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Biological Conservation 123 (2005) 373–380
BIOLOGICAL CONSERVATION
www.elsevier.com/locate/biocon

Population genetic structure of a disjunct population of Blanding's turtle (*Emydoidea blandingii*) in Nova Scotia, Canada

S.W. Mockford [a], L. McEachern [b], T.B. Herman [b,*], M. Snyder [b], J.M. Wright [a]

[a] *Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada*
[b] *Biology Department, Acadia University, 24 University Avenue, Wolfville, Nova Scotia, Canada B4P 2R6*

Received 16 February 2004; received in revised form 17 November 2004; accepted 26 November 2004

Abstract

The Nova Scotia population of Blanding's turtle (*Emydoidea blandingii*) is small and disjunct but surprisingly contains spatial structure. In this paper we explore the nature of that structure and attempt to identify the processes underlying it. Recent studies of the species in Nova Scotia have identified three discrete groups in what was previously thought to be a single panmictic population. There is no evidence of current movement between these groups. One group, at Kejimkujik National Park and National Historic Site, is restricted to the Mersey watershed; the other two groups are on the adjacent Medway watershed. The lack of current movement led to the prediction that there might be measurable genetic structure among these groups. Microsatellite analysis of five loci revealed significant genetic structure (F_{ST} = 0.042–0.124; $p < 0.05$) in pairwise comparisons between groups. Distance rather than watershed appears to be the principal determinant of spatial structure. Two separate analyses reveal no evidence of recent population bottlenecks. Population simulations suggest that this differentiation likely pre-dated human influence on the local landscape. In the face of rapid environmental change, understanding spatial structure in this population complex is essential so that we can match management scale to ecological scale in this long-lived, late maturing species.

1. Introduction

Blanding's turtle (*Emydoidea blandingii*) is a North American freshwater turtle with a main range centred on the Great Lakes and including Ontario, Quebec, Wisconsin, Illinois, Michigan, Ohio, Minnesota, Iowa, Indiana, Nebraska and Pennsylvania. Disjunct populations occur along the eastern seaboard in New York, Massachusetts, Maine and Nova Scotia (Herman et al., 1995, and references therein). While large populations occur in Michigan and Minnesota (Congdon and Gibbons, 1996; Pappas et al., 2000; Sajwaj et al., 1998) the species' distribution is patchy throughout its entire range, particularly on the periphery (McCoy, 1973).

Of the peripheral populations, the Nova Scotia population is the most isolated, occurring at the north-eastern limit of the species range. Within Nova Scotia, the known range of Blanding's turtle is restricted to the south-western region of the province's interior with confirmed sightings limited to the Medway and Mersey watersheds (Herman et al., in press; Fig. 1). This limited local distribution may reflect the region's relatively warm summer temperatures (Power, 1989; Standing et al., 1999).

The species was first described in Nova Scotia in 1953 (Bleakney, 1958) at Grafton Lake, in what would become Kejimkujik National Park and National Historic Site (KNP). Since the creation of KNP in 1968, sightings of Blanding's turtle have been opportunisitically recorded within the park. Before 1987, most of these sightings were coincidental to other work; in 1987 studies directed at the ecology and life history of Blanding's

* Corresponding author. Tel.: +1 902 585 1469; fax: +1 902 585 1059.
E-mail address: tom.herman@acadiau.ca (T.B. Herman).

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doi:10.1016/j.biocon.2004.11.021

