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Testing viability of oligonucleotide primers on microsatellite markers amplified by polymerase chain reaction on Dermacentor variabilis samples from the Bulloch county region of Georgia, USA

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in The Biology Department

By Danielle Souza

Under the mentorship of Dr. Quentin Fang

Abstract

Dermacentor variabilis, more commonly known as the wood tick, or the American dog tick, can be found all over North America. D. variabilis is a common tick in Bulloch county, Georgia, USA, and little is known about the population structure of this population of the species. Microsatellite markers are able to locate sections of the genome and can aid in estimating relatedness among individuals in a population. Six different oligonucleotide primer pairs were published for D. variabilis, however, published and non-published works indicated that not all of those primer pairs were working equally well for PCR. This study is to test all six primer pairs on DNA samples of D. variabilis collected from Bulloch County, to determine their efficiency at amplifying microsatellite markers with PCR. The primers tested were 3B, 5E, 6F, Est120, DVA28, and DVA31. Of these six primers, Est120 was determined to be the most effective at locating and amplifying microsatellite markers in DNA samples of ticks, from the Bulloch county population of Dermacentor variabilis.

Thesis Mentor: Dr. Quentin Fang

Honors Director: Dr. Steven Engel

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University Honors Program

Georgia Southern University
Acknowledgements

Thank you to Dr. Quentin Fang for his mentorship and assistance with the completion of this research. Thank you to Georgia Southern University graduate Miranda Wilson for her assistance with screening some of the tick samples. Thank you to previous students of Dr. Fang that collected the tick samples and performed the DNA extraction.

Introduction

To study the population genetics of the Bulloch county population of *Dermacentor variabilis*, microsatellite markers were used for genetic analysis, as they are able to estimate relatedness between members in a population (Selkoe et al. 2006). Microsatellites are also known as STRs, or short tandem repeats. Microsatellites are segments of repetitive DNA in a locus that are typically 1-6 base pairs long, and that typically repeat 5-40 times.

The segments surrounding the microsatellite location are called flanking regions, and that is where the primers, also known as oligonucleotides, can attach to target sequences (Selkoe et al. 2006). Once these oligonucleotide primers bind to the flanking regions, the microsatellite locus can be amplified using polymerase chain reaction (PCR). Therefore, certain oligonucleotide primers that are better suited to match the flanking regions in *D. variabilis*, will allow for greater amplification. PCR is additionally useful for small arthropods since they do not contain a large amount of DNA.

Microsatellite markers are helpful when studying population genetics
because they have a higher mutation rate than other regions of the locus. This higher mutation rate allows for better detection of polymorphic traits (Kloch et al. 2017). Polymorphic traits are traits that are comprised of two or more alleles. An example of polymorphic traits in ticks are body weight and body size (Cutullé et al. 2009).

Research on microsatellite markers in arthropods has improved the field of entomology, by furthering the understandings of phylogenetic and taxonomic relationships. Microsatellite markers also allow for species identification, biological control, pest control, and diagnosing arthropod vectors and their corresponding diseases and pathogens (Gariepy et al. 2007). *D. variabilis* is an invertebrate with an external skeleton, known as an arthropod, that is a vector of such diseases.

*Dermacentor variabilis* is a tick species of the family Ixodidae, one of the two families of ticks. Species in the family Ixodidae are classified as hard ticks because of their external shell called the scutum (Brites-Neto et al. 2015). *D. variabilis* is more commonly known as the wood tick, or the American dog tick (Krakowetz et al. 2010). Larva of this species are usually found on small animals such as squirrels and birds. Properly named, this tick is most commonly found on dogs, when the tick is in its adult life stage (Araya-Anchetta et al. 2015).

In its adult life stage, *D. variabilis* can also be found on larger mammals such as raccoons, cattle, horses, deer, and humans (Krakowetz et al. 2010). *D. variabilis* resides in the United States (Fig. 1), and predominantly in the Eastern half of the country, with major exceptions in California, and the Rocky Mountain
region (James et al. 2015). These areas are all highly wooded, grass-covered lands (Dharmarajan et al. 2011), where these ticks can easily pounce onto hosts. *D. variabilis* has also spread to Southern Canada and Northern Mexico (Krakowetz 2010) but has stayed confined within the North American continent.

