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Genetic Connectivity among Populations of the Threatened Bog Turtle (*Glyptemys muhlenbergii*) and the Need for a Regional Approach to Turtle Conservation

Kevin T. Shoemaker[1,2] and James P. Gibbs[1]

The threatened Bog Turtle (*Glyptemys muhlenbergii*) is considered among the most sedentary of turtles, yet with population sizes generally below 50 individuals, gene flow among populations is clearly necessary to maintain healthy levels of genetic diversity. Therefore, designing effective reserve networks for this species will require clarification of the rates of among-population gene flow over several spatial scales. We obtained genetic samples from a complex of 11 Bog Turtle populations within the Berkshire-Taconic region of Massachusetts and New York, and all individuals (n = 234) were genotyped across 15 microsatellite loci. Average multi-generation dispersal rates were inferred from population-level differences in allele frequencies using an approximate-likelihood approach, and recent dispersal rates were inferred using genetic assignment algorithms. Over small geographic distances (average inter-fen distance of ca. 1 km), among-population dispersal rates historically averaged between 0.25 and 0.5 effective migrants per population per year (ca. 1% of each population dispersing each year), and these dispersal rates appear to have persisted in recent decades. Over larger geographic distances (≥10 km), we infer that Bog Turtle populations in the Berkshire-Taconic region have experienced low rates of gene flow among populations according to a ''stepping-stone'' model. We conclude that (1) Bog Turtle populations with nearest-neighbor distances of <2 km should be managed as interconnected demographic units, (2) dispersal movements among adjacent populations may enhance regional population stability, and (3) gene flow over larger spatial and temporal scales probably requires dispersal among ''stepping stone'' habitats that may not harbor viable populations. Regional conservation planning for these and other small-bodied, endangered turtles should focus on establishing and maintaining networks of loosely connected population complexes to mimic historical connectivity patterns.

MAINTENANCE of gene flow among neighboring populations is critical for conservation of species that occur in small, discrete populations. In the short term, gene flow from neighboring populations can circumvent maladaptive genetic drift (''mutational meltdown'') and other deleterious effects of inbreeding (Dudash and Fenster, 2000). In the long term, among-population dispersal functions to retain large genetic effective population sizes and maintain adaptive genetic diversity (Sherwin and Moritz, 2000). From a metapopulation perspective, dispersal can effect re-colonization of extirpated populations and rescue populations from extirpation (Akçakaya et al., 2007). To this end, conservation reserve networks focused on augmenting among-population dispersal rates have been established or proposed for the Florida panther (Kautz et al., 2006), spotted owl (*Strix occidentalis*; Murphy and Noon, 1992), and other threatened species (e.g., Haig et al., 1998; Loucks et al., 2003).

Turtles are considered among the most threatened of the vertebrate orders, with nearly 50% of turtle species (ca. 40% of freshwater turtle species) currently listed as threatened or endangered by the International Union for the Conservation of Nature ''Red List'' (IUCN, 2010). Because these animals are thought to be poor long-distance dispersers, habitat fragmentation may place turtle populations at risk due to loss of genetic and demographic connectivity (Gibbons et al., 2000). Although rarely documented, dispersal limitation within fragmented landscapes may have played a key role in the decline of wetland-dependent turtles (Gibbs, 1993). Furthermore, those turtles that engage in long-distance dispersal within a fragmented landscape may be at increased risk of dispersal-related mortality (e.g., road mortality; Gibbs and Shriver, 2002). Some recent evidence suggests that turtles may engage in long-distance movements more frequently than previously believed (Howeth et al., 2008; Castellano et al., 2009), suggesting that turtle conservation efforts would benefit from a regional planning perspective. To date, there are few examples of multi-population habitat reserves proposed or created for the conservation of turtles and other wetland reptiles (Roe and Georges, 2007).

Designing and implementing habitat reserves and reserve networks requires a working knowledge of background rates of dispersal and the relevant spatial scales at which gene flow occurs (Haig et al., 1998). Animal movements are often studied via direct observation of tagged individuals over short (2–3 years) time periods (Nathan et al., 2003). However, effective dispersal rates as low as one individual per generation can theoretically reduce the potential for inbreeding depression and genetic drift (Mills and Allendorf, 1996), and such rare movements are unlikely to be detected via direct observation—especially for species with long generation times such as turtles (Jones, 2010). By comparing allele frequencies at neutral loci (genetic loci not subject to natural selection) across populations, researchers can infer dispersal rates in low gene flow contexts (Hellberg, 2009). Population genetics can be used to identify disperser individuals directly (Pearse and Crandall, 2004) or to infer historical dispersal by evaluating the rate of gene flow necessary to maintain observed genetic structure at migration-drift equilibrium (Nathan et al., 2003).

