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# Genetic Diversity in *Dermacentor andersoni* Populations in Western Montana

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**Genetic Diversity in *Dermacentor andersoni* Populations in Western Montana**

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**SIGNATURE PAGE**

This thesis for honors recognition has been approved for the  
Department of Life and Environmental Sciences.

Director Jennifer Nowiacka Date 4/29/16

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**Abstract**

Ticks have been made a top priority for analysis as disease vectors. Specifically the tick *Dermacentor andersoni* is a vector for Colorado Tick Fever, Rocky Mountain Spotted Fever, Bovine Anaplasmosis, and Powassan Encephalitis. This study was designed to assist in the utilization of the current protocols for a West Nile Virus vector distribution map in Montana and apply these to develop a *D. andersoni* distribution map. Samples were collected from across western Montana, and the 16S mitochondrial DNA gene was amplified. Six tick populations were tested, and there were sixteen different haplotypes found. However, when comparing these haplotypes there was no statistically significant difference with respect to the haplotype frequencies found between the populations, as well as no significant difference between the genetic diversity of tick populations on either side of the Continental Divide. This leads to the conclusion that either the genetic marker used is not an informative indicator between tick populations within 300 kilometers of one another, or that these tick populations are panmictic and therefore are not genetically isolated within the tested area.

## Introduction

Ticks, part of the subclass Acari, are one of the main arthropod vectors of disease to both humans and domestic animals (Jongejan and Uilenberg 2004). They are second for human disease vectors in the entire world, just behind mosquitos (Parola and Raoult 2001). In 2015, the Entomological Society of America declared tick research as one of its top priorities and conveyed the necessity to develop an integrated tick management system.

One area of tick management is focused on the ecological surveillance of tick habitats and the host landscape. In Montana there is a model of the state wide distribution of West Nile Virus, carried by the mosquito vector *Culex tarsalis*, that has been in use since 2009 (Hokit *et al.* 2013). This model shows the distribution of mosquito populations in Montana and tests each of these populations for WNV. The results of this modeling allow for the development of a risk map that can be used to help hikers, medical professionals, and researchers know where WNV is located within the state at any time. The WNV model is currently being adopted for assessing tick populations and their risk for disease (Dotson 2015).

Ticks (Archnida: Parasitiformes) are classified into three families: hard bodied ticks (*Ixodidae*), soft bodied ticks (*Argasidae*), and a less distinguishable family *Nuttalliellidae* (Bedford 1931). Hard bodied ticks are named so because of their sclerotized scutum on their dorsal side. This is lacking in soft-bodied ticks which are not sclerotized and *Nuttalliellidae* which have a pseudo-scutum that is partially sclerotized (Bedford 1931). Forty-four species of ticks are infectious to humans; 11 are soft-bodied and 33 are hard-bodied (Merten and Durden 2000).

Ticks become disease vectors by feeding on an infected host, which primarily consists of a wide variety of vertebrates (Sonenshine 1991). Most ticks feed in each one of their last three life stages: the larval, nymphal, and adult (Parola and Raoult 2001). This feeding process starts with a painless bite that pierces the skin of the host, allowing the ticks to take their blood meals (Parola and Raoult 2001). The ticks feed for several days and once ticks have finished ingesting a blood meal and are fully engorged, they release themselves and go about digesting the meal just consumed (Parola and Raoult 2001).

The state of Montana has four different types of ticks that are vectors for diseases such as Rocky Mountain Spotted Fever, Lyme Disease, and Tularemia. These ticks include the Rocky Mountain wood tick (*D. andersoni*), winter tick (*D. albipictus*), American dog tick (*D. variabilis*) and soft ticks (*Ornithodoros hermsii*) (Johnson, 2010). The Rocky Mountain wood tick is most prevalent in Montana (Merton and Durden, 2000).

