Sampling Spider Webs for the Detection of Black Widow DNA
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Abstract:

Ecological surveys are an important tool used to understand an environment. By identifying the overall diversity in a habitat, the ecological health can be assessed. In general, the greater the species richness the healthier the environment is. All spiders are predaceous and, as main predators of arthropods, a large diversity of spiders means a thriving insect population, indicating a healthier environment. Spiders, in general, are secretive, making them difficult to collect during ecological assessments. In addition to this, many spiders are very tiny, have similar colorations between species, and have both sexual and maturation dimorphism (the males and females, as well as the juveniles and adults look different from each other). This is why spiders often must be identified using either their genitals or DNA. To extract DNA from a spider or examine its genitals, not only does the spider have to be found and captured, but it often must then be killed, potentially damaging the ecosystem one is attempting to study. Collecting spider webs is considerably less difficult than collecting the spiders themselves, as the webs don’t move and many species build new webs each night, abandoning the web every morning. While not perfect, sampling web DNA is another tool that can be used to gain a more comprehensive understanding of an environment. Using a standardized DNA extraction protocol (Xu et al, 2015), DNA will be extracted from both abandoned and active webs collected in the environment, as well as from webs spun by known black widow spiders (*Latrodectus spp.*) in a laboratory setting. The DNA will be subsequently run in a PCR with primers that amplify the cytochrome c oxidase subunit 1 gene found in all eukaryotic mitochondrial DNA. The amplified DNA will be compared using gel electrophoresis and sequencing to assess how closely related the spiders that spun the webs may have been. It is expected that some, but not all, of the webs
collected will have possibly been spun by black widow spiders, proving the concept that DNA from a web collected in the environment can be used to identify the species of spider that spun the web.
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Background:

As some of the most abundant and voracious predators of insects, spiders are an important aspect of any environment. (Moulder, 1972) Without spiders eating the enormous amount of insects they consume every year, insects would be far more numerous. As one of the main competitors of our food crops, a surplus of insects could be a real detriment to human health. By describing and assessing the number and type of spider species found in an environment, the overall health of that environment can be better understood. Many spiders are very small weighing between 1-3 mg, (Nyffeler, 2000) have similar colorations and so DNA is the most reliable way to identify the species of spider. Another thing to keep in mind is that spiders are generally nocturnal and hide from researchers doing collections, while webs, on the other hand, are sedentary and usually out in the open to collect insects for food. In addition to this, some species of spider make more than one web a night, meaning it does very little, if any, harm to the spider to collect its web.

Spider webs are made from $\alpha$-sheet bonded fibroin proteins coated in sticky sericin. (Humenik, 2011) Proteins, by nature, do not have DNA in them. The DNA extracted from spider webs is DNA from cells shed by the spider onto the silk either during spinning or while walking on the strands. Because this DNA is not imbedded in tissues, it is exposed to the elements including UV radiation, causing the DNA to be easily degraded.

Research Question:

Can spider species be determined from DNA extracted from a web collected from the environment?
Hypothesis:

By extracting DNA from spider webs and comparing the PCR amplified DNA of a known species to an unknown environmental sample, one can better determine which species of spider spun the web.

Methods:

Web Collection: Web was collected from sites around campus by spooling it around sterile microbial transfer wands and placing it in a 1.5 mL eppendorf tube. Webs similar to black widow webs in structure and placement in the environment were selected for. Webs from a lab environment were collected from Dr. Chad Johnson’s black widow behavior lab at ASU West. The support structure a known black widow spider spun a web on was removed and placed in a 10 mL falcon tube.

