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Biological Conservation 99 (2001) 323–330

BIOLOGICAL CONSERVATION

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# Population genetic structure of Blanding's turtles (*Emydoidea blandingii*) in an urban landscape

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Received 21 March 2000; received in revised form 8 September 2000; accepted 16 October 2000

**Abstract**

Recent investigations of Blanding's turtles *Emydoidea blandingii* in urban landscapes in the Greater Chicago metropolitan area (GCMA) indicate that populations are small and isolated. This led to the prediction that two local populations of Blanding's turtles in the GCMA would have less within-population genetic variation than larger populations in Michigan, Nova Scotia, and Wisconsin. We further predicted that Blanding's turtles in Nova Scotia would be genetically differentiated from those in the species' main range. We tested these predictions using variation in randomly amplified polymorphic DNA. Levels of genetic variability as measured by percent polymorphism and mean percent band sharing were similar among populations in the GCMA, Nova Scotia, and Wisconsin, though genetic variation in Michigan was significantly higher. No unique bands were detected in GCMA, however, 16 were found in Michigan, five in Nova Scotia, and one in Wisconsin. As predicted, Blanding's turtles in Nova Scotia, which have been geographically isolated from the species' main range for 4000–8000 years, were genetically differentiated from all other populations in the study. Although it was not clear that recent isolation and population declines resulted in a loss of genetic variation, our results indicated that Blanding's turtles in the GCMA may be genetically depauperate. The use of management interventions to prevent the loss of genetic diversity in the GCMA is discussed. 

*Keywords:* Blanding's turtle; *Emydoidea blandingii*; Genetic variation; Urban development; Small population size; RAPD

## 1. Introduction

Population genetic theory predicts that small and isolated populations tend to lose genetic variation through genetic drift (Lacy, 1987; Lande and Barrowclough, 1987; Frankham, 1996; Bouzat et al., 1998). Because of their delayed sexual maturity, high juvenile mortality, narrow habitat requirements, and low vagility (Ernst et al., 1994), freshwater turtles that occur in intensely developed landscapes are highly vulnerable to isolation, small population size, and loss of genetic variation. Thus, as metropolitan areas continue to grow and rapidly encroach on remaining wetland habitats, a better understanding of the population genetic structure of freshwater turtles is desirable for the development of management interventions that may circumvent their extinction.

There are at least three biological reasons that make the preservation of genetic variation of wildlife populations one of the major goals of conservation biology. First, the loss of genetic variation may increase the probability of population extinction through a decline in fecundity and viability, i.e. inbreeding depression (Lacy, 1987; Ralls et al., 1988; Meffe and Carroll, 1994; Frankham, 1995). Second, populations with low levels of genetic variation, upon which natural selection can operate, may have reduced opportunities for future adaptation through evolutionary change (Lande and Barrowclough, 1987; Fahrig and Merriam, 1994; Meffe and Carroll, 1994; but see Lynch, 1996; Cheverud et al., 1994). Third, the preservation of genetic variation may play a key role in identifying evolutionary significant units for conservation, i.e. genetically distinct populations of management concern (Meffe and Carroll, 1994; Moritz, 1994).

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0006-3207/01/$ - see front matter 
PII: S0006-3207(00)00198-1

Several studies have investigated the genetic effects of fragmentation on wildlife populations (e.g. Ashley et al., 1990; Templeton et al., 1990; Wayne et al., 1992; Gibbs, 1998). However, to our knowledge no studies have evaluated the genetic consequences of isolation and small population size on freshwater turtles in an urban landscape. The Blanding's turtle *Emydoidea blandingii* offers a unique opportunity to investigate the population genetic structure of a freshwater species in an urban landscape. The species is semi-aquatic and inhabits marshes, ponds, streams, and other shallow vegetated waters and associated upland habitats in North America (Ernst et al., 1994). Its main range includes discontinuous populations that are centered on the Great Lakes, west to Nebraska, Iowa, and northeastern Missouri (Fig. 1). Peripheral populations also occur in New York, Massachusetts, New Hampshire, Maine, and Nova Scotia (Ernst et al., 1994; Harding, 1997). Moreover, Blanding's turtles in Nova Scotia have been disconnected from the main range for 4000–8000 years (Herman et al. 1994; Mockford et al. 1999).