*Dermacentor andersoni* was chosen for this study due to its prevalence and abundance across the state, and its known involvement with the transmission of Colorado Tick Fever (Brackney *et al.* 2010), Rocky Mountain Spotted Fever (Ricketts 1909), Bovine Anaplasmosis (Piesman and Eisen 2008), and Powassan Encephalitis (Jongejan and Uilenberg 2004). Colorado Tick Fever (Brackney *et al.* 2010), Rocky Mountain Spotted Fever (Paddock *et al.* 2002), and Powassan Encephalitis (Jongejan and Uilenberg 2004) can all affect humans. Symptoms can vary widely depending on the disease and the individual, but they can range from flu like symptoms, to rashes, and can even lead to death. Bovine Anaplasmosis primarily affects cattle and is estimated to cost the U.S. 300

million dollars every year due to infected livestock (Kocan *et al.* 2000). If a geographic distribution of these disease vectors were physically mapped out based on tick populations it could greatly help reduce outbreaks for all of these diseases. The distribution map would serve as a resource for healthcare facilities to aid in the diagnosis of patients, for ranchers in Montana so they know what precautions they need to take in order to protect their cattle, and for the general public.

Mitochondrial DNA can be an indicator of population differences within species, particularly between tick populations (Black and Piesman 1994). Specifically, the 16S ribosomal mitochondrial gene has been used to determine the differences between hard tick and soft tick families and subfamilies (Black and Peisman 1994). This gene should be ideal for this study in determining differences in population genetics because this region evolves quickly (Simon *et al.* 1994). Patterson *et al.* (2009) and de la Fuente *et al.* (2005) found that by isolating distinct fragments of the 16S gene, genetic differences between *D. andersoni* populations could be observed. Garringer (2014) found that the primers 16S+1 (Patterson *et al.* 2009) and D16S5 (de la Fuente *et al.* 2005) produced a DNA sequence around 360 base pairs that was found to be informative in determining gene flow, or migration rates, between *D. andersoni* populations.

In 2005, de la Fuente *et al.* found 14 different haplotypes, or specific single nucleotide polymorphisms observed in populations, in the 16S mitochondrial DNA in the Lake Como region of the Bitterroot Valley in Montana. Patterson *et al.* (2009) found five new haplotypes suggesting gene flow between populations in Saskatchewan and Alberta.

The goal of this study was to determine the level of migration and interaction of ticks across western Montana using genetic data. Samples of *D. andersoni* were collected

throughout western Montana and their 16S mitochondrial ribosomal DNA was amplified and sequenced to determine whether there is sufficient genetic diversity between samples to have unique gene pools across the state. This results of this study may give a better understanding of the distance over which the vector might transmit disease. Dotson (2015) tested for genetic diversity and gene flow between populations over a 30 kilometer area and found no statistical difference in genetic structure among the populations, suggesting high levels of gene flow between populations. Based on this study and Patterson *et al.* (2009), my hypothesis is that the distance may not have been great enough between sampling sites to find statistical evidence for limited gene flow. I believe that by increasing the sample region to a few hundred kilometers the gene flow between populations might become limited and there may be a statistical significance suggesting isolated populations. Another goal of this study is to see if the Continental Divide is a barrier to gene flow between tick populations. By determining the level of migration between tick populations in western Montana, population genetics can be used to determine gene flow among populations using variance analysis (Tabachnick and Black 1995). This information can then define the dispersal patterns of these vectors to better understand the transmission of these diseases between populations (Tabachnick and Black 1995). This information combined with environmental factors could allow the development of a map to show and predict the distribution of disease vectors in Montana.

