Spider web DNA: a new spin on noninvasive genetics of predator and prey.

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Running title: Detecting DNA in spider webs

Abstract
Noninvasive genetic approaches enable biomonitoring without the need to directly observe or disturb target organisms. Environmental DNA (eDNA) methods have recently extended this approach by assaying genetic material within bulk environmental samples without a priori knowledge about the presence of target biological material. This paper describes a novel and promising source of noninvasive spider DNA and insect eDNA from spider webs. Using black widow spiders (Latrodectus spp.) fed with house crickets (Acheta domesticus), we successfully extracted and amplified mitochondrial DNA sequences of both spider and prey from spider web. Detectability of spider DNA did not differ between assays with amplicon sizes from 135 to 497.
base pairs. Spider DNA and prey eDNA remained detectable at least 88 days after living organisms were no longer present on the web. Spider web DNA may be an important tool in conservation research, pest management, biogeography studies, and biodiversity assessments.

Introduction

As dominant predators of arthropod communities in natural and agricultural ecosystems, spiders are important ecological indicators that reflect habitat quality and change across trophic levels (Churchill 1997; Clausen 1986). Monitoring the species diversity and abundance of spider assemblages facilitates natural resource management (Pearce and Venier 2006). Spiders are enormously diverse (~ 44,000 described species; Platnick 2013) and difficult to identify. Morphological identification of spiders relies primarily on differences in copulatory organs (Huber 2004) and many complications can prevent identification such as the inability to identify juveniles, extreme sexual dimorphism, size differences between life stages, and genital polymorphisms (Barrett and Hebert 2005; Brennan et al. 2004; Huber and Gonzalez 2001). Other major issues include the ever decreasing availability of expertise necessary for traditional taxonomy as well as the significant training required to learn taxonomic skills (Hopkins and Freckleton 2002). In the face of such challenges to morphological taxonomy, genetic identification methods are growing in popularity because of decreasing costs and ease of use. DNA barcoding, the use of a short and standardized fragment of DNA to identify organisms, has gained significant traction within the last decade (Jinbo et al. 2011). In particular, the use of DNA barcodes for species identity and systematics of spiders has proven successful in multiple studies (Astrin et al. 2006; Barrett and Hebert 2005; Robinson et al. 2009). The most commonly used genetic marker is the cytochrome oxidase subunit I (COI) mitochondrial gene because of its
designation as the standard DNA barcode (Hebert et al. 2003). Mitochondrial markers are also ideal for detecting low quantity and quality DNA from environmental or gut samples because each cell contains hundreds to thousands of mitochondrial genomes (Hoy 1994) and there is a positive correlation between gene copy number and detection success (Agustí et al. 2003b; Chen et al. 2000).

Spiders have a great diversity of life histories and various sampling methods are employed in capturing them including vacuum sampling, sweep netting, pitfall traps, and visual searches. Experiments testing the efficacy of traditional spider sampling methods show high variability between methods as well as inconsistency across spatial and temporal scales (Churchill and Arthur 1999; Green 1999; Merrett and Snazell 1983). Sampling duration is also an important factor as short-term sampling has been found to reduce the number of recorded species by up to 50% (Riecken 1999). In this paper, we propose a new biomonitoring tool that would complement existing methods: DNA from spider web. While spider web has been found to effectively collect pollen, fungal spores and agrochemical sprays (Eggs and Sanders 2013; Samu et al. 1992), no study, to our knowledge, has assessed spider web as a potential source of genetic material. We hypothesized that spider web could simultaneously provide a noninvasive genetic sample (spider DNA) and an environmental DNA sample (prey DNA). Noninvasive genetic sampling uses extraorganismal material like feces, hair, and feathers from individual organisms for genetic analysis without the need to contact target organisms (Beja-Pereira et al. 2009). Environmental DNA (eDNA) sampling uses genetic material from environmental mixtures like water or soil without isolating target organisms or their parts (Turner et al. 2014).
Although noninvasive genetic sampling is most common for vertebrates, it has been successfully applied to arthropod exuviae and frass (Feinstein 2004; Petersen et al. 2006). Webs are an abundant and easily collected spider secretion that may provide spider DNA. Spider webs may also contain eDNA from captured prey and other local organisms, functioning as natural biodiversity samplers. This idea parallels recent molecular studies using mosquitos, ticks, leeches, and carrion flies to sample local animal biodiversity (Calvignac-Spencer et al. 2013, Gariepy et al. 2012, Schnell et al. 2012, Townzen et al. 2008). Previous studies have successfully used mitochondrial DNA markers to detect spider prey from gut contents, but this requires physically capturing and killing spiders (Agustí et al. 2003a; Sheppard et al. 2005). Furthermore, traditional taxonomic identification of spider prey items is time-consuming, subject to human error, and accurate only to the order level (Salomon 2011). Spider webs may provide a unique noninvasive opportunity to study arthropod communities without the need to directly observe spider or insect.

