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Genetic Diversity of *Dermacentor andersoni* in Western Montana via Inter-simple Sequence Repeats

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Abstract

Demacentor andersoni (Ixodidae) is one of the most important disease vectors in Montana. Ticks transmit more animal disease agents than any other blood-sucking arthropods. Ticks are the second most important vector in public health and the most important in veterinary medicine. *Dermacentor andersoni* is the vector of Rocky Mountain spotted fever, Colorado tick fever, tularemia, bovine anaplasmosis, and Powassan encephalitis. Population genetic studies of disease vectors can allow insight into the vectors contribution to the spread of disease and possible control strategies. This study used inter-simple sequence repeats (ISSRs) to assess genetic variation within and among populations. An analysis of molecular variance (AMOVA) and population pairwise fixation index (F_{ST}) were used to determine statistical differences in ISSR banding patterns. A regression analysis was performed to determine if there was a significant relationship between Euclidean distance and F_{ST} values. Significant variation was found between individuals and populations, though no significant variation was found between groups of populations. While no connection was found between geography and genetic variation, this analysis was limited by small population sizes for some sites. This analysis found that ISSRs could potentially be useful in determining genetic variation in *D. andersoni* populations.

Introduction

Currently only three major groups of ticks are found worldwide; soft bodied ticks (Argasidae), hard bodied ticks (Ixodidae), and Nuttalliellidae, which consists of a single species found in South Africa (Bedford 1931; Guglielmone et al., 2010). Soft bodied ticks lack the sclerotized scutum found in hard bodied ticks (Bedford 1931). *Nuttalliellidae* have a pseudo-scutum that is only partially sclerotized.

The Rocky Mountain wood tick *(Dermacentor andersoni)* is one of six tick species in Montana (Johnson 2009). *Demacentor andersoni* (Ixodidae) is one of the most important disease vectors in Montana. Ticks transmit the most animal disease agents of all blood-sucking arthropods (Johnson 2009). Ticks are the second most important vector in public health and the most important in veterinary medicine (Johnson 2009). *Dermacentor andersoni* is the vector of Rocky Mountain spotted fever, Colorado tick fever, tularemia, bovine anaplasmosis (Johnson 2009), and Powassan encephalitis (Jongejan and Uilenberg, 2004). Though Bovine anaplasmosis does not affect humans, it is estimated to cost the United States 300 million dollars every year due to infection (Kocan et al., 2000).

According to Tabachnick and Black (1995), "population genetic studies of arthropod disease vectors provide opportunities for understanding their role in arthropod-borne disease, and for developing more effective control strategies." Further, population genetic studies that analyze variation and extrapolate gene flow provide information about the capacity of the vector to transmit disease and about its breeding habits.

Dermacentor andersoni has only been studied genetically twice in small studies using 16S rDNA. For example, de la Fuente et al, (2004) only studied 54 individuals from the same location. Patterson (2009) studied two allopatric populations in Canada and called for further studies to "determine the extent of genetic variation and the vector potential of ticks from different populations throughout the range of *D. andersoni* in the United States and Canada". Koslosky (2016) and Dotson (2015) also agreed that further studies are necessary to determine genetic variation.

While microsatellite loci have not been found to be abundant in all arthropod genomes (Fagerber et al., 2001), ISSRs (inter-simple sequence repeats) are useful in arthropod genetics (Ochando et al., 2010). ISSRs are noncoding regions found between two of the same microsatellite regions. ISSRs have assessed variation in arthropod vector populations such as the *Aeides aegypti* mosquito (Abbot 2001). In this study, intra- and inter-population levels of genetic variation were assessed in *D. andersoni* populations using ISSRs. Information from this study can be used in the future to add to a GIS model of the distribution of *D. andersoni*. I hypothesized that there would be significant and distinguishable variation within and among populations of *D. andersoni* in Western Montana using ISSRs as a genetic marker.