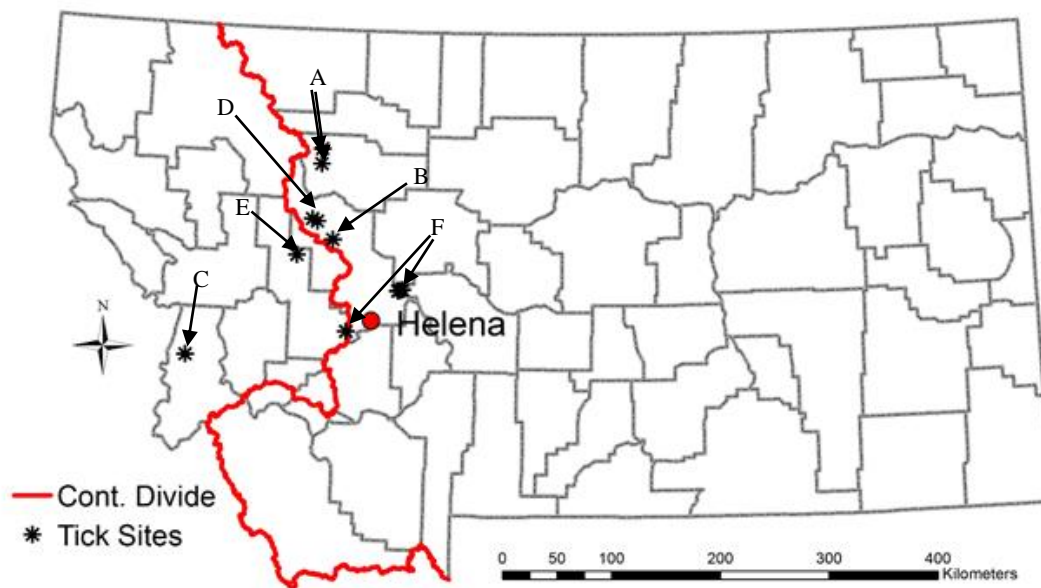
## **Materials and Methods**

### ***Sample Collection***

Samples were collected in western Montana (Figure 1), using a square meter of white cloth which was drawn through grasses and brush to collect questing ticks. In total,



the samples used for this experiment were gathered from twelve different sample sites. Several of these samples sites were within a few kilometers of one another, and based on the findings of Dotson (2015) study, these samples were shown to be part of populations that were not genetically distinct and had large amounts of gene flow between them. In an attempt to improve the informative nature of the data, a larger area was sampled. These ticks were captured live and stored in vials until returned to Carroll College where they were frozen in the  $-80^{\circ}\text{C}$  freezer for long-term storage.



**Figure 1:** Sampling sites where ticks were collected across western Montana. The sites are as follows Blackleaf (A), Dearborn (B), Blodgett (C), Woodlake (D), North Fork (E), Helena Local (F).

### *Isolation*

Morphological differences (Gregson, 1956) were used to identify ticks. These included: *D. andersoni* have spiracular plates which have moderately large goblets as opposed to the large ones of *D. albipictus* and the small ones of *D. variabilis*, and the

scutum is evenly rounded with large deep punctuations posteriorly instead of being pointed.

### ***DNA Extraction***

Samples were homogenized using the QIAGEN DNeasy® Blood and Tissue Kit. The bench protocol for animal tissues (Garringer, 2014; Dotson, 2015) was used. An adjustment had to be made for ticks due to *D. andersoni*'s sclerotized shell. The samples first had to be ground up using FastPrep Smart Solutions LTD bead beater for 35 seconds at a speed of 30 oscillations/minute. Then the QIAGEN (2006) protocols were used for DNA extraction and incubation for one and one half hours.

### ***PCR Amplification***

PCR amplification was performed on the extracted DNA. The D1625 primers used in de la Fuente *et al.* (2005) study and the D165 1 primers from the Patterson *et al.* (2009) were used to amplify a 360-bp fragment of the 16S rDNA gene. The primers sequences are:

D1625 (5'-GAATGCTAAGAGAATGGAAT-3')

D165 1 (5'-CGGTCTGAACTCAGATCAAGT-3')

Using the PCR procedure developed by de la Fuente *et al.* (2001), 50 µl PCR reactions were run using a Bio Rad Thermocycler. The thermal cycler conditions were as follows: initial denaturation occurred at 94 °C for 30s; followed by 35 cycles with denaturation at 94 °C for 30s, annealing at 52 °C for 30s, and elongation at 68°C for 1 min. A negative

control of DI water was used. To analyze the PCR products and qualitatively test for amplification, the products were run on 1% agarose electrophoresis gels.

### ***Sequencing***

The samples were sent to Macrogen in South Korea for sequencing using the primers listed above.

### ***Population and Phylogenetic Analyses***

The forward and reverse sequences were aligned using Condon Code Aligner (CodonCode Corporation, [www.codoncode.com](http://www.codoncode.com)). Once the two sequences were combined to form a consensus sequence, they were trimmed in order to give a 360-bp DNA fragment. These sequences were then aligned and compared to each other using Clustal X version 2.0 (Larkin *et al.* 2007). Next, Arelquin software was used for calculating nucleotide diversity, AMOVA testing pairwise FSTs, and calculation of number of haplotypes (Excoffier *et al.* 2010). AMOVA testing was used to determine if there was a significant difference between the populations on either side of the Continental Divide. Of the populations in this analysis, two were located on the west side of the Continental Divide and three were located to the east. The last group fell upon the divide line and was therefore excluded from the Continental Divide grouping for the AMOVA test. Neighbor-joining analysis was performed using PAUP\* 4.0a147 (Swofford, 2003) to construct a phylogenetic tree based on the genetic differences using default settings. All six different sample populations were compared for haplotype similarities and differences.