Once common throughout the northern two-thirds of Illinois (Garman, 1892), the Blanding's turtle is now rare in the Greater Chicago metropolitan area (GCMA) presumably from decades of urban development in the region and an associated loss of wetland habitats (Ludwig et al., 1992; Redmer and Kruse, 1998). Remaining Blanding's turtle populations in the GCMA persist in a mosaic of small insular habitat patches in which they are isolated and their numbers are low (Rubin, 2000). By contrast, larger populations in less developed areas remain in other parts of the species' range (Pappas and Brecke, 1992; Congdon and Gibbons, 1996; Mockford et al., 1999). Thus, we predicted that two small and isolated local populations of Blanding's turtles in the intensely developed GCMA would have less within-population genetic variation than larger populations in Michigan, Wisconsin, and Nova Scotia. We further predicted that the Nova Scotia population would be genetically differentiated from populations in the main range. We tested these predictions using variation in randomly amplified polymorphic DNA (RAPD) markers (Williams et al., 1990). RAPD markers have proved to be useful for evaluating population genetic structure (e.g. Kimberling et al., 1996; Nusser et al., 1996), determining levels of genetic variation (e.g. Hsiao and Lee, 1999; Maki and Horie, 1999), and assessing paternity (e.g. Gronemeyer et al., 1997) in natural populations.

## 2. Methods

### 2.1. Populations sampled

Blanding's turtles were sampled from five sites representing four geographic regions (Fig. 1): Pratts Wayne Woods (IL-PWW; 618.7 ha; north section) and West Chicago Prairie (IL-WCP; 123 ha) in the GCMA in northeastern Illinois; E. S. George Reserve (MI; 615 ha)

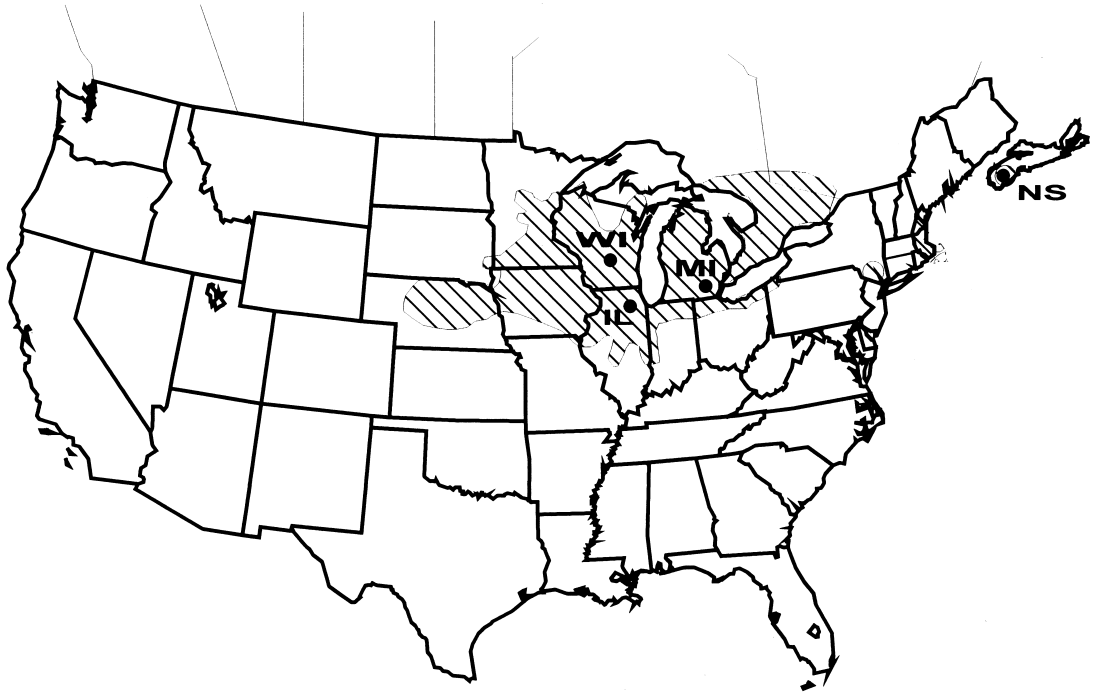


Fig. 1. Range of Blanding's turtle (after Ernst et al., 1994) and locations of populations sampled in the GCMA (IL), Michigan (MI), Nova Scotia (NS), and Wisconsin (WI).