The Bog Turtle, *Glyptemys muhlenbergii,* is a diminutive, wetland-obligate turtle endemic to the eastern United States

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that is generally recognized as one of the most threatened turtle species in North America (USFWS, 2001). Much Bog Turtle habitat has been lost to wetland drainage for agriculture or other forms of human land development, and many populations are currently threatened by invasive plants (Tesauro and Ehrenfeld, 2007). Conservationists are concerned that current rates of dispersal among extant populations may be unacceptably low due to extreme geographic isolation and the imposition of anthropogenic barriers to movement such as roads (Rosenbaum et al., 2007). Estimation of Bog Turtle dispersal rates has proven difficult using traditional capture–recapture methods (Jaycox and Breisch, 2006).

Our primary objective was to characterize background dispersal rates for Bog Turtles using genetic markers. Previous research has hinted that dispersal rates may be very low for this species (Jaycox and Breisch, 2006), supporting current conservation and management practices for small-bodied turtles which emphasize a population-level focus over a larger-scale, regional perspective (Carter et al., 1999; Morrow et al., 2001). In contrast, evidence for substantial current and historical gene flow among populations would implicate a larger-scale approach to Bog Turtle conservation. Bog Turtle core habitat (sedge meadows and rich fens) is naturally patchy in many parts of its range (Chase et al., 1989), and population stability and metapopulation resilience may benefit from a regional approach to Bog Turtle stewardship. To discriminate between these competing turtle conservation directives, and to fill an important knowledge gap for the globally threatened Bog Turtle, we used microsatellite (simple sequence repeats) allele frequencies to infer current and historical rates of gene flow at different scales of proximity. We present evidence for substantial genetic connectivity among Bog Turtle populations at local and regional scales, suggesting that conservation plans for small-bodied turtles should cover a larger spatial extent than previously believed.

MATERIALS AND METHODS

Study organism.—The Bog Turtle is a small freshwater turtle inhabiting shallow fens and wet meadows of the eastern United States (USFWS, 2001). Previous studies using radiotelemetry and capture–recapture (CR) methods have demonstrated small home range size (0.05 to 2 ha) and have suggested that inter-fen dispersal of Bog Turtles is uncommon (Carter et al., 2000). The longest straight-line movement distance for a Bog Turtle recorded in the literature was 2.7 km, representing one of very few recorded instances of a Bog Turtle presumably moving among discrete fen units (Carter et al., 1999).

Study region.—The Berkshire-Taconic (B-T) valley region comprises a complex of swamps, fens, and wet meadows extending from southeastern New York into southwestern Massachusetts. The B-T region supports >20 extant Bog Turtle colonies separated by distances ranging 0.1 to 10 km and a wide range of matrix habitat types and potential barriers to Bog Turtle movement (e.g., coniferous and deciduous forests, limestone ridges and outcroppings, streams, roads, pastures, agricultural fields, and residential developments; Jaycox and Novak, 2003). Seven of our study sites were drawn from the Focal Fen Complex (Fig. 1), a network of ca. 11 calcareous fens situated within the B-T region that supports several of the most abundant and

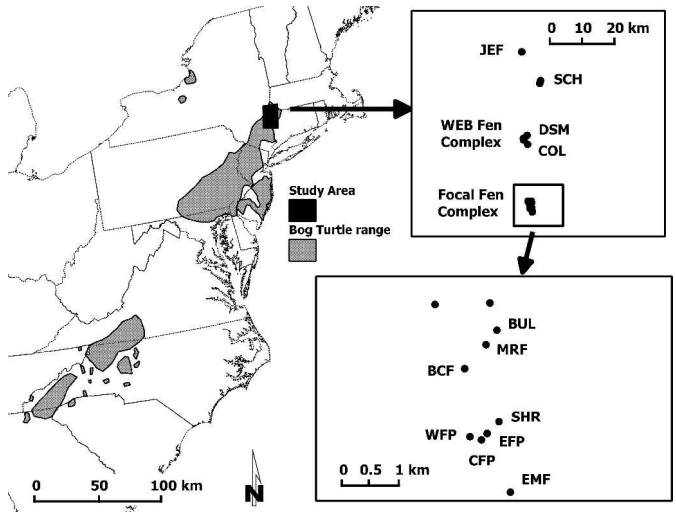

Fig. 1. Range map of the Bog Turtle, *Glyptemys muhlenbergii*, with insets detailing the locations of all sampled subpopulations. All study sites are identified by unique three-letter codes and (where applicable) are identified as belonging to one of two major fen complexes: the Focal Fen Complex and the WEB Fen Complex. Exact locations are withheld to protect populations from illegal poaching.

apparently viable Bog Turtle populations in New York (Jaycox and Breisch, 2006). Four additional sites were included to evaluate genetic structure at larger spatial scales: two sites (COL and DSM) within a separate complex of fens located ca. 20 km north of the Focal Fen Complex (the WEB Fen Complex), and two sites in Massachusetts located ca. 38 and 48 km (respectively) north of the Focal Fen Complex.