## Results

Of the 120 samples sequenced, 108 samples had sequences that were of a high enough quality and consistent with the *D. andersoni* 16S mitochondrial gene sequence, and had a total aligned length of 381 base pairs. The twelve sequences that were excluded had insertions of ten to twenty bases or lacked statistical certainty within their bases and, therefore, were excluded from the analysis. These sequences were then trimmed to approximately 360 base pairs for comparison (Appendix 1).

Nucleotide diversity within each population was relatively low (Table 1), with an average of 0.0055 differences per site.

Blackleaf (A)	0.004214
Dearborn (B)	0.004354
Blodgett (C)	0.004061
Woodlake (D)	0.006657
North Fork (E)	0.008088
Helena Local (F)	0.005403
<b>Average</b>	<b>0.005463</b>

Table 2 shows the number of samples or gene copies for each population tested as some tick sequences had to be excluded due to low quality. The number of total bases that were usable for this analysis was 359 as some nucleotide sites had to be excluded due to missing data at the beginning or end of sequences. Within the number of usable loci, the number that were polymorphic varied in number between populations.

**Table 2:** Basic properties for usable and Polymorphic Loci in each population.

Statistics	Blackleaf	Dearborn	Blodgett	Woodlake	North Fork	Helena Local
No. of gene copies	13	20	19	18	18	20
No. of loci	381	381	381	381	381	381
No. of usable loci	359	359	360	378	379	377
No. of polymorphic loci	4	5	5	5	6	9

There were a total of sixteen haplotypes found within ticks from the six sites that were tested. However, ten of these unique haplotypes were found within only one tick out of the 108 tested (Table 3). The other six haplotypes were spread out over the three populations tested and did not show any significant pattern (Table 3).

<b>Table 3.</b> Relative frequencies for haplotypes in the six tested tick populations. A = Blackleaf, B = Dearborn, C = Blodgett, D = Woodlake, E = Northfork, F = Helena Local.						
Haplotype	A (13)	B (20)	C (19)	D (18)	E (18)	F (20)
1	0.308	0.25	0.368	0.278	0.5	0.25
2	0.0769	0.15	0.105	0.111	0.0556	0.15
3	0.462	0.3	0.158	0.5	0.222	0.35
4	0.0769	0.15	0.263	0	0.111	0.05
5	0.0769	0	0	0	0	0
6	0	0.1	0	0	0	0
7	0	0.05	0	0	0	0
8	0	0	0.0526	0	0	0
9	0	0	0.0526	0	0	0
10	0	0	0	0.0556	0	0
11	0	0	0	0.0556	0	0
12	0	0	0	0	0.0556	0
13	0	0	0	0	0.0556	0
14	0	0	0	0	0	0.1
15	0	0	0	0	0	0.05
16	0	0	0	0	0	0.05

Table 4 shows a comparison of the pairwise  $F_{ST}$  values for all six populations. The low  $F_{ST}$  values indicate high similarity in genetic diversity among the six populations. All of the p-values (not shown) were above the 0.05 level of significance.

**Table 4:** Distance method: pairwise difference between the six tick populations tested. A = Blackleaf, B = Dearborn, C = Blodgett, D= Woodlake, E = Northfork, F = Helena Local

