Influence of sampling scheme on the inference of sex-biased gene flow in the American badger (*Taxidea taxus*)

E.M. Kierepka, E.K. Latch, and B.J. Swanson

Abstract: Population genetics has fueled a substantial growth in studies of dispersal, a life-history trait that has important applications in ecology and evolution. Mammals typically exhibit male-biased gene flow, so this pattern often serves as a null hypothesis in empirical studies. Estimation of dispersal using population genetics is not without biases, so we utilized a combination of population genetic methods and simulations to evaluate gene flow within the American badger (*Taxidea taxus* (Schreber, 1777)), a highly elusive and poorly understood mustelid. A total of 132 badgers captured between 2001 and 2002 were genotyped at nine microsatellite loci to investigate fine-scale genetic structure consistent with philopatry in females and dispersal in males. Resultant genetic patterns were largely consistent with a panmictic population with little evidence for sex-biased dispersal, and simulations confirmed that our sampling scheme did not substantially impact our statistics. An overall deficiency of heterozygotes was observed across the Lower Peninsula, which indicates either a Wahlund effect, mixing of separate populations, or inbreeding. Our study emphasizes the importance in deciphering between actual behavioral mechanisms and sampling effects when interpreting genetic data to understand other factors that influence dispersal like population density and territoriality.

Key words: Taxidea taxus, American badger, sex-biased dispersal, sampling, spatial autocorrelation.

Résumé : La génétique des populations a entraîné une augmentation marquée des études sur la dispersion, un caractère du cycle biologique qui offre d'importantes applications en écologie et en science de l'évolution. Les mammifères présentent typiquement un flux génétique biaisé vers les mâles, cette situation servant donc souvent d'hypothèse nulle dans les études empiriques. L'estimation de la dispersion à l'aide de la génétique des populations n'est pas sans biais. C'est pourquoi nous avons utilisé une combinaison de méthodes de génétique des populations et de simulations pour évaluer le flux génétique chez le blaireau d'Amérique (*Taxidea taxus* (Schreber, 1777)), un mustélidé très discret et méconnu. Un total de 132 blaireaux capturés de 2001 à 2002 ont été génotypés sur neuf sites microsatellites afin d'étudier la structure génétique fine associée à la philopatrie chez les femelles et à la dispersion chez les mâles. Les patrons génétiques en découlant concordaient globalement avec une population panmictique, mais très peu avec une dispersion biaisée selon le sexe. Des simulations ont en outre confirmé que notre schéma d'échantillonnage n'avait pas une incidence importante sur les statistiques obtenues. Un déficit global d'hétérozygotes a été observé à l'échelle de la péninsule inférieure du Michigan, ce qui indique soit un effet Wahlund, soit le mélange de populations distinctes, soit de la consanguinité. Notre étude souligne l'importance de distinguer les mécanismes comportementaux réels des effets d'échantillonnage au moment d'interpréter des données génétiques afin de comprendre les autres facteurs qui influencent la dispersion, tels que la densité de population et la territorialité.

Mots-clés : Taxidea taxus, blaireau d'Amérique, dispersion biaisée selon le sexe, échantillonnage, autocorrélation spatiale.

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Introduction

Natal dispersal and resultant patterns in gene flow has been recognized as a critical process in ecology and evolution because of its influence on internal population dynamics and interpopulation connectivity (Hanski 1999; Clobert et al. 2001; Heinz et al. 2006). Consequently, these broad applications have fueled considerable research across both theoretical and applied fields, particularly within birds and mammals (e.g., Lawson Handley and Perrin 2007; Clobert et al. 2009). As data on patterns of dispersal and gene flow continue to accumulate, generalities have emerged among taxa that can be used as core assumptions when investigating gene-flow patterns in unstudied species. In mammals, for example, predominately male-biased dispersal tends to generate contrasting patterns of genetic structure between the sexes. Typically, male-biased dispersal leads to high rates of gene flow and weak fine-scale genetic structure relative to females

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E.M. Kierepka* and B.J. Swanson. Department of Biology, Central Michigan University, Mount Pleasant, MI 48859, USA.
E.K. Latch. Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, WI 53211, USA.

Corresponding author: Elizabeth Kierepka (e-mail: Liz.Kierepka@gmail.com).

*Present address: Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, WI 53211, USA.

(e.g., Chappell et al. 2004; Solmsen et al. 2011). Although some exceptions to this scenario have been recorded (e.g., Pérez-González and Carranza 2009; Frantz et al. 2010), male-biased gene flow remains the most common dispersal pattern in mammals (Lawson Handley and Perrin 2007), and thus serves as a null hypothesis for unstudied mammal species.

Genetic methods are a powerful tool for estimating dispersal and have been fundamental to our understanding of sex-biased dispersal and underlying patterns of genetic structure, particularly in understudied species (Prugnolle and de Meeus 2002). Indirect dispersal estimation is not without its limitations and a number of authors have pointed out potential biases in dispersal estimation using genetic methods (Beerli 2004; Broquet and Petit 2009). Biases associated with sample collection (e.g., Latch and Rhodes 2006; Schwartz and McKelvey 2009) are of particular concern in conservation, where sampling is often opportunistic. Ideally, sample collection should occur at a scale appropriate to capture genetic variation across the biological process of interest (e.g., Gauffre et al. 2008). Considering that genetic structure can vary across both time and space (e.g., Bowen et al. 2005; Latch and Rhodes 2006), sample collection should be distributed across these variables and include samples from multiple seasons, sex classes, and age classes collected across the landscape. Opportunistically collected samples limit control over the sampling scheme, making it difficult to determine whether biases have been introduced. If sample collection does introduce a bias, departures from random mating may not correspond to expectations based on behavior or physical barriers to gene flow (Latch and Rhodes 2006; Cushman and Landguth 2010).