in southeastern Michigan; Kejimkujik National Park (NS; 40,300 ha) in southwestern Nova Scotia; and Sandhill Wildlife Area (WI; 3,704 ha) in central Wisconsin. Pratts Wayne Woods and West Chicago Prairie are separated by less than 8 km and located in the towns of West Chicago and Wayne in northwestern DuPage County, approximately 50 km west of downtown Chicago. Commercial and residential development (e.g. housing, industrial parks, roads, railroad yards, and railroads) comprises nearly 80% (Ludwig, 1995) of the area in DuPage County. Recent investigations have indicated that Blanding's turtles in Pratts Wayne Woods and West Chicago Prairie are isolated and population sizes are small (see Rubin, 2000). The E. S. George Reserve is located about 40 km northwest of Ann Arbor, Michigan, in southwest Livingston County. The E. S. George Reserve is operated as a wildlife sanctuary and research area by the University of Michigan. Kejimkujik National Park consists of two separate and distinct blocks of land, an inland portion of 38,100 ha and a coastal portion along the Atlantic of 2200 ha. The Sandhill Wildlife Area is part of a continuous 40,000 ha block of natural lands located in southwestern Wood County in the heart of a cranberry production region in Wisconsin. The Wisconsin Department of Natural Resources owns and manages Sandhill Wildlife Area as a wildlife demonstration area, and human activity is minimal. Population size estimates for all studied populations are shown in Table 1.

### 2.2. DNA extraction and RAPD amplification

Blood samples were taken from a total of 59 Blanding's turtles from the five populations (7–18 per population) for RAPD analysis (Table 1). Blood extraction techniques from turtles trapped in the GCMA and Wisconsin were similar to those discussed in Avery and Vitt (1984). A 1 ml sterile tuberculin syringe with a 26-gauge needle was used to draw approximately 100 μl of blood from either a forelimb or hindlimb. Samples were then stored in 500 μl of "Queen's" lysis buffer (Seutin et al., 1991). Blood samples stored in lysis buffer from Michigan and Nova Scotia were generously supplied by M. Osentoski at E. S. George Reserve and S. Mockford at Kejimkujik National Park, respectively. Seventy-five microliters blood samples were digested with 20 μl of proteinase K (20 mg/ml) in 500 μl of lysis buffer at 65°C overnight. DNA was purified through a standard phenol-chloroform extraction and then precipitated using ethanol. DNA quality was checked by electrophoresis on a 0.8% agarose gel. High molecular weight DNA samples were quantified with a Hoeffer DNA fluorometer and diluted to a working concentration of 15 ng/μl for further PCR analysis.

Table 1
Population size estimates and the number of individuals sampled for RAPD analysis in Blanding's turtle populations in the GCMA (IL-PWW and IL-WCP), Michigan (MI), Nova Scotia (NS), and Wisconsin (WI)

| Population | Size estimate | Turtles sampled |
|---|---|---|
| IL-PWW[a] | 36 | 7 |
| IL-WCP[a] | 25 | 18 |
| MI[b] | 186 | 8 |
| NS[c] | 132 | 14 |
| WI[a] | 308 | 12 |

[a] Rubin, (2000).
[b] Congdon and Gibbons, (1996).
[c] Herman et al., (1994).

Each RAPD reaction contained 0.5 units of *Taq* polymerase, 0.1 mM each deoxyribonucleotide triphosphate (dNTP), 0.2 μM of an Operon 10-base primer (from kits A and C; Operon Technologies, Alameda, CA), 1X PCR buffer (20 mM Tris–HCl, 50 mM KCl), 2 mM $MgCl_2$, 15 ng of DNA, and sterile water to a final volume of 25 μl. The DNA, PCR buffer, primer, $MgCl_2$, and water were combined to a volume of 15 μl, capped with 10 μl of liquid wax, placed in a thermal cycler and subjected to a "hot start" (Chou et al. 1992) for 5 min at 85°C. Following the hot start, *Taq* polymerase, dNTPs and water were added to a final volume of 25 μl. The samples were then subjected to the following PCR profile: (1) 3 min at 94°C; (2) 30 s at 94°C; (3) 30 s at 36°C; (4) 1.5 min at 72°C; and (5) 10 min at 75°C (Williams et al., 1990). Steps 2 to 4 were repeated 44 times. All reactions were run using the same thermal cycler. The reaction products were then loaded into a 0.8% agarose gel, separated via electrophoresis, stained with ethidium bromide, visualized under UV light, and photographed for subsequent scoring. Negative control reactions with no DNA template were used to check for contamination.