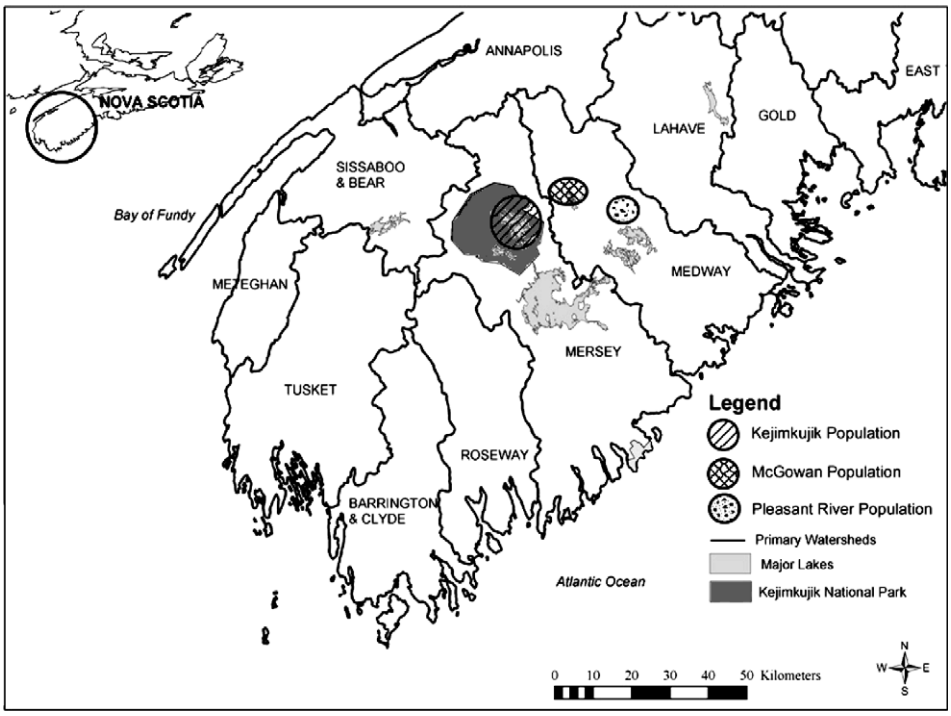

Fig. 1. Map of south-western Nova Scotia showing the location of three populations of Blanding's turtle, Kejimkujik National Park and National Historic Site and the major watersheds.

turtle began (Power, 1989; Power et al., 1994; Herman et al., 1995). As a result of these studies, the Nova Scotia population of Blanding's turtle was designated "threatened" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1993. This listing was based on the restricted geographic range of the Nova Scotia population, and an unstable age structure with apparently few juveniles or young adults. The province of Nova Scotia listed the species as "endangered" in 2001. Throughout most of its range elsewhere, the species is listed as locally threatened or endangered (Herman et al., in press). The World Conservation Union (IUCN) lists Blanding's turtle as LR/nt (Lower Risk/near threatened).

Although there have been scattered anecdotal sightings of the species throughout southwest Nova Scotia, it was long thought that the province contained a single panmictic population centred on KNP. The estimated size of the KNP concentration is 132 adults (95% CI 99.4–178.9; Herman et al., 1995), although recent unpublished estimates suggest the number may be lower. In 1996 a second concentration of Blanding's turtle was found at McGowan Lake (ML), approximately 15 km from KNP. Intensive capture, marking and radio telemetry conducted since 1996 has revealed no movement between these two groups (McNeil, 2002). The estimated size of the ML population is 79 adults (95% CI 59.9–116.5; McNeil, 2002). In 1999 a third concentration of Blanding's turtle was found at Pleasant River, approximately 25 km from KNP and 15 km from ML (PR; Caverhill, 2003). While recapture data are insufficient at present to accurately estimate the size of the PR concentration, 100 turtles, including 65 adults, have been marked since 1999. Although the three concentrations are close, they occupy two separate but adjacent watersheds. KNP is limited to the Mersey watershed, while both ML and PR occur on the Medway watershed (Fig. 1). With the exception of nesting, most activity in this species is restricted to water; we therefore reasoned that watershed, by mediating movements, might contribute to structure in this population.

Any measurable genetic structure will reflect the balance between genetic drift and selection, which will function to create differences between populations, and migration, which will function to homogenize the gene pool. Since generation time is so long in Blanding's turtle (~37 years in Michigan; Congdon et al., 1993; Congdon and van Loben Sels, 1993; possibly even longer in Nova Scotia, where maturation occurs as late as age 25), rates of migration sufficient to overcome genetic differences resulting from drift or local selection may not be detectable by field observation alone. The aims of this study were: (1) to compare population structure inferred from genetic analysis to the observed spatial structure and (2) to determine whether watershed may play a role in population structure of the species.