Deciphering between potential sampling biases and biologically relevant processes often requires comparisons with pre-existing life-history data or direct measures of dispersal to determine the biological relevance of significant statistical tests. For understudied species, such field data is often limited or completely absent, so another option is to draw on simulations to generate realistic situations for comparison. Although simulation studies have been more commonly used to evaluate statistics or model assumptions, authors have suggested that simulations can provide promising comparisons to empirical data (e.g., Epperson et al. 2010). One application of simulations is to explain deviations from generalities found across similar species. For example, Gauffre et al. (2008) assumed a large motorway would cause genetic subdivision in voles because such roads often act as impermeable barriers to many species of small mammals. Through simulations of barrier formation and effective population size, the authors showed that despite the field evidence for barrier effects of motorways, the large effective population size in voles prevented any genetic signature of isolation (Gauffre et al. 2008). Utilizing simulations to help distinguish among alternative hypotheses could be particularly useful for understanding gene-flow patterns in understudied species.

The American badger (*Taxidea taxus* (Schreber, 1777)) is a good candidate species for investigating the best practices for identifying and alleviating potential sampling biases when characterizing gene-flow patterns for two reasons. First, mammalian patterns of male-biased dispersal are well documented, providing an appropriate prediction for badgers. Little data exists for dispersal patterns in badgers, in large part due to their nocturnal, semifossorial lifestyle. Maximum dispersal distances of 125 km for males and 75 km for females suggest that badgers may exhibit the typical pattern of male-biased dispersal like other mammals (Messick and Hornocker 1981). Home-range size and dimorphism between sexes vary substantially across their range, but overall, spacing patterns in badgers appear to be consistent with mate defense polygyny (Minta 1993). Mate defense polygyny is considered a likely explanation for the high incidence of male-biased dispersal in mammals (Greenwood 1980), so this mating system may be expected to yield similar dispersal patterns in badgers. Second, sampling elusive species at low abundance necessitates opportunistic sample collection. For badgers, samples are most often collected from road-killed animals (most often collected during dispersal) or from trapping (during an annual fall–winter trapping season). We sampled animals in the Lower Peninsula of Michigan, where trapped animals were available for sampling. This was somewhat unique for the upper Midwest; badgers are of conservation concern in much of the region and under protection in most of the surrounding populations. The resulting opportunistic sampling regime calls for careful consideration of potential sampling biases to distinguish sampling effects from biologically relevant processes. In this study, we utilized simulations to develop null hypotheses for population genetic structure, given the sampling scheme. Comparisons between the patterns of genetic structure in the simulated populations and those observed in Michigan badgers permitted characterization of genetic structure and gene flow in light of the sampling scheme.

Materials and methods

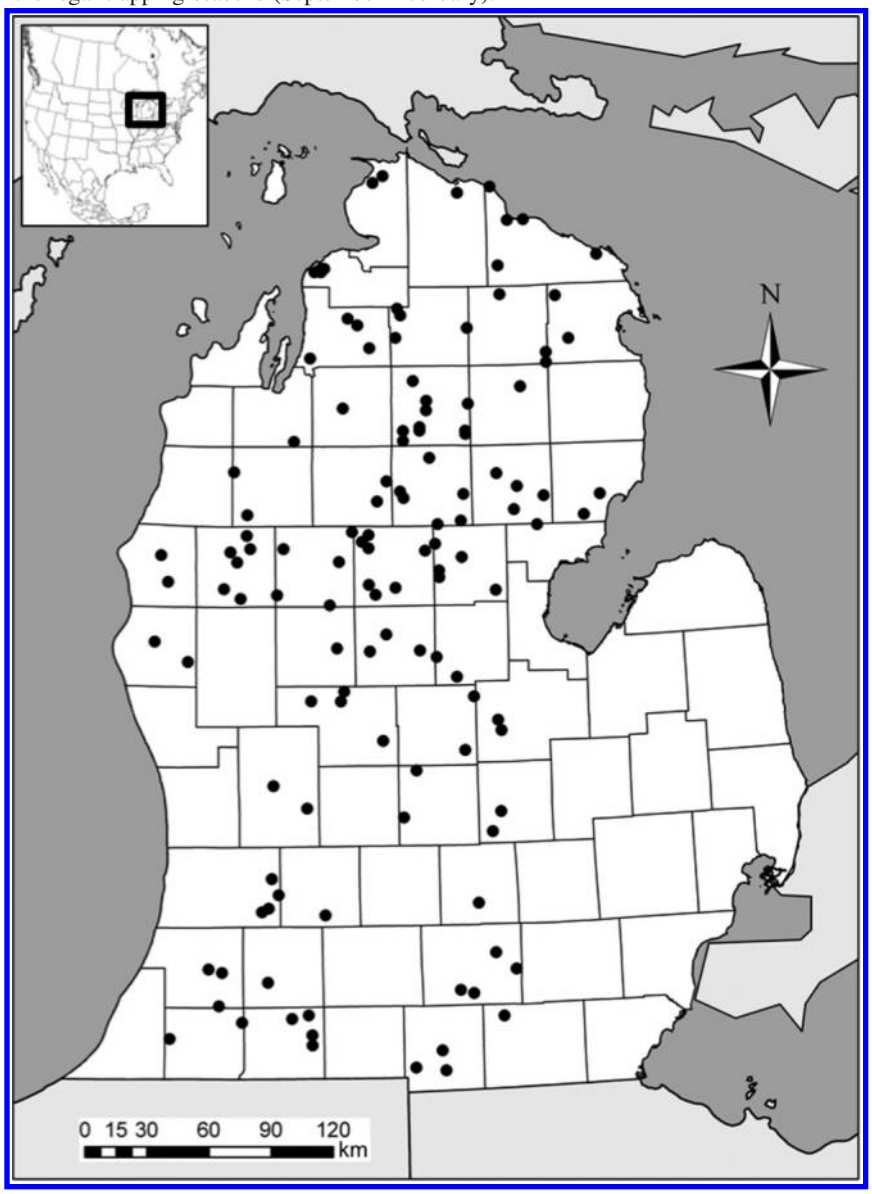
Sample collection

From 2001 to 2002, the Michigan Department of Natural Resources (DNR) obtained 132 (64 female, 68 male) badger tissue samples in the Lower Peninsula (Fig. 1), primarily in October and November. Unlike some genetic studies of mesocarnivores that rely on road-killed individuals, all 132 badgers were collected by trapping during the legal season. The location of each individual was estimated from the Michigan Public Land Survey System (PLSS) sections, which are 2.59 km^2 (1 square mile) in area. To obtain latitude and longitude coordinates for each individual, we randomly chose a point within each PLSS section using Hawth's tools (Beyer 2004) in ArcGIS version 9.2.

