Comparative analysis of courtship in *Agelenopsis* funnel-web spiders (Araneae, Agelenidae) with an emphasis on potential isolating mechanisms

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DEDICATION

I dedicate this dissertation to my parents, Joyce and Everett Payne, who always encouraged in me a love of books, knowledge and the natural world.

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ABSTRACT

The genus *Agelenopsis* (Araneae, Agelenidae) is a group of morphologically similar funnel-web spiders with overlapping habitat requirements and geographic ranges. Yet, molecular evidence suggests this group has undergone recent speciation with no evidence of hybridization. In this dissertation, I explored courtship divergence as a possible explanation for the formation and coexistence of these species. Courtship behavior patterns, sequences, and vibratory signals were compared across 12 *Agelenopsis* species and three related outgroup species. Courtship in *Agelenopsis* was found to be comparatively long but not completely species-specific. To investigate mechanisms of reproductive isolation within the genus, interspecific crosses were staged in the lab between species for two closely related species pairs, one pair being sympatric in distribution and the other peripatric. Vibratory courtship did not function as a reproductive isolating mechanism for either pair. In the sympatric species pair, males displayed significantly less courtship toward heterospecific females than to conspecific females and no interspecific copulations were attempted. Females did not reject males based on their vibratory courtship, rather males rejected females, apparently based on chemical cues. In contrast, there was interspecific courtship and copulation in the peripatric species pair. However, males appeared to encounter mechanical difficulties copulating with heterospecific females and no hybrid offspring were produced. Thus, the sympatric pair was reproductively isolated by barriers acting early in the mating

process, while the geographically isolated pair appeared to be isolated by barriers acting very late in the mating process. Though these results did not support a role for vibratory courtship in reproductive isolation, vibratory courtship appeared to be quite important in *Agelenopsis*. Males continued to court females even after females accepted them, and even behavior occurring late in the courtship sequence varied among species. In comparison to the two more distantly related outgroup species, vibratory courtship in *Agelenopsis* seems to be under relatively strong sexual selection for long, complex displays.

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INTRODUCTION

Courtship Function and Evolution

The selection of an appropriate mate is critical to the reproductive success of sexually reproducing animals. The system of communication between the sexes whereby animals choose their mates, courtship, is often found to be elaborate and species-specific. The evolution of courtship diversity is often the result of selection for choosiness at one of the two levels of mate choice. At the first level there must be discrimination of species and sex, followed by the selection of 'favorable' traits.

Species discrimination is an important process for closely related, sympatric taxa. When recently diverged species become sympatric, they may hybridize. If such hybrids are less fit than the parental taxa, there may be selection for divergence in courtship, via the process of reinforcement (Dobzhansky 1937, Mayr 1963). For example, reinforcement has lead to divergence in the calls of the green-eyed tree frog, in areas where recently diverged lineages are sympatric (Hoskin et al. 2005).

Regardless of sympatry with other species, within-species selection of mates is an important function of courtship. Courtship signals used in this context are subject to sexual selection. Sexual selection is known to produce rapid evolution of courtship signals because they are directly involved in the mating success of virtually all individuals. Also, there is often no clear optimum or limit for such signals to reach (West-Eberhard 1983). Sexual selection has lead to

conspicuous divergence in visual courtship displays between populations of the jumping spider *Habronattus pugillis* (Masta & Maddison 2002).

Courtship signals functioning in either level of mate choice may also evolve due to selection for transmission in the environment. For example, Seddon (2005) found that frequency characteristics of antbird loudsongs correlate with the level of the forest where the birds usually sing. Alternatively, courtship may evolve without any direct selection at all, via genetic drift or pleiotropy.

No matter which process leads to courtship divergence, differences in courtship behavior may facilitate species coexistence. Speciation is thought to require geographic isolation in most cases. If geographically isolated taxa eventually come into contact, the similarity of their courtship signals may influence whether or not they are able to coexist (Gröning & Hochkirch 2008). This dissertation focuses on courtship evolution in a genus of funnel-web spiders thought to have experienced recent speciation. These spiders typically engage in long courtship sequences involving chemical, vibratory and tactile signaling, providing an excellent opportunity to explore the role of courtship in speciation.

The genus *Agelenopsis*: Natural History and Speciation

Agelenopsis is a widespread genus in North America with 13 described species and one potential new species that is undescribed. These species are distinguished primarily by genitalic characters (Ayoub et al. 2005, Bennett &

Ubick 2005). Paison (1997) reviewed the distributions, life history, and genital morphology of the described species and constructed a simple phylogenetic hypothesis based on single trait differences in genital morphology. This review concluded that spiders of this genus are found in a variety of environments, ranging from arid habitats to leaf litter on forest floors to human disturbed habitats, and while some species are associated with particular types of habitat, others are not. In general, habitat requirements within species appear to be broad. Using two mitochondrial genes, Ayoub et al. (2005) reconstructed the speciation history of all but one described *Agelenopsis* species, as well as the potential new species. This molecular analysis revealed that some species with overlapping ranges are also very closely related, leaving open the question as to what allows for the coexistence of these species.

The courtship and pheromones of one species, *Agelenopsis aperta*, have already been analyzed (Papke, Riechert & Schulz 2001, Singer et al. 2000). Using headspace analysis of males, females and juveniles to identify potential pheromones, Papke et al. (2001) found that a compound found only in mature, virgin females, 8-methyl-2-nonanone, was able to attract and elicit courtship from males. Courtship analysis by Singer et al. (2000) found many different behavior patterns, with abdomen wagging and web flexing predominating. In successful courtships, males took shorter rests between bouts of activity and wagged their abdomens at a higher frequency compared to males in unsuccessful courtships, which suggests that females prefer more active, vigorous males (Singer et al.

2000). When female agelenid spiders accept a male, they enter a cataleptic state which enables the male to position the female properly for copulation (Gering 1953). In *A. aperta* this state is induced by a male-produced pheromone that functions within a few centimeters of the female (Becker, Riechert & Singer 2005). The Singer et al. (2000) study of *A. aperta* courtship found two patterns of catalepsis induction in *A. aperta*, one in which males court for a long period of time, induce catalepsis once and then mate (21% of matings) and another in which and another in which males court briefly before inducing catalepsis but then resume courtship for a period of time before mating (79% of matings). Given the relatively long and complex courtship sequence noted in *A. aperta*, we suspect that courtship could play an important role in species recognition within *Agelenopsis*.

Goals of the Dissertation Research

This dissertation focuses on the evolution of courtship and its potential role in maintaining species boundaries in the funnel-web spider genus, *Agelenopsis*. I investigate the evolution of courtship in this spider genus by first describing the courtship behavior of all but one described species in the genus and then analyze courtship in a historical context. This analysis reveals the patterns and processes of courtship divergence in these morphologically, ecologically and genetically similar spiders. I also test for reproductive isolation between closely related species directly.

Chapter I describes and compares the vibratory and tactile courtship behavior patterns and sequences of all but one described *Agelenopsis* species in addition to three species from other agelenid genera. These data are compared to determine the species-specificity and phylogenetic utility of courtship in these spiders. I also document the incidence of continued male courtship after female acceptance, both on the web and while mounted on the female, which may indicate strong sexual selection in this group.

Chapter II describes and compares the web-borne vibratory signals produced by courtship behavior patterns. Qualitatively, closely related species exhibit similar courtship sequences and use the same basic behavior patterns in courtship. I compare the amplitudinal, temporal and spectral characteristics of the resultant vibrations to determine whether quantitative differences in vibratory courtship signals exist among closely related species.

Chapter III investigates reproductive isolation within two groups of closely related *Agelenopsis* species, one being sympatric and the other peripatric. In this third chapter, I measure male response to web-borne chemical signals produced by same and different species females. I also stage interspecific and intraspecific matings within two species pairs and examined male and female behavior towards conspecifics and heterospecifics. Finally, I compare the success of inter- and intraspecific matings with specific interest in potential mechanical incompatibility in interspecific copulations.

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CHAPTER I

COMPARATIVE ANALYSIS OF COURTSHIP IN FUNNEL-WEB SPIDERS

This chapter is a modified version of an original research article to be submitted for publication by A. B. Galasso and S. E. Riechert.

My contributions to this paper include 1) co-formulation of the original research idea, 2) collection of most subjects and all data, 3) analysis of all data, 4) construction of figures and tables and 5) most of the writing.

Abstract

Courtship facilitates the selection and acquisition of appropriate mates. Courtship behavior may diverge among closely related species via a number of evolutionary mechanisms including reinforcement, sexual selection, drift and environmental adaptation. In this study, vibratory courtship evolution is examined in a genus of North American funnel-web spiders, *Agelenopsis* (Araneae, Agelenidae). The courtship behavior of all but one described *Agelenopsis* species and three species from related genera was described and compared. Though the ranges of many of the species overlap, courtship does not appear to be species-specific enough to function in species recognition. Also, attempts to use courtship to reconstruct phylogenetic relationships within the genus were marginally successful. Sexual selection factors may exert a strong force on courtship behavior within *Agelenopsis,* as courtship is long and continues even after female acceptance of the male. Furthermore, courtship after female acceptance shows a significant amount of divergence among species.

Introduction

The biological species concept holds that a species is a group of potentially interbreeding organisms (Mayr 1963). By this definition, our understanding of the process of speciation in a taxon requires delineation of the mechanisms that effect reproductive isolation and how such mechanisms have evolved (Coyne & Orr 2004). Courtship behavior is one mechanism animals use to mediate mate choice, thereby limiting the gamete wastage that typically occurs from interspecific matings. Courtship signals are known to be highly divergent among species in taxonomic groups that have undergone radiations such as the African cichlids and the Hawaiian *Drosophila* and *Laupala* crickets (Ritchie 2007). Divergence in courtship signals in such groups suggests that these traits often play an important role in rapid speciation.

A number of different evolutionary processes are associated with courtship signal divergence between closely related species, including sexual selection, ecological adaptation, genetic drift and reinforcement. In the systems mentioned in the previous paragraph, sexual selection is thought to have played a key role in signal divergence and speciation (Ritchie 2007). For example, *Laupala* crickets were found to have the highest speciation rate yet recorded in arthropods. In this group, closely related species show no ecologically distinguishable traits and are morphologically cryptic, but are distinguishable by the pulse rate of male courtship song (Mendelson & Shaw 2005). This pattern suggests that sexual selection on courtship song has driven speciation in the

genus. Similarly, among Lake Malawi rock-dwelling cichlids, Danley and Kocher (2001) found species within genera of these cichlids are nearly morphologically identical. They differ only in male secondary sexual characteristics, in particular nuptial coloration. The overall pattern in diversification within the Lake Malawi rock-dwelling cichlids suggests that sexual selection via female choice may play a key role in the later stages of a radiation following ecological diversification.

There are many systems in which ecological variables have mediated adaptive divergence in courtship behavior. For example, a meta-analysis of the relationship between bird song and habitat characteristics completed by Boncoraglio and Saino (2006) demonstrated that song frequency characteristics were lower in closed habitats, where lower frequencies are less attenuated and thus can be transmitted for greater distances. Thus, selection for detectabilty in different environments is another factor that can shape or constrain the characteristics of courtship signals.

Courtship might diverge via genetic drift, especially in small, isolated populations. A comparison of divergence in song (used primarily in reproductive contexts) and calls (used in a variety of contexts) to genetic divergence in greenish warblers found that both types of signals were correlated with genetic divergence, indicating a role for drift or other stochastic processes. However, songs showed an increase in length and complexity in areas of recent range expansion, while calls did not, indicating that sexual selection was an important factor in song evolution (Irwin, Thimgan & Irwin 2008). Other studies have found

weak or variable correlations between genetic distance and acoustic signal divergence, suggesting that genetic drift has influenced the evolution of sexual signals, but has probably not acted alone (Tietze et al. 2008, Päckert et al. 2009). It appears genetic drift often plays a significant, though perhaps, secondary role in courtship evolution, as seen in the fact that courtship behavior frequently retains a strong degree of phylogenetic signal. It is this factor that makes this trait a useful character in constructing phylogenies (Price & Lanyon 2002, de Quieroz & Wimberger 1993, Rendall & Di Fiore 2007).

These evolutionary forces (sexual selection, environmental adaptation, drift) may cause divergence of courtship in concert with speciation or independent of it. In contrast, reinforcement is an adaptive process that directly strengthens reproductive isolation between species in response to maladaptive hybridization (Dobzhansky 1937, Mayr 1963). In such cases natural selection can directly act to favor prezygotic isolating mechanisms. An example of a reinforcement mechanism comes from Coyne and Orr's (1989) examination of behavioral isolation in the genus *Drosophila*. Evidence suggests that behavioral isolation evolved faster in sympatric species than in allopatric species in this genus. Reinforcement has been a controversial hypothesis. Some authors have argued it is theoretically unlikely to occur for several reasons, including the expectation that mate recognition traits are under stabilizing selection, the possibility that selection may favor eliminating hybrid disadvantage instead of eliminating cross matings and the unlikelihood of both taxa coexisting long

enough for reinforcement to occur (Butlin 1987, Spencer, McArdle & Lambert 1986). However, recent evidence presented by Hoskin et al (2005) for the green-eyed tree frog suggests that it might have wider impact than acknowledged to date.

Study system: Agelenopsis *funnel-web spiders*

This study tests among the factors listed in Table I-1 that potentially underlie the divergence of courtship signaling in the North American spider genus *Agelenopsis* (Araneae, Agelenidae). The funnel-web spider genus *Agelenopsis* is an interesting group in which to examine the evolution of courtship. A recent phylogenetic study using two mitochondrial genes found that the monophyly of several species was not well supported, and it appeared that these species were the result of recent speciation (Ayoub et.al. 2005). This recent speciation provides an opportunity to ascertain which traits diverge during speciation events in the group. *Agelenopsis* species are morphologically quite similar (Figure I-1), and are distinguished primarily on the basis of their genital morphology (Chamberlin & Ivie 1941). While this kind of genitalic divergence was once thought to function to mechanically prevent interbreeding (the 'lock and key' hypothesis), it now seems more likely to be the result of sexual selection via female choice or sexual conflict (Arnqvist & Rowe 1995, Eberhard 1985), which may well extend to other aspects of the mating process. Many of the species have wide distributions throughout North America resulting in species range

overlaps and in some locations as many as five species can be found syntopically with overlapping breeding seasons (Paison 1997). Yet there is no evidence of hybridization occurring (Chamberlin & Ivie 1941, personal observation by Riechert). There is, thus, the potential for courtship evolution to be driven by reinforcement. Population numbers tend to be very large with only ground dispersal in evidence, weighing against a potential genetic drift explanation. However, molecular evidence indicates that range fragmentation during the Pleistocene played an important role in the history of *A. aperta*, suggesting that there might have been smaller, isolated *Agelenopsis* populations during past glacial maxima (Ayoub & Riechert 2004).

The courtship sequence in funnel-web spiders is largely vibratory and occurs on a substrate built by the female spider itself (the web). As a result, the communication channel between a male and female of a given species should be relatively private and constant across environmental conditions. Also, there is a great deal of habitat overlap between species (Paison 1997). We thus do not expect habitat differences to influence courtship patterns in this group.

Most of what is known about courtship and mate choice in this genus comes from studies of the southwestern desert species, *Agelenopsis aperta* (Gertsch). In this species, a methylated ketone pheromone both attracts males to female webs and elicits all elements of courtship (Papke et al. 2001). In this species' vibratory courtship, the male moves the across the web surface, wagging its abdomen as it drums its pedipalps (Singer et al. 2000). When

stationary the male will also flex the web by moving up and down. Males that take shorter rests and wag their abdomens more vigorously are more likely to copulate (Singer et al. 2000). Male size is also an indicator of courtship success as females have been shown to favor larger males (Riechert & Singer 1985). The successful male induces a quiescent state in the female called catalepsis, similar to death feigning, in which the female draws her legs up high and close to the body and is unresponsive. As the genitalia and copulatory mechanics of agelenids are complex, female catalepsis is likely necessary for copulation to be successful (Gering 1953). The cataleptic state has been shown to be induced by some unknown chemical cue released by the male (Becker, Riechert & Singer 2005). The pedipalps are apparently used in directing the chemical plume towards the female as males losing their palps in the final molt can only induce catalepsis in females by physical contact. Intact males can induce this state from a distance as far as 3.5 cm. While the females are in this state, males may drag them around on the web and engage in mount phase behaviors or abandon them while they continue to travel around the web producing vibratory courtship signals. Eventually the male positions the female on her side for mating (Singer et al. 2000). Copulation typically lasts for several hours (Singer & Riechert 1995).

The goal of this study is to quantitatively describe and compare courtship behavior across the genus *Agelenopsis*, as well as three species from other agelenid genera serving as outgroup samples. These data will be analyzed to

determine whether or not courtship is species-specific, as would be expected if courtship behaviors diverged due to reinforcement and were important in species recognition. Also, the prevalence of courtship after female acceptance, including courtship while mounted on the female and resumption of web phase after mounting will be assessed for these species. These behaviors occur too late in the courtship sequence for species recognition to be a likely function. They may instead represent the product of sexual selection and/or be involved in male readiness/preparation for sperm transfer. Finally, the degree of phylogenetic signal in courtship behavior will be determined. If there is strong phylogenetic signal to the courtship sequences, then behavioral data could be used to determine the phylogenetic affinity of *Agelenopsis actuosa,* the one species not included in the molecular phylogeny. These behavioral data could also resolve some of the higher level relationships within *Agelenopsis* that were poorly resolved by the mtDNA sequence data.

Methods

Collections and rearing conditions

Individuals of eleven *Agelenopsis* species, *Barronopsis texana*, *Hololena nedra*, and *Calilena californica* were collected from sites throughout the continental United States (Table I-2). For several species, individuals were collected from multiple locations. *Barronopsis texana, C. californica* and *H. nedra* were included in this study as outgroups as they are all part of the same agelenid

subfamily as *Agelenopsis*, Ageleninae (Bennett & Ubick 2005), and have ranges that overlap with *Agelenopsis* (Chamberlin & Ivie 1941, Chamberlin & Ivie 1942, Stocks 1999). Recent molecular work also supports a sister relationship between *Agelenopsis* and *Barronopsis* (Ayoub et al. 2005, Miller et al. 2010).

For most species, individuals were collected in the field as juveniles, reared in the lab, and then mated a few weeks after reaching maturity. For four species, the offspring of field collected individuals were reared to obtain individuals of known reproductive history and increase sample sizes. To determine if field-collected individuals differed in courtship behavior from individuals born in the lab, the five trials of *B. texana* with field-collected pairs (all copulated) were compared to thirteen trials of lab-bred pairs that copulated. The two groups were compared with Mann-Whitney-Wilcoxon tests for 214 variables measured in this study (described under *Quantitative description of web courtship*).

Small spiders were housed individually in round plastic condiment cups (3.0 cm height x 4.2–5.6 cm diameter), while larger (> 100 mg, approximately) spiders were housed individually in round plastic dishes (3.0 cm height x 11.0 cm diameter). Once per week, juveniles' containers were misted with water and they were fed a diet of termites, crickets, and adult *Drosophila*. Adults were fed crickets only, also once per week. Spiders were kept on a light-dark cycle of approximately 12L:12D, at temperatures of 20-25ºC.

Courtship trials

Courtship trials were conducted after methods described in Singer et al. (2000). Prior to the introduction of a male, each female was released into a transparent plastic arena that consisted of a cylinder (10.0 cm x 3.0 cm diameter) attached to round box (6.5 cm x 15.5 cm diameter), providing the structure necessary to building a web funnel and capture sheet equivalent to what an adult female would construct in nature. At the time of a courtship trial, the lid was removed from the arena, and the arena was placed in a larger plastic tub, so that the male or female could leave the arena without escaping into the laboratory. The male was introduced onto the center of the sheet portion of the web at trial initiation.