Survey methods and sample collection.—All New York study sites (n = 9) were surveyed one to two times per week from April 15 through August 15 of 2008 and 2009, during which turtles were hand-captured using visual and tactile methods (Whitlock, 2002). All turtles were uniquely marked by notching marginal scutes (method modified from Cagle, 1939). For each new capture, a blood sample (ca. 0.2 mL) was collected for DNA extraction. Blood was drawn from the caudal artery near the tail tip, and immediately placed in a preservative lysis buffer (buffer solution prepared following Longmire et al., 1997). At the Massachusetts study sites (n = 2) blood samples were collected in three separate visits in 2009, with turtles located via radiotelemetry. Blood samples were stored at −20°C prior to DNA extraction. Turtles were released at point of capture immediately after handling.

Genetic analysis.—DNA was extracted from blood samples using the DNeasy Blood and Tissue Kit (Qiagen Corporation, Valencia, CA). Each turtle was typed for 18 previously described microsatellite loci (King and Julian, 2004). Loci were selected from among those described by King and Julian (2004) based on consistent amplification success, polymorphic alleles over diverse Bog Turtle demes, and performance in multiplex PCR reactions (T. L. King, USGS, pers. comm.). Polymerase Chain Reaction (PCR) conditions followed the recommendations of King and Julian (2004), and PCR products were labeled with fluorescent tags and subsequently resolved using an automated capillary genetic analyzer (ABI 3730) by the core lab at the DNA Analysis Facility at Yale University (using Genescan 500-LIZ size standard). Allele peaks were visualized and called using

GENEMAPPER v.4.0 software (Applied Biosystems, Foster City, CA).

We tested for presence of null alleles and genotyping errors using MICRO-CHECKER (van Oosterhout et al., 2004), and used GENEPOP 4.0 (Raymond and Rousset, 1995) to test for deviations from Hardy-Weinberg (H-W) expectations and linkage disequilibrium among pairs of loci. Mean heterozygosity, allelic richness, and numbers of private alleles (alleles unique to a sampled population) were calculated for each locus using GenAlEx 6.2 (Peakall and Smouse, 2005). We tested for evidence of a historical bottleneck at any of the sampling sites using BOTTLENECK 1.2.02 (Cristescu et al., 2010), with the two-phased model of mutation (TPM) recommended by Luikart et al. (1998) for microsatellite data. We tested for differences in allelic richness and private alleles among sites using nonparametric bootstrap analyses, with samples rarefied to correct for differences in sample size among sites (R source code available on request). We assessed within-population inbreeding potential using likelihood-based relatedness analyses implemented in the software package ML-RELATE (Kalinowski et al., 2006). To minimize false-positives, pairs of individuals were only accepted as close-kin if the likelihood of the kin relationship was at least four log-likelihood units higher (ca. 2X the generally accepted cutoff for significance at $\alpha = 0.05$) than the likelihood of a non-kin relationship (individuals randomly sampled from the population). Unless otherwise mentioned, statistical analyses were performed in R (R Development Core Team, 2012) and significance of all statistical tests was based on a threshold of $\alpha = 0.05$.

We tested whether allele frequencies differed between pairs of fens using exact tests implemented in GENEPOP 4.0 (Raymond and Rousset, 1995) and using Weir and Cockerham's F_{ST} statistic (Φ_{ST}, Weir and Cockerham, 1984) calculated in FSTAT 2.9 (Goudet, 1995). We assessed regional genetic differentiation (genetic distance among fen complexes and among fens separated by >4 km) using Jost's D statistic (Jost, 2008). We further assessed local and regional genetic structure using Bayesian clustering algorithms (BAPS; Corander et al., 2004 and STRUCTURE 2.3.1.; Pritchard et al., 2000). We ran BAPS twice, first assigning individuals to clusters and then assigning sampling sites to clusters. Clustering results using STRUCTURE 2.3.1 were qualitatively similar to the BAPS results and are not discussed further. We assessed genetic isolation-by-distance (Wright, 1943) among all sampled sites by regressing pairwise F_{ST} values over geographic distance, with statistical significance determined using Mantel-type permutation tests (10,000 replications; Mantel, 1967), performed using R statistical software (''mantel.rtest'' function in the ade4 library).