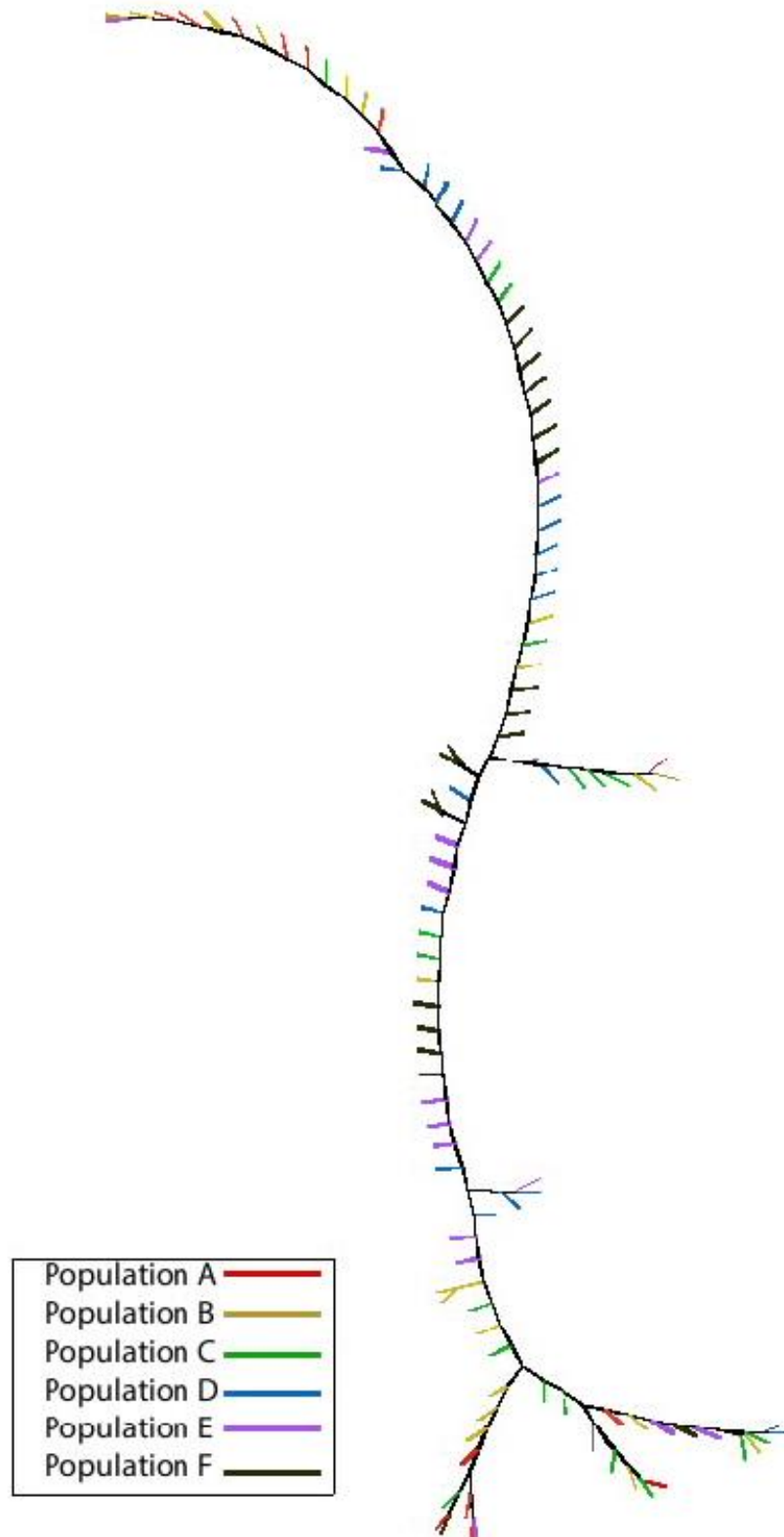
	A	B	C	D	E	F
A	0					
B	-0.02457	0				
C	0.05592	0.03727	0			
D	0.061	0.03914	-0.01413	0		
E	-0.0067	-0.01561	-0.0061	-0.01598	0	
F	0.01286	-0.00304	-0.02207	-0.05363	-0.04439	0

The results from the AMOVA (Table 5) indicate that approximately 98.51% of the genetic variation is distributed within populations, while approximately 4.55% of the variation is distributed among the populations separated by the Continental Divide. There is very little variation among populations within each of those groups.

**Table 5:** AMOVA results. Group east of the divide were tested against groups west of the divide.

Source of Variation	D.F.	Sum of Squares	Variance Components	Percentage of Variation
Among Groups	1	1.807	0.03409	4.55
Among Populations Within Groups	3	1.088	-0.02267	-2.96
Within Populations	83	62.696	0.75537	98.51
Total	87	65.591	0.76679	

Neighbor joining analysis showed that the individual relationships were scattered, with little to no separation among the individual populations based upon the 16S gene used in this



**Figure 3:** Neighbor-joining tree. Each color represents a different population. Colors are noted in the legend. Population A = Blackleaf, B = Dearborn, C = Blodgett, D= Woodlake, E = Northfork, F = Helena Local

## Discussion and Conclusion

Due to the lack of statistical evidence showing a significant difference between the tick populations, the hypothesis of finding relatively genetically isolated populations within western Montana was rejected. A cause could be that there is indeed a large amount of gene flow in between these tick populations. Ticks, when attached to their hosts, could have the potential to travel much farther than previously thought, and could cover these large distances between the populations.