![Map of D. variabilis in the US](image)

**Figure 1.** Distributions of *Dermacentor variabilis* in the US as of 2015 (James 2015).

*D. variabilis* is one of the more colorful species of ticks, appearing a spotted reddish-brown (Figs. 2 & 3). Males are typically 3 mm in length, unengorged females around 5 mm, and engorged “blood fed” females (Dharmarajan et al. 2011) can be anywhere from 10-15 mm (Brites-Neto et al. 2015). The life cycle of *D. variabilis* can take up to two years to complete and requires at least three different hosts. The ticks progress from an egg cluster, to larva, to a 6-legged nymph, to an 8-legged nymph, and then becomes an adult.
Adult females feed on a given host for anywhere up to two weeks, lay around 50,000 eggs, and then die. These eggs take approximately six weeks to hatch, and temperature conditions must be correct (Brites-Neto et al. 2015). Larva begin feeding on mice, and as they grow, are able to climb vegetation and host on larger animals, such as opossums, cats, dogs, or deer. Hosting on these animals can allow transmission of diseases to humans, which has caused the tick to become a common fear for many people.

*D. variabilis* is the primary vector for the pathogen of the bacterium *Rickettsia rickettsii* which causes Rocky Mountain spotted fever in humans (Krakowetz et al. 2010). Rocky Mountain spotted fever results in a rash, fever, muscle aches, abdominal pain, and can be fatal if left untreated. This tick can also be vector of the bacterium *Francisella tularensis* (Krakowetz et al. 2010), which causes tularemia (rabbit fever) in hares, and canine paralysis in dogs (Brites-Neto et al. 2015). This occurs if the tick is able to feed on the animal’s neck for an extended period of time. A common misconception about ticks is that all ticks are vectors for *Borrelia burgdorferi*, which causes Lyme disease. Although *D. variabilis* reside in regions where they are exposed to this bacterium, they are not competent vectors (Dharmarajan and Rhodes 2010).
Both samples are *Dermacentor variabilis* from the United States National Tick Collection which is housed at Georgia Southern University.
Knowing that *D. variabilis* is a vector for a potentially fatal disease, understanding the population genetics via microsatellite markers will allow for better biological control of this species, as well as an understanding of the potential prevalence of the disease. Oligonucleotides which are best matched to the flanking regions to amplify the microsatellite markers, has the potential to evaluate the genetic diversity of the Bulloch county population, as well as the diversity of natural enemy populations for pest control (Gariepy et al. 2007).

Most eukaryotes have an abundant number of microsatellites that are well studied. Some arthropods such as many honey bees, butterflies, moths, wasps, ants, and aphids have been well-studied to identify the microsatellite markers (Chambers and Meece 2007). However, the microsatellite markers of many mosquitos and ticks are underrepresented. It appears that mosquitos and ticks do not have as many microsatellite loci as other eukaryotes. Studies have been done on another species of tick, *Ixodes Scapularis*, to describe alternate methods to building libraries of microsatellite markers. It has also been shown that many of the microsatellites in *I. Scapularis* are codominant microsatellite markers at a single locus (Fagerberg et al. 2001). The same has not yet been done for *D. variabilis*.

This study is to test the efficiency of different oligonucleotide primers of microsatellite markers on *D. variabilis*. The results of this study are expected to show which of the published primer pairs are the most effective for PCR amplification and are suitable for further population genetics analysis.
Materials and Methods

Adult tick *D. variabilis* (engorged and not engorged,) were collected from the Bulloch County Animal Shelter or the Gateway Animal Hospital in Statesboro, Georgia. All ticks were picked up from animal bodies with a set of forceps. The samples were then transported to the laboratory and identified under microscope. The ticks were identified for sex and life stage and classified by what animal was their host. DNA was extracted from the samples using the Epicentre MasterPure™ Complete DNA and RNA Purification Kit.

Ticks were cleaned externally using deionized water, prior to being broken down with a Tissue and Cell Lysis Solution to soften the tough exterior. The ticks were then transferred to a glass slide and cut with a knife and set of forceps. The entirety of the tick, guts, and blood was placed back into 300 μl of 2X T & C (Tissue and Cell) Lysis Solution of the MasterPure™ Complete DNA and RNA Purification Kit. The tick was grinded with a sterile pestle by hand. Then, 1μl of Proteinase K (50μg/μl) was added to the tube, and the tube was incubated at 55 °C for 30 minutes on a heating block. The sample was continually grinded every 5 minutes. Then, after being cooled in an ice bath, 150μl of MPC Protein Precipitation Reagent was added to the samples and mixed vigorously for 10 seconds. To pellet the debris, the tube was centrifuged at 4°C for 10 minutes at 14,000 rpm on an Eppendorf microcentrifuge. The supernatant was transferred to another clean microcentrifuge tube and the debris pellet was discarded.

