SHORT COMMUNICATION

A preliminary examination of genetic variation in a peripheral population of Blanding's turtle, *Emydoidea blandingii*

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Abstract

Random amplified polymorphic DNA (RAPD) was used to compare the Nova Scotia population of Blanding's turtle (*Emydoidea blandingii*) with several populations from the species' main range. The Nova Scotia population is believed to have been isolated from the main range for 4000–8000 years. Cluster analysis using a neighbour-joining algorithm produced a dendrogram showing the Nova Scotia population clustering separately from those populations in the main range. Analysis of molecular variance shows 34.28% of total variance to be accounted for between the Nova Scotia population and populations in the main range. While this study is preliminary, the results suggest that the Nova Scotia population of Blanding's turtle may be important to the maintenance of genetic diversity in the species.

Keywords: Emydoidea blandingii, genetic variation, peripheral populations, random amplified polymorphic DNA (RAPD)

Received 25 March 1998; revision received 22 July 1998; accepted 22 July 1998

Introduction

Blanding's turtle (*Emydoidea blandingii* Holbrook [Emydidae]) is a North American freshwater turtle with a distribution centred on the Great Lakes (Fig. 1). The main range of *E. blandingii* includes southern Ontario and southwestern Québec, Wisconsin, Illinois, Michigan, Ohio, southeastern Minnesota, northern Iowa, northern Indiana, northeastern Nebraska and northwestern Pennsylvania (Herman *et al.* 1995). Blanding's turtle is distributed in patches throughout its range (McCoy 1973), which may be the most latitudinally compressed of all North American turtles.

Isolated populations of the Blanding's turtle occur outside the main range in Maine (Graham & Forsberg 1987), Massachusetts (Ernst & Barbour 1972), New York (Petokas & Alexander 1981), New Hampshire (Ernst & Barbour 1972), and Nova Scotia (Bleakney 1958; Dobson 1971). Of these disjunct populations the Nova Scotia population is the most isolated (Herman *et al.*, in press; Herman *et al.* 1995).

Phenotypic differences between populations of *E. blandingii* have been noted; these include size (Graham & Doyle 1977) and carapace colouration (Eaton 1989). These differences do not necessarily indicate genetic differences, as both size and colouration may be influenced by environmental factors. However, phenotypic differences coupled with spatial and temporal isolation may indicate genetic change. In this study we employed random amplified polymorphic DNA (RAPD; Williams *et al.* 1990) to examine the relationship between the Nova Scotia population of Blanding's turtle and populations in the species' main range.

Materials and methods

Samples were collected at Kejimkujik National Park, Nova Scotia ($n = 8$); St Lawrence Islands National Park, Ontario ($n = 3$); Harvard, Massachusetts ($n = 2$); Hennepin County, Minnesota ($n = 2$) and Walworth, Waukesha and Milwaukee Counties, Wisconsin ($n = 7$) (Fig. 1). Included in the Nova Scotia samples are five hatchlings collected from five different nests at emergence in the autumn of 1994. These hatchlings were raised at the Acadia

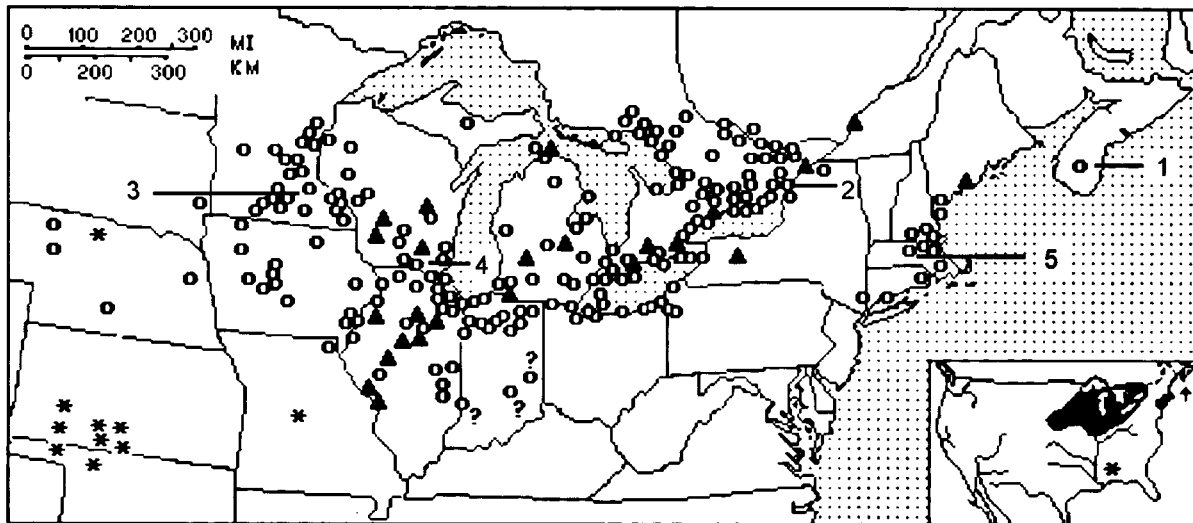Correspondence: M. Snyder. E-mail: marlene.snyder@acadiau.ca