Primers were chosen based on a screening process using 40 primers and one randomly chosen DNA sample from each population. From these initial 40 primers, 12 produced bright, distinct polymorphic bands and were chosen for further analysis (A07, A08, A10, A11, A16, A20, C02, C08, C11, C14, C17, C19). From this subset, 39 polymorphic bands were scored to create a presence (1)/absence (0) RAPD matrix. One variable individual was chosen as the minimum criterion for band polymorphism.

The consistency of RAPD reactions was examined through a series of repeatability tests. Using three randomly chosen DNA samples from each population, repeatability was tested within and between PCR runs. Under the same conditions, each randomly chosen DNA sample was subjected to three independent PCR runs. Furthermore, each sample was repeated three times within each run. Although unsuccessful amplifications occurred occasionally, RAPD patterns from all amplified test samples were 100% repeatable both within and among PCR runs. As an additional check on

repeatability for subsequent genetic analysis, individual PCR runs consisting of all samples were performed twice using the same thermal cycler.

### 2.3. Genetic analysis

RAPD polymorphisms were analyzed with the following assumptions: (1) bands from different loci do not comigrate; (2) each locus is a two allele system in which only one allele is amplifiable; and (3) alleles arise from identical mutations, among and within individuals (Black, 1993; Lynch and Milligan, 1994; Apostol et al., 1996). Levels of within-population genetic variation were assessed by the number of unique bands, percent polymorphism, and mean percent band sharing. A band was considered unique if it was only detected in a single population. Percent polymorphism was calculated by dividing the number of bands that exhibited polymorphism within a population by the total number of bands scored among all populations and then multiplying by 100. Percent band sharing was estimated using the following equation

$$\mathrm{BS} = 2N_{\mathrm{AB}}/(N_{\mathrm{A}} + N_{\mathrm{B}}) \times 100$$

where $N_{\mathrm{AB}}$ is the number of bands individuals A and B have in common, and $N_{\mathrm{A}}$ and $N_{\mathrm{B}}$ are the total number of bands scored for each individual, respectively (Wetton et al., 1987).

Comparisons of relative levels of within-population genetic variation were investigated using an analysis of variance (ANOVA) on mean percent band sharing (e.g. Trame et al., 1995). Prior to the ANOVA, the square root of each proportion was calculated and then transformed to its arcsine to account for binomial distributions formed from percentage data (Zar, 1999). Model assumptions of normality and homoscedasticity were then assessed using normal probability plots and the Levene's equality of variances test (Norusis, 1994), respectively, and were not violated. Differences between means were evaluated using a Bonferroni multiple comparison test (Norusis, 1994). Linear regression was used to check for possible correlations between estimated population size and levels of genetic variation (percent polymorphism and mean percent band sharing).

Genetic differentiation between Blanding's turtle populations was assessed using MANTEL-STRUCT (Miller, 1999). Developed for the analysis of RAPD generated presence/absence data, MANTEL-STRUCT employs a Mantel test (Mantel, 1967) to test the null hypothesis that there is no difference in within- and between-population genetic similarities or distances of individuals (Miller, 1999). Given that RAPD markers are dominant and, hence, allelic proportions are unknown, estimators of genetic differentiation that require information on allele frequencies, such as an $F_{\mathrm{st}}$ value (Wright, 1978) or its unbiased estimator $\theta$ (Weir, 1996) may be inappropriate in the analysis of RAPD data (Miller, 1999). Because MANTEL-STRUCT uses measures that do not require information on allele frequencies, it is likely a more robust estimator of genetic differentiation when using RAPD markers (Miller, 1999).

To assess genetic differentiation between populations, MANTEL-STRUCT first calculates interobservational genetic similarity or distance values. In the present study Dice's (1945) similarity index was used. MANTEL-STRUCT then constructs a large half matrix of all pairwise combinations between individuals, with within-population similarity values occurring in triangular submatrices along the diagonal of the matrix and between-population similarities occurring in rectangular off-diagonal submatrices. Congruent with the constructed genetic similarity matrix, MANTEL-STRUCT creates a second binary matrix that consists of 0′s and 1′s that correspond to the between and within-population similarities, respectively. The Mantel test is then used to calculate a correlation coefficient ($r$) of the two matrices that provides a measure of the amount of genetic differentiation between populations. Finally, MANTEL-STRUCT determines levels of significance between populations by a Monte Carlo procedure where the 0′s and 1′s of the binary matrix are randomly redistributed 1000 times.