These results are inconsistent with the results of de la Fuente *et al.* (2005) and Patterson *et al.* (2009) who found distinctive haplotypes in their study areas. Both studies found distinct haplotypes between the populations with a distance of 700 kilometers apart. However, the results found in this study confirm what was previously found by Dotson (2015). This study showed that there was no statistical difference in the haplotypes between the populations tested within our 300 kilometer testing area.

The nucleotide diversity found in this study was greater than what was found in Dotson (2015), and similar to the nucleotide diversity observed in Patterson *et al.* (2009). Patterson *et al.* (2009) showed an average nucleotide diversity of 0.00533 compared to 0.005463 found in this study (Table 1). There were also a number of polymorphic sites in each tick population (Table 2), however these mutations could not be linked to any specific tick population and many of these mutations were restricted to a single tick (Table 3). The statistical comparisons of the tick populations did not show any indication that there could be a genetic difference among the six populations of ticks tested (Table 4). Lastly, the AMOVA test showed that almost all of the genetic variability fell within



the tick populations rather than between the populations or by separation of the Continental Divide (Table 5).

The tick data that was excluded had two massive insertions. These insertions, however improbable, were found in four other ticks from that population. These sequences were compared to other possible tick species' 16S mitochondrial gene and no matches were found. Further analysis of ticks from the area may reveal that these insertions could in fact be included in the ticks' genome from the Blackleaf population, and this would suggest some genetic distinctiveness of that population.

Overall, no statistical evidence showed these tick populations to be isolated from one another, which would allow the production of a population map throughout Montana for disease awareness. Given the lack of genetic structure found in this study, these data cannot be used to construct a gene flow map. Other markers and further studies could provide a marker that would be effective.

Further research is needed to continue to analyze the data from the other tick sites collected within Montana. Increasing the sizes of the tick populations used in this study is also recommended. With further analysis, there could be a significant difference between the populations. Future directions for this research could also involve comparing haplotypes from year to year between populations to see if the haplotypes within populations change over time. Ticks of varying ages could also be compared to determine if there is a difference between older and younger ticks. Since this study suggests that the 16S mitochondrial gene is not a good indicator of genetic structure among these tick populations in Montana, researchers could look at other possible genetic markers, potentially somewhere in the D-loop (Shields and Kocker, 1991) or in microsatellite

regions (Chan and Wan, 2012). These areas have shown promise in being the most variable areas within the genome in accordance with more recent data about tick genetic variability.

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**Appendix 1 Tick Sequences****A=Blackleaf**

&gt;1A

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
 ATCTTCATTAAAAAAGTATCCTAATCCAACATCGAGGTCGCAAACATTTTGT  
 CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA  
 ATTATCATTAATAATGGATCATTTTATTAAATAAAAAGTTTATAATCTTTTTTA  
 GTTGCCCAACCAAAAATAATAATTTTAATATTAATAATTATTATTTTTAAAA  
 TTCTTAGGGTCTTCTTGTCCTTAATTTAAATAATTGTTTCTTCACAAATTA  
 TAAATTTAATTTTTAAGTTTAAAACAGTTTTTCCCTGAAATTC

&gt;2A

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
 ATCTTCATTAAAAAAGTATCCTAATCCAACATCGAGGTCGCAAACATTTTGT  
 CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA  
 ATTATCATTAATAATGGATCATTTTATTAAATAAAAAGTTTATAATCTTTTTTA  
 GTTGCCCAACCAAAAAGATAATAATTTTAATATTAATAATTATTATTTTTAAAA  
 TTCTTAGGGTCTTCTTGTCCTTAATTTAAATAATTGTTTCTTCACAAATTA  
 TAAATTTAATTTTTAAGTTTAAAACAGTTTTTCCCTGAAATTCCA

&gt;3A

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
 ATCTTCATTAAAAAAGTATCCTAATCCAACATCGAGGTCGCAAACATTTTGT  
 CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA

ATTATCATTAATAATGGATCATTTTATTAAATAAAAAGTTTATAATCTTTTTTA  
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>4A

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ATTATCATTAATAATGGATCATTTTATTAAATAAAAAGTTTATAATCTTTTTTA  
GTTGCCCCAACCAAAAGATAATAATTTTAATATTTAAAATTATTATCTTTAAAA  
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>5A

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GTTGCCCCAACCAAAAGATAATAATTTTAATATTTAAAATTATTATCTTTAAAA  
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>7A



CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
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GTTGCCCAACCAAAAAGATAATAATTTAATATTAATAATTATTATCTTTAAAA  
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>8A

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>11A

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>12A

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>13A

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>14A

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>15A

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>16A

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>17A

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>19A

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**B = Dearborn**

>1B

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>2B

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>3B

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>5B

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>6B

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&gt;7B

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&gt;8B

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&gt;9B

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>11B

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**C = Blodgett**

>1C

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CAT

>5C

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>7C

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&gt;8C

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&gt;9C

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CATC

&gt;11C

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CATTCA

>12C

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CATTCA

>13C

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GTTGCCCCAACTAAAAATAATAATTTTAATATTTAAAATTATTATTTTTAAAA  
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CATTC

>14C

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
ATCTTCATTAATAAAAGTATCCTAATCCAACATCGAGGTCGCAAACATTTTGT  
CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA  
ATTATCATTAATAATGGATCATTTTATTAAATAAAAAGTTTATAATCTTTTTTA  
GTTGCCCCAACCAAAAATAATAATTTTAATATTAATAATTATTATTTTTAAAA  
TTCTTAGGGTCTTCTTGTCCTTAATTTAAATAATTGTTTCTTCACAAATTA  
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>15C

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ATCTTCATTAATAAAAGTATCCTAATCCAACATCGAGGTCGCAAACATTTTGT  
CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA  
ATTATCATTAATAATGGATCATTTTATTAAATAAAAAGTTTATAATCTTTTTTA  
GTTGCCCCAACCAAAAGATAATAATTTTAATATTAATAATTATTATCTTTAAAA  
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TAAATTTAATTTTTAAGTTTAAAACAGTTTTTCCCTGAAATTCCATTCTCTTA  
GCA

>16C

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
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CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA  
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GTTGCCCCAACCAAAAAATAATAATTTTAATATTAATAATTATTATTTTTAAAA  
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TAAATTTAATTTTTAAGTTTAAAACAGTTTTTCCCTGAAATTCCATTCTCTTAG  
CATTC

>17C

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
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CTATATGTTCTATCAAAAATTATTACGCTGTTATCCCTAGAGTATTTTTATCAA  
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GTTGCCCCAACCAAAAAATAATAATTTTAATATTAATAATTATTATTTTTAAAA  
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>18C

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
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&gt;19C

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 GTTGCCCCAACCAAAAGATAATAATTTTAATATTTAAAATTATTATCTTTAAAA  
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 TAAATTTAATTTTTAAGTTTAAAACAGTTTTTCCCTGAAATTCCATTCCTTTAG  
 CATTCA

&gt;20C

CTCAGATCAAGTAGGACTTTAAAAGTTGAACAAACTTCTTTTTTTAAC  
 ATCTTCATTAAAAAAGTATCCTAATCCAACATCGAGGTCGCAAACACTATTTTGT  
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 GTTGCCCCAACCAAAAATAATAATTTTAATATTTAAAATTATTATTTTTAAAA  
 TTCTTAGGGTCTTCTTGTCCTTAATTTAAATAATTGTTTCTTCACAAATTTAAA  
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**D= Woodlake**

&gt;2D

CTCAGATCAA-GTAGGACTTTAAA-  
 GTTGAACAAACTTCTTTTTTTAACATCTTCATTAAAAAAGTATCCTAATCCAA  
 CATCGAGGTCGCAAACACTATTTTGTCTATATGTTCTATCAAAAATTATTACGCT

GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
 AA-

TATTAAAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>3D

CTCAGATCAA-GTAGGACTTTAAAA-  
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>4D

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
 AA-

TATTA AAAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>6D

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 AA-

TATTA AAAATTATTATTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>7D

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 GTTATCCCTAGAGTATTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTT  
 AA-  
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>8D

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 AA-  
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>9D

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 AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
 AA-  
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&gt;10D