Laboratory techniques

Genomic DNA was extracted using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Valencia, California, USA) and quantified using an Eppendorf Biophotometer (Brinkman Instruments Inc., Westbury, New York, USA). Nine polymorphic microsatellite loci previously developed in American badger (*Tt-1*, *Tt-2*, *Tt-3*, *Tt-4*: Davis and Strobeck 1998), wolverine (*Gulo gulo* (L., 1758); *Gg-234*: Duffy et al. 1998; *Gg-443*, *Gg-465*: Walker et al. 2001), American marten (*Martes americana* (Turton, 1806); *Ma-1*: Davis and Strobeck 1998), and American mink (*Neovison vison* (Schreber, 1777); *Mvis072*: Fleming et al. 1999) were amplified from each sample via polymerase chain reaction (PCR). These markers

Fig. 1. Locations of 132 trapped American badgers (*Taxidea taxus*) within the Lower Peninsula of Michigan. All individuals were captured between 2001 and 2003 in the legal trapping seasons (September–February).



were selected because they are highly polymorphic in western populations (Kyle et al. 2004). PCRs were carried out in 10 μL reactions containing 75 ng genomic DNA, 0.25 mmol/L dNTPs, 0.16 μmol/L of each primer (forward and reverse), and 0.5 units of *Taq* DNA polymerase in 1× *Taq* buffer. Each amplification involved an initial denaturation of DNA for 2 min at 94 °C followed by 31 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at the primer-specific temperatures (51 °C for locus *Gg-465*, 53 °C for the remaining eight loci), and extension of the DNA product for 30 s at 72 °C, followed by a final extension at 72 °C for 2 min. PCR products were size-scored with an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and analyzed using GENESCAN ANALYSIS version 3.1.2 and GENOTYPER version 2.0 (Applied Biosystems, Foster City, California, USA). For quality control, we repeated the above procedure for all observed homozygotes and 10% of heterozygotes for each locus to ensure accurate genotypes. In total, we repeated 521 genotypes and identified only 5 cases of allelic dropout, all at locus *Gg-443*. We also corrected two additional genotypes that were incorrectly called during initial genotyping owing to the presence of stutter bands. Thus, the total genotyping error rate for the data set was 7/521 or 1.3%.

Overall genetic structure

To test for deviations from random mating among the 132 badgers sampled from the Lower Peninsula, Fisher's exact test was used to test for significant departures of allele frequencies from expectations under Hardy–Weinberg equilibrium (HWE) and linkage equilibrium (LE) in GENEPOP version 4.3 (Raymond and Rousset 1995). To correct for multiple tests in HWE and LE tests, we employed the false discovery rate (FDR) method of Benjamini and Yekutieli (2001) to minimize type II errors (Narum 2006). Number of alleles per locus, observed and expected heterozygosities, and

F_{IS} values were quantified in the total data set and for each sex separately in SPAGeDi version 1.3 (Hardy and Vekemans 2002). Permutation tests for an excess of heterozygotes were undertaken using 10 000 simulations in SPAGeDi. A total population that is genetically structured (caused either by isolation by distance (IBD) or by the presence of discrete barriers to gene flow) is expected to contain an excess of heterozygotes (deviations from HWE and LE and positive F_{IS} values) relative to expectations for a single, randomly mating population (Wahlund effect; Wahlund 1928).

Two Bayesian clustering programs (spatially implicit and spatially explicit) were used to determine the number of distinct badger populations within the Lower Peninsula of Michigan. First, STRUCTURE version 2.3 (Pritchard et al. 2000) was used to partition individuals into clusters (K) without a priori information using the admixture and correlated alleles models. STRUCTURE was run for $K = 1$–10 using 500 000 iterations after a burn-in of 100 000 iterations. This process was repeated 10 times at each value of K. The most likely K was chosen as the one with the highest likelihood while maintaining a relatively low variance among runs (Pritchard et al. 2000). If likelihood values suggested $K > 1$, Evanno et al. (2005)'s ΔK method was used, where the true K has the highest second-order rate of change in the likelihoods between successive values of K. To corroborate the results from STRUCTURE, we compared the results with a spatially explicit, Bayesian program, GENELAND (Guillot et al. 2005). Like STRUCTURE, we performed runs from $K = 1$–10 with 10 runs at each K. Every GENELAND run consisted of 500 000 (100 000 stored, thinning = 5) iterations with a spatial uncertainty of 2.0 km. This uncertainty estimate was incorporated to account for any ambiguities concerning the public land survey coordinates. Each run assumed correlated alleles and allowed for null alleles within the data set. Post processing included calculation of posterior probabilities for each pixel of the 100 pixel × 100 pixel domain after a burn-in of 100 stored iterations.

Patterns of gene flow and sex-biased dispersal

For many species, genetic structure is a function of the spatial relationships between them, with genetic differentiation increasing with geographic distance (Wright 1943). This IBD can be estimated by regressing pairwise genetic distance on pairwise geographical distance. When both sexes disperse, the overall pattern of IBD may be strong and observed in the total sample. When only one sex disperses, the dispersing sex may generate stronger IBD patterns than the philopatric sex, resulting in differences in the slope of the regression (e.g., Knight et al. 1999). Sex-biased dispersal is common in mammals and most often males are the dispersing sex. Although limited field evidence exists for badgers, dispersal may be male-biased (Messick and Hornocker 1981). IBD and sex-biased dispersal in badgers were tested using a Mantel test of matrix correlation for the total population and for each sex separately. Pairwise matrices of genetic distance (F_{ij}; Loiselle et al. 1995) and Euclidean distance were generated in SPAGeDi and GenALEx version 6.3 (Peakall and Smouse 2006). F_{ij} was selected as the most appropriate genetic distance estimator for this study because of its low sampling variance and no assumption of HWE (Vekemans and Hardy 2004). Statistical significance for Mantel tests was assessed using a Pearson's correlation coefficient calculated in the program ZT with the Manteltester GUI frontend (Bonnet and Van de Peer 2002) after 100 000 randomizations.