An event-recording system (Noldus Observer 5.0, Wageningen, Netherlands) was used in coding the behavior during the courtship trials. Most of the trials were digitally recorded concurrently with the encoding (Sony HDR-HC3 HDV 1080i Handycam® camcorder New York, NY). This provided a back-up to the scoring. Repeated playbacks also facilitated description of new behavior patterns. A trial was scored as ended when one of the following occurred: a) the pair copulated, b) the male failed to move for one hour, c) the male failed to court within the first hour, d) the female did not enter catalepsis within two hours of courtship initiation, e) the male did not copulate within one hour of catalepsis induction or f) either the male or female spider left the arena. Observations were made of 190 unique pairings of conspecific individuals (Table I-2). All females

used in this study were virgins, as mated females are known to be less attractive to and less receptive to potential mates (Riechert & Singer 1995, Singer & Riechert 1995). Additionally, nine videotaped trials of *A. aperta* courtship recorded for the study reported in Singer et al. (2000) study were coded for use in this study.

Quantitative description of courtship

Behavior patterns observed were categorized following Singer et al. (2000), and several new behavior patterns were added that had not been observed in *A. aperta* courtship (Table I-3). Courtship was quantified by determining the frequencies of observed behavior transitions for all behaviors, the proportion of courtship spent on each behavior and the average durations of several common behaviors for each observation and then averaged across trials for each species. Common behaviors were those that constituted at least 10% of male web courtship in at least one species. Only trials in which courtship elements were exhibited were included in the analyses.

For these analyses, the courtship sequence was divided into two discrete segments: web phase (activity on the web, when the male was not mounted on the female) and mount phase (male activity while mounted on the female prior to copulation, female is always cataleptic during this phase). This was done because the two phases were distinct and some trials included only one phase, not both. Furthermore, the proportion of trials that included both phases varied

among species. Combining the two phases when calculating proportions of time and transition frequencies would have confounded differences among species in courtship success and differences in typical courtship behavior. While there were some transitions between the two phases, the vast majority of transitions were within one phase.

Transition matrices were used in analyzing the respective web phase and mount phase sequences. The relative frequency of each first-order transition was calculated for each trial, and these were averaged across all trials for each species. Common transitions were included in a kinematic diagram describing that species' web phase courtship sequence. Common transitions included those which occurred in at least a third of the trials including courtship elements and which constituted on average at least 1% of all transitions. Transitions only observed in only one trial were excluded. The same criteria were applied when developing the kinematic diagram of the mount phase for each species. Transitions between the two phases that met the above criteria were included in the mount phase diagrams.

In calculating the proportion of time spent on each behavior pattern, the total duration of a particular behavior pattern within the respective phases was divided by the total duration of that phase for each trial. Total duration of each phase included absolute time spent inactive (resting or mounting). These proportions were averaged for each species.

To quantify courtship differences between species, nonparametric Kruskal-Wallis tests were performed for each of the 214 variables included in the data set using the NPAR1WAY procedure in the Statistical Analysis System software (SAS 9.2, SAS Institute Inc., Cary, NC). For variables that differed significantly between species, post-hoc comparisons were computed using Dunn's test, implemented in the KW_MC SAS macro (Elliot and Hynan 2011), since the NPAR1WAY procedure does not calculate post-hoc tests. Nonparametric tests were used because the data were not normally distributed and species did not have equal variance-covariance matrices, in large part due to the high frequency of zero values in the data. There were many zero values because many behavior patterns and transitions did not occur in all species. This issue could not be eliminated by a data transformation.

Additionally, canonical discriminant analyses were also implemented using the SAS procedure CANDISC. This procedure produces a new set of variables that are a linear combination of the original variables. These new variables are created to maximize the amount of between-species variation explained while minimizing the within species variation. This procedure allows for differences between species to be described and visualized in a reduced number of dimensions, as many of the original variables were highly correlated. One canonical discriminant analysis was performed on the 78 mount phase variables and another was performed on the 136 web phase variables.

Relative durations of two courtship phases and incidence of mixed phase behavior

For each species, the mean durations of the web phase and the mount phase of courtship were determined for all trials in which copulation took place. These durations were compared across species with the ANOVA procedure in SAS and followed by Tukey-Kramer tests for post hoc comparisons. To meet the assumptions of ANOVA, web phase duration was square root transformed and mount phase duration was log transformed. These ANOVAs were performed both with and without the outgroup species

Also, for each species, the proportion of trials in which males transitioned back from mount phase to web phase stages of the courtship sequence was determined for each species. These frequencies were compared across species, both with and without the outgroup species, using Fisher's exact test in the FREQ procedure in SAS.

Tests to determine if species can be discriminated by courtship behavior

To determine whether or not male courtship is species-specific, nearest-neighbor discriminant analyses were performed separately on the web phase and the mount phase of the courtship sequence as well as on the full courtship sequence. These three analyses were performed only on trials (n = 101) that had both types of courtship, excluding *A. oregonensis* and *A. oklahoma* because only one trial from each of these species included both phases of courtship. Also, an

analysis of web phase only was done for all trials that included web phase, whether or not the male mounted the female. This analysis was performed on just the thirteen species used in the other analyses (*n* = 159), to determine whether including unsuccessful trials would reduce discriminability, and on all fifteen species (*n* = 169), to determine the species specificity of web courtship in *A. oklahoma* and *A. oregonensis*. Additionally, an analysis of the web phase of courtship was performed where different populations were coded as separate groups. This permitted detection and estimation of population differences in the courtship sequence exhibited for a species. Only populations at least 50 kilometers apart and represented by at least three pairings were included in the tests for population differences.

The variables included in the discriminant analyses were the latency to initiate courtship, the durations of prevalent behavior patterns, the proportion of each phase spent on each behavior pattern, and transitions between behavior patterns that occurred in at least a third of the pairings for at least one species. Tremulation behavior varied among species with respect to the concurrent presence or absence of abdomen bobbing (Table I-2). To include this aspect of tremulation in the discriminant analyses, the presence or absence of bobbing was coded as a dummy variable. The overall duration of web and mount phase as well as whether or not the male resumed courtship after mounting were considered for the analyses including only pairings in which mount phase occurred.

As there were a large number of potential variables (p = 67 – 148), a stepwise selection technique was performed using the STEPDISC procedure in SAS to determine what subset of the discriminating variables to include in each of the discriminant analyses. This variable selection technique starts with no variables in the model and then adds and removes variables according to their significance in an analysis of covariance, where the variables already selected act as covariates and the variable being considered is the dependent variable. At each step during variable selection, the variable in the model with the least power to discriminate among *Agelenopsis* taxa, as measured by Wilks' lambda, is examined, and if its significance level is below 0.25, it is removed. (Wilks' lambda is an inverse measure of the discriminating power of remaining variables which have not yet been selected.) If all the variables in the model meet the criteria to stay, then the variable with the best discriminating power not already in the model is added if its significance level is at least 0.15. When all variables in the model meet the criteria to stay and no new variables meet the criteria to enter, variable selection is complete. This process was repeated for each discriminant analysis separately.

Using the variables selected by the stepwise selection processes, nearest neighbor discriminant analyses were performed (DISCRIM procedure in SAS). This method classifies courtship and copulation sequences into species according to the nearest neighbor rule (Covert and Hart 1967). Mahalanobis distances are computed between pairs of trials based on the total sample

covariance matrix. Each trial is assigned to the taxon for which it has the highest posterior probability, which is based on the identities of its nearest neighbors (i.e. those with the smallest Mahalanobis distance from the trial of interest). A jackknife or "leave one out" method was used to cross-validate the results of the discriminant analyses. By this method, the observation (trial) of interest is "left out" so that it cannot be its own nearest neighbor: its classification is based only on the other observations. Additionally, posterior probabilities and posterior probability error rates were calculated for the cross-validation. For nonparametric classification methods, posterior probability error rate estimates have less variance than jackknife estimates, but they have a slightly optimistic bias (Lugosi & Pawlak 1994).

Phylogenetic analysis of courtship

The characters used for phylogenetic reconstruction included all the variables compared in the Kruskal-Wallis tests (See *Quantitative description of courtship* for details) as well as the durations of web phase and mount phase. Additionally, the three tremulation characteristics described in Table I-4 were coded as discrete, numeric characters, with *B. texana* character states coded as "?", since that species did not exhibit tremulation. Thus, nearly all the characters used in these analyses were in fact continuous measurements of various courtship characteristics. Continuous characters are often avoided or discretized in phylogenetic studies, but recently the program T.N.T (Tree Analysis Using

New Technology) has implemented algorithms that allow continuous characters to be analyzed as such (Goloboff, Mattoni & Quinteros 2006). These continuous characters were coded as a range going from the mean minus one standard error of the mean to the mean plus one standard error of the mean, as suggested by Goloboff, Mattoni and Quinteros (2006). In total there were 219 characters included in the analysis, 140 of which pertained to web phase courtship and 79 of which pertained to mount phase courtship.

Among species relationships were evaluated by performing maximum parsimony analysis using the program TNT 1.1 (Goloboff, Farris & Nixon 2008). The most parsimonious trees were determined by implicit enumeration with implied weights (K = 5) to down-weight homoplasious characters and reduce problems due to the scaling of different continuous characters. Thus, characters that changed more on a given tree were given less weight than more conserved characters, as the latter characters should be more reliable (Goloboff et al. 2008) Searches were performed with *Agelenopsis* species constrained to be monophyletic. Three analyses were conducted, one using all characters, one using just web phase characters and one using just mount phase courtship characters.

This analysis was also performed to examine within species variation for the four species that were collected from multiple populations. Since these analyses had more ‘taxa’, searching by implicit enumeration was no longer feasible. Heuristic searches were performed with 5 random seeds by tree

bisection reconnection (TBR) branch swapping with 1000 replications of random taxon addition sequences maintaining up to 10 trees per replication.

For each most parsimonious tree found, support was determined with 1000 bootstrap replicates and 1000 symmetric resampling replicates (P = 0.33), which are unaffected by differing weights (Goloboff et al. 2003). Bootstrap replicates were performed using constrained, heuristic searches by tree bisection reconnection (TBR) branch swapping with 100 replications of random taxon addition sequences maintaining up to 10 trees per replication. The consistency index (CI; Kluge and Farris 1969) and retention index (RI; Farris 1989) were also calculated for each tree to determine levels of homoplasy.

Results

General courtship features

With two exceptions, successful web phase courtship in *Agelenopsis* exceeds 45 minutes on average from first male movement to copulation (Figure I-2). Web phase duration significantly differed among *Agelenopsis* species (ANOVA: F (9, 62) = 2.81, p = 0.008). Post-hoc tests revealed this difference was due to the short web phase duration in *A. oregonensis* trials (6.14 ± 5.97 minutes), which differed significantly from five other *Agelenopsis* species.

In all *Agelenopsis* species males spent the majority of their time resting (76.6 ± 3.5 %, Table I-5). Periods of male activity typically involved alternating between two categories of behavior: movement around the web (with or without

an abdomen waggle) and stationary vibratory signaling (tremulate, web flex, bob abdomen) (Fig. I-3). Females also spent most (>80%) of their time resting (Table I-6). They did not exhibit much signaling behavior; rather they typically moved or retreated in response to male activity. *Agelenopsis* species could be separated into four broad clusters with similar web phase courtship (Figure I-4). See Appendix 1 for more details on the courtship pattern of each species.

Immediately prior to mounting the female, males often walked toward female, moved to be near female, or abdomen waggled toward female. In *A. actuosa* and *A. pennsylvanica*, it was rather common for males to contact females for some time immediately prior to mounting. This happened occasionally in several other species as well. Many males also engaged in tremulation or abdomen bobbing just prior to or even during the process of mounting, events that were particularly common in *A. emertoni* and *A. utahana*. Lunging was a less common method of initiating mounting, though it happened in three of four *A. oregonensis* matings.

While most courtship time was spent on web phase, all species engaged in some amount of mount phase, and this included behaviors involved in positioning the female (haul, adjust position, turned over), cleaning the palps in preparation for copulation (groom) and behaviors likely functioned to signal or stimulate the female (flex while mounted, bob while mounted, tremulate while mounted, bob tap, parallel rub) (Figure I-5, Table I-7). Just as most of web phase

was spent resting, most of mount phase was spent merely mounted on the female, not moving (61.5 ± 2.0%).

Courtship in the three outgroup species was generally rather similar in structure to courtship in *Agelenopsis*, but with a few notable differences. *Barronopsis texana*, a member of the sister genus to *Agelenopsis,* exhibited web phase sequences that were of similar duration to *Agelenopsis* species (46.6 ± 4.5 minutes). In contrast, the other outgroup species representing more distantly related agelenid genera had shorter successful courtship durations, with web phase durations of 8.58 ± 3.05 (*C. californica*) and 13.19 ± 3.66 minutes (*H. nedra*) (Figure I-2). The duration of web phase did differ significantly among species when outgroups were included (ANOVA: F (12, 85) = 3.60, p = 0.0002). Also, in terms of the courtship sequence, there was a general lack of stationary signaling behavior in *B. texana*.

Behavior patterns exhibited

Several new behaviors were described in this study (Table I-3). Stroke web was observed in only a few pairings, while the other behaviors were more common. The behavior tremulate includes the behavior described in *A. aperta* courtship as 'vibrate' (Singer et al. 2000), as well as other forms of whole body shaking. In some species, these tremulations are brief and uncommon, as in *A. aperta*, while in others they are much more common and may even form longer series of several bouts (Table I-4, Table I-5). Abdomen bobbing was not seen in

A. aperta courtship, but was seen in the courtship of several other species, though usually infrequently (Table I-5). Tremulate while mounted and bob while mounted were similar to web phase behaviors, but performed while mounted on the female. Bob tap was a behavior pattern only seen in mount phase in two species, and it did not strongly resemble any web phase behavior. Other behaviors recorded in this study were previously described in *A. aperta* courtship. Some behavioral definitions were slightly expanded during this study (Table I-3). Also, several uncommon behavior patterns in *A. aperta* courtship were not observed in this study, including wave legs, exaggerated walk, wrap, and spread front legs another locating behavior done while stationary. This might reflect rarity or differences between observers in the two studies.

Test for differences in rearing history

When comparing the courtship of lab-bred and field-collected *B. texana*, significant differences were only found for 12 variables (5.6% of the total), which is only slightly more than the number of differences expected by chance (5%). Only two variables differed significantly after the Holm's correction. In contrast, 78% of these variables differed significantly among species, indicating that any within species differences due to lab rearing are small compared to the between species differences that are the focus of this study.

Species differences in courtship variables

Significant differences were found in 167 of 214 courtship variables, far more than would be expected by chance alone (Table I-4). After Holm's correction for multiple comparisons, 113 variables differed significantly. In general, variables describing male behavior during web phase were more likely to differ than variables describing female behavior. During mount phase, the variables that did not differ tended to be those pertaining to functional behaviors like groom, haul and turned over. Of the significant pairwise comparisons, 63.3% of the variables discriminated among *Agelenopsis* species, while 32.3% only discriminated *Agelenopsis* species from one or more outgroups. The remaining variables only distinguished the outgroup species from one another.

A canonical discriminant analysis applied to the web phase courtship sequences accounted for 90.91% of the variation in the original variables useful for discrimination among species in six canonical variables (Figure I-6, Table I-9). The first six canonical variables captured variation in the extent of web flexing, tremulation, abdomen bobbing and abdomen wagging, as well as transitions between them (Table I-10). The variables that showed the highest overall multiple correlations with species grouping were those pertaining to these four behavior patterns (Table I-11). *Agelenopsis oklahoma* had very distinctive courtship sequences and was well distinguished from the other species by the first three canonical variables. The other species could be distinguished by the canonical variables to a more moderate degree. The outgroup species did not

appear to be any better distinguished by the canonical variables than the *Agelenopsis* species were.

The first three canonical variables from the analysis of mount phase explained 92.65% of the variance between species (Figure I-7, Table I-12). The first two canonical variables captured variation due to two mount phase behavior patterns only found in a few species: flexing while mounted and bob tap (Table I-13). The third variable primarily captured variation in two more widespread behavior patterns: parallel rub and bobbing while mounted. The variables that showed the highest multiple correlations with species groupings were those pertaining to these four behavior patterns as well as adjusting position and hauling (Table I-14). *Agelenopsis kastoni*, *A. emertoni*, *A. pennsylvanica*, *A. potteri* and *A. utahana* are not well-distinguished by any of the first three canonical variables. Again, the outgroup species did not appear to be better distinguished from *Agelenopsis* than the *Agelenopsis* species were from each other.

Assessing prevalence of courtship after female acceptance

Mount phase tended to be rather long and complex in *Agelenopsis* (Figure I-2). Each *Agelenopsis* species displayed two or three behavior patterns that appeared to serve a signaling function in mount phase. There were no significant differences in the duration of mount phase within *Agelenopsis* (ANOVA: F (9, 62) = 0.85, p = 0.57). However, when outgroups were included, species did differ in

the duration of mount phase (ANOVA: F (12, 85) = 3.29, p = 0.0006). *C. californica*'s mount phase was significantly shorter (2.52 ± 1.03 minutes) than the 11 *Agelenopsis* species' mount phase (genus as a whole: 14.47 ± 1.48 minutes) it was compared to, but not the other two outgroup species, which had intermediate durations (*B. texana*: 6.97 ± 0.94 minutes, *H. nedra*: 13.19 ± 3.66 minutes).

Resuming web phase courtship after mounting the female was also rather common (Figure I-8). The frequency of web phase courtship resumption differed significantly among *Agelenopsis* species (Fisher's exact test: $p < 0.0001$). It also differed among species when the outgroup species were included (Fisher's exact test: $p < 0.0001$). Resumption of web phase was extremely common in *B. texana*, occurring in all but one pairing in which mounting occurred. In contrast, the two more distantly related outgroup species did not display this pattern in any trials.

Species-specificity of courtship

For the analyses of pairings with both courtship phases, the stepwise variable selection procedure selected 54 of 145 variables for full sequence, 32 of 67 variables for mount phase only, and 27 of 78 variables for web phase only (Table I-14). For analysis of all pairings with web phase, excluding *A. oklahoma* and *A. oregonensis*, 39 of 77 variables were selected. For analysis of all pairings with web phase, 43 of 77 variables were selected. For analysis of web phase at the population level, 41 of 77 variables were selected. Using these variables, all

discriminant analyses found significant differences among the species (Wilks' $\lambda < 0.0001$, $P < 0.0001$).

Cross-validation of the discriminant analyses showed species were correctly classified based on male courtship in the majority of cases, but no analysis perfectly discriminated between the species (Figure I-9, Table I-16). When examining only trials where both web phase and mount phase occurred, both phases produced similar classification successes (73.3% and 67.7% correct, respectively). Combining both phases of courtship produced a better classification success rate of 95.9%. Including parings without mount phase and adding *A. oklahoma* and *A. oregonensis* pairings to the web phase analyses yielded a small decrease in classification success. All *A. oklahoma* trials were correctly classified, but no *A. oregonensis* trial was correctly classified. *Agelenopsis pennsylvanica* and *A. potteri* were frequently confused with each other in the web phase discriminant analyses, and they were sometimes confused with *A. emertoni*. These three species were also confused with *A. kastoni* in the mount phase analysis, but *A. kastoni* had distinct web phase courtship. *Agelenopsis naevia* and *B. texana* were frequently confused, and there were some misclassifications between these species and *A. actuosa*. Finally, a substantial fraction of *A. utahana* trials were misclassified as *H. nedra.*

Examining the posterior probabilities of group membership revealed that there was some uncertainty in the classification of most species in most of the analyses (Table I-17). However, the posterior probability error rate estimates

were generally higher than those from the jackknife cross-validation (Figure I-9), perhaps reflecting the optimistic bias of this estimator.