2. Materials and methods

2.1. Sampling

Blood was collected from adult turtles at KNP between 1995 and 2000 ($N = 43$), at McGowan Lake between 1996 and 2000 ($N = 40$) and at Pleasant River between 1999 and 2002 ($N = 27$). Ten to 100 μl of blood were drawn from the dorsal coccygeal vein (Haskell and Pokras, 1994) using a 1 cc syringe and a 26 gauge needle, and stored immediately in 1ml of lysis buffer (1.25% SDS, 300 mM Tris–HCl (pH 8.0), 500 mM EDTA, 5% sucrose). Blood in lysis buffer was stored at ambient temperature for up to 6 months until DNA extraction. Additionally, 20 samples collected at the E.S. George reserve in Michigan were included for comparison.

2.2. DNA extraction and polymerase chain reaction (PCR) amplification

DNA was extracted using a phenol–chloroform extraction modified from Jowett's *Drosophila* DNA extraction protocol (Jowett, 1986). Following extraction, DNA was suspended in 100 μl of TE (10 mM Tris–HCl (pH 8.0), 1 mM EDTA). Yield was estimated either by gel electrophoresis of extracted DNA and visual comparison with a known quantity of *Hin*dIII digested lambda DNA run on the same gel, or by UV spectrophotometry.

All samples were amplified using primers targeting four microsatellite regions previously identified in *E. blandingii* (Eb09, Eb11, Eb17, Eb19; Osentoski et al., 2002) and one from *Caretta caretta* (Cc7; FitzSimmons, 1997). PCR reactions were carried out using a MJ-100 thermocycler (M.J. Research, Inc.) under the following conditions: initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 45 s, a primer-specific annealing temperature for 45 s, and 72 °C (elongation) for 45 s followed by a final extension at 72 °C for 5 min. Genotypes were determined by subjecting ^{33}P-labelled PCR products to electrophoresis in 6% acrylamide gels following established procedures (Sambrook et al., 1989).

Allele sizes were determined by comparison to internal standards. Each gel included both M13 bacteriophage sequence and previously scored samples for comparison and to ensure consistent scoring. Any individual amplified sample for which the scoring was ambiguous was reamplified and rescored. In three cases the ambiguity could not be resolved and the score for that sample at that locus was entered as missing data.

2.3. Statistical analysis

Data analysis was conducted using FSTAT ver. 2.9.3.2 (Goudet, 1995) and Arlequin ver.1.1 (Schneider et al., 1997). Data were tested for evidence of genotypic disequilibrium and deviations from Hardy–Weinberg equilibrium. Expected and observed heterozygosities were calculated.

Population differentiation was measured by calculating pairwise F_{ST}. Probability values for these were derived by generating 10,000 distributions in which haplotypes were permuted between populations; p = the proportion of distributions where the F_{ST} value is equal to, or greater than, the observed value. The level of gene flow was calculated based on F_{ST}.

As an alternative method of describing population differentiation within Nova Scotia, total variance was partitioned into hierarchical components using analysis of molecular variance (AMOVA; Excoffier et al., 1992). This provides estimates of the percentage of total variance accounted for within populations and among populations. Statistical significance was determined by >1000 permutations of genotypes.

To reduce the probability of Type I error, a sequential Bonferroni correction was applied to tests involving multiple comparisons.

Evidence of recent population bottlenecks was sought using two tests in the program BOTTLENECK (Cornuet and Luikart, 1996). Following a bottleneck both the number of alleles and the gene diversity are reduced but the loss of gene diversity lags behind the loss of alleles. The program looks for an excess of gene diversity from that expected given the observed number of alleles. The first test was carried out under a two-phased model (TPM) and 90% of mutations followed the stepwise mutation model (SMM) and 10% of mutations followed the infinite allele mutation model (IAM). Statistical significance was determined using a Wilcoxon sign-rank test. The second test looked for a mode shift in the distribution of allele frequencies, reflecting a loss of low frequency alleles following a bottleneck. This test is qualitative rather than statistical but is useful in detecting recent bottlenecks (less than a few dozen generations) (Luikart et al., 1998).