Fig. 1 Past and present distribution of Blanding's turtle. Circles represent extant populations, stars represent fossil finds, triangles represent archeological finds (Herman *et al.* 1995). Sample locations: 1, Kejimkujik National Park, Nova Scotia (*n* = 8); 2, St Lawrence Islands National Park, Ontario (*n* = 3); 3, Hennepin County, Minnesota (*n* = 2); 4, Walworth, Waukesha and Milwaukee Counties, Wisconsin (*n* = 7); 5, Harvard, Massachussetts (*n* = 2).

University animal care facility until they were large enough for nondestructive blood sampling (≈ 50 g).

From adult turtles, ≈ 100 μL of blood was drawn from either the dorsal coccygeal vein (Haskell & Pokras 1994), the scapular vein/brachial artery (Avery & Vitt 1984), or the ventral caudal vein (C. Harvey-Clark, personal communication) using a 26-guage needle and a 1 mL syringe. In hatchlings, blood was collected by puncturing a venous plexus anterior and dorsal to the rear leg. Blood from the resultant bleeding was collected in heparanized capillary tubes. After collection, blood samples were placed in 300 μL of lysis buffer (1.25% SDS, 300 mM Tris-HCl (pH 8.0), 500 mM EDTA, 5% sucrose), agitated until no clots remained and stored at ambient temperature until they could be returned to the laboratory for extraction (Seutin *et al.* 1991).

DNA was extracted using a phenol–chloroform extraction modified from Jowett's *Drosophila* DNA extraction protocol (1986). Yields were estimated by running 2 μL of stock DNA solution in a 1% agarose gel containing ethidium bromide adjacent to a known quantity of *Hind*III-digested λDNA.

Primers used in the analysis were selected in a two-stage process. Initially, 100 primers synthesized at the Nucleic Acid-Protein Service Unit, University of British Columbia were screened using a single DNA sample. Side-by-side duplicates of each reaction were run to identify primers that would yield a potentially scoreable, repeatable, banding pattern. This initial screening revealed 33 primers that produced bands that were both potentially scoreable and repeatable.

These 33 primers were subjected to a second level of screening. At this second level each primer was used in four reactions. Each of the four reactions contained a single DNA sample from one of two individuals from each of two populations. From this second level of screening primers were selected if they:

(i) exhibited at least one band that was polymorphic between individuals or populations;
(ii) the polymorphic bands repeated in a minimum of three replicates;
(iii) the polymorphic bands were distinct enough in size from surrounding bands that they could be scored confidently;
(iv) the polymorphic bands were bright enough that presence/absence scoring would not be confounded by mere intensity differences.

This may favour loci separating the Nova Scotia population from populations in the main range but was necessitated by low levels of polymorphism overall (see the Results). Four primers yielding seven polymorphic bands were selected in this second level of primer scan and incorporated in the experiment.

PCR (Mullis *et al.* 1986) was performed in a total volume of 12.5 μL containing: 8 mM Tris-HCl (pH 9), 2 mM (NH$_4$)$_2$SO$_4$, 350 μM MgCl$_2$, 15 μg/mL BSA, 80 μM each dNTP, 40 ng of primer, 0.5 units *Taq* polymerase, and ≈ 1.2 ng of genomic DNA. Reaction conditions were: initial denaturation of DNA for 2 min at 94 °C; 39 cycles of 1 min denaturing at 94 °C, 1 min annealing at 35 °C, 2 min extension at 72 °C; 5 min final extension at 72 °C. These

were held at 4 °C until reaction products were run on agarose gels. All reactions were carried out in a MJ Research Inc. PTC-100 thermocyler. All reactions were conducted using a single primer.