Sex-biased dispersal is likely to arise as a mechanism to avoid breeding with close relatives and (or) kin competition (Perrin and Mazalov 2000). As such, the scale at which the sex bias in dispersal patterns occurs may be small because the dispersing sex may not have to move very far to avoid relatives. For example, Dharmarajan et al. (2009) found genetic differentiation between habitat patches as well as fine-scale genetic structure in females consistent with sex-biased dispersal in raccoons (*Procyon lotor* (L., 1758)), but no IBD across the entire study area. In these cases, spatial autocorrelations, which investigate the presence of genetic structuring at a range of geographic distance classes, may be better at identifying sex-biased dispersal. Indeed, they have proven to be useful in characterizing sex-biased dispersal patterns at very small scales in other mesocarnivores (e.g., Dharmarajan et al. 2009; Croteau et al. 2010). Thus, sex-biased dispersal was also assessed in our study using spatial autocorrelations. Using Euclidean geographic distance and F_{ij} genetic distance, spatial autocorrelations were conducted in GenALEx for the total population, males, and females by calculating a spatial autocorrelation coefficient (r) within predefined distance classes (Smouse and Peakall 1999). Although the choice of the number of size of distance classes is somewhat arbitrary, a minimum of 30 pairwise comparisons per distance class is recommended to allow for sufficient sample sizes to detect genetic structure at small distances (Fortin et al. 1989; Smouse and Peakall 1999). Distance classes of 10 km were used in this study in accordance with these requirements. Autocorrelation coefficients range from –1 to 1, where 0 indicates the absence of any relationship. Significance was assessed using both a permutation test and a bootstrap test. First, confidence limits for the null hypothesis of a random distribution of genotypes in space were generated by 10 000 random permutations of the data. Permuted values of r were then sorted and compared with the observed value. Observed correlations outside the permuted 95% confidence interval were considered to be statistically significant. Second, 95% confidence intervals around the observed r were estimated using 10 000 bootstraps of the data by sampling with replacement from comparisons within a given distance class. Confidence intervals that did not include zero indicated the presence of spatial genetic structure at the focal distance class. Although the bootstrap test is less powerful than the permutation test because of pseudoreplication, it is more conservative than the permutation test for small sample sizes (Peakall et al. 2003).

For species that exhibit sex-biased dispersal, the philopatric sex is expected to show a pattern of increasing genetic relatedness with increasing geographic proximity. This pattern was tested in two ways. First, to assess whether related individuals are more often found at small distance classes, relatives were identified by generating 10 000 pairs of unrelated individuals in KINGROUP version 2 (Konovalov et al. 2004) based on the observed allele frequencies in the total data set. Relatedness values (r_{xy}; Queller and Goodnight 1989) were then calculated for the simulated unrelated individuals and the resultant null distribution was used to identify pairs of putative relatives in the observed sample. Relatives were de-

fined as individuals that had r_{xy} values greater than 95% of the unrelated individuals' r_{xy} values ($r_{xy\text{-related}} > 0.435$). For all pairs of putative relatives, the Euclidean distance between the individuals was recorded in the total data set and for each sex separately. Second, differences between the sexes in the distribution of pairwise geographic distances between relatives (as defined above) were tested. Geographic distances for each pair of relatives were plotted and a two-sample Kolmogorov–Smirnov test was used to assess differences in the two distributions. If badgers do follow the typical mammalian dispersal pattern of male-biased dispersal, the distributions of geographic distances for related males and females should differ, with geographic distances in females peaking at smaller geographic distances than males.

Effect of sampling scheme

Badgers are highly elusive and are not heavily trapped within Michigan, so the availability of trapped individuals was limited. To investigate the role that sampling scheme played in our analyses, we simulated panmictic populations and compared results obtained from those populations to the observed data. One hundred independent, randomly mating populations with 3000 individuals were simulated in the program EASYPOP version 2.0.1 (Balloux 2001). Each population started with a possible 10 alleles per locus and then was run for 300 generations to achieve mutation–drift equilibrium. From each of the simulated populations of 3000 individuals, 132 individuals were randomly selected and assigned geographic locations and sexes from the observed data set. Therefore, the simulated data sets had the same geographic locations and sexes as the empirical data set, but were known to be panmictic. Mantel tests, spatial autocorrelations, Kolmogorov–Smirnov tests, F_{IS} estimates, and Bayesian clustering were then performed on the simulated samples as described above to characterize patterns of spatial genetic structure (or lack thereof) in panmictic populations with the observed sampling scheme.

Results

Overall genetic structure

After correction for multiple testing, significant departures from HWE were detected in two loci ($p_{Ma1} = 0.002$ and $p_{Gg\text{-}234} = 0.006$), while one pair of loci (*Tt-3* × *Mvis072*) exhibited a significant deviation from LE ($p < 0.001$). Despite these deviations from HWE and LE, all loci were retained for all analyses because (*i*) population substructure can cause deviations from HWE and LE, and thus we did not want to exclude potentially informative loci, and (*ii*) these same loci were not shown to deviate from HWE or LE in a study of badgers from the Pacific Northwest (Kyle et al. 2004), despite that study encompassing a lower sampling density (similar number of samples over a larger geographic scale) than our study. Genetic diversity estimates are provided in Table 1. F_{IS} values averaged over all loci ranged from 0.080 in the population of males to 0.098 in females and were statistically significant in all tests. In the total data set, F_{IS} estimates for individual loci ranged from 0.025 to 0.162. Although these values were calculated for a single locus in a single population, and thus do not permit statistical significance testing, applying the standard deviation from the overall F_{IS} value (averaged over all loci; 0.025) to the locus-specific estimates indicated that all loci but one (*Tt-4*) exhibited heterozygote deficiencies.