Finally, we used the program EASYPOP (Balloux, 2001) to model the Nova Scotia populations to examine the time required for the observed degree of population differentiation (F_{ST}) to have developed. The small sizes of the Nova Scotia population allowed us to model this directly. The model was run assuming random mating and an equal sex ratio. The migration model is a one-dimensional stepping stone, and the mutational model is mixed with 95% of mutations following the stepwise mutation model (SMM) and 5% of mutations following the K alleles model; the mutation rate was set at 0.0001. Population sizes reflected the estimates of the existing populations: 140, 80 and 200. The model was run for 20 generations with a migration rate of 99% ($M = 0.99$) to attain panmixia; the migration rate was then reduced to either 5% ($M = 0.05$) or 1%

($M = 0.01$) and run for a variable number of generations. Model output was converted to the Arlequin format and F_{ST} was calculated using Arlequin. This approach is constrained by not allowing for fluctuations in population size nor for the modelling of a bottleneck followed by population expansion.

3. Results

Mean heterozygosity across all samples and loci is 0.56 and ranges from 0.45 to 0.62 (Table 1). The number of alleles per locus ranges from 3 to 15. Four of fifteen (27%) loci/population in Nova Scotia showed significant departures from Hardy–Weinberg expectations before correction for multiple comparisons. After sequential Bonferroni correction, only one locus at one population deviated from Hardy–Weinberg expectations (Eb11 at McGowan Lake). Prior to correction 4 of 30 comparisons (13%) exhibited some linkage; after sequential Bonforroni correction, there was no significant linkage between loci.

Pairwise F_{ST} values were all positive, ranging from 0.043 to 0.318 (Table 2). All were significant after sequential Bonforroni correction. Pairwise F_{ST} values within Nova Scotia ranged from 0.043 to 0.124 while values between the Nova Scotia populations and Michigan ranged from 0.253 to 0.318. Estimates of gene flow based on F_{ST} range from 1.76 to 5.8 within Nova Scotia and from 0.54 to 0.61 between the Nova Scotia populations and Michigan.

AMOVA results provide a different perspective on population differentiation. Our results show the majority of variation in Nova Scotia to be within populations (93.4%) but with a statistically significant component among populations (6.6%; $p < 0.0001$).

There is evidence of genetic drift within individual units. The Pleasant River population is fixed for the 104 bp allele at locus Eb19 (Fig. 2) and the KNP population is near fixation for the 152 bp allele at locus Eb09 ($p(152) = 0.89$).

BOTTLENECK tests showed no evidence of recent bottlenecks in any of the three Nova Scotia populations ($p > 0.5$ in all populations). Allele frequency distribution

Table 1
The sample size (*n*), number of alleles, range of allele sizes (bp), observed heterozygosity (Ho), and expected heterozygosity (He) for five microsatellite loci in three Blanding's turtle populations in Nova Scotia and one population in Michigan

Primer	Pleasant River	Kejimkujik National Park	McGowan Lake	Michigan	Overall
Eb09					
n	27	43	40	20	130
#Alleles	5	5	9	11	15
Range	146–158	146–160	146–169	128–154	128–169
Ho	0.56	0.19	0.58	0.80	0.53
He	0.58	0.20	0.62	0.89	
Eb11					
n	26	43	40	20	129
#Alleles	6	10	6	9	12
Range	174–188	174–204	174–186	172–190	172–204
Ho	0.42	0.77	0.65	0.65	0.62
He	0.64	0.74	0.70	0.79	
Cc7					
n	27	43	40	20	130
#Alleles	3	4	4	4	5
Range	151–169	151–171	151–169	151–171	151–171
Ho	0.82	0.44	0.72	0.40	0.60
He	0.52	0.57	0.54	0.60	
Eb17					
n	27	42	40	20	129
#Alleles	3	3	4	5	5
Range	105–114	105–114	105–114	99–114	99–114
Ho	0.41	0.67	0.52	0.70	0.58
He	0.53	0.53	0.54	0.70	
Eb19					
n	27	42	40	20	129
#Alleles	1	3	3	3	3
Range	104	101–110	101–110	101–110	101–110
Ho	0.00	0.49	0.35	0.50	0.45
He	0.00	0.40	0.30	0.55	