Here, we tested the feasibility of extracting, amplifying and sequencing DNA of black widow spiders, *Latrodectus* spp. (Araneae: Theridiidae), and their prey, the house cricket *Acheta domesticus* (Orthoptera: Gryllidae), from black widow spider webs. Because extraorganismal DNA in spider webs is exposed to environmental degradation and may exist in short fragments, we used nested primer sets to test the effect of amplicon size on detection probability.

**Materials and methods**

**Web collection**

The black widow spider exhibit at the Potawatomi Zoo in South Bend, Indiana was inhabited by a single female western black widow spider (*Latrodectus hesperus*) before its death on November
19, 2011. The spider was fed 2 medium sized house crickets (*A. domesticus*), on a weekly basis by zookeepers. The exhibit measured 40 cm by 40 cm by 40 cm and contained a few twigs, a small piece of wood, and wood shavings lining its floor. 88 days after the death of the spider, a web sample was collected from the exhibit on February 15, 2012, which will be referred to as “Lhes_zoo”. The duration of inhabitance within the exhibit prior to the sample collection date is unknown. Three individual enclosures measuring 35 cm by 30 cm by 35 cm were constructed with plywood and acrylic sheeting. All enclosures were decontaminated with 10% bleach and installed at the Potawatomi Zoo in South Bend, Indiana.

Three female southern black widow spiders (*Latrodectus mactans*) were purchased from Tarantula Spiders (http://tarantulaspiders.com/). The spiders were hatched from egg sacs collected in Marion County, Florida, USA and raised on 2-3 housefly maggots (*Musca domestica*) twice per week before delivery to the Potawatomi Zoo. A single live *L. mactans* and a decontaminated branch for web building were placed into each enclosure on April 26, 2012 (Figure 1). Each *L. mactans* was immediately fed two medium-sized crickets by placing them onto web. Web samples were collected from each enclosure 11 days later on May 7, 2012, which will be referred to as “Lmac_1”, “Lmac_2”, and “Lmac_3”. All web samples were collected by twisting single-use, sterile plastic applicators to spool silk strands. No organism body parts or exuviae were visible in any web samples but cricket parts and spider feces were clearly evident on the bottom of the enclosures. Applicator tips were snipped into 1.5 mL microcentrifuge tubes using 10% bleach decontaminated scissors before storing at -20°C.

**DNA extraction**
DNA extractions from web samples were conducted using a modified extraction protocol for shed reptile skins (Fetzner 1999). One negative control containing no web was also extracted. 800 μL of cell lysis buffer (10 mM Tris, 10 mM EDTA, 2% sodium dodecyl sulfate [SDS], pH 8.0) and 8μL of proteinase K (20 mg/L) were added to 1.5 mL microcentrifuge tubes containing web samples followed by 10-20 inversions and incubation at 55°C for 4 hours. Upon reaching room temperature, 4 μL of RNase A (10 mg/mL) were added to each sample followed by 20 inversions. Samples were incubated at 37°C for 15 min and then brought back to room temperature. 300 μL of protein precipitation solution (7.5 M ammonium acetate) were added to each sample and vortexed for 20 seconds followed by incubation on ice for 15 min. Samples were then centrifuged at 16,873 rcf for 3 min. Supernatants were transferred to new 2 mL microcentrifuge tubes containing 750 μL of ice cold isopropanol and inverted 50 times before centrifugation at 14,000 rpm for 2 min. All supernatants were drained and 750 μL of 70% ethanol was added to each sample followed by centrifugation at 14,000 rpm for 3 min. All liquids were removed and samples were air dried. DNA pellets were rehydrated using 100 μL of low TE buffer (10 mM Tris, 0.1 mM EDTA).

Primer design

To detect *Latrodectus* DNA, we designed four nested primer sets based on an alignment of *Latrodectus* COI DNA barcoding sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank database. All four assays included the same forward primer but different reverse primers, producing amplicons of 135 bp, 257 bp, 311 bp, and 497 bp respectively (Table 1). To detect prey DNA, we designed a set of primers that specifically targets
the DNA barcoding region of the COI gene in *A. domesticus*, which produces an amplicon of 248 bp (Table 1).