Concordant results indicating a single genetic population were obtained from both Bayesian clustering programs. All 10 replicates for each program yielded the highest likelihood at $K = 1$, and decreasing likelihoods with higher variance among runs at successive Ks. For $K = 2$ runs, likelihoods were often similar to $K = 1$, and were characterized by a single large cluster (containing approximately 85% of the samples) and a much smaller cluster with individual membership that varied among runs. This is indicative of spurious clustering (Evanno et al. 2005; Latch et al. 2006; Frantz et al. 2009), and thus, $K = 1$ was selected as the most likely number of clusters.

Patterns of gene flow and sex-biased dispersal

None of the tests for IBD indicated any deviation from panmixia. In the total population, no pattern of IBD was observed (Mantel test, $r = 0.032$, $p = 0.135$). Although a significant spatial autocorrelation for the total population was observed at the smallest distance class ($p_{10\ km} = 0.020$), this was likely due to the presence of one putative parent–offspring pair. This pair consisted of a juvenile male and an adult male captured in the same location within 2 days of one another, and with a relatedness value of 0.6 that far exceeds the value of $r = 0.435$ used as a cutoff for related individuals, and was the highest pairwise relatedness value in the entire data set. Once the juvenile of this pair was removed from the analysis, the spatial autocorrelation was no longer significant ($p_{10\ km} = 0.072$).

Similarly, none of the tests indicated any sex biases in patterns of gene flow. Neither males nor females exhibited correlations between genetic and geographic distance ($r_{males} = 0.058$, $p = 0.114$; $r_{females} = 0.020$, $p = 0.281$). At a smaller scale, no pattern of spatial autocorrelation was observed in the total data set and for either sex (Fig. 2). Females exhibited only a single positive spatial autocorrelation at a scale of 60 km ($p_{60\ km} = 0.026$). Males had a positive spatial autocorrelation at 40 km ($p_{40\ km} = 0.016$) and at the smallest distance class ($p_{10\ km} = 0.028$), although the latter again was likely due to the inclusion of a parent–offspring pair and was no longer significant when the juvenile from the pair was removed ($p_{10\ km} = 0.065$). Only males exhibited a negative autocorrelation at the large distance classes (150 km; $p_{males} = 0.011$). Males also showed a slight negative autocorrelation at 100 km ($p = 0.023$). The lack of any strong trends in the genetic correlations at any distance class suggests a very large genetic population (>150 km), although the possibility of significant genetic structure below the smallest distance class used in this study (<10 km) cannot be ruled out. Finally, no male-biased dispersal was inferred from Kolmogorov–Smirnov tests to compare the distributions of pairwise geographic distances between related males and females ($D = 0.081$, $p = 0.901$).

Effect of sampling scheme

In the simulated data sets, no departures from panmixia were detected using Mantel tests (all $p > 0.115$) or Kolmogorov–Smirnov tests (all $p > 0.562$). Spatial autocorrelations in the simulated panmictic populations incorrectly

Table 1. Allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and deviation from random mating (F_{IS}) over nine microsatellite loci within all Lower Peninsula American badgers (*Taxidea taxus*), as well as males and females separately.

Locus	*n*	A_R	H_O	H_E	F_{IS}	*p*
Total						
Ma-1	132	3.999	0.455	0.540	**0.162**	**0.002**
Gg-465	131	7.969	0.519	0.576	0.102	0.042
Gg-234	132	8.885	0.598	0.694	**0.142**	**0.006**
Tt-1	132	5.000	0.652	0.681	0.047	0.240
Tt-2	129	4.969	0.589	0.651	0.098	0.073
Tt-3	129	9.984	0.705	0.783	0.103	0.015
Tt-4	132	11.923	0.667	0.681	0.025	0.513
Gg-443	127	9.000	0.685	0.745	0.085	0.061
Mvis072	132	6.000	0.705	0.755	0.071	0.091
Overall	132	7.525	0.619	0.678	**0.091**	**<0.001**
Males						
Ma-1	68	3.971	0.515	0.515	0.009	0.515
Gg-465	67	7.000	0.507	0.594	0.153	0.031
Gg-234	68	6.969	0.603	0.683	0.124	0.064
Tt-1	68	5.000	0.662	0.695	0.056	0.284
Tt-2	67	3.985	0.627	0.636	0.022	0.442
Tt-3	66	9.000	0.606	0.757	**0.206**	**0.002**
Tt-4	68	9.941	0.647	0.656	0.021	0.439
Gg-443	66	9.000	0.712	0.753	0.062	0.204
Mvis072	68	6.000	0.721	0.748	0.043	0.303
Overall	68	6.763	0.622	0.671	**0.080**	**0.001**
Females						
Ma-1	64	3.953	0.391	0.557	**0.306**	**0.003**
Gg-465	64	6.859	0.531	0.555	0.051	0.307
Gg-234	64	6.906	0.594	0.701	0.161	0.023
Tt-1	64	4.951	0.641	0.659	0.036	0.381
Tt-2	62	3.840	0.548	0.656	0.172	0.043
Tt-3	63	8.968	0.810	0.783	–0.026	0.714
Tt-4	64	10.902	0.688	0.702	0.029	0.393
Gg-443	61	8.000	0.656	0.733	0.114	0.070
Mvis072	64	5.998	0.688	0.749	0.091	0.129
Overall	64	6.725	0.616	0.677	**0.098**	**<0.001**