Table 2
Pairwise differentiation (F_{ST}) and gene flow (N_m) between three populations in Nova Scotia and one population in Michigan

Pairwise comparison	Approximate distance (km)	F_{ST}	p value	N_m
Kejimkujik vs McGowan Lake	15	0.042	0.00201 ± 0.0004	5.8
McGowan Lake vs Pleasant River	15	0.042	0.00750 ± 0.0008	5.7
Kejimkujik vs Pleasant River	25	0.124	<0.0001 ± 0.0000	1.76
Michigan vs Kejimkujik	Approx. 1510	0.289	<0.0001 ± 0.0000	0.61
Michigan vs McGowan	Approx. 1510	0.253	<0.0001 ± 0.0000	0.74
Michigan vs Pleasant River	Approx. 1510	0.318	<0.0001 ± 0.0000	0.54

All p values are significant after sequential Bonferroni correction.

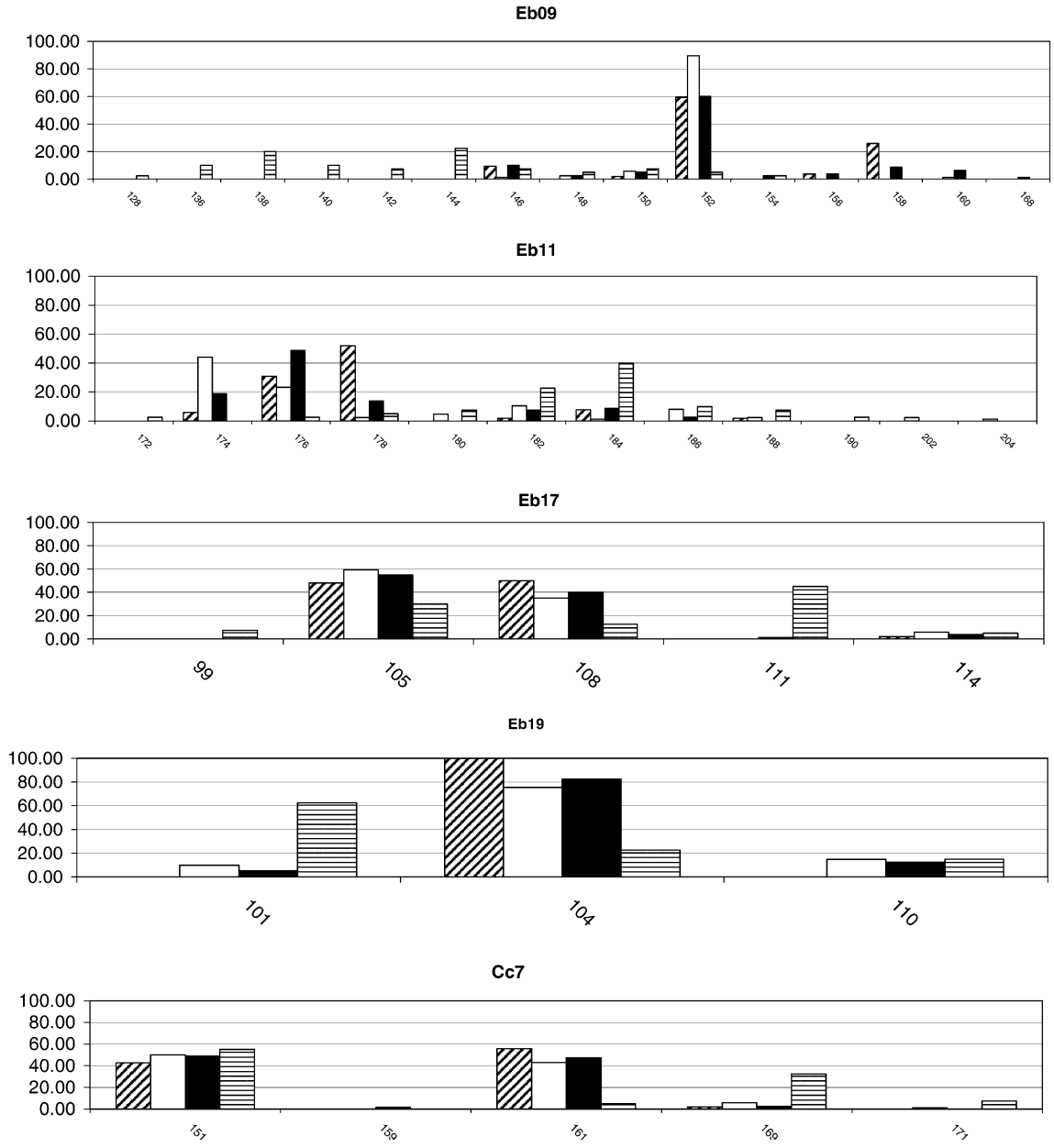

Fig. 2. Frequency of individual alleles for five microsatellite loci in three Nova Scotia subpopulations and one population in Michigan. X-axis is allele sizes in basepairs. Y-axis is allele frequency. Black bars represent McGowan Lake (ML); white bars represent Kejimkujik National Park (KNP); bars with horizontal lines represent Michigan (MI); bars with angled lines represent Pleasant River (PR).

did not deviate significantly from the L-shaped distribution expected under mutation-drift equilibrium.

In EASYPOP simulations conducted with a 5% migration rate F_{ST} values approximating those actually found in Nova Scotia were not attained even after 40 generations (approx. 1500 years). The more restrictive migration rate of 1% produced values approximating actual values in 11 generations (approx. 400 years).

4. Discussion

Analysis of five microsatellite loci in Blanding's turtle shows significant genetic differentiation over short geographic distances in Nova Scotia. The lower F_{ST} values within the Nova Scotia population complex relative to those between Nova Scotia and Michigan presumably reflect the higher level of gene flow occurring between these local populations. Based on the extensive present range disjunction, we assume that there is no current gene flow between Nova Scotia and Michigan.