To recover the supernatant, 500μl of isopropanol was added. The tube was then inverted 30-40 times before being centrifuged at 4°C for 10 minutes at
14,000 rpm to pellet the nucleic acids. After rinsing with 1.0 ml of 75% ethanol and 1.0 ml of 100% ethanol, the tube was vacuum dried to remove the alcohol completely. Then, the pellet was resuspended in 50μl of Tris-HCl (10 mM) buffer. The extracted DNA was stored at -20 °C for short term storage, or at -75 °C for long term storage.

The microsatellite loci were amplified using polymerase chain reaction (PCR). Phire Hot Start II DNA Polymerase (Thermo-Fisher Scientific, Waltham, MA) was used for PCR reaction. Each PCR reaction was carried out in a 25 μl volume solution, containing a mixture of 1x DNA polymerase buffer (10mM Tris-HCl, pH 8.5, 50 mM KCl), 2.5 mM MgCl₂, 200 μM of each dNTP, 2.5U/100μl of DNA Polymerase, 0.2 μM of each primer, and 0.5 μl (~0.1μg-0.5μg) of the DNA template. A negative control (sterile water in the place of template DNA) was always run with each PCR reaction. PCR product (5μl of the 25μl reaction volume) was visualized on a 1% agarose gel containing a 1kb standard DNA ladder. PCR reactions were performed on a MJ PT-200 or on a Bio-RAD C-1000 Thermocycler. The reaction cycle consisted of an initial step at 98°C for 30 seconds, followed by 35 cycles of denaturation at 98°C for 36 seconds, annealing at 60°C for 8 seconds, and extension at 72°C for 30 seconds. Finally, the reaction PCR tubes went to final extension for 7 minutes.

Six different published primer pairs (Dharmarajan et al. 2009, Leo et al. 2012) were used for microsatellite amplification. The primer names and sequences are listed in Table 1. The Promega 1 kb DNA ladder was used during gel electrophoresis. The Promega 1 kb DNA ladder has thirteen blunt end
fragments between 250 bp and 10,000 bp. The 1,000 bp and 3,000 bp bands have increased intensity relative to the other bands for easier comparison and identification.

Agarose gel electrophoresis was run for each DNA extraction and again for each PCR reaction. GelRed was used for DNA staining. DNA bands were visualized with a FotoDyne Documentation system.

Table 1. Published primer pair names and sequences used for microsatellite amplification.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Original Primer Name</th>
<th>Primer Sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKSAT_3B_F</td>
<td>3B (Leo et al. 2012)</td>
<td>CGA AGC TTT CTC TGC CTA AAC T</td>
</tr>
<tr>
<td>TKSAT_3B_RC</td>
<td>3B (Leo et al. 2012)</td>
<td>ACC CAT AGC CAC TCA GCA AC</td>
</tr>
<tr>
<td>TKSAT_5E_F</td>
<td>5E (Leo et al. 2012)</td>
<td>CAC TTT CGT AAG GAG GAT TTA A</td>
</tr>
<tr>
<td>TKSAT_5E_RC</td>
<td>5E (Leo et al. 2012)</td>
<td>AGG ATA CCA GAT GGT CAC TTG C</td>
</tr>
<tr>
<td>TKSAT_6F_F</td>
<td>6F (Leo et al. 2012)</td>
<td>TCA CTA TAG GGC GAA TTG GGT A</td>
</tr>
<tr>
<td>TKSAT_6F_RC</td>
<td>6F (Leo et al. 2012)</td>
<td>CTA AAG GGA ACA AAG CTG GAG</td>
</tr>
<tr>
<td>TKSAT_Est120_F</td>
<td>Est120 (Leo et al. 2012)</td>
<td>GGG ATG TTT TGG TCG ATG TTA T</td>
</tr>
<tr>
<td>TKSAT_Est120_RC</td>
<td>Est120 (Leo et al. 2012)</td>
<td>TGT AGA CTT AGC TCG CTT AGA G</td>
</tr>
<tr>
<td>TKSAT_DVA28_F</td>
<td>DV_28 (Dharanajan et al. 2009)</td>
<td>CGT CTT CCC TAG GCC ACT C</td>
</tr>
<tr>
<td>TKSAT_DVA28_RC</td>
<td>DV_28 (Dharanajan et al. 2009)</td>
<td>ACC ATC AGT CAC CTT CGT TGC</td>
</tr>
<tr>
<td>TKSAT_DVA31_F</td>
<td>DV_31 (Dharanajan et al. 2009)</td>
<td>CTA TTC TTT CTT TCG CTT ACC</td>
</tr>
<tr>
<td>TKSAT_DVA31_RC</td>
<td>DV_31 (Dharanajan et al. 2009)</td>
<td>CGT CAT CAA ATA CGA AGT GAC</td>
</tr>
</tbody>
</table>
**Results**