**DNA amplification**

All DNA samples were amplified in polymerase chain reactions (PCR) of 20 μL containing 13.28 μL of ddH₂O, 2 μL of 5 PRIME® 10x Taq Buffer advanced, 2 μL of 5 PRIME® Magnesium Solution at 25 mM, 0.4 μL of dNTP at 2.5 mM, 0.12 μL of 5 PRIME® Taq DNA polymerase at 5 U/μL, 0.6 μL of forward and reverse primers at 10 μM, and 1.0 μL of DNA template using Eppendorf Mastercycler® pro thermocyclers. Cycling conditions were as follows: 94°C/5 min, 55X (94°C/20 s, 54.4°C/35 s, 72°C/30 s), 72°C/7 m, 4°C/hold. Each *Latrodectus* spp. primer set was used to amplify all DNA samples with 10 technical replicates to measure detection probability for different amplicon sizes. All DNA samples were amplified with 2 technical replicates using the *A. domesticus* primer set. Negative control reactions to detect contamination were included in every batch. Gel electrophoresis was conducted using 5 μL of PCR product mixed with 3 μL of loading dye and 10 μL of ddH₂O. Multiple wells were loaded with 5 μL of 100 bp ladder (Promega) on each gel. Technical replicates showing amplicons of the expected size were pooled and purified using ExoSAP-IT (Affymetrix). Sanger sequencing using ABI BigDye chemistry (Life Technologies) was conducted on an ABI 3730xl 96-capillary sequencer by the University of Notre Dame Genomics Core Facility. Sequencing chromatograms were primer- and quality-trimmed in Sequencher (ver. 5.0; Gene Codes Corp.). BLASTn searches of the NCBI GenBank database (http://www.ncbi.nlm.nih.gov; Benson *et al.* 2012) were used for taxonomic identification of COI barcode sequences.
Results

All extraction and PCR negative controls produced no amplification. Using the nested primer sets, we successfully amplified 135 bp, 257 bp, 311 bp, and 497 bp of *Latrodectus* spp. COI from web DNA samples (Figure 2). With the exception of zero amplification for the 265 bp PCR assay from two samples, 2-10 technical replicates of each PCR assay successfully amplified from all samples. DNA sequences obtained from enclosure samples, “Lmac_1”, “Lmac_2”, and “Lmac_3”, were confirmed by NCBI BLAST to be *L. mactans* and DNA from the zoo exhibit sample, “Lhes_zoo”, was confirmed to be *L. hesperus*. Amplicon size had no effect on PCR success based on the number of successful PCR replicates (ANOVA, F = 1.941, d.f. = 3, P = 0.194). We also successfully amplified 248 bp of *Acheta domesticus* COI from eDNA samples. Both PCR duplicates from all four web samples were positive and all resulting DNA sequences were confirmed by NCBI BLAST to be *A. domesticus*. The zoo exhibit web sample, “Lhes_zoo”, was collected 88 days after the death and removal of both spider and prey, demonstrating substantial persistence of web DNA. All DNA sequences generated in this study are provided in Table S1 (Supplementary Data).

Discussion

The present study represents, to our knowledge, the first demonstration of spider web as a source of noninvasive genetic material. Spider web is an ideal source of noninvasive genetic material for spiders because web can be found and collected without direct observation of target organisms. Unlike most spiders, which are small, mobile, and elusive, webs are relatively large, stationary, and usually clearly visible, making sample collection more efficient. Spider webs may also remain after the inhabitant moves or dies, which increases detection probability, especially for the
more elusive spider species. Webs can also exist in great abundance. For example, web coverage
may reach up to more than 50% of land area in agricultural fields (Sunderland et al. 1986). Spider
webs have already been utilized by citizen scientists to assess spider biodiversity through visual
analysis of web structure (Gollan et al. 2010). It could be possible to implement similar citizen
science initiatives to collect web samples for DNA analysis.