RAPD patterns produced by the four primers were recorded as 22, 1 × 7 vectors of 1 and 0. Initial analysis treated each band as a phenotypic marker and tested the null hypothesis that bands were independently distributed across regions. Small sample sizes prevented the use of any statistic that made use of the chi-square distribution to test significance. To overcome this, the populations from the species' main range were combined and tested against the Nova Scotia population. This allowed for the use of Fisher's exact test to analyse 2 × 2 contingency tables with regions on one side and presence and absence of bands on the other. The alpha of 0.05 was adjusted for multiple comparisons; alpha' = alpha/(7) = 0.007.

Analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was employed to examine the organization of variance among individuals, populations, and regions. Originally developed for the analysis of mitochondrial DNA haplotypes, this procedure can be directly applied to RAPD phenotypes (Huff *et al.* 1993; Haig *et al.* 1994). Twenty-two seven-component vectors were treated as phenotypes and analysed using WINAMOVA 1.55 produced by Dr Laurent Excoffier, University of Geneva.

Additionally, a dissimilarity matrix between individuals was produced using RAPDPLOT (Black 1995):

$$D = 1 - (2N_{AB}/(N_A + N_B))$$

This matrix was then used as input into the NEIGHBOR program in PHYLIP 3.5c (Phylogeny Inference Package; Felsenstein 1993). A neighbour-joining (Saitou & Nei 1987) dendrogram was produced. To allow for the occurence of similar trees 50 iterations of NEIGHBOR were run with random order of input and a consensus tree was calculated from the resulting trees using the CONSENSE program in PHYLIP 3.5c.

Results

The 33 primers produced a total of 156 potentially scoreable bands (range 2–9; mean 4.73). Of these, only seven

bands, i.e. 4.5%, were polymorphic. No population-specific bands were found for 22 individuals from five populations.

Three of seven polymorphic bands varied significantly from the null hypothesis that bands were independently distributed across regions. Two bands produced a *P*-value less than 0.05 and one band produced a *P*-value less than the adjusted α of 0.007. On average, one band in 12 would be expected to produce a *P*-value less than 0.05 by chance alone if all bands were independently distributed (Rice 1989).

The seven bands produced 11 RAPD phenotypes. Six of 11 phenotypes were represented in the eight Kejimkujik samples; three Ontario samples were separate phenotypes; two Minnesota samples were separate phenotypes; two Massachusetts samples were separate phenotypes; and seven Wisconsin samples represented only two phenotypes.

Analysis using AMOVA allowed partitioning of overall phenotypic variance into three levels (Table 1). Populations from Ontario, Wisconsin, Massachussetts and Minnesota were treated as a single region representing the species' main range, and the Nova Scotia population was treated as a region of its own. Approximately 55% of total variance is accounted for among individuals within populations. Approximately 11% of total variance occurs among populations within regions suggesting that there is partitioning of populations within the main range. The remaining 34% of variance is among regions, suggesting strong partitioning between regions.

The unrooted consensus tree clearly separates the Nova Scotia population from those populations in the main range (Fig. 2). The Massachusetts samples do not separate completely from the Nova Scotia population. The bifurcation between the cluster containing the Nova Scotia population and Mass02, and the cluster containing the remaining populations, occurred in 100% of 50 replicate trees.

Discussion

Six of 11 RAPD phenotypes were represented in the Nova Scotia population. This seems to indicate that the Nova Scotia population is not suffering from a lack of

Variance component	Variance	% of total	P-value
Among regions	0.539	34.28	<0.002
Among populations/within regions	0.170	10.78	<0.002
Among individuals/within populations	0.864	54.95	<0.002