## 3. Results

Levels of within-population genetic variation as measured by mean percent band sharing were similar among the majority of populations studied, with the exception of Michigan. Mean percent band sharing ranged from 66.6±2.7% in Pratts Wayne Woods, 60.4±1.4% in West Chicago Prairie, 57.3±2.1% in Wisconsin to 55.6±1.2% in Nova Scotia, with no significant differences ($P < 0.05$) between the two GCMA populations, Pratts Wayne Woods and West Chicago Prairie, or among West Chicago Prairie, Wisconsin, and Nova Scotia (Fig. 2). Mean percent band sharing, however, was significantly lower in Michigan with a mean percent band sharing of 47.0±2.7% ($F_{4,54} = 10.616$, $P < 0.001$; Fig. 2). Percent polymorphism was also similar among four of the five populations, ranging from 25.6% in Pratts Wayne Woods, 33.3% in West Chicago Prairie, 35.9% in Nova Scotia to 38.5% in Wisconsin. Michigan was again the most variable population with 69.2% of the bands exhibiting polymorphism. No unique bands were detected in either GCMA population, however, 16 were found in Michigan, five in Nova Scotia, and one in Wisconsin. Levels of within-population genetic variation were not related to size (Fig. 3). No significant relationships between estimated population size and mean percent band sharing ($r^2 = 0.529$, $P = 0.359$) or percent polymorphism ($r^2 = 0.463$, $P = 0.432$) were found.

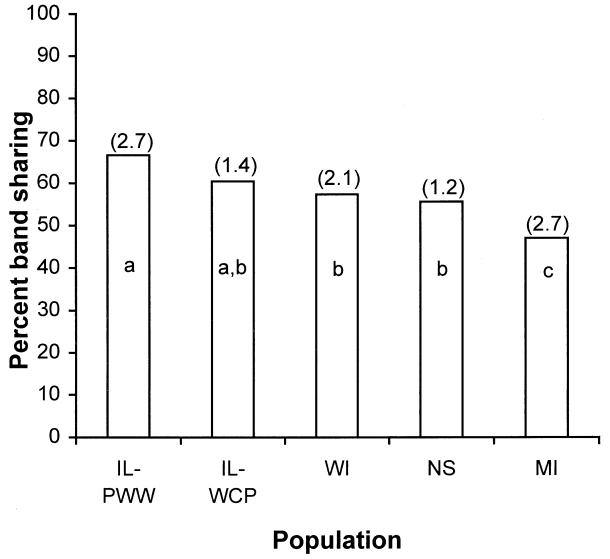


Fig. 2. Mean percent band sharing of Blanding's turtle populations in the GCMA (IL-PWW and IL-WCP), Michigan (MI), Nova Scotia (NS), and Wisconsin (WI). Means with the same letter are not significantly different using a Bonferroni adjusted $\alpha$ ($\alpha = 0.05 / 5 = 0.01$) for multiple comparisons on mean percent band sharing. Standard errors are in parentheses.

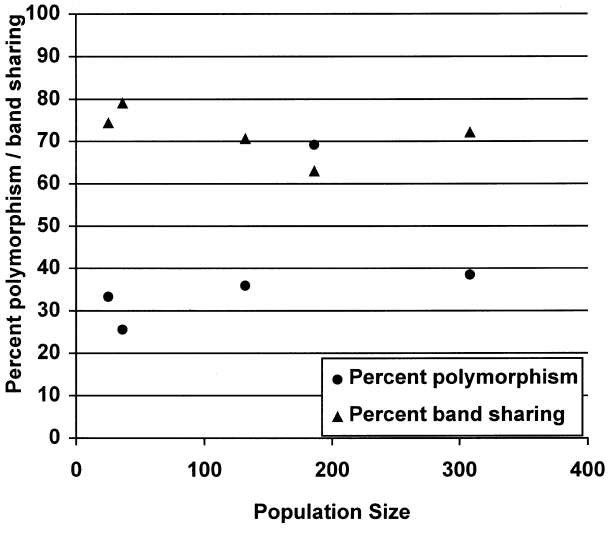


Fig. 3. Scatter plot of within-population genetic variation as measured by percent polymorphism and percent band sharing versus population size estimates of Blanding's turtles.