Materials and Methods

Sample Collection

Samples were collected from 26 sites in Western Montana using one square meter of white cloth drawn over grasses and brush to collect questing ticks. The twelve sites selected for this analysis are found in **Table 1** along with location name and sample size. Samples of smaller than 10 individuals were excluded from the analysis. Sites where one tick was found in 15-person minutes were sampled for a full 60-person minutes. Sample sites were chosen to provide varying

distances between sites. Several high yield sample sites were used from previous studies found by Koslosky (2016) and Dotson (2015). Prospecting new high elevation ravines and mountains determined other sites while previous personal experience determined areas that potentially could be good tick habitat. Ticks were captured live and stored in vials to be returned to Carroll College for storage in a -80 ˚C freezer.

Table 1. Site labels along with their location name, and sample size.

Site Label	Location Name	Sample Size
1	North Fork Blackfoot River Trail	52
2	Beaver Creek C	37
3	Beaver Creek Pipeline	19
4	Blackleaf Wildlife Refuge	37
5	Teton Pass	17
6	Woodlake Campground	26
7	Dearborn Trail	41
8	Alice Creek A	25
9	Holland Falls	12
10	Blodgett Trail	13
11	York Bridge	10
12	Black Tail Mountain	11

Isolation

Identity of tick species was determined by morphological differences. These included: white markings on the dorsal shield, short palpi roughly long as the basis capituli, spiracular plates with a dorsal prolongation, a short cornua of the basis capituli, and goblets of the spiracular plates are large and less numerous than *D. variabilis.*

DNA Extraction

DNA was extracted using the QIAGEN DNeasy® Blood and Tissue Kit and the QIAGEN (2006) Bench Protocol: Animal Blood with the following modifications. Individual ticks were homogenized with Lysing Matrix A tubes from MP Biomedicals containing 500 μL of Buffer AL in the FastPrep Smart Solutions LTD for two cycles of 35 seconds at a speed of 30 oscillations/minute. Three hundred microliters of this homogenate were used in the proteinase K digest. This digest was incubated for one hour and vortexed every 20 minutes. The QIAGEN (2006) protocol Bench Protocol: Animal Blood was then followed from step three through seven with a modification to the elution volume to be of 100 μL Buffer AE.

PCR Primer Determination and Optimization

PCR primers were chosen from screenings of 15 randomly selected samples and 10 ISSR primers for the arthropod *Culex tarsalis* derived from Rasgon et al. (2006). Primer CTISSR4 (5'-$(AG)_8$TGA-3' was selected for high level of variability, some parity of banding, and clarity of banding in gels. This primer was subjected to annealing temperature trials using a temperature gradient from 68 ˚C to 58 ˚C to find the best second cycle annealing temperature for amplification.

PCR Amplification

Extracted DNA was amplified by PCR using *C. tarsalis* primers described above. Primers were rehydrated to 100 μM for storage as a stock concentration. This stock concentration was diluted to a working concentration of 10 μM. The master mix (**Table 2**) and the thermocycler

protocol (**Table 3**) used to amplify ISSR sequences in 25 µL reactions were adapted from Rasgon et al. (2009).

Table 2. PCR master mix reagents and quantity of reagent per reaction.

Master mix reagents	**Quantity per reaction (25µL reaction)**
PCR Certified Water	17.5µL
10x Standard *Taq* Reaction Buffer Mg Free	2.5µL
CTISSR Primer	2.0µL
Taq DNA Polymerase	0.5µL
25mM $MgCl_2$ Solution	1µL
25mM dNTP Solution	0.5µL
D. andersoni template DNA	1µL

Table 3. Thermocycler protocol for PCR reactions.