When populations of *A. naevia*, *A. oklahoma*, *A. pennsylvanica* and *A. utahana* were coded separately, there were still significant differences between populations/species (Wilks' $\lambda < 0.0001$, $P < 0.0001$), but overall classification accuracy decreased to 53.8% of all trials (Figure I-9, Tables I-18 & 1-19). The *A. naevia* and *A. pennsylvanica* populations were frequently misclassified and did not appear to be distinguishable at the population level. One *A. oklahoma* population (Kansas) could be correctly classified without error, while all but one pairing of the other population were misclassified into other species. Similarly, one *A. utahana* population (Utah) could be correctly classified in all but one case, but only one trial in the other population was correctly classified. It appears that there is not a high degree of population-level differentiation in courtship in these four species.

Phylogenetic analyses

All analyses found one best tree (Figures I-10 & I-11). In general, most nodes within *Agelenopsis* were not well supported. The trees with the most well-supported nodes were those based on mount phase characters alone (Figures I-10C and I-11C). Most of the well-supported relationships in the courtship-based trees were also found in the mtDNA-based trees obtained by Ayoub et al. (2005).

Five trees grouped *A. utahana* and *A. oregonensis*, but the support for this relationship was only high in analyses that included mount phase. In the population-level trees, the Washington *A. utahana* population formed a clade with *A. oregonensis*, making *A. utahana* paraphyletic with respect to *A. oregonensis* (Figure I-11). The mtDNA-based analyses also found that *A. utahana* and *A. oregonensis* formed a well-supported clade, but different analyses showed conflicting support for the monophyly of *A. utahana*. The mtDNA-based trees found the *A. utahana*/*A. oregonensis* clade to be basal to all the other *Agelenopsis* species, but four of the courtship-based trees did not include this relationship.

All four analyses involving web phase supported a clade consisting of *A. emertoni*, *A. pennsylvanica* and *A. potteri* (Figures I-10A-B & I-11A-B). In the species-level analyses, *A. pennsylvanica* and *A. potteri* formed a well-supported clade, but when *A. pennsylvanica* was separated into populations, one population grouped with *A. emertoni* and the other two populations grouped with *A. potteri*, and those relationships were not well-supported. The trees based on mount phase alone grouped these three species into a clade with *A. kastoni* and *A. oklahoma*, but this clade was only well-supported in the species-level tree (Figures I-10C and I-11C). The positions of *A. kastoni* and *A. oklahoma* vary among the trees based on web phase. Similarly, in the mtDNA phylogenetic analyses, *A. emertoni*, *A. pennsylvanica* and *A. potteri* formed well-supported

clade with *A. kastoni* as its sister group. The position of *A. oklahoma* was ambiguous in the mtDNA trees.

The trees based on mount phase alone found a well-supported clade consisting of *A. aperta*, *A. aleenae* and *A. spatula*, with *A. aleenae* and *A. spatula* forming a less well-supported clade (Figures I-10C and I-11C). This result is in agreement with the mtDNA analyses, which found *A. aleenae* and *A. spatula* to form a monophyletic species group, with *A. aperta* as the likely sister group to *A. aleenae* and *A. spatula* and another, undescribed species not included in this study. However, these three species were found to be sister to the rest of *Agelenopsis*, which is not a relationship found in any of the mtDNA-based analyses. The species level trees including web phase tended to group these three species together, though there was not much support for this relationship.

Agelenopsis actuosa was the only *Agelenopsis* species not included in the mtDNA study. In this study, the position of *A. actuosa* varied; in different trees it was found to be sister to *A. aperta*/*A. spatula* and *A. naevia*. The only one of those relationships that was well-supported was the sister relationship with *A. naevia* found in the mount phase-only trees. In the population-level mount phase tree, it formed a clade with the Arkansas *A. naevia* population, but not the Kansas *A. naevia* population (Figure I-11C).

Discussion

General features of funnel-web spider courtship

Funnel-web spider courtship consists of two phases: web phase and mount phase. Mounted courtship prior to copulation has also been noted in the bowl-and-doily spider, *Frontinella pyramitela* (Suter & Renkes 1984). Like funnel-web spiders, bowl-and-doily spiders exhibit signaling behavior during mount phase that is very similar in form to their web phase behavior. Web phase behavior in funnel web spiders typically consists of males cycling between a few common behavior patterns, usually one or two forms of movement and one or two stationary signaling behavior patterns. Signaling behavior usually involves shaking or up-and-down movements of the body or abdomen. Similar courtship behavior patterns have been reported in a variety of web building spiders, including an African mygalomorph funnel-web spider (*Thelechoris karschi*, Coyle & O'Shields 1990), cellar spiders (*Pholcus phalangiodes*, Bartos 1998), the golden orb-weaving spider (*Nephila clavipes*, Christenson et al. 1985), the bowl-and-doily spider (Suter & Renkes 1984), and other funnel-web spider species (*Hololena adnexa*, Fraser 1987; *Coelotes terrestris*, *Tegenaria parietina*, Krafft 1978).

Females were generally not very active and some females remained completely inactive until the males reached them in the funnel of their webs. Other females responded to male activity with movement, retreating or

occasionally aggression, but obvious signaling behaviors were relatively rare. Female receptivity behavior patterns or postures have been described in several spider species (Eberhard & Huber 1998, Quirici & Costa 2005, Uetz, Roberts & Taylor 2009). In funnel-web spiders, receptive females assume a cataleptic posture, but this does not usually occur until the male contacts the female.

Agelenopsis courtship. Within the genus *Agelenopsis*, there appear to be four clusters of species with similar web phase courtship (Figure I-4), and these clusters largely coincide with phylogenetic relationships. One cluster of species consists of *A. emertoni*, *A. pennsylvanica* and *A. potteri*, which all exhibited tremulation series with abdomen bobbing. These three species were found to form a closely related species group in the Ayoub et al. (2005) molecular phylogeny and all are found primarily in the eastern United States. They were confused with one another in the discriminant analyses and grouped together in the phylogenetic analyses. Within this cluster *A. potteri* and *A. pennsylvanica* were most similar.

Another cluster consisted of southwestern species that exhibited web flexing – *A. aperta*, *A. aleenae*, *A. spatula*, and *A. oklahoma*. *Agelenopsis aleenae* and *A. spatula* formed a well-supported clade in the molecular phylogeny, and *A. aperta* often appeared sister to them. Despite qualitative similarity in courtship, these species were rather well-distinguished in the discriminant analyses, perhaps mirroring the higher levels of interspecific

molecular divergence Ayoub et al. (2005) found in the western species. Furthermore, these three species appeared sister to the rest of *Agelenopsis* in the mount phase-based trees, further indicating the distinctiveness of their courtship within the genus. *Agelenopsis oklahoma* often appeared sister to the other three species in the molecular phylogenetic analyses, but this relationship was less well-supported. Similarly, *A. oklahoma* courtship was quite distinctive and its position in the courtship-based trees was variable.

Another cluster was comprised of a closely related pair of species, *A. oregonensis* and *A. utahana*. Males of both species were rather active during web phase, spending relatively high proportions of time tremulating and abdomen bobbing. These species grouped together in most courtship-based trees and all mtDNA-based trees in the Ayoub et al. (2005) study. Both species had relatively short web phase, compared to other *Agelenopsis* species.

The other three *Agelenopsis* species, *A. actuosa*, *A. naevia* and *A. kastoni*, displayed web phase consisting of high proportions of abdomen wagging and movement and varying frequencies of single tremulations. There was some misclassification among these species in the nearest-neighbor discriminant analysis. This cluster probably does not form a clade, though phylogenetic position *of A. actuosa* was not determined in the molecular phylogeny. In the molecular phylogeny there was a well-supported sister relationship between *A. kastoni* and the *A. emertoni*/*A. pennsylvanica*/*A. potteri* clade, but the position of *A. naevia* was not well-resolved (Ayoub et al. 2005). *Agelenopsis actuosa* and *A.*

naevia do show a unique mount phase behavior, bob tap, which might indicate a sister relationship between the two.

Outgroup species. The three outgroup species had web phase courtship surprisingly similar to that of *Agelenopsis* species. *B. texana* web phase consisted mostly of abdomen wagging and physical contact, and was often misclassified into the *A. actuosa*/*A. naevia*/*A. kastoni* cluster. *Calilena californica* and *H. nedra* males mostly alternated between movement and tremulation, with some abdomen bobbing, similar to *A. utahana* and *A. oregonensis*. The most distinctive characteristic of these two species' courtship is that it was shorter in duration than that of most *Agelenopsis* species. These observations of *H. nedra* are similar to those of Fraser (1987), who described the courtship of *Hololena adnexa* males as searching for the female and vibrating by "vigorously pumping his legs and abdomen in the vertical plane without palpal drumming", with one to six bouts of vibration at a time. The time from introduction onto the female's web to contact with the female averaged 32.8 minutes in *H. adnexa.*

The outgroup species differed more from *Agelenopsis* in terms of the relative frequencies of different behavior patterns and their sequence rather than using qualitatively different behavior patterns unknown in *Agelenopsis*. Given that similar behavior patterns have been described for other web-building spiders, it may be that there are a limited number of movement types that are likely to evolve in communicative displays. For example, even though abdomen bobbing

was not part of *A. aperta* courtship (Singer et al. 2000), a similar behavior, pump abdomen, was described in *A. aperta* agonistic interactions (Riechert 1978).

Possible mechanisms of courtship divergence in Agelenopsis*: Drift and phylogeny*

If courtship were evolving via drift alone, strong phylogenetic signal would be expected. Though courtship is similar among closely related species, phylogenetic reconstructions based on courtship did not clearly resolve the higher level relationships left unresolved in the molecular phylogeny of this genus and there were moderately high levels of homoplasy, as indicated by the CI and RI values for the courtship trees. One example of homoplasy is the absence of bobbing while mounted in *A. aleenae*, *A. spatula*, *A. aperta* and *B. texana*, which is sister to *Agelenopsis* and the presence of it in all other species in this study. If the mtDNA phylogeny is accurate, then this behavior pattern appears to have been lost twice or gained multiple times.

Most of the courtship characters used in the phylogenetic analyses were not qualitative differences in display behavior patterns, but were instead quantitative changes in the proportion of courtship spent on various behaviors or behavioral transitions. Stuart, Hunter and Currie (2002) found that qualitative differences in behavior patterns have been successfully used to reconstruct phylogenies congruent with phylogenies obtained from other data, but little use has been made of quantitative differences. Quantitative differences in behavior

are common at the population level, and there is often substantial genetic variation for these traits (Foster 1999). This study did not find a sufficient diversity of behavior patterns to produce a phylogeny based on purely qualitative differences.

Possible mechanisms of courtship divergence in Agelenopsis*: Reinforcement*

The molecular phylogeny produced by Ayoub et al. (2005) found three monophyletic species groups, which suggested recent speciation. If courtship played an important role in the speciation process, one would expect to see distinct differences in courtship within these closely related groups. However, as previously discussed, closely related species had rather similar courtship. Discriminant analyses of the full courtship sequence had a high degree of classification accuracy overall, though there were some errors. However, since mount phase only occurs after the female accepts the male and assumes a cataleptic posture, web phase is more likely to be used in species identification than mount phase. Yet, the web phase does not appear to be sufficiently species-specific to accomplish this function. Furthermore, many species with similar courtship behavior are also sympatric. For example, *A. emertoni*, *A. pennsylvanica*, *A. potteri* all have overlapping ranges and *A. aperta* co-occurs with *A. spatula*, *A. aleenae* and *A. oklahoma*.

Reinforcement is thought to result in a pattern of greater divergence in isolating traits in areas of sympatry than in allopatry (Noor 1999). Two closely

related species, *A. oregonensis* and *A. utahana*, have ranges that overlap in the Pacific Northwest, a region where other species have been found to come into secondary contact. Thus, reinforcement should be likely in this area. Yet, phylogenetic reconstructions based on courtship actually placed *A. utahana* from Washington as sister to *A. oregonensis*, also from Washington. This indicates that *A. utahana* courtship is more similar to *A. oregonensis* courtship where their ranges overlap, the opposite of what would be expected if there were selection on courtship to avoid hybridization. Furthermore, half of the *A. oregonensis* pairings observed did not involve any web phase at all, which strongly suggests web phase is not important in maintaining reproductive isolation between these two species.

Thus, the results of this study do not indicate that vibratory courtship has evolved via reinforcement in *Agelenopsis*, and they also do not support a major role for courtship in the recent speciation events in the genus. However, it is possible that there are more subtle differences in the temporal and frequency characteristics of vibratory signals in closely related species, and it is those characteristics, rather than gross differences in behavior patterns and sequences that are important in the formation and maintenance of species boundaries. This question will be addressed in future work.

Possible mechanisms of courtship divergence in Agelenopsis*: Sexual selection*

Several lines of evidence support a role for sexual selection in the evolution of *Agelenopsis* courtship. These include the continuation of male courtship after female acceptance of the male, divergence in mount phase comparable to web phase, and lengthy courtship and copulations in comparison to the more distantly related outgroups.

Previous work on *Agelenopsis* (Gering 1953, Singer et al. 2000) had found that males spend a significant amount of time mounted on the female before copulation. Fraser (1987) found *H. adnexa* pairs spent nearly ten minutes in this 'precopulation' phase. Past authors have emphasized the male's efforts to position the female during this period, but males also spend a substantial proportion of mount phase on behaviors that appear to serve a signaling function. Such mount phase has been observed in other arthropods, including backward and forward rocking in medflies (Briceno, Ramos and Eberhard 1996) and head nodding and antennal stroking in the Japanese beetle (Barrows and Gordh 1978), but in both of these species females may reject or dislodge males that have mounted prior to copulation, so males must persuade females to cooperate with their efforts. In funnel-web spiders, mounted females are cataleptic so there is little need for males to persuade females to allow copulation at that stage. Thus, mount phase in agelenids more closely resembles 'copulatory courtship', which is thought to reflect male attempts to influence cryptic female choice (Eberhard 1994).

Mount phase was found to be as species-specific as web phase, and trees based on mount phase showed similar levels of homoplasy (as measured by CI and RI) as trees based on web phase. It has been theorized that displays early in courtship are more likely to be involved in species recognition, and thus more likely to be conserved, while later displays are more likely to evolve rapidly under sexual selection (West-Eberhard 1983). In a phylogenetic study of courtship displays in storks, Slikas (1998) found earlier displays to be less homoplasious than later displays, in agreement with this prediction. However, funnel-web spider courtship does not seem to follow this pattern. One explanation for this result is that both web and mount phase have evolved primarily under sexual selection, and not as a result of selection for species recognition. Another possible explanation is that the web phase data are more 'noisy' because of interactions with the female, variation in female receptivity and variation in the topography of the web, and this noise has reduced phylogenetic signal. However, analyses of just male web phase, not including female behavior, only slightly improved phylogenetic resolution and did not substantially improve CI or RI (data not shown). Finally, changes in mount phase may be correlated with changes in web phase, such as the evolution of flexing while mounted occurring only in a clade of species that engage in web flexing prior to mounting. However, most mount phase signaling behaviors cannot be explained by such simple correlations.

In the majority of species studied, at least some males stopped mount phase and resumed web phase before copulating. This pattern is puzzling, as some males do not successfully remount and copulate during the observed time. In nature, delaying copulation in this way could allow a rival male to supplant the first male and deny him the opportunity to mate. Singer et al. (2000) hypothesized that web phase might serve a physiological function that takes some time to accomplish. This was supported by the observation that males that resumed web phase were males that had mounted the female earlier in courtship. However, this does not seem to be true in the other species examined, and some males mount and copulate without any web phase at all. It is possible that courtship does serve a physiological function by arousing males and/or females, but individuals vary greatly in their initial arousal level and courtship requirements. Alternatively, males may provide additional web phase to influence cryptic female choice. In the Australian redback spider, females exhibit a courtship duration threshold, and males that copulate without reaching that threshold are at a greater risk of being cannibalized after only a brief copulation. Also, females are more likely to accept copulations from rival males if the first male to mate has a short copulation (Stoltz and Andrade 2010). In *A. aperta*, most females mate only once and mated females cease to produce the male attraction pheromone (Singer and Riechert 1995, Papke et al. 2001). Perhaps this decrease in female receptivity and attractiveness is mediated by courtship, and males that mate after brief courtships risk female remating.

The frequency of resumption of web phase and courtship duration varies among species and the reason for this variation is unclear. One interesting trend is that the species with high frequencies of web phase resumption also tend to be those species that often abdomen waggle. Abdomen wagging is an exaggerated version of silk laying behavior and may actually involve silk production. It is possible that males resume web phase to lay silk as deterrent to other males. Males of several spider species lay silk during copulation (Aisenberg et al. 2008) and extracts of male *Schizocosa ocreata* silk inhibit the courtship of other males (Ayyagari & Tietjen 1986). It is also possible that receptivity in the lab setting varied somewhat across species. Another possibility is that the intensity of sexual selection on male courtship varies across species. There is little information available on species besides *A. aperta* with which to evaluate these possibilities.

Resumption of web phase after mounting was not seen in any males from the more distantly related outgroups, *H. nedra* and *C. californica*. Also, both species exhibited shorter web and mount phase durations than species in the *Agelenopsis*/*Barronopsis* clade. Additionally, copulation duration in *A. aperta* averages more than 14 hours, while copulation in *H. adnexa* averaged less than three hours (Singer and Riechert 1995, Fraser 1987), and informal observations in this study concur that copulation in *Agelenopsis* is usually quite long. Taken together, these observations suggest that there has been a greater degree of sexual selection on courtship in *Agelenopsis* and *Barronopsis*.

Conclusions

Overall, the results of this study indicate vibratory courtship may be less important in speciation and species recognition and more important in sexual arousal and reducing female receptivity to remating attempts. Further research on the functions of long courtship sequences, long copulations and resumption of web phase courtship is needed. This study found that closely related species predominately utilize the same behavior patterns in courtship, though courtship data were unable to clarify higher level phylogenetic relationships within *Agelenopsis*. Future work will quantify the vibratory signals produced by these behavior patterns to determine if their temporal and frequency characteristics differ between species, which could indicate a role for these signals in species recognition.

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Appendix I

Table I-1. Potential mechanisms of courtship divergence and evidence that would support each mechanism in *Agelenopsis*.

Mechanism	Definition	Evidence in favor
Sexual selection	Selection for traits that directly aid an organism in obtaining mates and their gametes. These traits may be involved in mate choice or intrasexual competition for mates	Divergence throughout the courtship sequence Courtship long, complex
Reinforcement	The evolution of prezygotic reproductive isolation due to natural selection against the production of unfit hybrids	Courtship species-specific Greater divergence among closely related species Greater divergence in areas of sympatry Greater divergence early in the courtship sequence
Genetic drift	Changes in gene frequency due solely to chance	Courtship characters show strong phylogenetic signal

Table I-2. Collecting localities for the species included in this study, with sample sizes.