Genetic differentiation among populations corresponded with geographic distance and degree of isolation. Consistent with their long-term geographic isolation, Blanding's turtles in Nova Scotia were significantly differentiated from populations in the main range, having the lowest between-population similarity values; ranging from 0.358 to 0.443 (MANTEL-STRUCT, $P < 0.001$; Table 2). Although there was no detectable genetic differentiation among Pratts Wayne Woods, Michigan, and Wisconsin, significant differentiation was revealed between West Chicago Prairie in the GCMA and the Michigan and Wisconsin populations (Table 2). Despite a lack of recently detectable movement between sites in the GCMA (Rubin, 2000), no genetic differentiation was revealed between the geographically proximate Pratts Wayne Woods and West Chicago Prairie populations (Table 2).

Table 2
Dice's (1945) similarity values calculated by MANTEL-STRUCT between populations (below diagonal) and *P*-values (indicating genetic differentiation) after a Monte Carlo procedure where the data was randomly redistributed 1000 times (above diagonal) for Blanding's turtles in the GCMA (IL-PWW and IL-WCP), Michigan (MI), Nova Scotia (NS), and Wisconsin (WI)

| Population | IL-PWW | IL-WCP | MI | NS | WI |
|---|---|---|---|---|---|
| IL-PWW | – | 0.889 | 0.139 | 0.001 | 0.642 |
| IL-WCP | 0.771 | – | 0.001 | 0.001 | 0.004 |
| MI | 0.687 | 0.633 | – | 0.001 | 0.333 |
| NS | 0.402 | 0.358 | 0.406 | – | 0.001 |
| WI | 0.746 | 0.690 | 0.688 | 0.443 | – |

## 4. Discussion

The population genetic structure of Blanding's turtles in an urban landscape in the GCMA was assessed and compared with larger populations in Michigan, Nova Scotia, and Wisconsin. Population size estimates indicated that the GCMA populations were smaller relative to the other populations in less developed areas (Table 1). Thus, according to population genetic theory, Blanding's turtles in the GCMA would likely be more vulnerable to the loss of genetic variation.

Within-population genetic variation as measured by mean percent band sharing and percent polymorphism revealed that variation in the GCMA was not significantly lower than in Nova Scotia or Wisconsin. Thus, irrespective of locale, degree of isolation, or population size, levels of within-population genetic variation were similar within the GCMA, Nova Scotia, and Wisconsin. The Michigan population, however, was significantly more genetically diverse than the other populations studied. Hence, assuming that the Michigan population is representative of "large" genetically diverse populations, then populations in the GCMA, Nova Scotia, and Wisconsin may all be genetically depauperate.

One possible explanation for this phenomenon is that the number of individuals that actually contributed to a given gene pool, i.e. the effective population size, was likely only a fraction of the total size. This is exemplified by recent studies indicating that Blanding's turtles do not become reproductive until between 11 and 20 years old (Congdon and van Loben Sels, 1993) and only 40–60% of adult females reproduce in a given year (Congdon et

al., 1993). Thus, effective population sizes were likely small in all populations, with the possible exception of Michigan.

In addition, the lower level of genetic variation exhibited in Wisconsin may be tied to prior anthropogenic events, such as past land use practices. In the early 1900s Sandhill Wildlife Area was drained for agricultural production, resulting in the widespread and rapid loss of wetland habitat. Consequently, the population may have suffered a severe demographic bottleneck in the recent past, which may have resulted in a decrease in genetic variation.

Similarly, founder effects and isolation may have also been responsible for the low level of genetic variation reported in Nova Scotia. Endemic to the Great Lakes region (Schmidt, 1938), Blanding's turtles extended their range during the Pleistocene in response to glacial advances (Preston and McCoy, 1971; Jackson and Kaye, 1974). In addition to moving south (Jackson and Kaye, 1974), Blanding's turtles during the Pleistocene apparently migrated along a "steppe corridor" (Schmidt, 1938) to the northeastern United States and Nova Scotia (Preston and McCoy, 1971). Because of its geographic location, Nova Scotia was probably not easily accessible to Blanding's turtles. Thus, it is probable that the genetic structure of the modern Nova Scotia population was influenced by past founder effects and continued low densities. Furthermore, the occurrence of genetic drift in Nova Scotia, which results in the fixation of alleles and ultimately lower genetic variation due to isolation and small population size was evident in this study given that the Nova Scotia population was genetically differentiated from each population sampled in the main range. This finding is consistent with results reported by Mockford et al. (1999) in a RAPD study that examined the genetic relationship between Blanding's turtles in Nova Scotia and the main range. The present study and results of Mockford et al. (1999) clearly indicate that the Nova Scotia population is genetically unique compared to those from the main range and, therefore may be considered an important evolutionary unit of conservation.