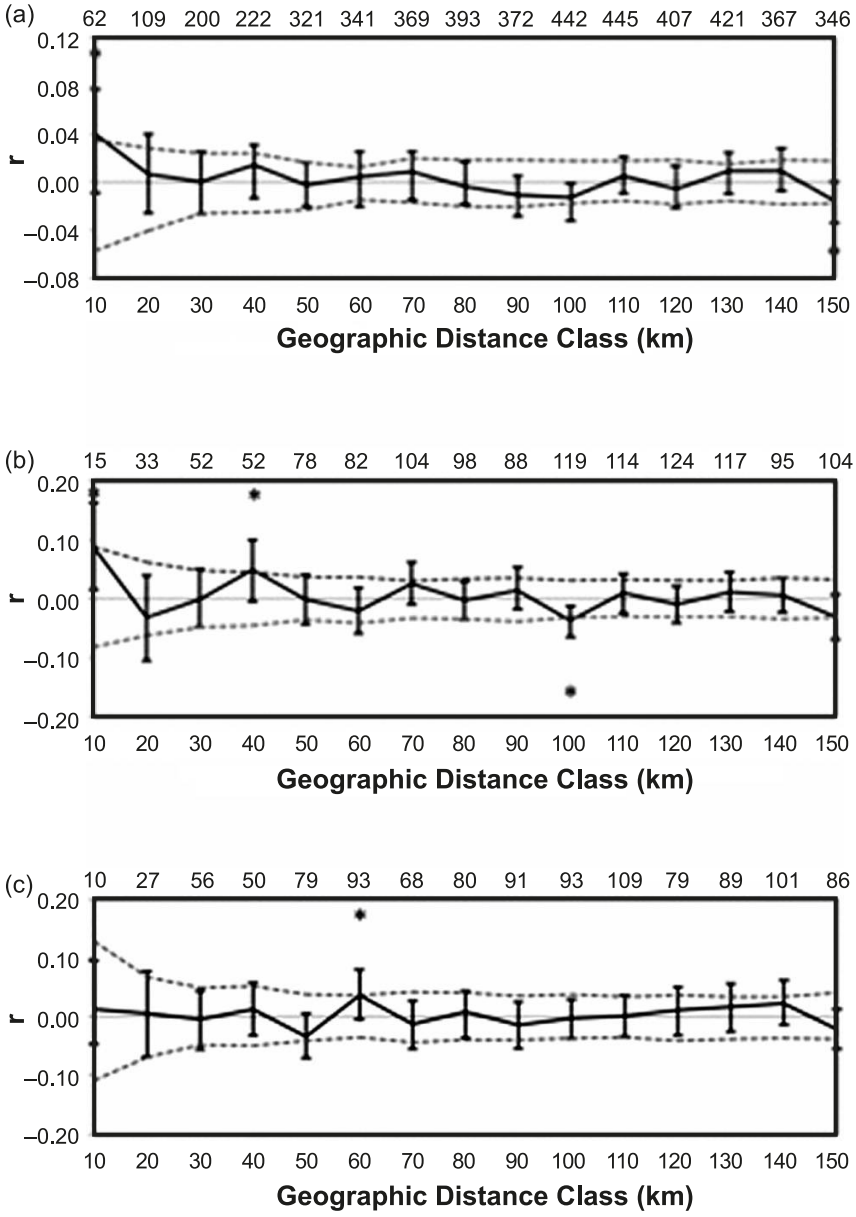
Note: Significant F_{IS} values are in boldface type. Sample sizes (*n*) correspond to the number of individuals that were genotyped in each category.

inferred spatial genetic structure (exhibited type I error) in the range of 1%–14% for any distance class, with the maximum rate of 14% corresponding to the 10 km distance class in the total data set (Fig. 3). The frequency of erroneous, positive deviations from panmixia were not correlated with the number of pairwise comparisons within each distance class (Spearman rank correlation: $r_S = 0.056$, $p = 0.604$) despite the variability in sample sizes among distance classes. Bayesian clustering results indicated a lack of genetic structure in the simulated populations, as expected; the highest likelihood was found at $K = 1$ for all 100 of the simulated populations. As expected for panmictic populations, concordance between expected and observed heterozygosity resulted in F_{IS} values that were not significantly different from zero in any of the 100 simulated populations.

Discussion

Proper interpretation of spatial genetic structure analyses requires careful consideration of biological factors such as demography and life history, as well as potential biases that can create false patterns of spatial genetic structure. Badgers in Michigan present a challenge for studying spatial genetic structure on a regional scale because of their elusive nature and low densities. In addition, relevant data on dispersal is limited and only available from populations inhabiting markedly different landscapes (e.g., British Columbia: Kinley and Newhouse 2008; Wyoming: Messick and Hornocker 1981). Because badger ecology is known to vary widely among habitats, even the limited data available are unlikely to be strong predictors of badger ecology in Michigan. Opportunistic sample collection was a necessity in this case and resulted in a geographic distribution of samples that was not uniform. The problems of diffuse sampling and lack of biological data is fairly common in elusive or rare mammals, so we incorporated simulations to assist in distinguishing patterns caused by biologically relevant processes and potential biases in

Fig. 2. Correlograms produced from the spatial autocorrelations of the total data set of American badgers (*Taxidea taxus*) (*a*), males (*b*), and females (*c*). Each correlogram displays both statistical tests performed in GenALEx: bootstrap and permutation tests. Error bars around each *r* represent bootstrapped 95% confidence intervals and observed *r* values were considered significant when the bars did not include zero. Broken lines are 95% permutational confidence intervals around each observed *r*. If the observed *r* fell outside the broken lines, then *r* was considered significant in the permutation test. The number of pairwise comparisons within each distance class is given above each data point.