One hundred thirteen *D. variabilis* tick samples were tested with PCR for the 3B primer pair (Table 2) and 45.13% of the samples resulted in positive bands, of which 35 ticks showed homozygous bands and 16 ticks showed heterozygous bands. The upper band size is about 525 bp and the lower band size is about 450 bp (Fig. 4).

Sixty-one *Dermacentor variabilis* tick samples were tested with PCR for the 5E primer pair (Table 2) and 16.40% of the samples resulted in positive bands, of which 4 ticks showed homozygous bands and 6 ticks showed heterozygous bands. The upper band size is about 1,000 bp and the lower band size is about 750 bp (Fig. 5).

Sixty-one *Dermacentor variabilis* tick samples were tested with PCR for the 6F primer pair (Table 2) and 16.40% of the samples resulted in positive bands, of which 6 ticks showed homozygous bands and 4 ticks showed heterozygous bands. The band size is about 750 bp (Fig. 6).

Sixty-one *Dermacentor variabilis* tick samples were tested with PCR for the Est120 primer pair (Table 2) and 86.89% of the samples resulted in positive bands, of which 51 ticks showed homozygous bands and 2 ticks showed heterozygous bands. The band size is about 750 bp (Fig. 7 and Fig. 8).

One hundred thirteen *Dermacentor variabilis* tick samples were tested with PCR for the DVA28 primer pair (Table 2) and 23.89% of the samples resulted in positive bands, of which 15 ticks showed homozygous bands and 12 ticks
showed heterozygous bands. The upper band size is about 750 bp, and the lower band size is about 500 bp. The homozygous band size is also at about 750 bp (Fig. 9).

Eighty-five Dermacentor variabilis tick samples were tested with PCR for the 5E primer pair (Table 2) and 48.24% of the samples resulted in positive bands, of which 32 ticks showed homozygous bands and 9 ticks showed heterozygous bands. The upper band size is about 750 bp, and the lower band is about 700 bp (Fig. 10).

Table 2. Percentages of primer effectiveness at detecting microsatellite markers.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Ticks showed Homozygous band</th>
<th>Ticks showed Heterozygous band</th>
<th>Number of Tick samples used for PCR</th>
<th>% of Ticks showed Homozygous band</th>
<th>% of Ticks showed Heterozygous band</th>
<th>% of Ticks with positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3B</td>
<td>35</td>
<td>16</td>
<td>113</td>
<td>30.97</td>
<td>14.16</td>
<td>45.13</td>
</tr>
<tr>
<td>5E</td>
<td>4</td>
<td>6</td>
<td>61</td>
<td>6.56</td>
<td>9.84</td>
<td>16.40</td>
</tr>
<tr>
<td>6F</td>
<td>6</td>
<td>4</td>
<td>61</td>
<td>9.84</td>
<td>6.56</td>
<td>16.40</td>
</tr>
<tr>
<td>Est120</td>
<td>51</td>
<td>2</td>
<td>61</td>
<td>83.61</td>
<td>3.28</td>
<td>86.89</td>
</tr>
</tbody>
</table>
Table 3. Voucher identification numbers for *D. variabilis* ticks screened with 3B, 5E, 6F, Est120, DVA28, and DVA31. See List of Samples in the appendices for more information on sex, life stage, locality, date collected, collector, and host of each sample.