Species	*Collecting locality*	*Pairings attempted*	*Pairings with courtship*	*Pairings with copulation*
A. actuosa	Rochester, WA	10	8	4
A. aleenae	Las Vegas, NM	13	12	4
A. emertoni	Knox County, TN	19	16	11
	Woodward, OK	2	2	0
	Russellville, AR	2	2	1
	Gainesville, FL	1	1	0
A. kastoni	Andersonville, TN	9	9	6
	Powell, TN	1	1	1
	Gatlinburg, TN	3	2	0
	Kingsport, TN	1	1	1
A. naevia	Morrilton, AR	7	5	4
	Lawrence, KS	6	6	4
A. oklahoma	Wichita Falls, TX	4	3	0
	Meade, KS	5	4	1
A. oregonensis	Silver Creek, WA	10	6	4
A. pennsylvanica	Meade, KS	5	5	1
	Knob Noster, MO	6	6	5
	Topeka, KS	5	5	5
	Lawrence, KS	3	3	2
A. potteri	Dane County, WI	10	8	4
A. spatula	Quitaque, TX	7**	7**	1
	Canyon, TX	1	1	1
A. utahana	Alta, UT	12	7	3
	Leavenworth, WA	3	3	3
	Cle Elum, WA	9	6	3
B. texana	Knoxville, TN	27	25	19
C. californica	CA	6	6	3
H. nedra	Mabton, WA	7	7	4

** Includes one cross between Canyon and Quitaque *A. spatula*

Table I-3. Ethogram of spider courtship behavior. Descriptions of most behavior patterns are modified from Singer et al. (2000). New behavior patterns described in this study are indicated with an asterisk (*). Most behavior patterns were coded as states with durations recorded. Those behavior patterns that were coded as events are indicated with the letter E.

Behavior	Description
Web Phase	
Abdomen waggle	Spider waves abdomen parallel to the web while walking across the surface of the web, often includes palpal drumming. It may be associated with laying silk and may also include brief stops of less than three seconds.
Bites web[E]	Spider bites web with mouthparts
Bob abdomen*	Spider alternately raises and lowers abdomen. It varies from a slow, distinct raising and lowering to fast, indistinct, low amplitude up and down movement.
Chase	Spider runs after partner on the web.
Contact	Spider makes physical contact with partner.
Drum	Spider drums pedipalps while stationary, typically at a slow rate.
Flinch[E]	Spider jerks body without moving in response to contact or sudden movement by partner
Joust	Spider raises or strikes at partner with first pair of legs.
Lunge[E]	Spider launches body in direction of partner
Move	Spider walks across the surface of the web.
Orient to partner[E]	Spider turns around and faces partner while partner is within 3 cm.
Palpate	Spider slowly and deliberately walks across the web, exploring the web with first two pair of walking legs while drumming pedipalps.
Rest	No detectable movement.
Retreat	Spider runs in opposite direction of approaching partner.
Stroke web*	Spider, typically male, strokes the web rapidly with his front two pair of walking legs. Typically occurs at beginning of courtship sequence.
Tremulate*	Spider vibrates entire body, sometimes violently, may drum pedipalps briefly at start.
Walk toward partner	Late in courtship, spider walks in direction of partner, ultimately standing right above (for male) or right below (for female) partner.
Web flex	Male flexes web with walking legs, while raising his body; then retracts his legs while lowering his body. Each web flex is comprised of several syllables. Usually the male also vibrates his legs and body.
Mount Phase	
Bob tap*	While mounted to the female, male bobs abdomen distinctly while tapping or jerking his front pair of legs. May be synchronized.
Bob while mounted*	Male bobs abdomen while mounted to the female.
Haul	Male hauls cataleptic female around on web.
Mount	Male holds cataleptic female under his body ventral side down.

Table I-3 (continued)

Behavior	Description
Parallel rub	Male rapidly rubs forelegs parallel to and against each other while mounted to the female.
Tremulate while mounted*	Male tremulates while mounted to the female.
Turn over	Male turns cataleptic female on her side and maintains contact with her.
Web flex while mounted	Male web flexes while mounted to the female, typically with fewer syllables than in web phase.
Palpal insertion	Male inserts pedipalp into the female's epigynum, and expands and contracts the palpal bulb.
Both Phases	
Adjust position	Spider picks up legs and sets them down in same location.
Catalepsis	Female collapses and appears unconscious with legs held up against her body.
Groom	Spider rubs pedipalps or walking legs against other legs or pulls palp through chelicerae.

Table I-4. Characteristics of tremulation in each species studied.

	Usually occurs late in courtship when male is near female or in funnel	Sometimes occurs in a series of tremulation bouts	Each bout of tremulation begins with distinct bob of the abdomen
A. actuosa	No	No	No
A. aleenae	Yes	No	No
A. aperta	Yes	No	No
A. emertoni	No	Often	Yes
A. kastoni	No	No	No
A. naevia	Yes	No	No
A. oklahoma	No	No	No
A. oregonensis	No	Occasionally	No
A. pennsylvanica	No	Often	Yes
A. potteri	No	Often	Yes
A. spatula	Yes	No	No
A. utahana	No	Occasionally	No
B. texana	No tremulation	No tremulation	No tremulation
C. californica	No	No	No
H. nedra	No	Often	No

Table I-5. Mean percent of web phase of courtship sequence spent on various male behaviors by species. Means are based on all pairings except eight in which the male mounted the female without any species-typical web phase behavior. RE: rest, TR: tremulate, WF: web flex, BA: bob abdomen, DM: drum palps, AW: abdomen waggle, MV: move, WT: walk toward partner, RT: retreat, PP: palpate, SW: strokes web, AP: adjusts position, GR: groom, CN: contact, JT: joust, CH: chase, LG: lunge, FL: flinch, BW: bites web, OM: orients to mate. A dash indicates that the behavior pattern was never observed.

	A. actuosa	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
RE	70.2	88.2	54.4	82.2	80.5	80.3	88.1	73.1	83.9	86.4	79.5	52.6	64.3	84.4	83.7
TR	0.1	0.1	0.3	4.5	2.0	0.1	1.7	10.6	7.5	4.2	0.2	13.3	-	3.4	5.9
WF	-	8.4	11.3	-	-	-	5.2	-	-	-	7.3	-	-	-	-
BA	0.5	-	-	<0.05	0.1	0.5	<0.05	1.7	0.3	<0.05	0.8	22.5	<0.05	1.6	7.1
DM	-	-	0.3	<0.05	<0.05	0.1	<0.05	-	-	-	-	0.2	0.5	0.2	0.3
AW	14.1	2.0	23.2	0.4	12.6	5.3	1.5	-	4.2	4.2	5.5	0.2	15.5	-	-
MV	8.2	0.2	0.4	3.1	1.5	3.4	2.2	10.1	2.3	0.4	4.5	3.1	1.5	8.8	1.0
WT	<0.05	0.1	<0.05	<0.05	<0.05	0.2	-	-	0.1	0.1	0.1	-	0.1	-	-
RT	0.1	<0.05	<0.05	<0.05	<0.05	0.1	0.9	-	<0.05	<0.05	0.1	-	0.1	0.1	<0.05
PP	-	-	-	<0.05	<0.05	<0.05	-	-	-	-	-	-	0.2	-	0.2
SW	-	-	-	<0.05	-	-	-	-	-	-	-	-	-	0.3	-
AP	<0.05	-	0.2	0.2	0.1	0.4	-	-	<0.05	<0.05	0.1	0.1	0.1	-	0.1
GR	0.2	0.4	2.6	0.7	2.2	1.1	0.9	-	0.2	0.1	0.6	0.1	5.0	-	-
CN	6.5	-	3.8	8.5	0.6	8.9	0.3	4.6	1.7	4.0	1.1	7.1	12.4	0.9	0.5
JT	-	-	-	-	-	<0.05	-	0	<0.05	<0.05	-	-	<0.05	0.3	-
CH	-	-	-	-	-	-	-	<0.05	-	-	-	-	<0.05	-	-
other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.6
LG	-	-	-	0.3	0.1	0.1	0.1	0.3	0.1	-	-	0.2	0.8	0.2	0.7
FL	0.5	0.1	0.3	0.2	0.4	0.6	0.1	-	0.4	0.4	0.1	0.2	1.8	0.3	0.2
BW	7.1	0.7	13.3	-	19	1.0	6.9	-	3.3	4.9	11.1	1.4	16.1	-	-
OM	-	-	-	<0.05	-	0.1	-	-	-	0.1	0.1	-	0.1	-	-

Table I-6. Mean percent of web phase spent on various female behaviors by species. Means are based on all pairings except eight in which the male mounted the female without any species-typical courtship behavior. RE: rest, CT: catalepsis, TR: tremulate, BA: bob abdomen, DM: drum palps, AW: abdomen waggle, MV: move, WT: walk toward partner, RT: retreat, PP: palpate, SW: strokes web, AP: adjusts position, GR: groom, CN: contact, JT: joust, CH: chase, LG: lunge, FL: flinch, BW: bites web, OM: orients to mate. A dash indicates that the behavior pattern was never observed.

	A. actuosa	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
RE	84.2	95.0	85.3	93.1	89.9	84.0	97.7	87.5	89.2	97.6	96.7	89.1	74.8	96.4	78.8
CT	13.3	2.7	12.9	3.8	5.8	13.3	-	-	3.5	0.8	1.7	4.7	18.7	1.2	20.2
TR	-	<0.05	-	-	-	0.1	<0.05	-	-	-	-	-	-	-	-
BA	1.1	<0.05	-	<0.05	-	<0.05	-	-	-	-	0.1	-	-	-	-
DM	-	0.1	-	-	<0.05	<0.05	<0.05	-	-	-	<0.05	-	0.8	<0.05	-
AW	-	0.2	<0.05	0.1	0.4	-	<0.05	-	0.1	-	0.3	-	1.5	<0.05	<0.05
MV	0.9	0.2	1.8	1.1	3.0	1.6	1.9	10.2	6.7	1.3	0.9	5.1	1.4	2.1	0.5
WT	<0.05	<0.05	-	<0.05	<0.05	<0.05	-	-	<0.05	<0.05	-	-	<0.05	-	-
RT	-	0.1	0.8	0.1	0.6	0.2	0.1	2.2	0.3	0.1	0.4	0.1	0.3	<0.05	-
PP	0.1	0.4	-	-	-	-	-	-	<0.05	-	-	-	0.1	-	-
SW	-	-	-	-	-	-	-	-	<0.05	0.6	-	-	-	-	-
AP	0.2	<0.05	0.2	0.9	0.2	0.5	0.2	0.2	0.1	-	0.1	0.8	0.5	0.1	0.2
GR	-	<0.05	-	-	-	-	-	-	0.1	-	<0.05	-	<0.05	-	-
CN	-	0.1	-	0.5	<0.05	<0.05	-	-	0.1	-	<0.05	-	2.4	-	<0.05
JT	0.1	<0.05	<0.05	0.4	0.1	0.4	-	-	0.1	0.1	0.2	-	0.3	0.2	<0.05
CH	-	<0.05	-	-	<0.05	<0.05	-	-	-	<0.05	0.1	-	<0.05	<0.05	-
other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2
LG	0.3	-	-	0.4	-	1.9	0.4	-	0.1	-	0.5	-	0.4	0.3	-
FL	0.6	0.1	-	1.0	2.8	1.7	0.6	0.3	0.7	0.5	2.4	0.3	1.7	0.3	0.5
BW	-	0.2	-	0.1	0.1	-	0.3	-	-	0.1	-	0.2	0.3	-	-
OM	-	-	-	0.2	0.2	-	0.1	-	0.1	0.3	0.6	0.2	1.0	-	-

Table I-7. Mean percent of mount phase spent on various male behaviors by species. Averages based only on pairings with mount phase. MT: mounted, TO: turned over, FM: flexes while mounted, BM: bobs while mounted, TM: tremulates while mounted, PR: parallel rubs, BT: bob taps, HL: hauls, AP: adjusts position, GR: grooms palps. A dash indicates that the behavior pattern was never observed.

	A. actuosa	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
MT	72.2	54.6	65.5	63.7	55.1	66.2	54.5	57.6	60.0	52.1	72.7	63.9	63.2	67.0	62.0
TO	4.0	6.6	2.2	4.3	1.2	3.3	0.7	4.3	3.5	3.7	0.4	1.8	9.0	12.8	4.1
FM	-	3.1	3.0	-	-	-	-	-	-	-	7.3	-	-	-	-
BM	1.0	-	-	13.7	15.4	0.7	3.8	3.6	12.0	10.4	-	8.2	-	2.0	3.1
TM	-	-	-	0.3	0.1	0.6	-	1.3	0.1	0.9	-	0.8	-	-	5.3
PR	1.4	2.6	5.7	1.7	3.3	1.6	0.9	0.2	2.2	1.6	0.8	0.3	5.7	1.7	-
BT	14.5	-	-	-	-	2.3	-	-	-	-	-	-	-	-	-
HL	4.2	5.5	6.2	2.6	5.6	3.7	3.8	18.3	5.9	2.9	6.4	7.4	1.8	8.8	10.8
AP	0.7	0.8	6.5	2.1	0.6	3.7	0.8	7.0	2.4	1.6	4.2	5.7	1.7	2.1	9.2
GR	2.1	9.9	11.5	11.1	18.7	17.9	35.5	7.8	13.8	27.0	8.4	11.9	18.5	5.7	5.0

Table I-8. Interspecific comparisons for courtship variables. Interspecific comparisons for courtship variables. X^2 and *p* values are from Kruskal-Wallis tests comparing agelenid species. Species with different letters were found to differ significantly for that variable in Dunn's test. Transitions are indicated with a dash and transition variables represent the proportion of all behavioral transitions that are that particular transition (e.g. AW-AW represents proportion of all web transitions consisting of the transition from one bout of abdomen wagging to another).

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
Web Phase																	
AW Duration*	85.90	<0.0001	AB	ABC	A	BC	ABC	ABC	ABC	BC	BC	ABC	ABC	BC	ABC	C	C
BA Duration*	47.78	<0.0001	AB	B	B	B	AB	AB	AB	AB	B	B	A	B	AB	AB	AB
CN Duration*	38.72	0.0004	AB	A	AB	AB	AB	B	AB	AB	AB	AB	AB	B	AB	AB	AB
MV Duration*	48.46	<0.0001	AB	ABC	AB	ABC	ABC	ABC	A	ABC	ABC	ABC	AB	B	ABC	ABC	C
RE Duration*	79.53	<0.0001	BC	AB	C	A	ABC	ABC	AB	ABC	AB	A	ABC	C	ABC	ABC	ABC
TR Duration*	130.90	<0.0001	BCD	BCD	BCD	ABCD	BCD	CD	ABCD	ABCD	A	A	ABCD	ABCD	D	ABCD	ABCD
WF Duration*	160.27	<0.0001	C	A	AB	C	C	C	A	BC	BC	BC	A	C	C	C	C
AP Proportion*	20.04	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW Proportion*	104.71	<0.0001	AB	ABC	A	BC	AB	ABC	ABC	BC	BC	BC	ABC	BC	AB	C	C
BA Proportion*	51.84	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CH Proportion	25.09	0.034	B	B	B	B	B	B	B	A	B	B	B	B	AB	B	B
CN Proportion*	43.94	<0.0001	AB	A	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	B	AB	AB
DM Proportion	24.38	0.041	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GR Proportion*	44.48	<0.0001	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	A	B	B
JT Proportion	17.06	NS															
MV Proportion*	50.10	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
PP Proportion	9.74	NS															
RE Proportion*	40.97	0.0002	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RT Proportion	23.27	NS															
SW Proportion	33.24	0.003	B	B	B	AB	B	B	AB	AB	AB	AB	AB	AB	B	A	B
TR Proportion*	138.29	<0.0001	AB	AB	AB	BC	BC	AB	BC	BC	C	BC	AB	C	A	BC	BC
WF Proportion*	160.20	<0.0001	C	A	A	C	C	C	AB	BC	BC	BC	AB	BC	C	C	C
WT Proportion	26.93	0.020	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
BW Count*	45.45	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
FL Count	22.69	NS															

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
LG Count	28.84	0.011	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
OM Count	9.74	NS															
fAP Proportion	16.32	NS															
fAW Proportion	19.80	NS															
fBA Proportion*	49.28	<0.0001	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B
fCH Proportion	11.13	NS															
fCN Proportion	18.60	NS															
fCT Proportion*	39.61	0.0003	AB	AB	AB	AB	AB	AB	A	AB	AB	AB	AB	AB	B	AB	AB
fDM Proportion	24.84	0.036	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
fGR Proportion	10.92	NS															
fJT Proportion	31.85	0.004	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
fMV Proportion	22.71	NS															
fPP Proportion	21.52	NS															
fRE Proportion	30.81	0.006	AB	AB	AB	AB	AB	AB	A	AB	AB	AB	AB	AB	B	AB	AB
fRT Proportion*	53.23	<0.0001	B	AB	AB	AB	A	AB	AB	AB	AB	B	AB	B	AB	B	B
fSW Proportion	6.41	NS															
fTR Proportion	14.28	NS															
fWT Proportion	3.94	NS															
f BW Count	8.57	NS															
f FL Count	30.40	0.007	AB	A	AB	AB	AB	B	AB	AB	AB	AB	AB	AB	AB	AB	AB
f LG Count	19.54	NS															
f OM Count	25.30	0.032															
Latency	32.00	0.004	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW-AW*	89.91	<0.0001	A	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	A	B	B
AW-BW*	46.99	<0.0001	AB	AB	AB	B	A	AB	AB	AB	AB	AB	AB	AB	AB	B	B
AW-CN*	62.29	<0.0001	AB	B	AB	AB	AB	AB	B	AB	AB	AB	AB	AB	A	B	B
AW-DM	28.32	0.013	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW-fAP*	44.67	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW-fBA*	61.10	<0.0001	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
AW-fFL*	45.67	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW-fMV*	51.34	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW-fRT*	83.87	<0.0001	A	AB	AB	B	A	AB	B	B	AB	AB	AB	AB	AB	B	B
AW-fWU*	61.23	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AW-GR*	83.11	<0.0001	B	B	AB	B	AB	B	B	AB	AB	AB	AB	AB	A	B	B
AW-MV*	53.34	<0.0001	A	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	B	A	A
AW-PP*	57.79	<0.0001	A	B	B	AB	AB	AB	B	AB	AB	AB	AB	AB	AB	B	B
AW-TR*	79.17	<0.0001	BC	BC	BC	BC	A	BC	AB	BC	BC	BC	BC	BC	C	BC	BC
AW-WF*	149.23	<0.0001	C	A	A	C	C	C	C	C	BC	BC	AB	BC	C	C	C
BA-AW*	46.02	<0.0001	AB	B	B	B	A	B	B	AB	AB	AB	AB	AB	B	B	B
BA-BA*	55.14	<0.0001	B	B	B	B	B	B	B	AB	AB	AB	AB	A	B	B	AB
BA-MV*	49.69	<0.0001	AB	B	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	B	AB	A
BA-TR*	77.12	<0.0001	B	B	B	B	B	B	B	AB	B	B	B	A	B	AB	B
BW-AW*	46.45	<0.0001	AB	AB	AB	B	A	AB	B	AB	AB	AB	AB	AB	AB	B	B
BW-BW	35.34	0.001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
BW-MV	35.51	0.001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CN-AW*	67.62	<0.0001	AB	B	AB	AB	AB	AB	B	AB	AB	AB	AB	AB	A	B	B
CN-fRT	34.32	0.0018	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CN-LG	27.47	0.0167	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CN-MV*	49.85	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CN-TR	27.12	0.019	AB	AB	AB	A	AB	AB	AB	AB	AB	AB	AB	AB	B	AB	AB
DM-MV	21.64	NS															
GR-AW*	48.94	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GR-GR*	43.14	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GR-MV	29.31	0.010	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JT-TR*	39.83	0.0003	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
MV-AW*	55.08	<0.0001	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	B	A	A
MV-BA*	40.72	0.0002	AB	B	B	AB	AB	B	B	AB	AB	AB	AB	AB	B	A	AB