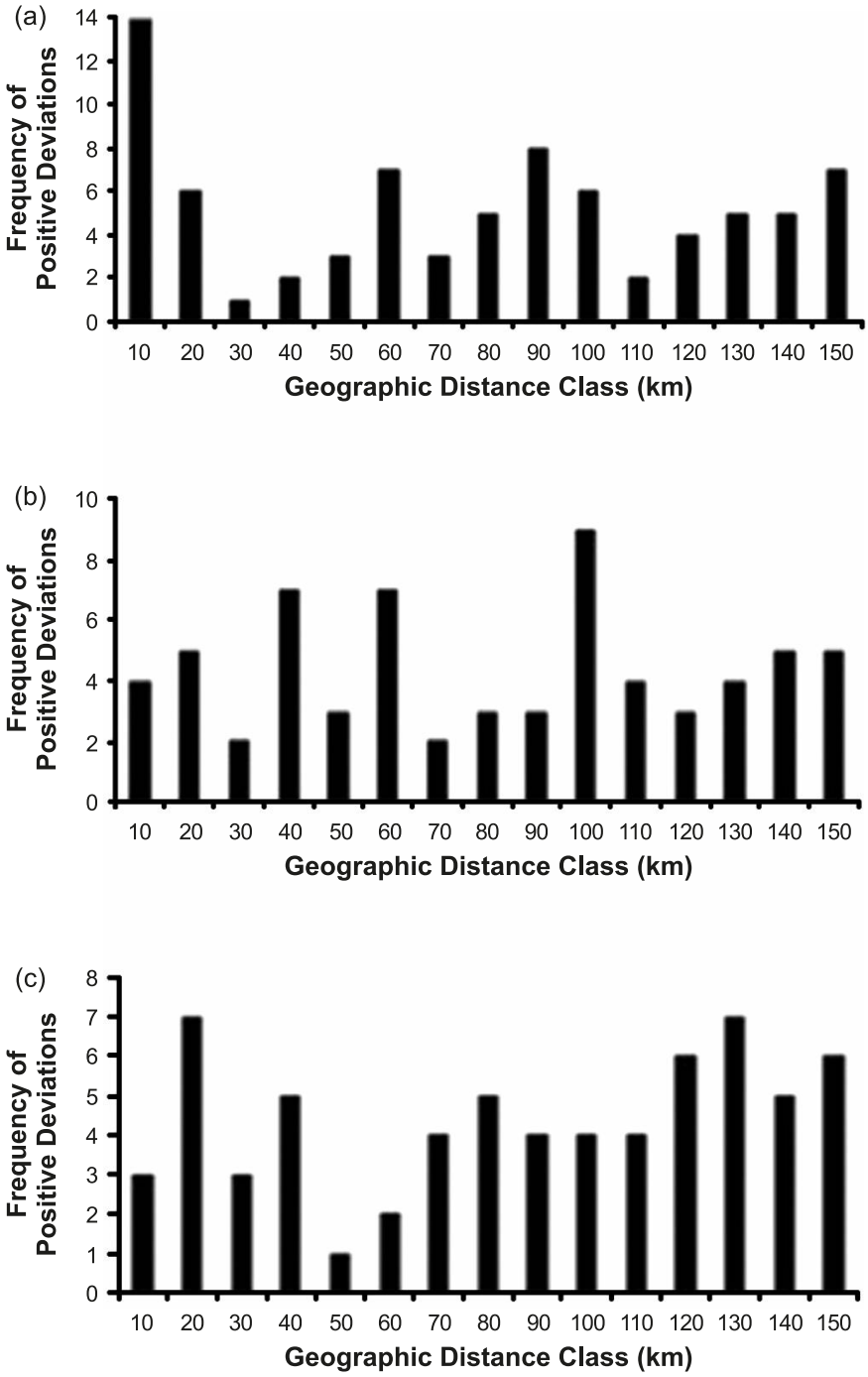
sampling. In this study, simulations were designed as a null hypothesis to evaluate how opportunistic sampling of a randomly mating population could impose false genetic structure. Comparisons between the observed data and the simulated randomly mating populations suggested that the patterns of genetic structure and gene flow we observed were biologically relevant and not driven by the sampling scheme.

Overall genetic structure

The bulk of our analyses suggest that the population of badgers in Michigan is largely panmictic. No spatial genetic structure was detected using any of the methods employed, within the total population or within either sex. Signatures of IBD or spatial autocorrelation were absent. Only the F_{IS} statistic deviated from the patterns of spatial genetic structure under panmixia and it showed a deficiency of heterozygotes in the total population. The observed F_{IS} values far exceeded those obtained for the simulated panmictic populations. In our simulated panmictic populations, heterozygosity did not deviate from Hardy–Weinberg expectations (F_{IS} = –0.006, SD = 0.013).

There are two potential mechanisms for the pattern of heterozygote deficiency we observed: genotyping error and (or) null alleles and demographic or mating system processes. Stochastic genotyping errors such as allelic dropout were

Fig. 3. Number of erroneous, positive deviations from panmixia detected in spatial autocorrelations for the 100 simulated total data set of American badgers (*Taxidea taxus*) (*a*), males (*b*), and females (*c*). Criteria for rejecting the null hypothesis of panmixia were identical to methods utilized for the observed data set.



very low in our study (1.3%) and were minimized by replicating genotypes. Highly reproducible errors such as null alleles do cause Hardy–Weinberg disequilibrium; however, demographic or mating system processes including population substructure, inbreeding, or selection at or near microsatellite loci could also cause deviations from HWE (Dakin and Avise 2004). Genotyping errors differ from demographic processes in their effects across multiple loci; demographic processes are expected to affect all loci similarly, whereas genotyping errors should have variable effects over loci. Examination of F_{IS} estimates for individual loci reveal a similar pattern of heterozygote deficiencies across all loci, making genotyping error an unlikely mechanism for the observed heterozygote deficiency. If we use the highest estimate provided by one of the few studies to objectively quantify the rate of occurrence for known null alleles in wild populations (0.5%–7.8%; Pemberton et al. 1995), the chance that all nine loci used in this study contained null alleles is minute (1.07×10^{-10}).

Demographic or mating system processes such as cryptic

population substructure or inbreeding are most likely responsible for the deficiency of heterozygotes in badgers in Michigan. These processes fall into three distinct categories. First, badgers in Michigan may exhibit very weak spatial genetic structure that went undetected in our analyses. Weak genetic structure can be caused by high levels of gene flow among populations or by recent population divergence. It is known that assignment methods are not always able to identify population genetic structure when genetic differentiation is weak (Pritchard et al. 2000; Latch et al. 2006). However, given that badgers are highly mobile and relatively tolerant of human encroachment (Warner and Ver Steeg 1995; Duquette 2008), the possibility of weak genetic subdivision or multiple populations seems questionable.

Second, the badger population in Michigan may be composed of a mixture of genetically discrete units (Wahlund effect). Nuclear data such as microsatellites exhibit positive values of F_{IS} when Wahlund effects are present, attributable to a deficiency of heterozygotes relative to expectations under conditions of random mating. It is possible that the contemporary population of badgers in Michigan represents recent recolonization from multiple source populations (e.g., Indiana and Ohio to the south, Ontario to the east), or recolonization that supplemented a remnant Michigan badger population. The problem with the recent recolonization scenario is twofold. Ontario's endangered population is geographically isolated from Michigan and contains very few individuals (<100), making it an unlikely source for Michigan. To the south, historical records of badgers in Indiana and Ohio are concentrated along Michigan's southern boundary (Lyon 1932; Duquette 2008), suggesting that Indiana and Ohio probably have maintained connectivity with Michigan.