DNA extraction: DNA was extracted using a method developed by Xu et. al (2015). After webs were collected and placed in the 1.5 mL eppendorf tube, 800 μL of cell lysis buffer composed of 10mM Tris, 10mM EDTA and 2% SDS, and 16 μL proteinase K were added to the sample. The sample was inverted 10-20 times and incubated at 55°C for 4 hours. 4 μL of RNase A was then added and the sample was incubated at 37°C for 15 minutes. 300 μL of 7.5 M ammonium acetate was added and the sample was vortexed for 20 seconds and then incubated on ice for 15 minutes. The web samples were then centrifuged on the highest setting for 3 minutes and the supernatant was poured off and kept in another 1.5 mL eppendorf tube. 750 μL of ice cold isopropanol was added to the supernatant, the sample was inverted 50 times and then centrifuged on the highest setting for 2 minutes. The supernatant was drained off and 750 μL of 70% ethanol was added then the sample was centrifuged on high for 3 minutes. The supernatants
were drained off and the samples were air dried in the hood. When working with the DNA later it must be rehydrated with 30 μL of TE buffer.

PCR: Primers, DNA extracted from spider webs, PCR water and Mastermix were mixed into PCR tubes. The amount of PCR water and DNA template was adjusted according to how concentrated the DNA was so that the overall volume of the PCR product was no more than 40 μL and there was a high enough concentration of DNA in the tube to allow for amplification. The samples were and put in in the PCR machine for the time periods given in Xu et. al (2015). The cycling conditions were as follows: 94 °C for 5 minutes, 94 °C for 20 seconds, 54.4 °C for 20 seconds, 72 °C for 30 seconds, steps 2-4 55 times, 72 °C for seven minutes and held at 4 °C indefinitely.

Agrose Gel Extraction: Using a black light to florese the syber green dye, the bands of amplified DNA was cut out of agrose gel. The band was put into a 1.5 mL eppendorf tube with equal amounts of gel and binding buffer and the sample was heated at 50-60°C until the agar band melted. The liquid sample was poured over a special filter column and centrifuged for 1 minute at the highest setting. The DNA was bound to the screen of the column and the binding buffer that flows through the filter was thrown away. 700 μL of wash buffer was poured over the filter column to remove any remaining gel, and then the sample was centrifuged to dry it. Elution buffer was poured over the filter column to wash the DNA into a fresh 1.5 mL eppendorf tube and then the sample was sent to be sequenced.
**Data Results:**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Target Taxon/Purpose</th>
</tr>
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<tbody>
<tr>
<td>Lat_COI_F1</td>
<td>GAATTAGGGCAACCGGG AAG</td>
<td>Laterodectus species/ Forward primer</td>
</tr>
<tr>
<td>Lat_COI_R1</td>
<td>AGGAAGCTAAATCAATTTC AAACCC</td>
<td>Laterodectus species/ Reverse primer 1</td>
</tr>
<tr>
<td>Lat_COI_R2</td>
<td>CCAGCTTCAAACCTTTCC C</td>
<td>Laterodectus species/ Reverse primer 2</td>
</tr>
<tr>
<td>Lat_COI_R3</td>
<td>ACAGAGACTTCTTCTATGT CCTTAA</td>
<td>Laterodectus species/ Reverse primer 3</td>
</tr>
<tr>
<td>Universal Forward</td>
<td>GGTCAACAATATCATAAA GATATTGG</td>
<td>Arthropods/ Universal Forward primer</td>
</tr>
<tr>
<td>Universal Reverse</td>
<td>TCAGGGTGACCAAAAAA TCA</td>
<td>Arthropods/ Universal Reverse primer</td>
</tr>
</tbody>
</table>

*Figure 1: Bands of genomic DNA*
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Figure 2: PCR results with samples collected from the West side of H (HW) building, and the Southeast side of F (FSE) building
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Figure 3: PCR results from webs collected from the Southwest side of R building (RSW) and from Dr. Chad Johnson’s black widow lab (+C)