Table 1 Analysis of molecular variance (AMOVA) for 22 individuals in five populations grouped into two regions. Region 1 is the Nova Scotia population; region 2 contains the remaining four populations. *P*-value is the probability of obtaining a larger variance by chance alone under the null hypothesis that the variance is zero. *P*-value is estimated from 500 sampling permutations
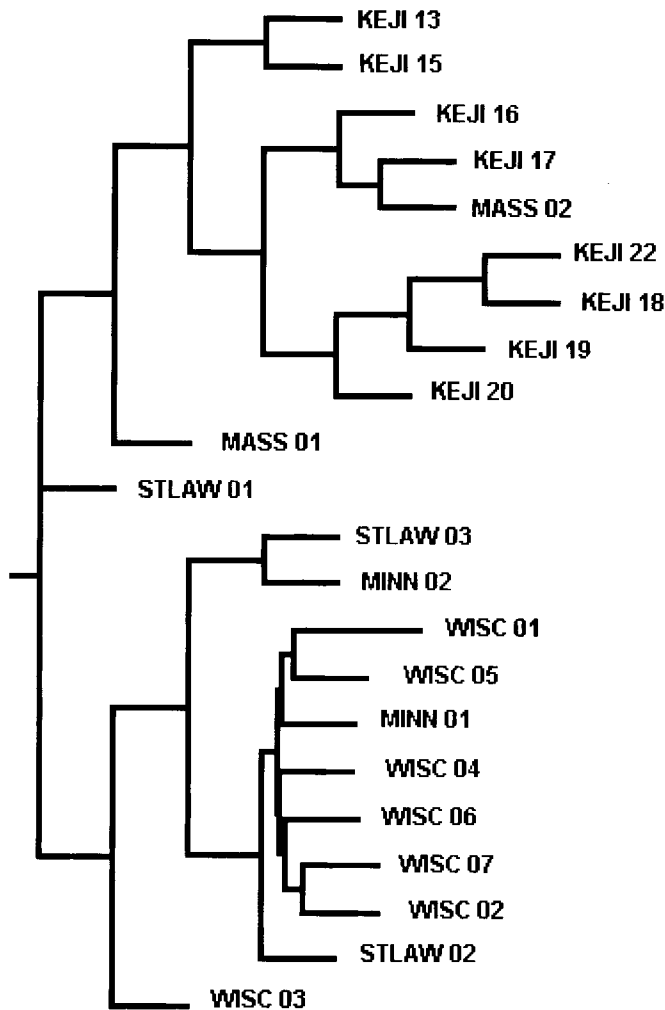
Fig. 2 Neighbour-joining dendrogram. Consensus tree of 50 replicates of the NEIGHBOR program in PHYLIP 3.5c. Replicates were performed using random input of data to allow for the occurrence of similar trees. KEJI = Kejimkujik National Park, Nova Scotia; STLAW = St Lawrence Islands National Park, Ontario; WISC = Wisconsin; MINN = Minnesota; MASS = Massachussetts.

within-population variation. This is consistent with the assumption that the Nova Scotia population has been gradually reduced to its current size and has therefore not experienced founder effects or a genetic bottleneck.

When compared to populations in the main range as a whole using AMOVA, 34% of total variance is accounted for among regions. This in itself does not mean that the Nova Scotia population contributes significantly to the overall genetic diversity in the species; a small population subject to genetic drift may differ from those populations in the species' main range while having low within-population variation. However, this large degree of partitioning between the Nova Scotia population and populations in and around the species' main range coupled with the variation found within the Nova Scotia population suggests that the Nova Scotia population may contribute significantly to the overall variation in the species. This suggests that the Nova Scotia population may be an important evolutionary unit of the species.

Levels of polymorphism found in Blanding's turtle were low (4.5% of 156 bands). In RAPD analysis of other reptile species polymorphisms among scorable bands ranged from greater than 21% in adders (Tegelstrom & Hoggren 1994) to 76% in black rat snakes and 56% in eastern massasauga rattlesnakes (Gibb et al. 1994). This apparent lack of polymorphism is not totally unexpected in studies of Testudines, as low rates of mutation have been found in mitochondrial DNA (Avise et al. 1992; Bowen et al. 1993) and more recently in nuclear DNA (FitzSimmons et al. 1995).

While small sample sizes make this study preliminary in nature, data presented here suggest that the Nova Scotia population of Blanding's turtle represents a significant proportion of the total genetic variation in the species. As a consequence, this population should be considered in the maintenance of genetic diversity in the species. Further study is being conducted to examine more closely the role of peripheral populations in the overall genetic variation in Blanding's turtle.

Acknowledgements

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