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
MV-BW	28.94	0.011	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MV-CN*	50.68	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MV-fAP	28.15	0.014	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MV-fMV	27.97	0.014	AB	AB	A	AB	AB	B	AB	AB	AB	AB	AB	AB	AB	AB	AB
MV-fRT	21.87	NS															
MV-GR	34.24	0.002	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MV-MV	24.02	0.046	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MV-TR*	120.78	<0.0001	AB	AB	AB	A	AB	AB	A	A	A	A	AB	A	B	A	A
MV-WF*	98.89	<0.0001	B	A	A	B	B	B	AB	AB	AB	AB	A	AB	B	B	B
RT-TR	20.93	NS															
RT-WF*	40.55	0.0002	B	AB	AB	B	B	B	B	AB	AB	AB	A	AB	B	B	B
TR-AW*	75.17	<0.0001	B	B	B	B	A	B	B	B	B	AB	AB	B	B	B	B
TR-BA*	74.57	<0.0001	B	B	B	B	AB	B	B	AB	B	B	B	A	B	B	B
TR-CN	28.12	0.014	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
TR-DM	29.51	0.009	B	B	B	AB	B	B	B	AB	AB	AB	AB	AB	B	A	B
TR-fAP	33.18	0.003	AB	AB	A	AB	AB	A	AB	AB	AB	AB	AB	AB	B	AB	AB
TR-fFL	23.06	NS															
TR-fMV*	80.66	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
TR-fRT	29.10	0.010	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
TR-MV*	119.35	<0.0001	B	B	B	A	AB	B	AB	AB	AB	AB	B	A	B	AB	A
TR-SW*	54.66	<0.0001	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
TR-TR*	94.92	<0.0001	D	D	D	ABC	ABCD	D	CD	ABCD	BCD	BCD	D	AB	D	ABCD	A
TR-WF*	116.93	<0.0001	C	BC	AB	C	C	C	BC	A	BC	BC	BC	BC	C	C	C
WF-AW*	135.29	<0.0001	BC	A	A	BC	BC	BC	BC	BC	BC	BC	AB	BC	C	BC	BC
WF-CN*	58.80	<0.0001	BC	BC	A	BC	BC	BC	AB	AB	AB	AB	AB	AB	C	BC	BC
WF-fAP	35.22	0.001	AB	AB	A	AB	AB	AB	AB	AB	AB	AB	AB	AB	B	AB	AB
WF-fCN*	47.76	<0.0001	B	B	B	B	B	B	B	B	B	B	A	B	AB	B	B
WF-fMV*	88.86	<0.0001	BC	A	AB	BC	BC	BC	ABC	ABC	ABC	ABC	ABC	ABC	C	BC	BC
WF-MV*	130.42	<0.0001	CD	AB	ABC	CD	CD	CD	A	BCD	BCD	BCD	AB	BCD	D	CD	CD
WF-TR*	126.81	<0.0001	C	BC	AB	C	C	C	A	BC	BC	BC	AB	BC	C	C	C

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
WF-WF*	53.52	<0.0001	AB	A	AB	AB	AB	AB	AB	AB	AB	AB	A	AB	B	AB	AB
fAP-AW	39.87	0.0003	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
fAP-fMV	29.57	0.009	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
fAP-MV	15.91	NS															
fAP-TR*	47.32	<0.0001	AB	AB	AB	AB	A	AB	AB	AB	AB	A	AB	AB	B	AB	AB
fAP-WF*	78.70	<0.0001	BC	ABC	A	BC	BC	BC	AB	ABC	ABC	ABC	AB	ABC	C	BC	BC
fBA-AW*	48.08	<0.0001	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B
fFL-TR	30.54	0.006	AB	AB	AB	AB	A	AB	AB	AB	AB	AB	AB	AB	B	AB	AB
fJT-TR	23.16	NS															
fLG-RT	32.11	0.004	AB	AB	B	B	B	B	A	AB	AB	AB	AB	AB	B	AB	B
fMV-AW*	47.94	<0.0001	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	A	B	B
fMV-fAP	22.46	NS															
fMV-fMV	18.70	NS															
fMV-LG	9.89	NS															
fMV-MV	24.41	0.041	ABC	BC	BC	AB	ABC	ABC	BC	ABC	A	ABC	BC	ABC	C	ABC	ABC
fMV-RT	15.87	NS															
fMV-TR*	63.97	<0.0001	ABC	BC	BC	AB	ABC	BC	ABC	ABC	A	ABC	BC	ABC	C	ABC	ABC
fMV-WF*	89.66	<0.0001	C	ABC	A	C	C	C	ABC	B	B	B	AB	B	C	C	C
fRT-AW*	78.19	<0.0001	B	AB	AB	B	A	AB	AB	AB	AB	AB	AB	AB	A	B	B
fRT-fMV	21.82	NS															
fRT-fRT	32.34	0.004	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
fRT-TR*	66.82	<0.0001	B	B	B	B	A	B	AB	B	B	B	B	B	B	B	B
fRT-WF*	117.92	<0.0001	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B
fWU-fMV*	51.52	<0.0001	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Mount Phase																	
AP Proportion*	40.37	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
BM Proportion*	84.80	<0.0001	AB	AB	AB	A	A	AB		AB	A	A	AB	A	B	AB	AB
BT Proportion*	81.06	<0.0001	A	BC	BC	BC	BC	AB		BC	BC	BC	BC	BC	C	BC	BC
FM Proportion*	100.48	<0.0001	B	A	A	B	B	B		B	B	B	A	B	B	B	B

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
GR Proportion	18.06	NS															
HL Proportion*	46.96	<0.0001	ABC	ABC	ABC	BC	ABC	ABC		A	ABC	ABC	ABC	ABC	C	ABC	AB
MT Proportion	6.06	NS															
PR Proportion*	46.58	<0.0001	AB	AB	AB	AB	AB	AB		B	AB	AB	AB	B	A	AB	B
TM Proportion	28.78	0.007	A	A	A	A	A	A		A	A	A	A	A	A	A	A
TO Proportion	24.77	0.025	A	A	A	A	A	A		A	A	A	A	A	A	A	A
f WU Count*	68.72	<0.0001	AB	AB	AB	AB	AB	AB	B	AB	AB	AB	AB	AB	A	B	B
AP-AP	18.67	NS															
AP-BM*	42.92	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
AP-BT*	55.11	<0.0001	A	BC	BC	BC	BC	AB		BC	BC	BC	BC	B	C	BC	BC
AP-FM*	76.30	<0.0001	B	B	B	B	B	B		B	B	B	A	B	B	B	B
AP-HL	27.09	0.012	A	A	A	A	A	A		A	A	A	A	A	A	A	A
AP-PR	25.18	0.022	A	A	A	A	A	A		A	A	A	A	A	A	A	A
AP-TO*	19.87	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
AP-TM	26.96	0.013	AB	AB	AB	AB	AB	AB		AB	AB	AB	AB	AB	A	AB	B
AW-fCT	20.08	NS															
BM-AP*	52.75	<0.0001	AB	AB	AB	AB	AB	AB		AB	A	AB	AB	AB	B	AB	AB
BM-AW	31.12	0.003	AB	AB	AB	AB	AB	AB		AB	AB	A	AB	AB	B	AB	AB
BM-BM*	44.88	<0.0001	AB	AB	AB	AB	AB	AB		A	AB	AB	AB	A	B	AB	AB
BM-GR*	40.45	<0.0001	A	B	B	B	B	B		B	B	B	B	B	B	B	B
BM-HL*	55.46	<0.0001	AB	AB	AB	AB	A	AB		AB	AB	AB	AB	AB	B	AB	AB
BM-PR	32.93	0.002	AB	AB	AB	AB	AB	AB		AB	AB	A	AB	AB	B	AB	AB
BM-TO*	40.31	<0.0001	AB	AB	AB	A	A	AB		AB	AB	A	AB	AB	B	AB	AB
BT-AP*	59.04	<0.0001	A	B	B	B	B	AB		B	B	B	B	B	B	B	B
BT-HL*	71.11	<0.0001	A	BC	BC	BC	BC	AB		BC	BC	BC	BC	BC	C	BC	BC
BT-MV*	53.49	<0.0001	A	B	B	B	B	B		B	B	B	B	B	B	B	B
BT-PR*	80.97	<0.0001	A	B	B	B	B	B		B	B	B	B	B	B	B	B
BT-TO*	55.23	<0.0001	A	B	B	B	B	B		B	B	B	B	B	B	B	B

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
CN-fCT	28.05	0.009	A	A	A	A	A	A		A	A	A	A	A	A	A	A
FL-PR	8.07	NS															
FM-AP*	70.40	<0.0001	B	AB	A	B	B	B		B	B	B	A	B	B	B	B
FM-AW*	37.91	0.0003	AB	A	AB	AB	AB	AB		AB	AB	AB	AB	AB	B	AB	AB
FM-HL*	86.38	<0.0001	BC	A	AB	BC	BC	BC		BC	BC	BC	A	BC	C	BC	BC
FM-TO	33.05	0.002	B	B	B	B	B	B		B	B	B	A	B	B	B	B
GR-AP	21.34	NS															
GR-HL	15.72	NS															
GR-PI	12.34	NS															
GR-TO	20.50	NS															
HL-AP	26.26	0.016	A	A	A	A	A	A		A	A	A	A	A	A	A	A
HL-AW	14.30	NS															
HL-BM*	77.60	<0.0001	ABC	BC	BC	ABC	A	ABC		ABC	AB	ABC	ABC	ABC	C	ABC	ABC
HL-BT*	86.45	<0.0001	A	BC	BC	BC	BC	AB		BC	BC	BC	BC	BC	C	BC	BC
HL-FM*	85.29	<0.0001	BC	A	AB	BC	BC	BC		BC	BC	BC	A	BC	C	BC	BC
HL-HL	24.99	0.023	A	A	A	A	A	A		A	A	A	A	A	A	A	A
HL-PR	23.29	0.038	B	A	AB	AB	AB	AB		AB	AB	AB	AB	AB	AB	AB	B
HL-TO	19.12	NS															
HL-TM	30.98	0.003	A	A	A	A	A	A		A	A	A	A	A	A	A	A
LG-fCT	20.89	NS															
MV-BM	22.88	0.043	AB	AB	AB	AB	AB	AB		AB	AB	AB	AB	AB	A	B	AB
PR-AP	27.87	0.010	A	A	A	A	A	A		A	A	A	A	A	A	A	A
PR-BM*	66.68	<0.0001	AB	AB	AB	B	B	AB		AB	B	B	AB	AB	A	AB	AB
PR-BT*	56.84	<0.0001	A	BC	BC	BC	BC	AB		BC	BC	BC	BC	BC	C	BC	BC
PR-FM*	88.84	<0.0001	BC	A	AB	BC	BC	BC		BC	BC	BC	A	BC	C	BC	BC
PR-HL	12.41	NS															
PR-MV*	53.15	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
PR-PR*	45.97	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
PR-TO	17.51	NS															

Table I-8 (continued)

Variable	X^2	*p*-value	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
TO-AP	11.41	NS															
TO-BM*	33.78	0.001	AB	AB	AB	A	AB	AB		AB	AB	A	AB	AB	B	AB	AB
TO-GR	21.26	NS															
TO-HL	18.72	NS															
TO-PI	17.57	NS															
TM-AP	21.96	NS															
TM-HL	23.34	0.038	AB	AB	AB	A	AB	AB		AB	AB	A	AB	AB	B	AB	AB
VB-fCT	17.52	NS															
WT-HL*	41.76	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
fCT-AP*	46.45	<0.0001	AB	B	B	B	B	B		A	B	B	B	B	B	B	B
fCT-AW	30.58	0.004	A	A	A	A	A	A		A	A	A	A	A	A	A	A
fCT-BM*	64.42	<0.0001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
fCT-CN	17.84	NS															
fCT-HL*	41.24	<0.0001	AB	AB	AB	AB	AB	A		AB	AB	AB	AB	AB	B	AB	AB
fCT-MV*	33.26	0.001	A	A	A	A	A	A		A	A	A	A	A	A	A	A
fCT-PR*	37.21	0.0004	A	A	A	A	A	A		A	A	A	A	A	A	A	A

*Significant differences between species after Holm's test to correct for multiple comparisons.

Table I-9. Canonical correlations from web phase canonical discriminant analysis. Eigenvalues, proportion of variation in the model explained per eigenvalue and cumulative proportion of variation explained is displayed for each of the 14 canonical correlations.

	Canonical correlation	Eigenvalue	Proportion	Cumulative proportion
1	1.00	393.79	0.59	0.59
2	0.99	93.99	0.14	0.73
3	0.99	42.98	0.064	0.79
4	0.99	35.60	0.053	0.84
5	0.98	26.24	0.039	0.88
6	0.97	17.99	0.027	0.91
7	0.96	13.02	0.019	0.93
8	0.96	11.43	0.017	0.95
9	0.95	9.55	0.014	0.96
10	0.95	9.09	0.014	0.97
11	0.93	6.35	0.001	0.98
12	0.92	5.24	0.008	0.99
13	0.90	4.10	0.006	1.00
14	0.83	2.25	0.003	1.00

Table I-10. Total web phase canonical structure. Values shown are correlations between the original variables and the canonical variables. For each canonical variable, the original variables with loadings > 0.3 are shown. Abbreviations are the same as in Table I-8.

	Canonical variable					
	1st	2nd	3rd	4th	5th	6th
AW-WF	-0.57		0.47			
MV-WF	-0.39					
TR-WF	-0.62	0.45				
WF-AW	-0.56		0.47			
WF-fMV	-0.50					
WF-MV	-0.67	0.35				
WF-TR	-0.48					
WF-WF	-0.39					
fAP-WF	-0.37					
fMV-WF	-0.34					
fRT-WF	-0.39		0.35			
WF Proportion	-0.61					
WF Duration	-0.85					
AW-AW		0.40				
AW-CN		0.33				
CN-AW		0.31				
MV-AW		0.34				
MV-TR		-0.31	-0.57			
TR-MV		-0.41	-0.49			
fRT-AW		0.32				
AW Duration		0.34				
TR Duration		-0.32		0.49		0.38
AW-fRT			0.41			
fMV-AW			0.32			
TR Proportion			-0.31			
AW Proportion			0.37			
GR Proportion			0.32			
AW-TR				0.54		
TR-AW				0.57		
TR-fMV				0.42		
fMV-TR				0.32		
fRT-TR				0.45		
CN-fRT					0.32	
TR-BA					0.32	
MV-BA						0.35
TR-SW						0.36
JT Proportion						0.34
SW Proportion						0.34

Table I-11. Squared multiple correlations between web phase courtship variables and species grouping. Only variables with squared multiple correlations > 0.3 are shown.

Courtship variable	Squared multiple correlation
AW-AW	0.47
AW-fRT	0.46
AW-fBA	0.30
AW-TR	0.48
AW-WF	0.72
MV-TR	0.55
BA-TR	0.37
TR-AW	0.55
TR-fMV	0.41
TR-MV	0.57
TR-BA	0.44
TR-WF	0.71
WF-AW	0.74
WF-fMV	0.34
WF-MV	0.62
WF-TR	0.46
WF-WF	0.30
fMV-TR	0.30
fMV-WF	0.35
fRT-TR	0.37
fRT-WF	0.53
TR Proportion	0.32
AW Proportion	0.38
WF Proportion	0.66
BA Proportion	0.32
TR Duration	0.68
WF Duration	0.81

Table I-12. Canonical correlations from mount phase canonical discriminant analysis. Eigenvalues, proportion of variation in the model explained per eigenvalue and cumulative proportion of variation explained is displayed for each of the 14 canonical correlations.

	Canonical correlation	Eigenvalue	Proportion	Cumulative proportion
1	1.00	1171.48	0.79	0.79
2	1.00	131.03	0.088	0.88
3	0.99	76.21	0.051	0.93
4	0.99	38.47	0.026	0.95
5	0.98	20.66	0.014	0.97
6	0.97	18.31	0.012	0.98
7	0.97	14.29	0.001	0.98
8	0.93	6.18	0.004	0.99
9	0.89	3.74	0.003	0.99
10	0.87	3.01	0.002	1.00
11	0.81	1.92	0.001	1.00
12	0.81	1.89	0.001	1.00
13	0.69	0.89	0.006	1.00

Table I-13. Total mount phase canonical structure. Values shown are correlations between the original variables and the canonical variables. For each canonical variable, the original variables with loadings > 0.3 are shown. Abbreviations are the same as in Table I-8.

	Canonical variable		
	1st	2nd	3rd
FM-AW	0.45		
FM-HL	0.52		
HL-FM	0.31		
HL-PR	0.35		
PR-FM	0.72		
AP-BT		0.39	
BT-AP		0.44	
BT-HL		0.78	
BT-MV		0.55	
BT-PR		0.66	
BT-TO		0.56	
HL-BT		0.85	
PR-BT		0.68	
WT-fCT		0.32	
fCT-HL		0.32	
BT Proportion		0.66	
BM-BM			-0.31
HL-BM			-0.45
PR-AP			0.30
PR-MV			0.31
PR-BM			0.37
BM-HL			-0.41
fCT-AP			-0.34
WU Count			0.41
HL Proportion			-0.35
BM Proportion			-0.37

Table I-14. Squared multiple correlations between mount phase courtship variables and species grouping. Only variables with squared multiple correlations > 0.3 are shown.

Courtship variable	Squared multiple correlation
AP-FM	0.66
AP-HL	0.30
AP-BM	0.31
AP-TM	0.30
BT-HL	0.66
BT-MV	0.45
BT-PR	0.64
BT-TO	0.34
FM-AP	0.49
FM-HL	0.77
FM-TO	0.36
HL-BT	0.81
HL-FM	0.72
HL-BM	0.56
PR-BT	0.52
PR-FM	0.74
PR-MV	0.33
PR-PR	0.35
PR-BM	0.52
BM-AP	0.38
BM-GR	0.33
BM-HL	0.44
BM-BM	0.64
BM-TO	0.33
TO-BM	0.32
fCT-AP	0.34
fCT-HL	0.34
fCT-MV	0.36
fCT-BM	0.59
fWU Count	0.36
FM Proportion	0.61
HL Proportion	0.54
AP Proportion	0.36
BM Proportion	0.41
BT Proportion	0.51

Table I-15. Variables chosen by stepwise selection for each discriminant analysis. Only variables selected for at least one discriminant analysis are shown. Abbreviations for behavior patterns are the same as in Tables 4 and 6, except TRB which is tremulation with bobbing.