We identified first-generation migrants using GeneClass2 (Piry et al., 2004), which was run using Rannala and Mountain's (1997) formulation to calculate the ratio of genotype likelihoods at the site of sample origin vs. other potential source populations. This test statistic was compared to a null distribution derived using the method of Paetkau et al. (2004) with 10,000 resampled individuals and $\alpha = 0.005$ (to minimize spurious results).

Simulation modeling.—Starting from a panmictic population in which genotypes were randomly sampled from observed global mean allele frequencies, and using information on Bog Turtle abundance, survival rate, and recruitment at our study sites (Shoemaker, 2011), we generated equilibrium microsatellite datasets (1000 years were sufficient to reach migration-drift equilibrium) under alternative plausible models of historical migration. We modeled four local-scale effective migration scenarios (1, 0.33, 0.2, and 0.02 individuals exchanged per year among the populations in the Focal Fen Complex) and three regional connectivity scenarios (Focal Fen Complex, WEB Fen Complex, and MA sites each exchange a single migrant with its nearest neighbor every 10, 20, and 100 years). We ran each dispersal model under a range of plausible effective population sizes (1X, 2X, and 3X current estimated abundance) and microsatellite mutation rates (drawn from Dallas, 1992 and Whittaker et al., 2003). A total of 25 equilibrium microsatellite datasets was generated per scenario (4 local dispersal rates × 3 regional dispersal rates × 3 abundance scenarios × 3 mutation scenarios = 108 scenarios), and summary statistics were compared with those calculated from the observed data (this approach is referred to as ''approximate likelihood;'' Beaumont et al., 2002). To gauge the overall data likelihood of each parameter combination, we chose a suite of four summary statistics with which to gauge the approximate likelihood of each simulation scenario: F_{ST} within the Focal Fen Complex (intended to assess genetic connectivity at a local scale); Jost's D among the four regional-scale ''populations'' (each population complex representing a single unit, intended to assess genetic connectivity at a regional scale); mean allelic richness per population; and mean number of private alleles per population. For each test statistic (e.g., F_{ST} within the Focal Fen Complex), the approximate likelihood of the observed test statistic was computed using the formula for a Gaussian distribution with mean and standard deviation derived from the simulation replicates ($n = 25$). Source code (in the R language for statistical computing; R Development Core Team, 2012) for the simulation models and approximate likelihood calculations is available on request.

RESULTS

In total, 234 tissue samples were collected from 11 fens in southeastern New York and southwestern Massachusetts (Table 1). Loci *Gmu*D28 and *Gmu*D93 did not amplify well, and were subsequently removed from the analyses. We found no evidence for null alleles or genotyping errors among the remaining 16 loci using MICRO-CHECKER. Allele frequencies within individual fens conformed to Hardy-Weinberg expectations, and exact tests for linkage disequilibrium indicated that loci *Gmu*D114 and *Gmu*D88 were linked for most populations. Therefore we removed locus *Gmu*D88, leaving 15 total microsatellite loci in the analyses (Table 2). These 15 loci exhibited low to moderate allelic diversity, with alleles per locus ranging from 2 to 11 (Table 2). Results from BOTTLENECK showed no evidence of a historical bottleneck for any of the study populations under either the Infinite-Allele Model (IAM) or the Stepwise-Mutation Model (SMM).

Allele frequencies differed among sites within the Focal Fen Complex and among fen complexes at a regional level ($P < 0.001$; exact test in GENEPOP), and global F_{ST} was larger than expected from a panmictic population (Mantel tests in FSTAT). All pairwise F_{ST} values were significant except for four pairs within the Focal Fen Complex involving site CFP, for which sample size was very small ($n = 5$). Comparison of allelic richness among populations, rarefied to account for

Table 1. Site-Specific Summary Statistics for Bog Turtle Genetic (Microsatellite) Samples Collected in the Berkshire-Taconic Region of New York and Massachusetts in 2008 and 2009.

Site code	*n*	Mean # alleles (sd)	H_O* (sd)	H_E* (sd)	# private alleles
TMR fen complex					
WFP	31	4.4 (0.6)	0.47 (0.06)	0.51 (0.06)	1
CFP	5	2.9 (0.4)	0.53 (0.08)	0.48 (0.06)	0
EFP	15	2.9 (0.4)	0.52 (0.08)	0.45 (0.06)	0
MRF	13	3.1 (0.4)	0.47 (0.07)	0.46 (0.05)	0
EMF	25	4.4 (0.5)	0.58 (0.06)	0.52 (0.06)	1
SHR	47	4.4 (0.6)	0.54 (0.07)	0.52 (0.06)	1
BUL	18	3.9 (0.5)	0.52 (0.07)	0.50 (0.06)	1
WEB fen complex					
DSM	25	4.4 (0.6)	0.49 (0.07)	0.50 (0.06)	1
COL	13	3.7 (0.4)	0.50 (0.07)	0.51 (0.06)	0
MA sites					
SCH	24	4.3 (0.6)	0.47 (0.06)	0.48 (0.06)	3
JEF	18	4.2 (0.6)	0.51 (0.08)	0.48 (0.08)	4