Alternatively, it is possible that badgers exhibit temporal variation in social organization, which could result in a Wahlund effect for samples collected during times when social groups are admixed. For example, in the Wild Turkey (*Meleagris gallopavo* L., 1758), seasonal variation in social structure resulted in an ability to detect population structure among localized winter flocks, but no evidence for genetic structure among sampling locations during the spring when Wild Turkeys exist in mixed assemblages of genetically differentiated winter flocks (Latch and Rhodes 2006). Badgers exhibit home-range site fidelity between years (Minta 1993; Warner and Ver Steeg 1995; Duquette 2008); however, home-range contractions between seasons or age-specific genetic structure could result in a Wahlund effect. Although available data do not permit evaluation of these alternatives in the present study, either mechanism could cause the heterozygote deficiency we observed in Michigan badgers. If the lack of detectable genetic structure among individuals is due to a Wahlund effect, then the magnitude of the heterozygote deficiency will be directly proportional to the variance partitioned among the admixed population. In practical terms, this means that the F_{IS} value may be more informative than the F_{ST} value in regard to the level of genetic structure among subpopulations (e.g., Latch and Rhodes 2006). In this study, the presence of a Wahlund effect would suggest that 9% of the total genetic variance is partitioned among the component subpopulations ($F_{IS} = 0.091$).

The third process potentially responsible for the deficiency of heterozygotes is inbreeding. Badgers in Michigan may be isolated from other populations of badgers and experiencing some degree of inbreeding. Genetic variation is very low in Michigan compared with other badger populations (Kyle et al. 2004) and is on par with levels of genetic variation found in an isolated, endangered population of 200–600 animals in British Columbia (Kyle et al. 2004). The Michigan badger population, like the British Columbia population, is a peripheral population within the species range and both may exhibit reduced genetic diversity as a result (Brown 1984). Reduced genetic diversity in the peripheral Michigan badger population may be further exacerbated by a lack of gene flow imposed by its geographic location on a peninsula. Any gene flow that did occur would most likely be from Indiana and Ohio, which are also thought to be small populations. The combination of peripherality, isolation, and small population size could result in inbreeding within the Michigan badger population.

Inbreeding is difficult to distinguish from a Wahlund effect because both are expected to cause a deficiency of heterozygotes across loci. If inbreeding is the cause of the heterozygote deficiency, then we would expect continued isolation to result in the accumulation of inbreeding. Careful monitoring of badger populations for increased genetic relatedness among individuals over time or physiological evidence of inbreeding would be required to address this hypothesis.

Patterns of gene flow and sex-biased dispersal

Our data showed similar rates of gene flow between the sexes. These findings are somewhat surprising, given that patterns of female philopatry and male-biased dispersal are common in mammals (Greenwood 1980) and specifically in solitary mesocarnivores (Cegelski et al. 2006; Dharmarajan et al. 2009; Croteau et al. 2010). One explanation for the similarity in gene-flow rates between males and females is a lack of power to detect philopatry given the presence of only a single population potentially characterized by high gene flow. In a single population, the ability to detect sex-biased dispersal is reduced to a point where only a high skew between philopatric and dispersive individuals can be detected, particularly when using population-based assignment indices (Favre et al. 1997; Goudet et al. 2002; Dalerum et al. 2007). When the bias in dispersal rates between the sexes is not strong, using individual-based assignment methods as we have done in this study alleviates some of the reductions in power common when using population-based statistics. Thus, although it is possible that male-biased gene flow is present in badgers in Michigan but was undetected in our study, it is unlikely that the bias in dispersal between the sexes is strong.

Like other mesocarnivores, badgers exhibit mate defense polygyny (Minta 1993), a trait thought to promote male-biased dispersal (Greenwood 1980). However, many other factors likely influence dispersal besides mating systems (e.g., Pusey and Wolf 1996; Ferraras et al. 2004), so a combination of other life factors could cause relatively equal rates of realized gene flow in males and females. One such factor is the interaction between low population density of badgers in Michigan and territoriality among females. Michigan does not contain large tracts of optimal badger habitat (i.e., grasslands, prairies, and scrub–steppe) and occurs at the eastern edge of the badger's geographic range, two factors that predict low population densities (Brown 1984). Low population

densities are thought to promote higher female dispersal rates in territorial species because the chance of encountering aggressive conspecifics is reduced (Wolff 1997), but empirical examples in carnivores are rare (brown bear, *Ursus arctos* L., 1758: Støen et al. 2006; American black bear, *Ursus americanus* (Pallas, 1780): Costello et al. 2008). For badgers, evidence for female territoriality has been found in less dense populations (i.e., limited home-range overlap in females; Goodrich and Buskirk 1998), as well as in dense populations where female badgers temporally avoid one another despite home-range overlap (Minta 1993). Low population densities combined with female territoriality could promote dispersal and reduce regional spatial genetic structure, but the relationships among population density, territoriality, and dispersal remain largely untested in solitary mesocarnivores.

Badgers are a highly cryptic species with little life-history information, so this study was designed to provide insight into patterns of spatial genetic structure and gene flow while minimizing the effects of compulsory opportunistic sampling. We documented a single population of badgers in Michigan without any signal of sex-biased dispersal. Although these findings are surprising, and in contrast to the expected pattern of sex-biased dispersal and spatial genetic structure for mammals, other factors like territoriality and low population density may promote high rates of dispersal and correspondingly weak spatial genetic structure in Michigan badgers. Simulations do not replace relevant life-history data; however, in this case they have allowed us to extract relevant biological data regarding patterns of gene flow and spatial genetic structure in badgers by identifying and accounting for potential sampling biases. While opportunistic sampling for species like badgers provides robust sample sizes that otherwise would not be feasible through other collection techniques, biases that affect population genetic results can be introduced into the data set. Therefore, understanding potential sampling biases requires careful consideration, especially when genetic patterns are contrary to expectations as observed in our study.

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