Variable	Full Trial[A]	Mount Phase[A]	Web Phase[A]	Web Phase[B]	Web Phase[C]	Web Phase[D]
Web Phase						
AW Duration						X
BA Duration	X			X	X	X
MV Duration			X	X	X	X
RE Duration	X				X	X
TR Duration	X		X	X	X	X
WF Duration	X		X	X	X	X
AP Proportion	X					
AW Proportion				X	X	
BA Proportion	X					
CH Proportion					X	
CN Proportion						X
JT Proportion	X		X	X	X	
MV Proportion			X	X	X	X
PP Proportion	X					
RE Proportion						X
SW Proportion					X	X
TR Proportion			X	X	X	X
WF Proportion	X		X	X	X	X
WT Proportion						X
TRB	X		X	X	X	X
AW-AW				X	X	X
AW-BW				X	X	X
AW-GR				X		
AW-MV				X		
AW-PP	X		X	X	X	X
AW-WF	X		X	X	X	X
BA-AW	X			X	X	X
BA-BA	X			X	X	X
BA-MV	X		X	X		X
BA-TR	X			X	X	
BW-AW				X	X	X
BW-MV					X	X
CN-AW				X		
CN-MV				X	X	X
CN-TR	X		X	X	X	X
DM-MV			X			
GR-MV	X					
JT-TR	X			X	X	X
MV-AW					X	
MV-BA	X		X	X	X	X
MV-BW					X	X
MV-MV				X	X	
MV-TR					X	X
MV-WF	X		X	X	X	X

Table I-15 (continued)

Variable	Full Trial[A]	Mount Phase[A]	Web Phase[A]	Web Phase[B]	Web Phase[C]	Web Phase[D]
RT-WF			X	X	X	X
TR-AW	X		X		X	X
TR -BA	X		X	X	X	X
TR -CN	X					
TR -DM				X		
TR -MV	X		X	X	X	X
TR -SW				X	X	X
TR - TR	X		X	X	X	X
TR -WF	X		X	X	X	X
WF-AW	X		X	X	X	X
WF-CN	X		X		X	X
WF-MV			X	X	X	X
WF-TR	X		X	X	X	X
WF-WF			X	X	X	
latency	X		X			
Mount Phase						
AP Proportion		X				
BM Proportion	X					
FM Proportion	X					
GR Proportion		X				
HL Proportion	X	X				
TM Proportion	X	X				
AP-AP	X					
AP-BT		X				
AP-FM		X				
AP-HL		X				
AP-PR		X				
AP-BM	X	X				
AP-TO		X				
AP-TM	X	X				
BM-AW	X	X				
BM-GR	X	X				
BM-TM		X				
BT-AP		X				
BT-MV	X	X				
BT-PR	X	X				
BT-TO		X				
FM-AP	X	X				
FM-AW	X					
FM-HL	X	X				
HL-AP	X	X				
HL-BT	X	X				
HL-FM	X	X				
HL-HL		X				
HL-BM	X	X				
HL-TM	X	X				
MV-BM	X	X				
PR-AP		X				

Table I-15 (continued)

Variable	Full Trial[A]	Mount Phase[A]	Web Phase[A]	Web Phase[B]	Web Phase[C]	Web Phase[D]
PR-BT	X					
PR-BM		X				
PR-TO		X				
TO-AP		X				
TO-BM	X					
Resumption of Web Phase	X	X				

[A] Trials with both courtship phases, all species except *A. oregonensis* and *A. oklahoma*.
[B] Trials with web phase courtship, all species except *A. oregonensis* and *A. oklahoma*.
[C] Trials with web phase courtship, all species.
[D] Trials with web phase courtship, all species, four of which were broken up into populations.

Table I-16. Cross-validation classification results for species-level discriminant analyses. Number shown is percent of all trials for each species classified into each species. Correct classifications are in bold. A dash indicates zero percent.

	Predicted group															
Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*	Other/Ties
A. actuosa																
Full trial[A]	**100.0**	-	-	-	-	-	na	na	-	-	-	-	-	-	-	-
Mount Phase[A]	**100.0**	-	-	-	-	-	na	na	-	-	-	-	-	-	-	-
Web Phase[A]	**-**	-	-	-	25.0	-	na	na	-	-	-	-	75.0	-	-	-
Web Phase[B]	**50.0**	-	-	-	12.5	12.5	na	na	-	-	-	-	25.0	-	-	-
Web Phase[C]	**37.5**	-	-	-	12.5	25.0	-	-	-	-	-	-	25.0	-	-	-
A. aleenae																
Full trial[A]	-	**100.0**	-	-	-	-	na	na	-	-	-	-	-	-	-	-
Mount Phase[A]	-	**100.0**	-	-	-	-	na	na	-	-	-	-	-	-	-	-
Web Phase[A]	-	**100.0**	-	-	-	-	na	na	-	-	-	-	-	-	-	-
Web Phase[B]	-	**84.3**	-	-	-	-	na	na	-	-	15.4	-	-	-	-	-
Web Phase[C]	-	**100.0**	-	-	-	-	7.7	-	-	-	-	-	-	-	-	-
A. aperta																
Full trial[A]	-	-	**100.0**	-	-	-	na	na	-	-	-	-	-	-	-	-
Mount Phase[A]	-	12.5	**62.5**	-	-	-	na	na	-	-	12.5	-	12.5	-	-	-
Web Phase[A]	-	-	**100.0**	-	-	-	na	na	-	-	-	-	-	-	-	-
Web Phase[B]	-	-	**88.9**	-	-	-	na	na	-	-	-	-	-	-	-	11.1
Web Phase[C]	-	-	**88.9**	-	-	-	-	-	-	-	-	-	-	-	-	11.1

Table I-16 (continued)

Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*	Other/Ties
	Predicted group															
A. emertoni																
Full trial[A]	-	-	-	**100.0**	-	-	na	na	-	-	-	-	-	-	-	-
Mount Phase[A]	-	-	-	**30.8**	30.8	-	na	na	23.1	7.7	-	-	-	-	-	-
Web Phase[A]	-	-	-	**92.3**	-	-	na	na	-	-	-	-	-	-	-	7.7
Web Phase[B]	-	-	-	**85.7**	-	-	na	na	14.3	-	-	-	-	-	-	-
Web Phase[C]	-	-	-	**90.5**	-	-	-	-	4.8	4.8	-	-	-	-	-	-
A. kastoni																
Full trial[A]	-	-	-	-	**100.0**	-	na	na	-	-	-	-	-	-	-	-
Mount Phase[A]	-	-	-	-	**85.7**	-	na	na	-	14.3	-	-	-	-	-	-
Web Phase[A]	-	-	-	-	**100.0**	-	na	na	-	-	-	-	-	-	-	-
Web Phase[B]	-	-	-	-	**91.7**	-	na	na	-	-	-	-	-	-	-	8.3
Web Phase[C]	8.3	-	-	-	**91.7**	-	-	-	-	-	-	-	-	-	-	-
A. naevia																
Full trial[A]	-	-	-	-	-	**66.6**	na	na	-	-	-	-	33.3	-	-	-
Mount Phase[A]	-	-	-	-	-	**77.8**	na	na	-	-	-	-	11.1	11.1	-	-
Web Phase[A]	11.1	-	-	-	-	**55.6**	na	na	-	-	-	-	33.3	-	-	-
Web Phase[B]	18.2	-	-	-	-	**45.5**	na	na	-	-	-	-	27.3	9.1	-	-
Web Phase[C]	18.2	-	-	-	-	**36.4**	-	9.1	-	-	-	-	36.4	9.1	-	-
A. oklahoma																
Web Phase[C]	-	-	-	-	-	-	**100.0**	-	-	-	-	-	-	-	-	-
A. oregonensis																
Web Phase[C]	-	-	-	-	-	33.3	-	**-**	-	-	-	-	33.3	-	33.3	-

Table 1-16 (continued)

Actual group	Predicted group															
	A. actuosa	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*	Other/Ties
A. pennsylvanica																
Full trial[A]	-	-	-	-	-	-	na	na	**100.0**	-	-	-	-	-	-	-
Mount Phase[A]	-	-	-	16.7	16.7	-	na	na	**41.7**	16.7	-	-	8.3	-	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	**58.3**	41.7	-	-	-	-	-	-
Web Phase[B]	-	-	-	-	-	-	na	na	**41.2**	58.8	-	-	-	-	-	-
Web Phase[C]	-	-	-	5.9	-	-	-	-	**35.3**	58.8	-	-	-	-	-	-
A. potteri																
Full trial[A]	-	-	-	-	-	-	na	na	20.0	**80.0**	-	-	-	-	-	-
Mount Phase[A]	-	-	-	40.0	20.0	-	na	na	20.0	**20.0**	-	-	-	-	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	100.0	**-**	-	-	-	-	-	-
Web Phase[B]	-	-	-	-	-	-	na	na	62.5	**37.5**	-	-	-	-	-	-
Web Phase[C]	-	-	-	-	-	-	-	-	50.0	**50.0**	-	-	-	-	-	-
A. spatula																
Full trial[A]	-	-	-	-	-	-	na	na	-	-	**100.0**	-	-	-	-	-
Mount Phase[A]	-	-	-	-	-	-	na	na	-	-	**100.0**	-	-	-	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	**100.0**	-	-	-	-	-
Web Phase[B]	-	12.5	12.5	-	-	12.5	na	na	-	-	**50.0**	-	-	12.5	-	-
Web Phase[C]	-	37.5	12.5	-	-	12.5	-	-	-	-	**25.0**	-	-	12.5	-	-
A. utahana																
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	**100.0**	-	-	-	-
Mount Phase[A]	-	-	-	11.1	-	-	na	na	-	-	-	**88.9**	-	-	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	-	**88.9**	-	-	11.1	-
Web Phase[B]	-	-	-	-	-	6.7	na	na	-	-	-	**73.3**	-	-	20.0	-
Web Phase[C]	-	-	-	-	-	-	-	6.7	-	-	-	**66.7**	-	-	26.7	-

Table I-16 (continued)

	Predicted group															
Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*	Other/Ties
B. texana																
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	-	**100.0**	-	-	-
Mount Phase[A]	-	-	-	-	-	27.3	na	na	-	-	-	-	**72.7**	-	-	-
Web Phase[A]	14.6	-	-	-	-	18.2	na	na	-	-	-	-	**77.3**	-	-	-
Web Phase[B]	8.0	-	-	-	4.0	24.0	na	na	-	-	-	-	**64.0**	-	-	-
Web Phase[C]	24.0	-	-	-	-	28.0	-	4.0	-	-	-	-	**44.0**	-	-	-
C. californica																
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	-	-	**100.0**	-	-
Mount Phase[A]	-	-	-	-	-	33.3	na	na	-	-	-	-	33.3	**33.3**	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	-	-	33.3	**66.7**	-	-
Web Phase[B]	16.7	-	-	-	-	-	na	na	-	-	-	-	-	**50.0**	16.7	-
Web Phase[C]	-	-	-	-	-	16.7	-	33.3	-	-	-	-	-	**50.0**	-	-
H. nedra																
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	-	-	-	**100.0**	-
Mount Phase[A]	-	-	-	-	-	-	na	na	-	-	-	-	-	33.3	**66.7**	-
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	-	-	-	-	**100.0**	-
Web Phase[B]	-	-	-	-	-	-	na	na	-	-	-	-	-	-	**100.0**	-
Web Phase[C]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**100.0**	-

[A] Trials exhibiting both courtship phases, all species except *A. oregonensis* and *A. oklahoma*.
[B] Trials exhibiting at least web phase courtship, all species except *A. oregonensis* and *A. oklahoma*.
[C] Trials exhibiting at least web phase courtship, all species.

Table I-17. Mean posterior probabilities for species-level discriminant analyses. Number shown is the mean posterior probability for group membership from the jackknife cross-validation. Posterior probabilities for the correct group are in bold. A dash indicates a probability of zero.

	Predicted group														
Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
A. actuosa															
Full trial[A]	1.00	-	-	-	-	-	na	na	-	-	-	-	-	-	-
Mount Phase[A]	0.96	-	-	-	-	0.04	na	na	-	-	-	-	-	-	-
Web Phase[A]	-	-	-	-	0.19	-	na	na	-	-	-	-	0.81	-	-
Web Phase[B]	0.33	-	-	-	0.17	0.22	na	na	-	-	-	-	0.28	-	-
Web Phase[C]	0.27	-	-	-	0.08	0.26	-	-	-	-	-	-	0.38	-	-
A. aleenae															
Full trial[A]	-	1.00	-	-	-	-	na	na	-	-	-	-	-	-	-
Mount Phase[A]	-	0.77	0.23	-	-	-	na	na	-	-	-	-	-	-	-
Web Phase[A]	-	1.00	-	-	-	-	na	na	-	-	-	-	-	-	-
Web Phase[B]	-	0.91	-	-	-	-	na	na	-	-	0.09	-	-	-	-
Web Phase[C]	-	0.95	-	-	-	-	0.05	-	-	-	-	-	-	-	-
A. aperta															
Full trial[A]	-	-	1.00	-	-	-	na	na	-	-	-	-	-	-	-
Mount Phase[A]	-	0.16	0.61	-	-	-	na	na	-	-	0.11	-	0.13	-	-
Web Phase[A]	-	-	1.00	-	-	-	na	na	-	-	-	-	-	-	-
Web Phase[B]	-	-	0.92	-	-	-	na	na	-	-	0.06	-	0.03	-	-
Web Phase[C]	-	-	0.89	-	-	-	-	-	-	-	0.06	-	0.05	-	-

Table I-17 (continued)

Actual group	Predicted group														
	A. actuosa	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
A. emertoni															
Full trial[A]	-	-	-	1.00	-	-	na	na	-	-	-	-	-	-	-
Mount Phase[A]	-	-	-	0.39	0.17	-	na	na	0.29	0.04	-	-	0.08	-	-
Web Phase[A]	-	-	-	0.96	-	-	na	na	0.04	-	-	-	-	-	-
Web Phase[B]	-	-	-	0.90	-	-	na	na	0.10	-	-	-	-	-	-
Web Phase[C]	-	-	-	0.93	-	-	-	-	0.04	0.03	-	-	-	-	-
A. kastoni															
Full trial[A]	-	-	-	-	1.00	-	na	na	-	-	-	-	-	-	-
Mount Phase[A]	-	-	-	0.10	0.69	-	na	na	0.11	0.10	-	-	-	-	-
Web Phase[A]	0.09	-	-	-	0.97	-	na	na	-	-	-	-	-	-	-
Web Phase[B]	-	-	-	-	0.93	0.04	na	na	-	-	-	-	0.03	-	-
Web Phase[C]	0.05	-	-	-	0.95	-	-	-	-	-	-	-	-	-	-
A. naevia															
Full trial[A]	-	-	-	-	-	0.52	na	na	-	-	-	-	0.48	-	-
Mount Phase[A]	-	-	-	-	-	0.54	na	na	-	-	-	-	0.37	0.09	-
Web Phase[A]	0.09	-	-	-	-	0.47	na	na	-	-	-	-	0.44	-	-
Web Phase[B]	0.14	-	-	-	-	0.39	na	na	-	-	-	-	0.38	0.06	-
Web Phase[C]	0.14	-	-	-	0.04	0.24	-	-	-	-	-	-	0.50	0.07	-
A. oklahoma															
Web Phase[C]	0.05	-	-	-	-	-	**0.95**	-	-	-	-	-	-	-	-
A. oregonensis															
Web Phase[C]	-	-	-	-	-	0.23	-	**-**	-	-	-	-	0.45	-	0.33

Table 1-17 (continued)

Actual group	Predicted group														
	A. actuosa	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
A. pennsylvanica															
Full trial[A]	-	-	-	-	-	-	na	na	1.00	-	-	-	-	-	-
Mount Phase[A]	-	-	-	0.17	0.24	-	na	na	0.36	0.12	-	-	0.04	-	-
Web Phase[A]	-	-	-	0.10	-	-	na	na	0.61	0.29	-	-	-	-	-
Web Phase[B]	-	-	-	0.08	-	-	na	na	0.45	0.47	-	-	-	-	-
Web Phase[C]	-	-	-	0.10	-	-	-	-	0.46	0.43	-	-	-	-	-
A. potteri															
Full trial[A]	-	-	-	-	-	-	na	na	0.23	0.77	-	-	-	-	-
Mount Phase[A]	-	-	-	0.26	0.26	-	na	na	0.28	0.20	-	-	-	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	1.00	-	-	-	-	-	-
Web Phase[B]	-	-	-	0.06	-	-	na	na	0.68	0.27	-	-	-	-	-
Web Phase[C]	-	-	-	0.06	-	-	-	-	0.55	0.39	-	-	-	-	-
A. spatula															
Full trial[A]	-	0.33	-	-	-	-	na	na	-	-	0.67	-	-	-	-
Mount Phase[A]	-	0.15	0.19	-	-	-	na	na	-	-	0.83	-	-	-	-
Web Phase[A]	-	-	0.11	-	-	-	na	na	-	-	0.89	-	-	-	-
Web Phase[B]	-	-	0.21	0.13	-	0.14	na	na	-	-	0.36	-	0.04	0.09	-
Web Phase[C]	-	0.42	0.18	-	-	0.09	-	-	-	-	0.15	0.04	0.04	0.09	-
A. utahana															
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	1.00	-	-	-
Mount Phase[A]	-	-	-	0.08	0.05	-	na	na	0.03	-	-	0.84	-	-	-
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	-	0.92	-	-	0.08
Web Phase[B]	-	-	-	-	-	0.04	na	na	-	-	-	0.80	-	-	0.16
Web Phase[C]	-	-	-	-	-	0.03	-	-	-	-	-	0.73	-	-	0.21

Table I-17 (continued)

	Predicted group														
Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia*	*A. oklahoma*	*A. oregonensis*	*A. pennsylvanica*	*A. potteri*	*A. spatula*	*A. utahana*	*B. texana*	*C. californica*	*H. nedra*
B. texana															
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	-	1.00	-	-
Mount Phase[A]	-	-	-	-	-	0.15	na	na	0.02	-	-	-	0.83	-	-
Web Phase[A]	0.07	-	-	-	-	0.15	na	na	-	-	-	-	0.78	-	-
Web Phase[B]	0.06	-	-	-	0.03	0.16	na	na	-	-	-	-	0.75	-	-
Web Phase[C]	0.19	-	-	-	-	0.18	-	0.04	-	-	-	-	0.59	-	-
C. californica															
Full trial[A]	-	-	-	-	-	0.07	na	na	-	-	-	-	0.01	0.92	-
Mount Phase[A]	-	-	-	-	-	0.22	na	na	-	-	-	-	0.48	0.30	-
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	-	-	0.03	0.97	-
Web Phase[B]	0.10	-	-	-	0.08	0.21	na	na	-	-	-	-	-	0.45	0.12
Web Phase[C]	-	-	-	-	0.08	0.09	-	0.22	-	-	-	-	-	0.50	0.11
H. nedra															
Full trial[A]	-	-	-	-	-	-	na	na	-	-	-	0.10	-	-	0.90
Mount Phase[A]	-	-	-	-	-	-	na	na	0.09	-	-	-	0.03	0.32	0.56
Web Phase[A]	-	-	-	-	-	-	na	na	-	-	-	-	-	-	1.00
Web Phase[B]	-	-	-	-	-	-	na	na	-	-	-	0.08	-	-	0.92
Web Phase[C]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00

[A] Trials exhibiting both courtship phases, all species except *A. oregonensis* and *A. oklahoma*.
[B] Trials exhibiting at least web phase courtship, all species except *A. oregonensis* and *A. oklahoma*.
[C] Trials exhibiting at least web phase courtship, all species.

Table I-18. Cross-validation classification results for population-level discriminant analyses. Number shown is percent of all trials for each species classified into each species. Correct classifications are in bold. A dash indicates zero percent.