Genetic structure found in this study confirms the existence of the apparent spatial structure of the Nova Scotia population noted in field studies. Field observations initially led us to hypothesize a role for watershed in determining this structure. While it is not statistically meaningful to test this with only three populations, the microsatellite data imply that distance, rather than watershed, is a better predictor of differentiation (Table 2). This is consistent with similar structure found in *Pseudemys scripta* where genetic structure was detected between groups separated by distances of less than 30 km; geographic proximity rather than intervening habitat type appeared to be the major determinant of genetic divergence (Scribner et al., 1986). Conversely, Souza et al. (2002) found genetic structure at fine geographic scale (<5 km) in *Hydromedusa maximiliani* that reflected the physical structure of local river drainages.

Since Blanding's turtle has an exceptionally long generation time and is largely aquatic, with limited overland travel (Power, 1989), this structure likely reflects naturally occurring landscape heterogeneity rather than recent anthropogenic landscape change such as road construction or land development. This is supported by the lack of any evidence of recent bottlenecks that may have resulted from population subdivision caused by anthropogenic changes.

In simulations this structure developed over a time frame of 400–1100 years.. The first permanent European settlement in Nova Scotia occurred in the early 17th century; development of the study area did not begin until the late 18th century. This suggests that genetic structure occurred over a time frame that exceeds European influence on the Nova Scotia landscape.

