for Bog Turtles will require identification of which habitat and microhabitat characteristics are most influential for Bog Turtle nest-site selection and reproductive success and at what critical scales these characteristics should be measured and monitored.

We should also note that recent observations from a nesting-habitat expansion project at one of our study sites (SMF) confirm that female Bog Turtles have nested in restored open-canopy nesting habitat at this site (J. Tesauro, pers. comm.). Additionally, Travis et al. (2018) studied the effects of differing grazing treatments at another of our focal fens (SHF) after this study was completed and observed Bog Turtles using restored areas for nesting. Ideally, future research will assess the effectiveness of nesting-habitat improvement for Bog Turtles by performing rigorous, long-term monitoring of individual females at multiple sites where nesting-habitat improvement efforts are planned or underway; management treatments should be implemented at varying distances from existing nesting areas and should be monitored over many years to assess the short- and long-term effectiveness of these treatments. We stress that, although nest-site fidelity may imply certain challenges for nest-site improvement efforts, nesting-site management remains a promising tool for conservation of Bog Turtles and other freshwater turtles.

We found evidence of fidelity to particular patches of nesting habitat in Bog Turtles, but no evidence supporting a pattern of natal homing. In addition, we found anecdotal evidence for interyear variation in fidelity to a nesting-habitat patch. Because nest-site fidelity is likely incomplete, our results leave open the possibility that creation or expansion of nesting habitats can be a valid conservation tool. With a better understanding of the mechanisms behind nest-site selection by female turtles, managers should be better equipped to prioritize habitat restoration actions within and across sites.

Acknowledgments.—We thank J. Tesauro, A. Sirois, A. Whitlock, A. Myers, M. TerAvest, A. Krofta, S. Lauricella, and B. Raphael for invaluable assistance and support in the field. We also thank J. Tesauro, C. Zimmerman, A. Breisch, J. Jaycox, and The Nature Conservancy for having the foresight to establish long-term field data collection efforts at our study sites. We thank G. Amato, S. Gaughran, and R. Hersch for support in the American Museum of Natural History's Sackler Institute of Comparative Genomics. We appreciate the review of previous drafts of this manuscript by A. Tuininga, E. Hekkala, J. Wehr, R. Zappalorti, and one anonymous reviewer. All research activities were completed under New York State Department of Environmental Conservation Endangered/Threatened Species Scientific Licenses 195 and 225 and Fordham University Institutional Animal Care and Use Committee approval (Protocol C-10-06r-b). Generous support was given by Fordham University Graduate School of Arts and Sciences, Clare Boothe Luce Foundation, Sigma Xi, and American Museum of Natural History's Science Research Mentoring Program, which was supported by the National Science Foundation Award 0833537, Christopher C. Davis, The Shelby Cullom Davis Charitable Fund, The Pinkerton Foundation, the Doris Duke Charitable

Foundation, the Solon E. Summerfield Foundation, Inc., and the Adolph and Ruth Schnurmacher Foundation.

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Accepted: 25 May 2020.

Appendix 1

Site descriptions.—The 'TMR Complex' contains 11 fens all within 2 km of each other. Within the TMR Complex, the four nesting study sites (CFP, EFP, WFP, and SHR) are mineral-rich sloping fens composed primarily of calcareous wetland plant communities that are 0.2, 0.4, 0.8, and 4.1 ha, respectively, in size. These fens are discrete wetlands that are geographically distinct and separated by a matrix of coniferous and deciduous forests, steep embankments, and ridges; a low-traffic road separates CFP, EFP, and WFP from SHR. Because Bog Turtle movement and gene flow occurs between these fens (Shoemaker and Gibbs, 2013) despite the fens not being connected via continuous wetlands, we refer to this association of fens as a habitat complex.

Approximately 13 km north of the TMR Complex, the 'AMF Complex' is a habitat complex with six discrete fens within a 2 km area. The AMF Complex includes two study sites (SHF and SHA) separated by upland and wetland forest, cropland, pasture, and light residential/commercial land use, including a road with moderate traffic. SHF has approximately 1.6 ha of mineral-rich sloping fen habitat with an intermittent drainage stream dividing the habitat into two sections. Approximately 1 km from SHF, the site SHA has approximately 0.6 ha of fen habitat with an unpaved farm access road bisecting the habitat into two distinct areas.

Four kilometers west of the AMF Complex is the site SMF. Found within a 110-ha continuous wetland complex, SMF has approximately 2.5 ha of habitat where Bog Turtles are found. This area of the wetland complex is characterized by red maple (*Acer rubrum*) swamps with seepage and shrub fens and an area of wet pasture primarily composed of reed canary grass (*Phalaris arundinacea*) that is actively grazed by cattle.

Derived from site descriptions given in Sirois et al. (2014), in Massachusetts, SCH is incorporated in an approximately 200-ha continuous wetland complex within which Bog Turtles occur in approximately 5.0 ha. Over 10 km from SCH, JEF contains approximately 4.0 ha of habitat in which Bog Turtles occur within an approximately 60-ha wetland complex. The habitat types of these two sites include red maple swamps with sloping seepage and shrub fens. JEF additionally has large areas of wet meadow and large stands of *Phragmites* sp. and has periodic flooding due to beaver activity.