* H_O denotes observed heterozygosity. H_E denotes expected heterozygosity, computed based on Hardy-Weinberg (HW) expectation.

differences in sample size, suggested that allelic richness was substantially lower at the two sites with lowest estimated abundance (sites EFP and MRF; Fig. 2). Furthermore, these two low-abundance sites had a much higher proportion of close-kin pairs than other sites (from ML-RELATE), with 20 close-kin pairs out of 105 possible at site EFP and eight close-kin pairs out of 78 possible at site MRF compared to 70 close-kin pairs out 2333 possible for all other sites combined (G-test; $P < 0.001$).

A strong pattern of isolation-by-distance (IBD) was apparent within the B-T region after the two populations with lowest estimated abundances (Shoemaker, 2011) were removed from the analysis (Fig. 3; $R^2 = 0.70$). The IBD relationship was substantially weakened when the low-abundance populations were included in the analysis ($R^2 = 0.32$). However, Mantel permutation tests indicated that both IBD relationships were unlikely to be observed by chance ($P < 0.001$). In contrast, IBD was not apparent for sites separated by <5 km ($P = 0.23$). Similarly, the BAPS clustering algorithm grouped the individual Bog Turtle samples into eight genetically distinct clusters in which regional origin was successfully resolved (e.g., Massachusetts populations were nearly always successfully differentiated from New York populations) but local origin within population complexes was not successfully resolved (e.g., populations within the Focal Fen Complex were not successfully differentiated). Considering only those sampling locations within the Focal Fen Complex, the BAPS group-clustering algorithm grouped most fen units into a single cluster, with two of the smaller sites (sites EFP and MRF) and a more distant, high-abundance site (site EMF) designated as genetically distinct.

The approximate likelihood of the observed F_{ST} for the Focal Fen Complex was maximized at dispersal rates of

Table 2. Locus-Specific Summary Statistics for 15 Microsatellite Loci from 10 Bog Turtle Populations in the Berkshire-Taconic Region of New York and Massachusetts (*n* = 234).

Locus*	Mean # alleles (sd)	Mean H_O** (sd)	Mean H_E** (sd)	F_{IT}	F_{IS}	F_{ST}
*Gmu*A18	1.82 (0.12)	0.20 (0.05)	0.18 (0.05)	−0.110	−0.005	0.095
*Gmu*B08	1.36 (0.15)	0.04 (0.02)	0.04 (0.02)	−0.085	−0.021	0.058
*Gmu*B67	2.64 (0.15)	0.45 (0.05)	0.44 (0.04)	−0.027	0.142	0.164
*Gmu*B91	2.00 (0.00)	0.34 (0.05)	0.32 (0.04)	−0.084	−0.026	0.054
*Gmu*D107	6.82 (0.38)	0.74 (0.03)	0.74 (0.01)	0.003	0.097	0.094
*Gmu*D114	3.36 (0.34)	0.62 (0.04)	0.57 (0.03)	−0.097	−0.003	0.086
*Gmu*D121	4.09 (0.21)	0.52 (0.04)	0.50 (0.03)	−0.048	0.005	0.050
*Gmu*D16	4.91 (0.48)	0.66 (0.05)	0.64 (0.02)	−0.029	0.064	0.090
*Gmu*D40	6.00 (0.30)	0.79 (0.03)	0.76 (0.01)	−0.047	0.023	0.067
*Gmu*D55	5.00 (0.54)	0.63 (0.04)	0.61 (0.03)	−0.024	0.051	0.073
*Gmu*D70	3.18 (0.26)	0.56 (0.04)	0.53 (0.03)	−0.051	0.045	0.092
*Gmu*D79	2.00 (0.23)	0.12 (0.03)	0.14 (0.04)	0.150	0.235	0.100
*Gmu*D87	5.82 (0.30)	0.72 (0.05)	0.72 (0.02)	−0.009	0.089	0.097
*Gmu*D89	3.18 (0.30)	0.47 (0.06)	0.49 (0.06)	0.051	0.204	0.160
*Gmu*D90	2.64 (0.20)	0.44 (0.06)	0.41 (0.06)	−0.091	0.073	0.150

* All loci originally described by King and Julian (2004).
** H_O denotes observed heterozygosity. H_E denotes expected heterozygosity, computed based on Hardy-Weinberg (HW) expectation.