An alternative explanation of current genetic structure, which we do not favour, involves more recent but nearly total separation of the Nova Scotia concentrations of Blanding's turtle; this would allow for the rapid genetic divergence of groups. However, we know that individual turtles, particularly males, are capable of occasional extensive aquatic and terrestrial travel (>15 km; Power, 1989) with no apparent barriers other than distance. It therefore seems more likely that there is some current gene flow between groups, but that the long generation time of the species precludes our detecting it in field studies.

The role of conservation genetics is not only to approach a system from the view of biological interest, but to provide information that will contribute to conservation planning (Taylor and Dizon, 1999). Genetic structure in a population has serious implications for management and conservation.

Small individual units are more susceptible to the effects of stochastic events that increase the risk of local extinction. However, this risk may be countered by migration from other units in the complex – the rescue effect (Brown and Kodric-Brown, 1977). Although individual units within the complex are likely to lose genetic variation as a result of genetic drift, the overall genetic diversity in the population will remain high as a result of individual units drifting independently of each other (Varvio et al., 1986). Genetic variation within units may also be lost as a result of inbreeding, which in small populations can occur even with random mating (Keller and Waller, 2002).

In such a population complex, overall genetic variation may actually be increased as a result of different selective pressures between populations. There is some evidence for this within component groups of the Nova Scotia population of Blanding's turtle. Mean clutch size differs significantly between KNP and ML as do nesting substrates and selection of nesting sites (McNeil, 2002). While differences in nest site selection may simply reflect behavioural plasticity, a genetic component in clutch size cannot be discounted.

The effects of population subdivision on conservation planning for Blanding's turtle in Nova Scotia are compounded by the fact that the component units of the population are afforded varying degrees of protection. The KNP concentration of Blanding's turtle is contained within a national park. While there are still potential threats within this environment, such as campsite and beach development and the impacts that these may have on both the species and its predators, there is a regulatory regime that ensures that conservation and management issues will be considered in development decisions.

The ML concentration of Blanding's turtle occurs in an area that is a mix of corporate forestry, private and provincial government holdings. Additionally, McGowan Lake water levels are controlled by the provincial power company. This has resulted in varying levels of protection within the same landscape. Recently Bowater

Mersey Paper Company Limited, under its unique areas program, protected more than 100 hectares (247 acres) containing important Blanding's turtle summer and winter sites as well as nesting areas. Subsequently, the province of Nova Scotia placed land use restrictions on substantial amounts of adjacent Crown land. The power company has agreed to adjust lake flow regimes to the extent possible to accommodate turtle nesting requirements.

The greatest conservation challenge is faced by the PR concentration of Blanding's turtle, which occurs almost entirely within a working landscape dominated by agriculture, small scale forestry and cottage development. The diversity of land use, and the large number of individual landholders in this area require well-organized stewardship efforts to achieve co-existence between people and turtles.

These three sub-populations of Blanding's turtle, and especially their habitats, are at varying degrees of risk. If this population were panmictic, as originally thought, loss of individual turtles or habitat components would be important but the influence at the genetic level would be diluted across the landscape. Additionally, resilience of the system to disturbance would be enhanced by mobility of individuals. However, in a subdivided population, a similar reduction in turtles or habitat could lead to local extinction and consequently a significant loss in overall numbers of individuals and genetic variation. In the face of rapid environmental change, understanding spatial structure in this population complex is essential so that we can match management scale to ecological scale in an attempt to conserve this long-lived, late maturing species.

Acknowledgements

We thank Parks Canada for funding analysis of samples and for assistance with the collection of samples. We also thank Jeffie McNeil, Brennan Caverhill, Lorraine Standing, Donna Hurlburt, Eric Alcorn and the staff at Keimkujik National Park for assistance with collecting samples. Samples from the E.S. George reserve were provided by Matt Osentoski and Justin Congdon. Scholarship support was provided to both S.W. Mockford and L. McEachern by the Natural Sciences and Engineering Research Council of Canada.

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