	Predicted group													
Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia* AR	*A. naevia* KS	*A. oklahoma* KS	*A. oklahoma* TX	*A. oregonensis*	*A. pennsylvanica* EKS	*A. pennsylvanica* WKS	*A. pennsylvanica* MO	*A. potteri*
A. actuosa	**25.0**	-	-	-	12.5	12.5	12.5	-	-	-	-	-	-	-
A. aleenae	-	**92.3**	-	-	-	-	-	-	7.7	-	-	-	-	-
A. aperta	-	-	**88.9**	-	-	-	-	-	-	-	-	-	-	-
A. emertoni	-	-	-	**90.5**	-	-	-	-	-	-	-	-	4.8	4.8
A. kastoni	-	-	-	-	**100.0**	-	-	-	-	-	-	-	-	-
A. naevia AR	-	-	-	-	-	**40.0**	40.0	-	-	-	-	-	-	-
A. naevia KS	16.7	-	-	-	-	-	**16.7**	-	-	-	-	-	-	-
A. oklahoma KS	-	-	-	-	-	-	-	**100.0**	-	-	-	-	-	-
A. oklahoma TX	-	33.3	-	-	-	-	-	-	**33.3**	-	-	-	-	-
A. oregonensis	-	-	-	-	-	-	33.3	-	-	**33.3**	-	-	-	-
A. pennsylvanica EKS	-	-	-	16.7	-	-	-	-	-	-	**16.7**	-	16.7	50.0
A. pennsylvanica WKS	-	-	-	-	-	-	-	-	-	-	-	**60.0**	-	20.0
A. pennsylvanica MO	-	-	-	16.7	-	-	-	-	-	-	16.7	-	**50.0**	33.3
A. potteri	-	-	-	-	-	-	-	-	-	-	25.0	37.5	12.5	**12.5**
A. spatula	-	38	12.5	-	-	25.0	-	-	-	-	-	-	-	-
A. utahana UT	-	-	-	-	-	-	-	-	-	16.7	-	-	-	-
A. utahana WA	-	-	-	-	-	-	11.1	-	-	55.6	-	-	-	-
B. texana	12.0	-	-	-	-	8.0	24.0	-	-	-	-	-	-	-
C. californica	-	-	-	-	-	-	-	-	-	16.7	-	-	-	-
H. nedra	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table I-18 (continued)

	Predicted Group						
Actual group	*A. spatula*	*A. utahana* UT	*A. utahana* WA	*B. texana*	*C. californica*	*H. nedra*	Other/Ties
A. actuosa	-	-	-	37.5	-	-	-
A. aleenae	-	-	-	-	-	-	-
A. aperta	-	-	-	11.1	-	-	-
A. emertoni	-	-	-	-	-	-	-
A. kastoni	-	-	-	-	-	-	-
A. naevia AR	-	-	-	20.0	-	-	-
A. naevia KS	-	-	-	50.0	16.7	-	-
A. oklahoma KS	-	-	-	-	-	-	-
A. oklahoma TX	-	-	-	-	-	-	33.3
A. oregonensis	-	-	33.3	-	-	-	-
A. pennsylvanica EKS	-	-	-	-	-	-	16.7
A. pennsylvanica WKS	-	-	-	-	-	-	20.0
A. pennsylvanica MO	-	-	-	-	-	-	-
A. potteri	-	-	-	-	-	-	-
A. spatula	**12.5**	-	-	-	-	-	12.5
A. utahana UT	-	**83.3**	-	-	-	-	-
A. utahana WA	-	-	**11.1**	-	-	11.1	11.1
B. texana	-	-	-	**56.0**	-	-	-
C. californica	-	-	-	-	**66.7**	16.7	-
H. nedra	-	-	-	-	-	**100.0**	-

Table I-19. Mean posterior probabilities for population-level discriminant analyses. Number shown is the mean posterior probability for group membership from the jackknife cross-validation. Posterior probabilities for the correct group are in bold. A dash indicates a probability of zero.

	Predicted group													
Actual group	*A. actuosa*	*A. aleenae*	*A. aperta*	*A. emertoni*	*A. kastoni*	*A. naevia* AR	*A. naevia* KS	*A. oklahoma* KS	*A. oklahoma* TX	*A. oregonensis*	*A. pennsylvanica* EKS	*A. pennsylvanica* WKS	*A. pennsylvanica* MO	*A. potteri*
A. actuosa	**0.19**	-	-	-	0.14	0.09	0.07	-	-	-	-	-	-	-
A. aleenae	-	**0.95**	-	-	-	-	-	-	0.05	-	-	-	-	-
A. aperta	-	-	**0.84**	-	-	-	0.05	-	-	-	-	-	-	-
A. emertoni	-	-	-	**0.92**	-	-	-	-	-	-	0.02	-	0.03	0.03
A. kastoni	0.03	-	-	-	**0.93**	-	-	-	-	-	-	-	-	-
A. naevia AR	-	-	-	-	0.04	**0.26**	0.27	-	-	-	-	-	-	-
A. naevia KS	0.10	-	-	-	-	-	**0.18**	-	-	-	-	-	-	-
A. oklahoma KS	-	-	-	-	-	-	-	**0.83**	0.17	-	-	-	-	-
A. oklahoma TX	-	0.33	-	-	-	-	-	0.23	**0.43**	-	-	-	-	-
A. oregonensis	-	-	-	-	-	-	0.30	-	-	**0.24**	-	-	-	-
A. pennsylvanica EKS	-	-	-	0.06	-	-	-	-	-	-	**0.19**	0.14	0.24	0.37
A. pennsylvanica WKS	-	-	-	0.02	-	-	-	-	-	-	0.29	**0.36**	0.07	0.26
A. pennsylvanica MO	-	-	-	0.17	-	-	-	-	-	-	0.16	0.07	**0.25**	0.34
A. potteri	-	-	-	-	-	-	-	-	-	-	0.27	0.15	0.26	**0.31**
A. spatula	-	0.27	0.30	-	-	0.17	-	-	-	-	-	-	-	-
A. utahana UT	-	-	-	-	-	-	-	-	-	0.09	-	-	-	-
A. utahana WA	-	-	-	-	-	-	0.08	-	-	0.35	-	-	-	-
B. texana	0.12	-	-	-	-	0.07	0.19	-	-	-	-	-	-	-
C. californica	0.06	-	-	-	0.13	-	0.16	-	-	0.08	-	-	-	-
H. nedra	-	-	-	-	-	-	-	-	-	0.08	-	-	-	-

Table I-19 (continued)

	Predicted Group					
Actual group	*A. spatula*	*A. utahana* UT	*A. utahana* WA	*B. texana*	*C. californica*	*H. nedra*
A. actuosa	-	-	-	0.51	-	-
A. aleenae	-	-	-	-	-	-
A. aperta	-	-	-	0.11	-	-
A. emertoni	-	-	-	-	-	-
A. kastoni	-	-	-	0.39	0.10	0.10
A. naevia AR	0.05	-	-	0.38	-	-
A. naevia KS	-	-	-	0.61	0.10	-
A. oklahoma KS	-	-	-	-	-	-
A. oklahoma TX	-	-	-	-	-	-
A. oregonensis	-	-	0.19	0.13	-	0.14
A. pennsylvanica EKS	-	-	-	-	-	-
A. pennsylvanica WKS	-	-	-	-	-	-
A. pennsylvanica MO	-	-	-	-	-	-
A. potteri	-	-	-	-	-	-
A. spatula	**0.15**	-	0.04	0.01	0.06	-
A. utahana UT	-	**0.88**	0.03	-	-	-
A. utahana WA	-	0.03	**0.21**	0.04	-	0.30
B. texana	-	-	-	**0.62**	-	-
C. californica	-	-	-	-	**0.35**	0.23
H. nedra	-	-	-	-	-	**0.92**

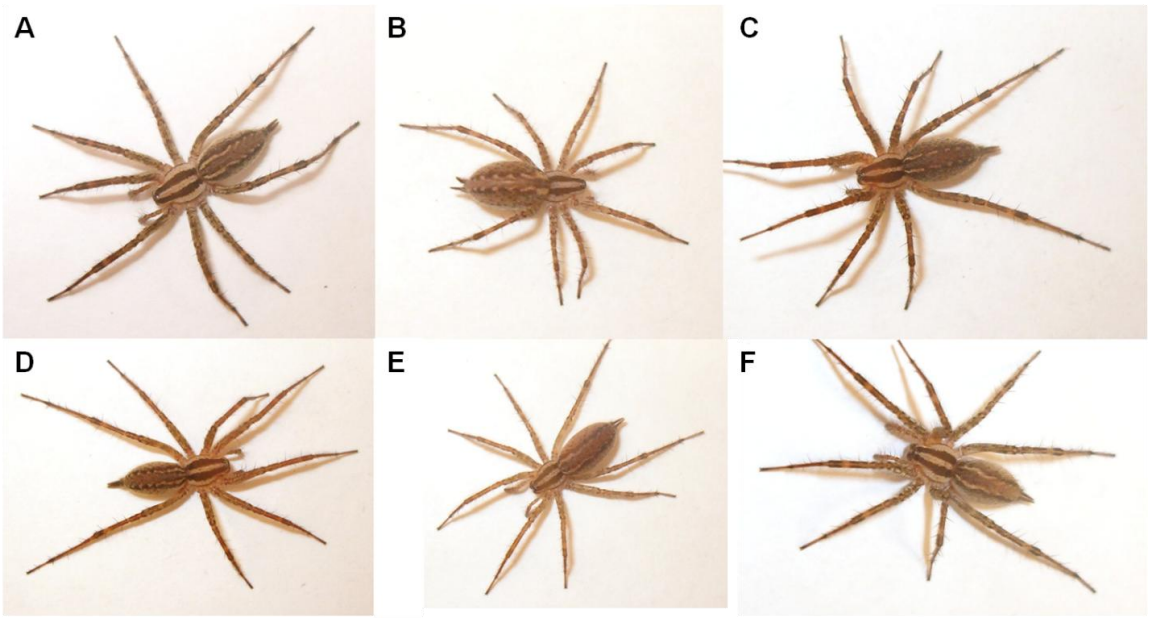
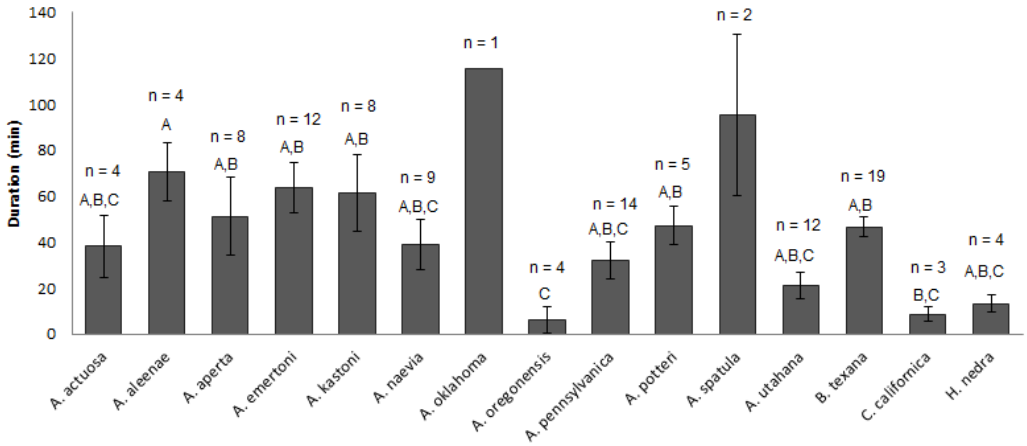


Figure I-1. Photographs of six *Agelenopsis* species including (A) *A. aleenae*, (B) *A. aperta*, (C) *A. emertoni*, (D) *A. pennsylvanica*, (E) *A. potteri* and (F) *A. spatula*. Photographs are not on identical scales, but these species do overlap in body size. Photographs taken by Jason R. Jones.

(A)



(B)

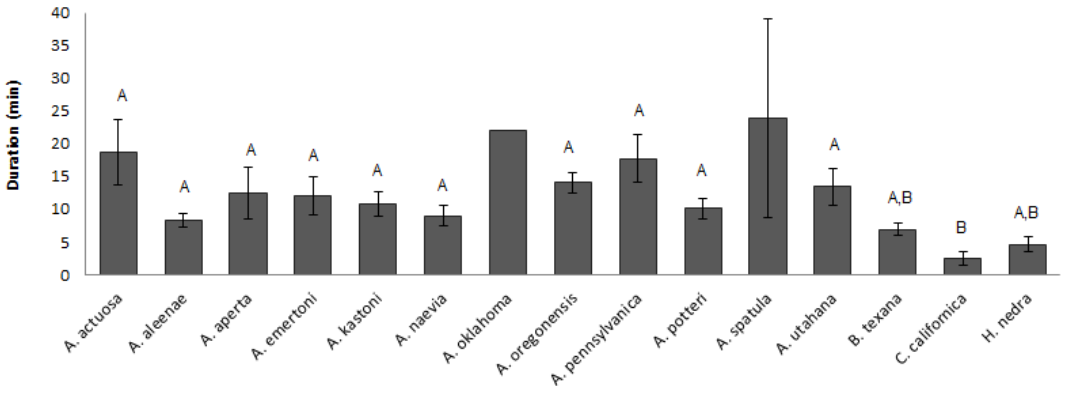
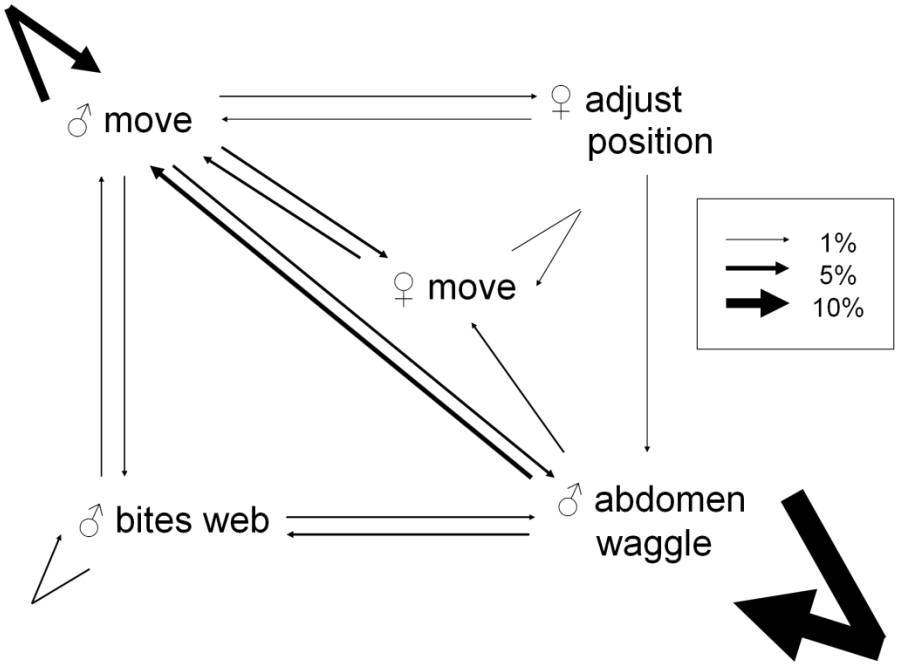


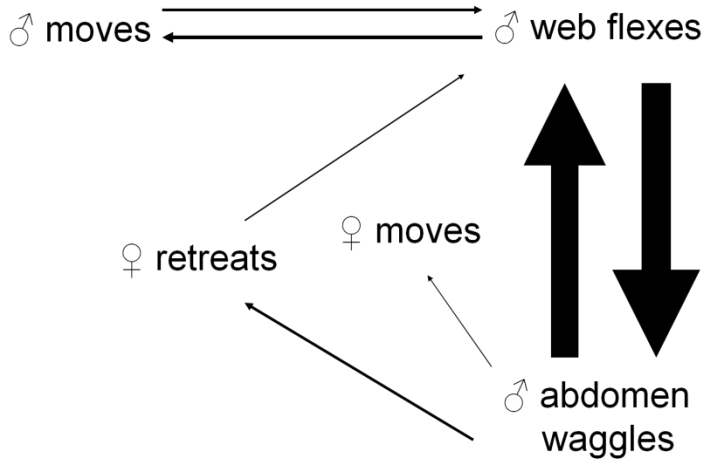
Figure I-2. Mean durations in minutes of (A) web and (B) mount phase for successful courtships, by species. Sample sizes for mount phase are the same as those shown for web phase.

Figure I-3. Kinematic diagrams showing common behavioral transitions in courtship on the web, while the male is not mounted on the female. The width of the arrow reflects the relative frequency of the transition. Transition frequencies were averaged across all trials in which courtship occurred for the respective species: (A) *A. actuosa*, (B) *A. aleenae*, (C) *A. aperta*, (D) *A. emertoni*, (E) *A. kastoni*, (F) *A. naevia*, (G) *A. oklahoma*, (H) *A. oregonensis*, (I) *A. pennsylvanica*, (J) *A. potteri*, (K) *A. spatula*, (L) *A. utahana*, (M) *B. texana*, (N) *C. californica*, (O) *H. nedra*.

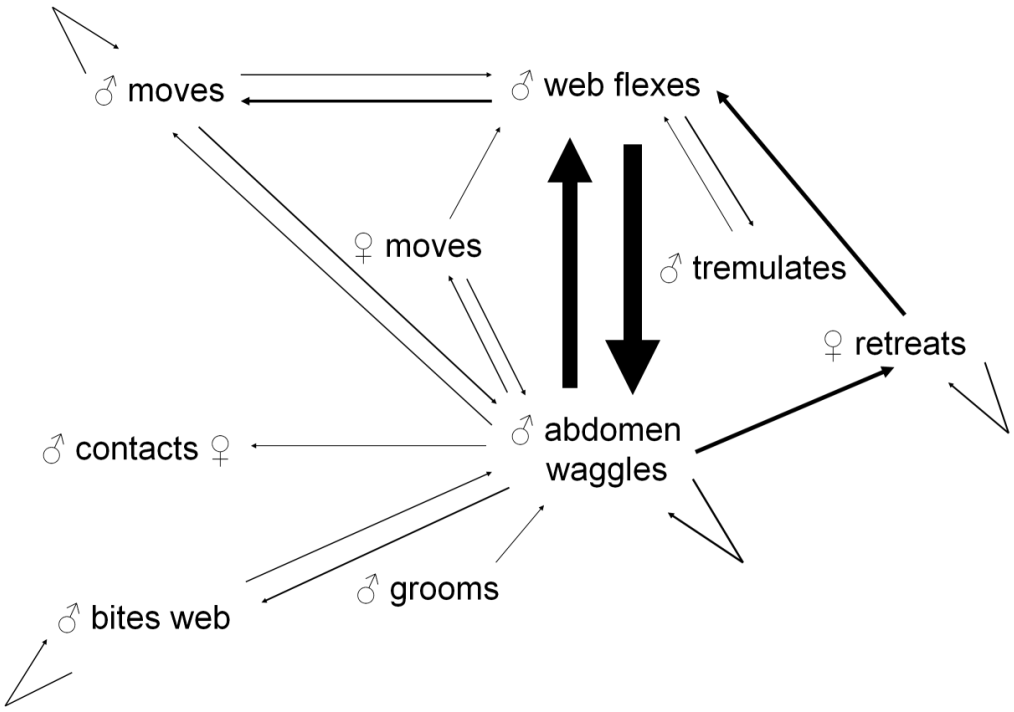
(A)