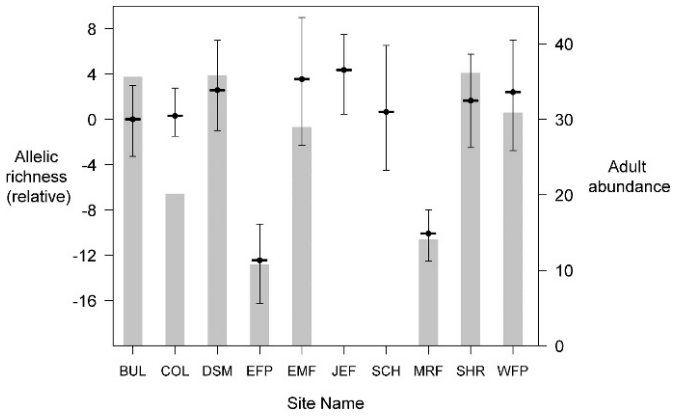

Fig. 2. Graphical summary of differences in allelic richness among ten Bog Turtle (*Glyptemys muhlenbergii*) populations from the Berkshire-Taconic valley region of New York and Massachusetts (see Fig. 1 for description of site acronyms), averaged for 15 microsatellite loci. Samples were rarefied to correct for differences in sample size among sites, and error bars represent 95% bootstrap confidence intervals. For comparison, adult abundance estimates (derived from Shoemaker, 2011) are overlayed as gray bars (abundance estimates were not available for sites JEF and SCH).

approximately 0.33 effective migrants per population per year (Fig. 4A). The approximate likelihood of the observed Jost D statistic among fen complexes separated by 10 km or more (genetic differentiation among more distant fen complexes) was maximized at a regional connectivity rate of one effective migrant between nearest-neighbor fen complexes per ten years, although the observed Jost D value was also somewhat consistent with a scenario of one effective migrant per 20 years (Fig. 4B). Effective population size and mutation rate had a smaller effect on local and regional genetic structure, although observed local genetic differentiation was slightly more likely under effective population sizes 2–3X greater than current estimates (Fig. 4A). Observed private alleles and per-site allelic diversity were equally plausible under a wide range of simulated scenarios, and simulated private allele totals were nearly always lower than observed values for all scenarios.

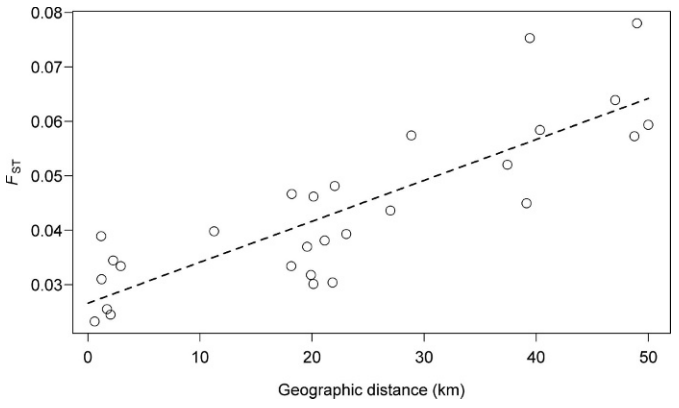

Fig. 3. Genetic isolation-by-distance (IBD) for Bog Turtle (*Glyptemys muhlenbergii*) populations ($n = 8$) within the Berkshire-Taconic valley region of New York and Massachusetts. A linear relationship (gray dashed line) explained ca. 70% of the variation in genetic distance (F_{ST}). A Mantel permutation test indicated that this IBD relationship was unlikely to be observed by chance ($P < 0.001$). The two lowest-abundance populations were removed for this analysis: see text for details.