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<tbody>
<tr>
<td>HW</td>
<td>4.8</td>
<td>0.095</td>
<td>0.078</td>
<td>1.22</td>
<td>-1.24</td>
</tr>
<tr>
<td>FSE</td>
<td>13.5</td>
<td>0.269</td>
<td>0.229</td>
<td>1.18</td>
<td>2.28</td>
</tr>
<tr>
<td>RSW</td>
<td>7.7</td>
<td>0.155</td>
<td>0.095</td>
<td>1.62</td>
<td>1.84</td>
</tr>
<tr>
<td>+C</td>
<td>19.6</td>
<td>0.392</td>
<td>0.301</td>
<td>1.30</td>
<td>0.68</td>
</tr>
<tr>
<td>-C</td>
<td>1.5</td>
<td>0.029</td>
<td>0.017</td>
<td>1.76</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

Analysis: The DNA extraction recommended by Xu et al (2015) worked for spider webs with strong structural support webbing. The extraction was relatively caustic and disintegrated webs lacking the tough support structures found in black widow webs. So while the extraction worked for widow-type webs as well as orb webs, it degraded the DNA of cellar spiders.
The primers given in Xu et al (2015) were not capable of amplifying DNA extracted from the +C webs, webs known to be spun by black widow spiders in a laboratory setting. It was because of this, despite some success with these primers with the RSW template, that no real conclusions can be drawn from this experiment. The experiment must be run again with either optimized PCR conditions to ensure amplification, or completely new primers developed independently of Xu.

The reasons some of the primers used did not amplify are many and varied. PCR cycling conditions given in Xu et al (2015) had suspiciously low durations of time for each of the temperatures. Normally in PCR, the temperatures will be held for a few minutes at a time, but in Xu et al the durations were for only a few seconds. The short duration of time these temperatures were held at may have not allowed sufficient time for the DNA template to unravel, or for the primer sets to anneal to the complementary strand. In the future the PCR conditions will have to be adjusted and optimized to ensure amplification occurs.

Another reason this PCR may not have amplified the DNA was because of possible contamination. There appeared to be slight but minimal contamination in the extraction chemicals used. The nanodrop for the negative control registered a .029 concentration of nucleic acid. Because this concentration was so low in comparison to the other DNA extractions and because there was no band of DNA on the gel testing for the detection of DNA, it was thought the nanodrop information was not enough to denote definitive contamination and the experiment was continued.

Another reason the PCR may not have amplified the DNA template was possible contamination of the DNA sample itself. According to the nanodrop, the ratio of 260/280 on all the samples indicated some possible protein contamination, and the 260/230 ratio indicated
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possible salt contamination. Both proteins and salts can interfere with PCR and cause errors and a reduction in amplification. It was not surprising to see salts and proteins in the DNA samples because they were coming from environmental sources, where many salts and proteins can accumulate on the web. In the future more chemical rinses will have to be performed to further clean the samples.

The universal arthropod primers amplified all the DNA templates tested. This was to be expected as the only known sources of silk in the environment would have come from an arthropod of some sort. It is also possible these primers were amplifying insect or other DNA blown onto the web and not necessarily the spider DNA. Only one primer that was amplified in RSW and all the universal primer bands were cut from a gel electrophoresis gel and sent to be sequenced.

Conclusion:

Claim: In this experiment, because the primers used by Xu et al (2015) did not amplify the positive control no real conclusions about the species of spider could be reached. The primers must be able to amplify a known black widow species DNA to be sure the primers work in the way expected. In theory, the band of RSW DNA amplified by the latrodectus primers and extracted from the gel could be sequenced, blasted, and compared against an online database of the complete latrodectus genome, but without knowing for sure the primers were amplifying black widow DNA it may not be illuminating to do so.

Evidence: When looking at figure 3, it can be seen in the lanes labeled +C 1-3 that there are no bands of DNA fluorescing in the gel. This means the primers did not amplify the genomic DNA of a known black widow spider during PCR.
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Reasoning: If amplification had occurred, there would be bright bands of DNA fluorescing in the lanes labeled +C1-3, such as those found in all the lanes using universal primers.

Merit: The DNA extraction process worked well on webs with strong structural strands. Being able to extract this DNA is a step in the direction of non-invasive ecological sampling, enabling scientists to investigate spiders and their DNA without killing the spider itself.
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