We hypothesize that spider web DNA originates either from microscopic pieces of fecal matter,
setae, and exuviae adhered to silk strands or directly from the silk gland exudate, which may
contain cells and mitochondria shed from silk glands. Because black widow spiders are orb
weavers that generate large three-dimensional cobwebs consisting of sheets dotted with glue
droplets (Zevenbergen et al. 2008), they were ideal to use in this experiment. Certain black
widow spiders like *L. mactans* and *L. hesperus* are common venomous pests so spider web DNA
could be a particularly useful tool for pest surveillance (Lewitus 1935). Because webs are easier
to find and collect than live spiders, spider web DNA could also help monitor low density
populations and determine invasive fronts of invasive widow spiders such as the brown widow,
*Latrodectus geometricus*, in southern California and the Australian redback, *Latrodectus hasseltii*,
in New Zealand and Japan (Vetter et al. 2012; Vink et al. 2011). Besides pests and invasives,
many spider species like the red katipo (*Latrodectus katipo*) are threatened or endangered and
hundreds if not thousands more are listed as “Data Deficient” but are probably at risk of decline
(Sirvid et al. 2012). Spider web DNA could be particularly useful in easily providing occurrence
and genetic diversity data for these rare species of concern. As a noninvasive biomonitoring
method, spider web DNA could be used for conservation and taxonomy without sacrificing
organisms that are already threatened by human disturbance. The collection and genetic analysis
of spider webs could also serve spider biogeography studies, which require large-scale sampling across wide geographic ranges (Garb et al. 2004). Even silk from organisms that do not weave webs such as tarantulas and moth larvae may still yield viable DNA, but further experimentation is needed. This may be applicable towards molecular studies of trapdoor spiders, which construct burrows using silk but are extraordinarily difficult to capture for genetic sampling (Cooper et al. 2011).

Although the efficacy of spider web eDNA needs to be validated with samples from the field, this is the first demonstration that DNA of other insects can be extracted from spider webs. Spider predation can serve as a useful proxy to monitor local arthropod biodiversity. In some environments such as temperate forests, approximately 40% of arthropod biomass is annually consumed by spiders (Moulder and Reichle 1972). Although spider predation cannot be concluded from the mere presence of DNA on spider web, it does indicate the local proximity of those organisms. The ability to target particular species could be useful in monitoring low density populations of pest, invasive, or endangered insects. Future work using massively parallel sequencing on spider web eDNA could reveal entire assemblages of arthropods in a cost-effective manner, especially with the rapid advancement and decreasing costs of such technologies (Shokralla et al. 2012). Spider web eDNA may complement traditional assessment methods of local arthropod biodiversity and potentially reveal previously undiscovered biodiversity through improved sensitivity and sampling effort (Neilsen and Laurence 2000). Such information regarding species diversity is critically important in conservation planning and environmental impact assessments (Kremen et al. 1993, Rosenberg et al. 1986).
In conclusion, we provide the first demonstration that noninvasive DNA of spider and its prey can be extracted from spider web and be used to identify organisms to species. This method is low-cost, efficient, and does not require significant taxonomic expertise. Spider web DNA is a promising tool for the biomonitoring of spiders and other arthropods, especially if combined with the power of massively parallel sequencing.

**Author Contributions**
CCYX and CRT designed the research. CCYX, CRT, IJY, and DB performed the research.
CCYX and CRT wrote the paper with help from IJY and DB.

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**Data Accessibility**

All DNA sequences generated in this study are provided in Table S1 (Supplementary Data) and will be archived in NCBI Genbank before publication of this manuscript.

**Table 1.** PCR primers designed to amplify the DNA barcoding region of the cytochrome oxidase subunit I gene of target species. All *Latrodectus* spp. primer sets are nested and use the same forward primer.

| Primer name | Sequence (5’-3’) | Size (bp) | Amplicon (bp) | Target taxon |
|-------------|------------------|-----------|---------------|--------------|
| Lat_COI_F1  | GAATTAGGGCAACCGGAAG | 20        | -             | *Latrodectus* spp. |
| Lat_COI_R1  | AGGAACACTAATCAATTCTCAACCC | 25        | 135           | *Latrodectus* spp. |
| Lat_COI_R2  | CCAGCTCCAACCCAACC | 18        | 257           | *Latrodectus* spp. |
| Lat_COI_R3  | ACAGAACTTCTCTCTATGCTCTTCAA | 26        | 311           | *Latrodectus* spp. |
| Lat_COI_R4  | GCCCCTGCTAATACAGGTAAT | 21        | 497           | *Latrodectus* spp. |
| Adom_F      | TGCTGGATTCCGAAATTGAT | 20        | -             | *A. domesticus* |
| Adom_R      | CCCGCAAGAACAAGGTAAAGA | 25        | 248           | *A. domesticus* |
Figure 1. Southern black widow spider (*Latrodectus mactans*) with its prey house cricket (*Acheta domesticus*) trapped in spider web.
**Figure 2.** Success in detecting the mtDNA cytochrome c oxidase subunit I (COI) locus of *Latrodectus* spp. from web samples as measured by the number of positive PCRs out of 10 replicates. Samples “Lmac_1”, “Lmac_2”, and “Lmac_3” were tested for *Latrodectus mactans* while “Lhes_zoo” was tested for *Latrodectus hesperus* using the same nested “Lat_COI” primer sets.