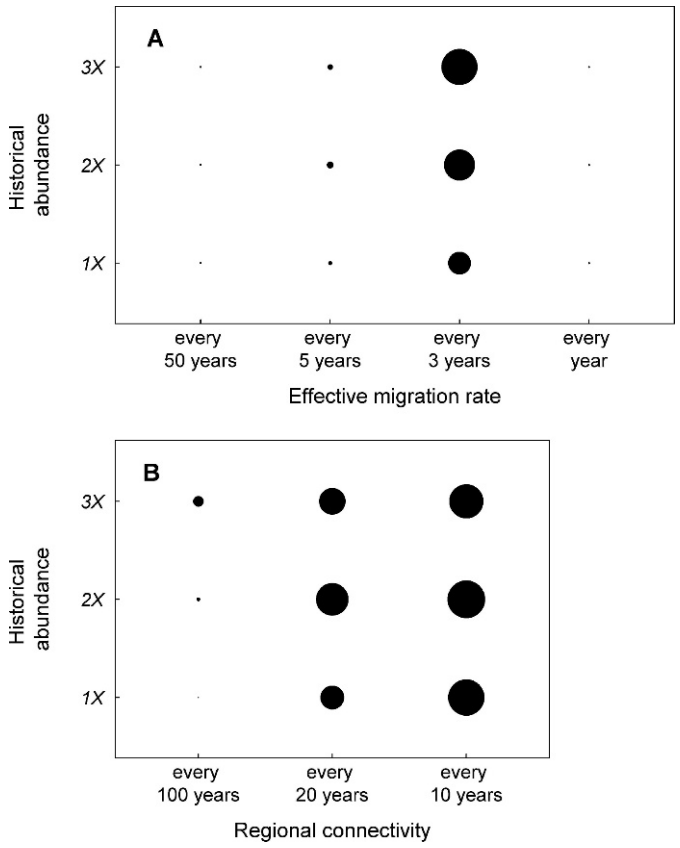

Fig. 4. Approximate likelihood of observed local (A; F_{ST}) and regional (B; Jost's D) genetic differentiation among Bog Turtle (*Glyptemys muhlenbergii*) populations in the Berkshire-Taconic region of New York and Massachusetts, under alternative scenarios of effective migration and historical abundance (from 1X to 3X current estimated levels). Higher-likelihood scenarios correspond to larger circles.

Approximately 75 to 100 years of complete genetic isolation would be necessary for genetic drift to produce F_{ST} values larger than those observed at the Focal Fen Complex, and approximately 125 years would be required under a scenario of incomplete genetic isolation (one effective migrant per population per ten years).

Nine sampled individuals (5 male, 4 female) were identified as first-generation migrants using GeneClass2. Similarly, 13 sampled individuals (8 male, 4 female, 1 juvenile) were likely to be close-kin (parent, offspring, or sibling) with an individual from a different fen unit within the Focal Fen Complex, four of which were also identified as migrants by GeneClass2. Overall, the proportion of putative dispersers (individuals originating in another population) in the sample from the Focal Fen Complex was 0.06 (9/148), while the proportion of putative dispersers within the three centrally located sites (sites WFP, SHR, and EFP) approached 0.10 (3/31, 3/47, and 1/15, respectively).

DISCUSSION

Assuming Bog Turtle populations at the Focal Fen Complex were in migration-drift equilibrium, we infer that among-population dispersal has occurred at a mean rate of approximately 0.33 individuals per year (approximately 1% of each population per year) over recent centuries (Fig. 4). Furthermore, Bayesian clustering algorithms, likelihood-based assignment tests and kinship tests suggest that

the Focal Fen Complex remains inter-connected via infrequent gene flow. With inter-fen distances at the Focal Fen Complex well within the range of the longest known straight-line distances traveled by Bog Turtles (Carter et al., 1999), we suggest that similar complexes of occupied fens with nearest-neighbor distances of 1–2 km or less are likely to be genetically linked, and that such population complexes may be most effectively managed as a single unit. Estimates of Bog Turtle gene flow at larger scales (e.g., 2 to 5 km) remain unavailable; it would be instructive to sample from a complex of Bog Turtle populations separated by larger mean nearest-neighbor distances although it is unknown whether such a complex exists for study.

Among-population dispersal may have been unable to counteract genetic drift at the lowest-abundance sites (<15 to 20 reproductive adults) within the Focal Fen Complex (sites MRF and EFP). Allelic richness was substantially lower at these sites (Fig. 2) despite some evidence of gene flow. Furthermore, genetic distance (pairwise F_{ST}) was much higher for pairs involving the lowest-abundance sites than for other pairs within the Focal Fen Complex, and a strong regional pattern of isolation-by-distance emerged only after these sites were removed from the analysis. Finally, a Bayesian site-clustering algorithm assigned these small sites to their own respective clusters, while designating a single cluster for most remaining fens in the Focal Fen Complex. These results raise concern that low-abundance sites may be vulnerable to loss of adaptive genetic diversity via strong genetic drift (Pearson et al., 2009), despite being situated within a genetically connected population complex.