<table>
<thead>
<tr>
<th></th>
<th>1709</th>
<th>Dan 2455</th>
<th>R012</th>
<th>R024</th>
<th>R128</th>
<th>R023</th>
<th>R160</th>
<th>R209</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1710</td>
<td>Dan 2456</td>
<td>R014</td>
<td>R121</td>
<td>R129</td>
<td>R135</td>
<td>R161</td>
<td>R214</td>
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<tr>
<td></td>
<td>1332</td>
<td>Dan 2458</td>
<td>R015</td>
<td>R122</td>
<td>R130</td>
<td>R136</td>
<td>R162</td>
<td>3612</td>
</tr>
<tr>
<td></td>
<td>1333</td>
<td>Dan 2459</td>
<td>R016</td>
<td>R123</td>
<td>R131</td>
<td>R142</td>
<td>R182</td>
<td>7000</td>
</tr>
<tr>
<td></td>
<td>1334</td>
<td>Dan 2460</td>
<td>R017</td>
<td>R124</td>
<td>R133</td>
<td>R144</td>
<td>R183</td>
<td>7002</td>
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<tr>
<td></td>
<td>1335</td>
<td>Dan 2461</td>
<td>R020</td>
<td>R125</td>
<td>R134</td>
<td>R155</td>
<td>R184</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1336</td>
<td>Dan 2472</td>
<td>R021</td>
<td>R126</td>
<td>R137</td>
<td>R158</td>
<td>R206</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1337</td>
<td>Dan 2473</td>
<td>R022</td>
<td>R127</td>
<td>R138</td>
<td>R159</td>
<td>R213</td>
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</tbody>
</table>

Table 4. Voucher identification numbers for additional *D. variabilis* ticks screened with 3B, DVA28, and DVA31F. See List of Samples in the appendices for more
information on sex, life stage, locality, date collected, collector, and host of each sample.

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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<td>M26</td>
<td>M65</td>
<td>M70</td>
<td>M80</td>
<td>5140</td>
<td></td>
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<td>M61</td>
<td>M66</td>
<td>M75</td>
<td>5136</td>
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<td>M67</td>
<td>M77</td>
<td>5137</td>
<td>5142</td>
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<tr>
<td>M63</td>
<td>M68</td>
<td>M78</td>
<td>5138</td>
<td>5143</td>
<td></td>
</tr>
<tr>
<td>M64</td>
<td>M69</td>
<td>M79</td>
<td>5139</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Voucher identification numbers for additional *D. variabilis* ticks screened with 3B and DVA28. See List of Samples in the appendices for more information on sex, life stage, locality, date collected, collector, and host of each sample.

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>5144</td>
<td>5314</td>
<td>19</td>
<td>5325</td>
<td>5433</td>
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<td>18</td>
<td>5324</td>
<td>5414</td>
<td>5467</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4. This gel was run with the 3B primer pair. The left lane is for tick R123. This tick is heterozygous, indicated by the two bands present in the lane. The 1 kb ladder is on the right, indicating that the upper band is at approximately 525 bp and the lower band is at approximately 450 bp.

Figure 5. This gel was run with the 5E primer pair. The lanes read in sequential order, ticks 2458, 2459, 2460, 2461, 2472, and 2473. The 1 kb ladder is on the right, indicating that these ticks are heterozygous, indicated by the two bands present in each lane. The upper band sizes are approximately 1,000 bp and the lower band sizes are approximately 750 bp.
Figure 6. This gel was run with the 6F primer pair. This lane is for tick R020. The 1 kb ladder is on the right. This tick is homozygous, indicated by the single band present in the lane. The band size is approximately 750 bp.

Figure 7. This gel was run with the Est120 primer pair. The lanes read in sequential order, ticks 2455, 2456, 2458, 2459, 2460, 2462, 2472, and 2473. Figure 7 shows that these ticks are homozygous, indicated by the single bands present in each lane. The 1 kb ladder is on the right, indicating that the band sizes are approximately 750 bp.

Figure 8. This gel was also run with the Est120 primer pair. The lanes read in sequential order, ticks R014, R015, R016, R017, R020, R021, and R022. R020 did not show up as well as the other ticks. Figure 8 shows that these ticks are
homozygous, indicated by the single bands present in each lane. The 1 kb ladder is on the right, indicating that the band sizes are all at approximately 750 bp.

Figure 9. This gel was run with the DVA28 primer pair. The lanes read in sequential order, ticks R136, R142, and R144. R136 and R144 are heterozygous, indicated by the double bands present in lanes 1 and 3. The 1 kb ladder is on the right, indicating that the upper bands are at approximately 750 bp, and the lower bands are at approximately 500 bp. R142 in lane 2 is homozygous, indicated by the single band present in the lane. This band size is approximately 750 bp.