Cycle	**Step and Temperature**
Cycle 1 (1x)	Step 1 (2 minutes): 94 ˚C
Cycle 2 (13x)	Step 1 (30 seconds): 94 ˚C Step 2 (30 seconds): **CTISSR4**-64 ˚C Step 3 (1 minute): 72 ˚C
Cycle 3 (36x)	Step 1 (30 seconds): 94 ˚C Step 2 (30 seconds): 55 ˚C Step 3 (1 minute): 72 ˚C
Cycle 4 (1x)	Step 1 (10 minutes): 72 ˚C Step 2 (Hold): 4 ˚C

Gel Electrophoresis and Visualization of Banding Patterns

PCR products were run in a 1% agarose, 25 cm by 25 cm, 50 mL TBE gel stained with SYBR Safe DNA gel stain for six hours at 125 volts. Gels were then visualized using Trans-UV light from a BioRad Molecular Imager® Gel Doc™ XR+ System and Quantity One® Gel Doc XR software.

Statistical Analysis of ISSR Banding Patterns

I used a Restriction Fragment Length Polymorphism (RFLP) analysis on the ISSR banding patterns using Arlequin version 3.5.2.2. This analysis included two tests, an Analysis of Molecular Variance (AMOVA) and a Population Pairwise Fixation Index (F_{ST}) to determine if a statistical difference occurred in the ISSR banding patterns of *D. andersoni* populations (Excoffier *et al*. 2010). For this analysis, groups were divided as follows; Big Belt Mountains: sites 2, 3, and 11, Western Front Range: sites 1, and 4-8, Flathead Valley: 11, and 12, and Bitterroot Mountains: site 10 (**Table 1**).

Regression Analysis

A regression analysis was performed in Microsoft Excel comparing the F_{ST} values between sites to the Euclidean distance between sites.

Results

Out of 26 sites, 12 sites with 10 individuals or more were selected for the analyses. These sites totaled 300 individuals. Results of the AMOVA analysis are shown in **Table 4**. Most of the genetic variation was found between individuals within populations (92.86%) while the smallest amount of variation (1.64%) was found between groups of populations. A relatively small amount of variation was found between populations within groups (5.5%). There was no significant variation found between groups. The results of the pairwise difference F_{ST} analysis are found in **Table 5** and visualized in **Figure 1**. Sites 7 and 8, which occur directly adjacent to each other were

genetically distinct populations. The most geographically isolated population was the most similar to all other populations. Regression analysis of Euclidean distance and F_{ST} values resulted in a p-value of 0.063.

Table 4. Summary statistics of the AMOVA analysis.

Source of Variation	d.f.	Sum of Square	Variance Components	Percentage of Variation	P-value
Among Groups	3	24.31	0.047	1.64	0.183±0.012
Among Populations within Groups	8	54.82	0.158	5.5	< 0.001
Within Populations	288	770.99	2.67	92.86	< 0.001

Table 5. Matrix of significant pairwise difference F_{ST} P-values ($p<0.05$). Significant values are marked with a plus and insignificant with a minus.

Site Label	1	2	3	4	5	6	7	8	9	10	11	12
1		-	+	+	+	-	+	+	+	+	+	-
2	-		-	+	-	-	+	+	+	-	-	-
3	+	-		+	+	-	+	+	+	+	+	-
4	+	+	+		+	+	+	+	+	+	+	-

5	+	-	+	+		+	+	+	+	+	+	-
6	-	-	-	+	+		+	+	+	+	+	-
7	+	+	+	+	+	+		+	+	+	+	+
8	+	+	+	+	+	+	+		+	+	+	+
9	+	+	+	+	+	+	+	+		-	-	-
10	-	-	-	-	+	-	+	+	-		-	-
11	+	-	-	+	+	+	+	+	-	-		-
12	-	-	-	-	-	-	+	+	-	-	-	