Population genetics research is often initiated to identify movement rates, and in many cases these studies rely upon simple formulae to estimate dispersal rates (e.g., $F_{ST} = 1/[4N_m+1]$; Slatkin, 1985; Whitlock and McCauley, 1999). However, estimation of dispersal rates from genetic data can be problematic due to assumptions that are rarely met in nature (e.g., Wright's island model, discrete generations, absence of mutation, equal population sizes; see Whitlock and McCauley, 1999). Fortunately, key demographic drivers of genetic drift (e.g., abundance, recruitment, and survivorship) have been estimated at our study sites (Shoemaker, 2011), enabling us to build a more realistic demographic/genetic model for estimation of dispersal rates. However, we retained the assumptions that (1) populations were at migration-drift equilibrium (MDE), (2) population sizes were stable over time, (3) populations exchanged migrants symmetrically, and (4) the system was closed (i.e., no additional historical or undiscovered populations within the study area). We cannot rule out a non-equilibrium scenario at the Focal Fen Complex; recent barriers to movement (i.e., 75 to 100 years of isolation) could produce the observed genetic differentiation (F_{ST}) results. However, our study landscape has experienced consistent and extensive agricultural use since the 18th century and there is no evidence of major changes to our study landscape over the past 75 to 100 years (Cronon, 1983; Vispo and Knab-Vispo, 2009). Therefore, we believe the MDE assumption is reasonable in this case.

In seeming contrast to genetic evidence which implies high connectivity among the fens of the Focal Fen Complex, ten years of capture–recapture (CR) research involving >25 individual surveyors have failed to record a single Bog Turtle outside its site of origin (Shoemaker, 2011). A similar paradox has been noted for the congeneric wood turtle (*Glyptemys insculpta*), where population genetics research concluded that turtle populations at Delaware Water Gap National Park in Pennsylvania for which years of CR research had failed to detect migration were effectively panmictic (Castellano et al., 2009). Using estimated capture rates for Bog Turtles at the Focal Fen Complex (annual adult capture rates ca. 0.70 for most years; Shoemaker, 2011), we calculate that hypothetical adult dispersers from 2006, 2007, or 2008 would have been detected with >90% probability (provided they dispersed within our four long-term study sites) using our methods. However, we were unlikely to have detected migration of juveniles (lower capture efficiency; Shoemaker, 2011) or turtles migrating to or from locations other than our four long-term study sites. Therefore, juvenile-dominated dispersal, which has been reported for the closely related wood turtle (Castellano et al., 2009) and some other reptiles (e.g., Welsh et al., 2010), may help resolve this paradox. In addition, rare and episodic periods of high dispersal could explain the apparent disparity between population genetics and CR data at our study sites, as such episodes may go undetected by CR methods (Slatkin, 1985). In fact, there is some evidence that Bog Turtle dispersal may spike during periods of drought (Faega, 2010).

At a regional scale, our data were consistent with a scenario of moderate historical connectivity within the Berkshire-Taconic valley. In our simulation models, individual turtles were allowed to disperse among sites situated 10 to 20 km apart, well above the longest-observed dispersal distance for Bog Turtles. A more realistic model of regional connectivity for Bog Turtles would incorporate intermediate ''stepping-stone'' populations, each separated from its nearest neighbor by a reasonable dispersal distance. Such a stepping-stone model would be consistent with the observed pattern of genetic isolation by distance (Wright, 1943; Slatkin, 1993). Furthermore, a stepping-stone model may help to explain why our simulation model tended to underestimate allelic diversity and numbers of private alleles at plausible historical levels of effective population size, mutation, and migration: the intermediate ''stepping stone'' wetlands (and undisovered populations; see below) which were left out of the model may have provided the additional effective population size necessary to support regional allelic richness more consistent with observed values.

Although it is likely that many of the ''stepping-stone'' populations that contributed to the observed regional isolation-by-distance pattern are no longer extant, we suspect that some undiscovered extant populations remain within the B-T region. Several populations have been discovered in other parts of the B-T region in recent years (J. Tesauro, Environmental Defense Fund, pers. comm.), and as part of this study a new, relatively abundant population was discovered (site BUL) within the well-studied Focal Fen Complex. Conservation of inter-connected complexes of Bog Turtle populations depends upon knowledge of where population complexes and ''stepping-stone'' wetlands persist. Consequently, there is a critical need to document extant but undiscovered Bog Turtle sites.

In this study, we have documented substantial genetic connectivity among discrete bog turtle sites at two spatial scales. Therefore we suggest expanding the focus of Bog Turtle conservation planning to include (1) population complexes and (2) regional networks of population complexes (e.g., preserving ''stepping stone'' wetlands as critical dispersal habitat). Ultimately, most effective would be a

loosely connected regional network of Bog Turtle complexes, which the genetic evidence suggests was the norm prior to European settlement. Despite the time and effort involved in building such a network, many components are already in place. Several populations within the B-T region appear to be stable and viable for the next century (Shoemaker, 2011), and we have shown that at least one extant complex (Focal Fen Complex) continues to support moderate inter-population gene flow. Timely implementation of regional-scale conservation planning for small-bodied turtles such as the Bog Turtle has great potential to preserve and maintain viable population networks with high genetic diversity and evolutionary potential.

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