Despite an absence of movement (Rubin, 2000), and therefore gene flow, between Pratts Wayne Woods and West Chicago Prairie populations in the GCMA, no genetic differentiation was revealed by the Mantel test. The lack of genetic differentiation between Pratts Wayne Woods and West Chicago Prairie was likely related to the short period that these populations have been isolated, the long generation time of Blanding's turtles (Congdon et al., 1993), and the relatively slow microevolutionary rates reported for turtles in general (Bickham, 1981; Avise et al., 1992; FitzSimmons et al., 1995). On the other hand, significant genetic differentiation was revealed between Blanding's turtles in West Chicago Prairie and those in Michigan and Wisconsin, whereas no differentiation was found between the latter two populations. This outcome was most likely related to the sampling effects associated with small population size, which may have resulted in the loss of RAPD bands, through random chance alone, from West Chicago Prairie that were originally shared with both Michigan and Wisconsin.

Based on the findings of the present study, it is not clear that Blanding's turtles in the GCMA have lost genetic variation as a result of recent isolation and small population size. Given that microevolutionary rates are relatively low in turtles (Bickham, 1981; Avise et al., 1992; FitzSimmons et al., 1995), the current lower level of genetic variation in the GCMA (relative to Michigan) may be tied to historical demographic bottlenecks or founder effects. Again, as noted earlier, our conclusion that Blanding's turtles in the GCMA, Nova Scotia, and Wisconsin are genetically depauperate is based on the assumption that the Michigan population is representative of most "large" populations from a genetic perspective. If Michigan is instead the exception, then our results suggest that Blanding's turtles in the GCMA are not genetically atypical of most Blanding's populations. However, the lack of unique bands in the GCMA compared to the Michigan, Nova Scotia, and Wisconsin populations suggests that Blanding's turtles in Pratts Wayne Woods and West Chicago Prairie may have lost some genetic variation as a result of isolation and small population size, justifying further investigation.

Deleterious effects related to the loss of genetic variation are likely to be exaggerated in populations that are small and isolated due to a greater sensitivity to both demographic and environmental stochasticity (Gilpin and Soulé, 1986). Thus, conservation strategies for Blanding's turtles and presumably other freshwater turtle species in landscapes conducive to population declines and isolation should incorporate management interventions aimed at the preservation of genetic variation. One possible strategy in areas where migration is limited, such as in urban centers, may involve translocations or human-facilitated movement of animals between populations to promote gene flow. The translocation of wildlife between populations to bolster genetic variation, however, should only occur under special circumstances where signs of inbreeding depression are evident, such as reduced hatchability rates and lower hatchling survival. In the GCMA there is currently no evidence to indicate a reduction in fitness. Moreover, results of the present study indicated that Blanding's turtle populations in the GCMA were not genetically different and, therefore translocations to promote gene flow between populations will likely have little or no influence on the maintenance of genetic variation in the region. Thus, management interventions to acquire and maintain quality habitat that promotes population increases and migration are likely to be the

most beneficial to the persistence and maintenance of genetic variation of Blanding's turtles in the GCMA.

## Acknowledgements

We thank T. Anchor and D. Ludwig for their assistance in collecting blood samples in Illinois; R. Thiel for supplying demographic data and assistance in collecting blood samples in Wisconsin; S. Mockford and M. Osentoski for supplying blood samples from Nova Scotia and Michigan, respectively; J. Brawn, J. Hartz-Rubin, C. Phillips, and an anonymous reviewer for helpful comments and suggestions on the manuscript. Funds for this study were provided by the University of Illinois at Urbana-Champaign Department of Natural Resources and Environmental Sciences, Council for Food and Agricultural Research (C-FAR), Forest Preserve District of DuPage County, and Lincoln Park Zoological Gardens.

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