Figure 10. This gel was run with the DVA31 primer pair. The lanes read in sequential order, ticks M77, M78, M79, and M80. Figure 10 shows that M77, M79, and M80 in lanes 1, 3, and 4 respectively are homozygous, indicated by the single bands present in each lane. The 1 kb ladder is on the right, indicating that
the homozygous bands are at approximately 750 bp. M78 in lane 2 is heterozygous, indicated by the double bands present. The upper band is at approximately 750 bp, and the lower band is at approximately 700 bp.

**Discussion**

The most effective primer pair for amplifying microsatellite markers in the tick *D. variabilis* is TKSAT Est120. The sequence for the forward Est120 primer is “GGG ATG TTT TGG TCG ATG TTA T” and the sequence for the reverse primer is “TGT AGA CGT AGC TCG CGT AGA G” as shown in Table 1. This primer amplified 86.89% of *D. variabilis* tick samples. Among them, 83.61% of the detected microsatellite markers were homozygous and 3.28% of the detected microsatellite markers were heterozygous, as shown in Table 2.

The second most effective primer at detecting microsatellite markers was TKSAT DVA31. The sequence for the forward DVA31 primer is “CTA TTC TTC CTT TCG CTT ACC” and the sequence for the reverse primer is “CGT CAT CAA ATA CGA AGT GAC” as shown in Table 1. This primer detected microsatellite markers in the *Dermacentor variabilis* tick samples 48.24% of the time. 37.65% of the detected microsatellite markers were homozygous and 10.59% of the detected microsatellite markers were heterozygous, as shown in Table 2.

The third most effective primer at detecting microsatellite markers was TKSAT 3B. The sequence for the forward 3B primer is “CGA AGC TTT CTC TGC CTA AAC T” and the sequence for the reverse primer is “ACC CAT AGC CAC TCA GCA AC” as shown in Table 1. This primer detected microsatellite
markers in the *Dermacentor variabilis* tick samples 45.13% of the time. 30.97% of the detected microsatellite markers were homozygous and 14.16% of the detected microsatellite markers were heterozygous, as shown in Table 2.

The other three primers were not effective at detecting microsatellite markers in the tick genomes. The DVA28 primer only detected microsatellites in 23.89% of the samples. The 5E and 6F primers only detected microsatellites in 16.40% of the tick DNA samples, as shown in Table 2.

In all of the primers except for 5E, there were more homozygous microsatellite markers than heterozygous markers. Future research should be done with population genetics to determine why this is the case. Possible explanations include that most of these ticks were found in Bulloch county, and thus may have a small breeding pool and/or intense sexual selection, so that most of the alleles that show up are homozygous in this region of southeast Georgia. Also, certain locations the ticks were extracted from, could promote further distribution towards being homozygous. Ticks were pulled off of different dog breeds in either an animal hospital or animal shelter.

However, even though many showed up homozygous, they were at various base pair locations. Some microsatellite markers appeared as small as 450 bp while other were as large as around 1,000 bp, as seen in Figures 4-10.

In five out of the six primers tested, more than 50% of the ticks screened did not locate microsatellite markers. All of the ticks screened were collected and the DNA extracted by previous Georgia Southern University students under
Fang’s direction. These ticks may have had the DNA improperly or poorly extracted. The tick DNA had been stored in the freezer, for a minimum of twelve years, and for some as long as over twenty years. Some DNA samples had not been resuspended after extraction and some test tubes were left slightly open, all of which could have damaged the DNA samples and made them unsuitable for PCR and microsatellite detection studies.

Further studies should be done on the Bulloch county population of *D. variabilis* to determine the population structure. Based on the results from this study, the Est120 primer would be the best primer choice for microsatellite analysis. Future research should also be done testing the Est120 primer on DNA from other species of ticks, to determine if Est120 is the best microsatellite primer for other tick species as well; especially those in the family Ixodidae. Conservation and genomic biologists should complete more studies to determine if having a large population of homozygous individuals of *D. variabilis* is only common in Bulloch county, or if that same population structure is found in other populations across the state of Georgia and the country. Understanding the population structure may lead to further insight on ecology and behavior, tick-host relations, and the vector biology of *D. variabilis*.

**References Cited**


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Appendices

List of Dermacentor Samples in Dr. Fang's Lab