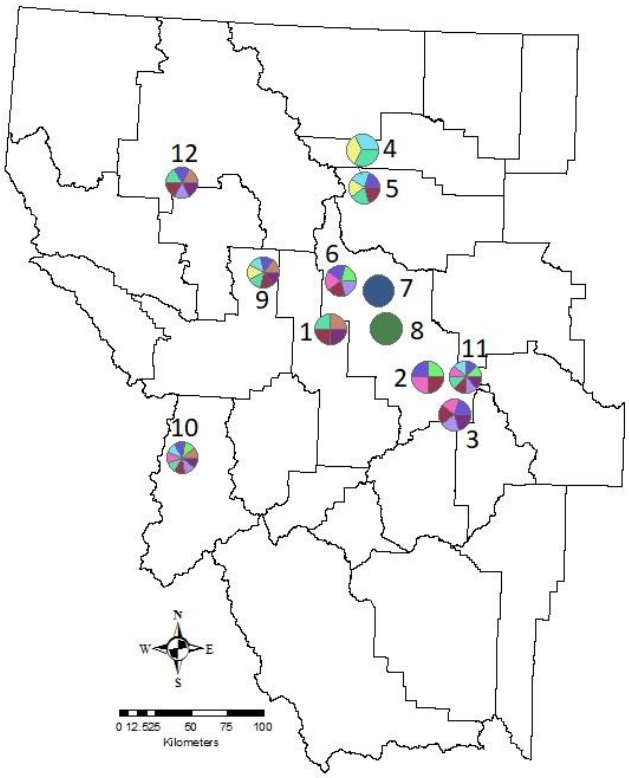

Figure 1. Visualization of pairwise differences in populations across Western Montana. The underlying lines denote divisions between counties. Shared colors indicate no significant difference in respect to F_{ST} values. Site numbers and locations are listed in Table 1.

Discussion

The hypothesis that there would be significant and distinguishable variation within and among populations of *D. andersoni* in Western Montana using ISSRs as a genetic marker is partially accepted and partially rejected. There were significant amounts of variation found among individuals and there was significant variation found

between populations within groups. However, there was no significant variation among groups of populations. This suggests that there is no relationship between the genetic variation and geographic relationships. The two genetically distinct populations (sites 7 & 8 in **Table 5, Figure 1**) reside directly next to one another while the most geographically isolated population shares the most genetic markers with other populations. There was no significant relationship determined from the regression analysis of F_{ST} and Euclidean distance which suggests no relationship between site distance and genetic variation. These results could be due to small sample size artifacts or marker behavior as there was a large sample size disparity between some populations.

Typically, there is a relationship between genetic variation and geography. Migration between populations can be limited by geographical barriers (Manel et al., 2003). Or migration is limited simply by individual range. Therefore, we tend to see either a relationship between genetic variation and geographical barriers or, Euclidean distance. However, there was no significant relationship to genetic variation found with geographical barriers or Euclidean distance.

This assessment has found a genetic marker with significant genetic variation between individuals. These analyses suggest that the populations of *D. andersoni* in Montana are panmictic though two groups were found to be genetically distinct and close together. Previous studies from Koslosky (2016) and Dotson (2015) came to the same conclusion that the populations in Montana could be panmictic. This could be due to movements of mammal hosts of *D. andersoni*, with individuals moving large distances while feeding on host species. It is highly unlikely that a tick would travel back to its original population location after feeding off its host for multiple days.

In the future, larger scale research with ISSRs in *D. andersoni* would be advised to determine if this genetic marker is valuable and if there is a significant relationship between geography and genetic variation. Further, analyses could determine whether *D. andersoni* populations in Montana are truly panmictic. This assessment was limited by small sample sizes in certain populations. Collecting at the same sites over multiple years could help by increasing sample size for a future analysis. Sites where ticks are found in lower density could benefit from multiple collections. These analyses would need to test and account for temporal effects. Further, I would advise using multiple ISSR primers to increase the number of bands per individual and therefore gain a larger understanding of the genetic variation. However, it is possible that ISSRs could be too variable in individuals to distinguish relationships as there was a high degree of variation at the individual level. A larger sample size of populations and the use of more primers could potentially allow the creation of a gene flow map and a landscape genetics model.

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