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GTTGAACAAACTTCTTTTTTTAACATCTTCATTAATAAAGTATCCTAATCCAA  
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GTTATCCCTAGAGTATTTTTATCAAATTATCATTATAATGGATCATTTTATTA  
AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
AA-  
TATTAATAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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TCCCTGAAATTCCATTCTC-TTAG-CATTCA

&gt;11D

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GTTGAACAAACTTCTTTTTTTAACATCTTCATTAATAAAGTATCCTAATCCAA  
CATCGAGGTCGCAAACACTATTTTGTCTATATGTTCTATCAAAAATTATTACGCT  
GTTATCCCTAGAGTATTTTTATCAAATTATCATTATAATGGATCATTTTATTA  
AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAATAATAATTTT  
AA-  
TATTAATAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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&gt;12D



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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
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 AA-  
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>13D

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
 AA-  
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>14D

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AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
AA-

TATTAATAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>15D

CTCAGATCAA-GTAGGACTTTAAAA-  
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AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
AA-

TATTAATAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>16D

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GTTGAACAAACTTCTTTTTTTAACATCTTCATTAAAAAAGTATCCTAATCCAA  
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AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAGATAATAATTTT  
AA-

TATTAATAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCTTAATTTAAAT  
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>17D

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AA-

TATTAATAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCTTAATTTAAAT  
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>18D

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GTTATCCCTAGAGTATTTTTATCAAATTATCATTATAATGGACCATTTTATTA  
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AA-

TATTA AAAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
AATTGTTTCTTCACAAATTA AAATAAATTTAATTTTTAAGTTTAAAACAGTTTT  
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>19D

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GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAGATAATAATTTT  
AA-

TATTA AAAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>20D

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GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAGATAATAATTTT  
AA-  
TATTA AAAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT

AATTGTTTCTTCACAAATTA AAAATAAATTTAATTTTTAAGTTTAAAACAGTTTT  
 TCCCTGAAATTCCATTCTC-TTAG-CATTCA

**E = Northfork**

>1E

CTCAGATCAA-GTAGGACTTTAAAA-  
 GTTGAACAAACTTCTTTTTTTAACATCTTCATTA AAAAAAGTATCCTAATCCAA  
 CATCGAGGTCGCAA ACTATTTTGTCTATATGTTCTATCAAAAATTATTACGCT  
 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTTAGTTGCCCAACCAAAGATAATAATTTT  
 AA-  
 TATTAAAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
 AATTGTTTCTTCACAAATTA AAAATAAATTTAATTTTTAAGTTTAAAACAGTTTT  
 TCCCTGAAATTCCATTCTC-TTAG-CATTCA

>2E

CTCAGATCAA-GTAGGACTTTAAAA-  
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 CATCGAGGTCGCAA ACTATTTTGTCTATATGTTCTATCAAAAATTATTACGCT  
 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTTAGTTGCCCAACCAAAGATAATAATTTT  
 AA-  
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>3E

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATT AATAATGGATCATTTTATTA  
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 AA-  
 TATTA AAATTATTATCTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>4E

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 AA-  
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&gt;5E

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AA-  
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&gt;6E

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AA-  
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&gt;8E

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
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 AA-  
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>9E

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 AA-  
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 TCCCTGAAATTCCATTCCT-TTAG-CATTCA

>11E

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ATGGATCATTTTATTAATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACC  
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>12E

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GTTATCCCTAGAGTATTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
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AA-

TATTAATAATTATTATTTTAAAATTCTTAGGGTCTTCTTGTCCTTAATTTAAAT  
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>13E

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GTTATCCCTAGAGTATTTTATCAAATTATCATTAAATAATGGATCATTTTATTA

AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACTAAAAATAATAATTTT  
 AA-  
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 TCCCTGAAATTCCATTCTC-TTAG-CATTCA

>14E

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTATAATGGATCATTTTATTA  
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 AA-  
 TATTAATAATTATTATTTTTAAAATTCTTAGGGTCTTCTTGTCTTAATTTAAAT  
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>15E

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 AA-

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>16E

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
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 AA-

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>17E

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 GTTATCCCTAGAGTATTTTTATCAAATTATCATTAAATAATGGATCATTTTATTA  
 AATAAAAAGTTTATAATCTTTTTTAGTTGCCCAACCAAAAAATAATAATTTT  
 AA-

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>19E

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>20E

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**F = Helena Local**

>1F

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AA-  
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AA-



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