♂ move
♀ adjust position
♀ move
1%
5%
10%
♂ bites web
♂ abdomen waggle

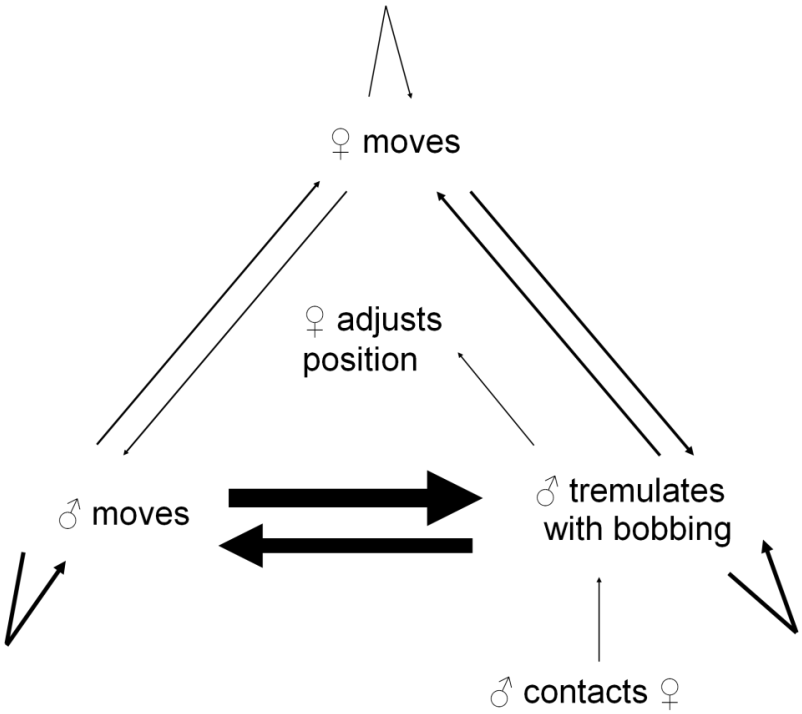
(B)


♂ moves
♂ web flexes
♀ retreats
♀ moves
♂ abdomen waggles

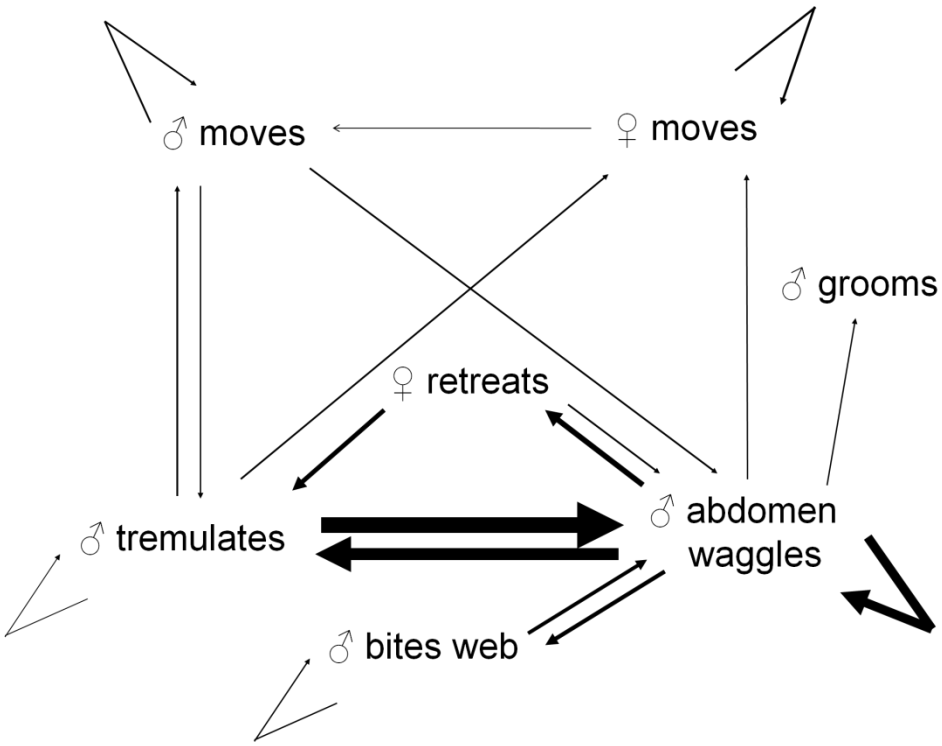
(C)


♂ moves
♂ web flexes
♀ moves
♂ tremulates
♀ retreats
♂ contacts ♀
♂ abdomen waggles
♂ grooms
♂ bites web

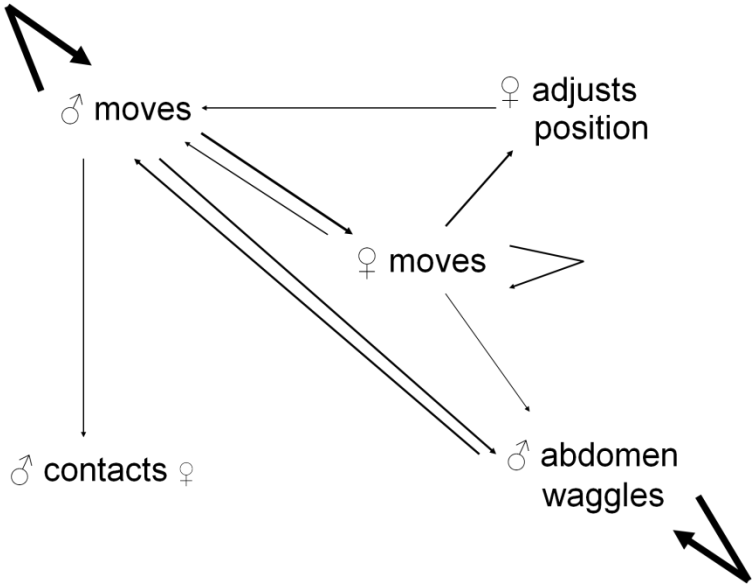
(D)


♀ moves
♀ adjusts position
♂ moves
♂ tremulates with bobbing
♂ contacts ♀

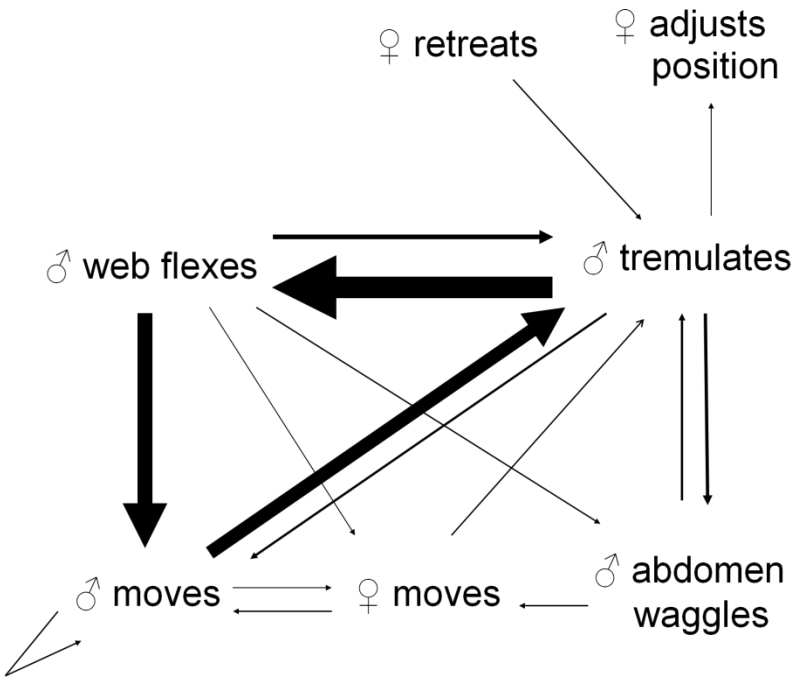
(E)


♂ moves
♀ moves
♂ grooms
♀ retreats
♂ tremulates
♂ abdomen waggles
♂ bites web

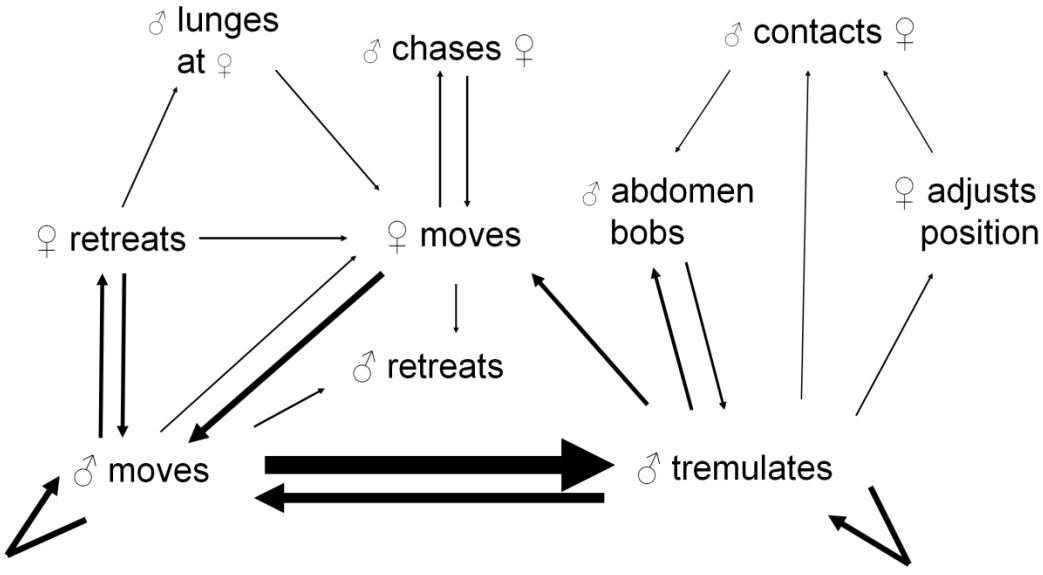
(F)


♂ moves
♀ adjusts position
♀ moves
♂ contacts ♀
♂ abdomen waggles

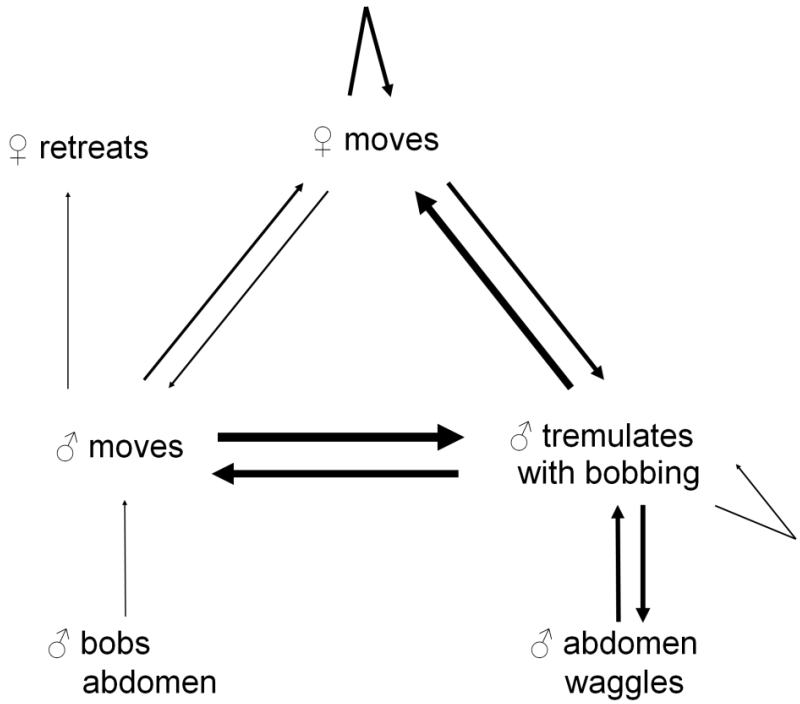
(G)


♀ retreats
♀ adjusts position
♂ web flexes
♂ tremulates
♂ moves
♀ moves
♂ abdomen waggles

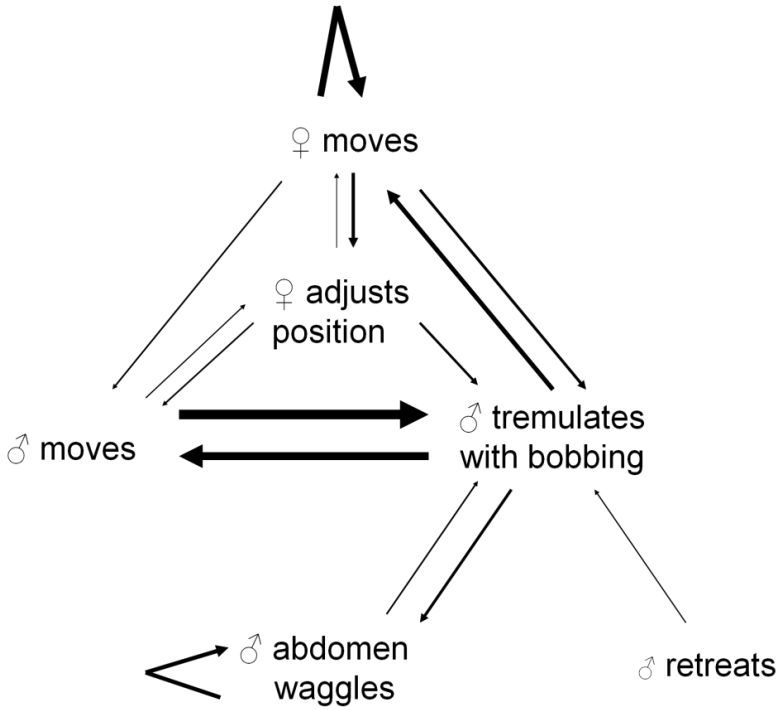
(H)


♂ lunges at ♀
♂ chases ♀
♂ contacts ♀
♀ retreats
♀ moves
♂ abdomen bobs
♀ adjusts position
♂ retreats
♂ moves
♂ tremulates

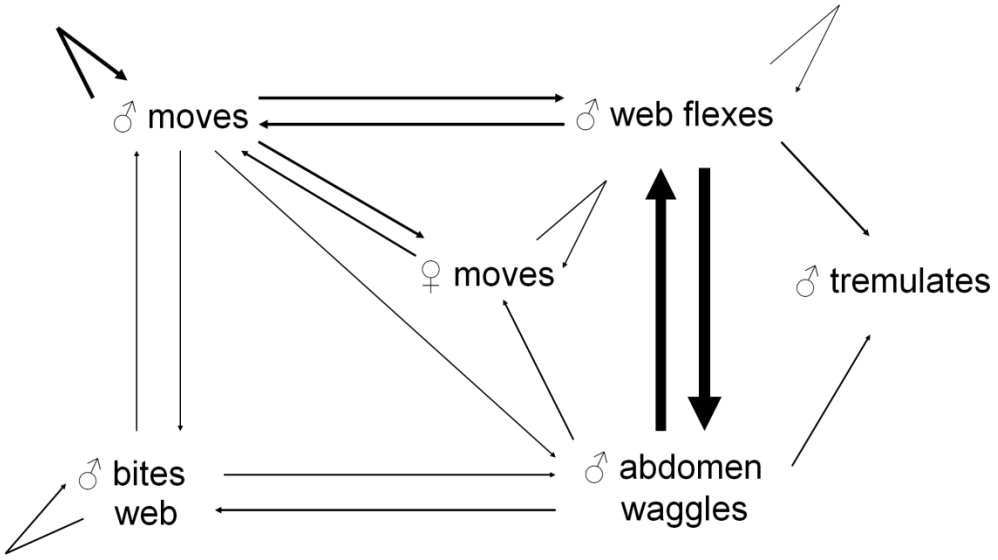
(I)


♀ retreats
♀ moves
♂ moves
♂ tremulates with bobbing
♂ bobs abdomen
♂ abdomen waggles

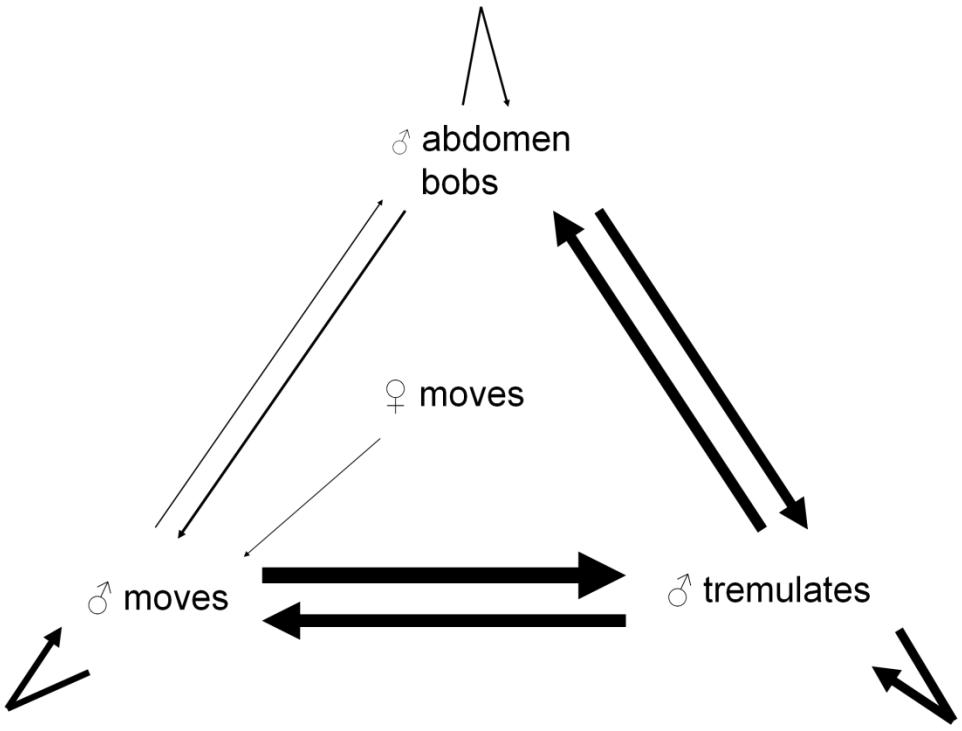
(J)


♀ moves
♀ adjusts position
♂ moves
♂ tremulates with bobbing
♂ abdomen waggles
♂ retreats

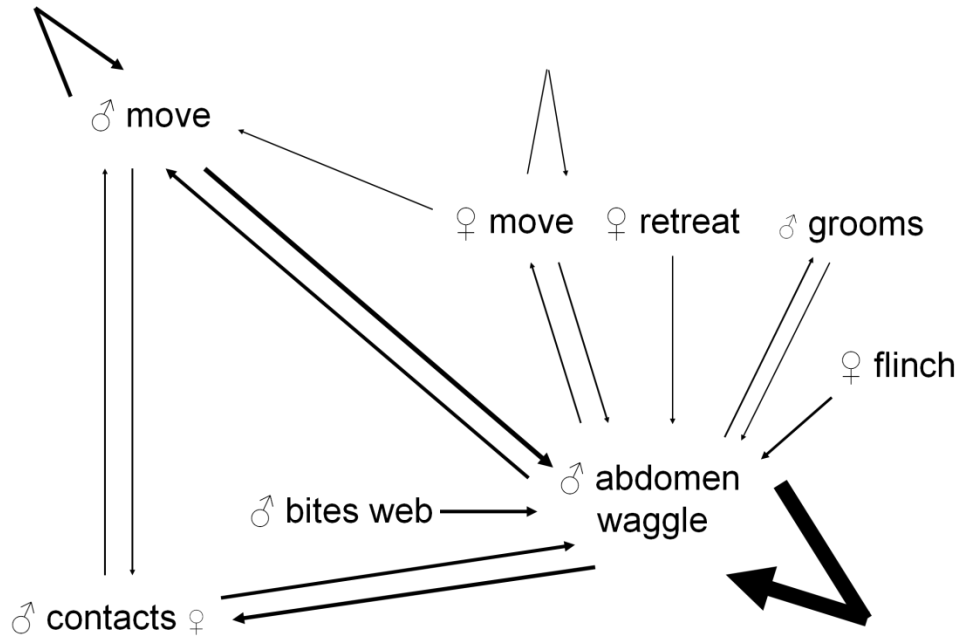
(K)


♂ moves
♂ web flexes
♀ moves
♂ tremulates
♂ bites web
♂ abdomen waggles

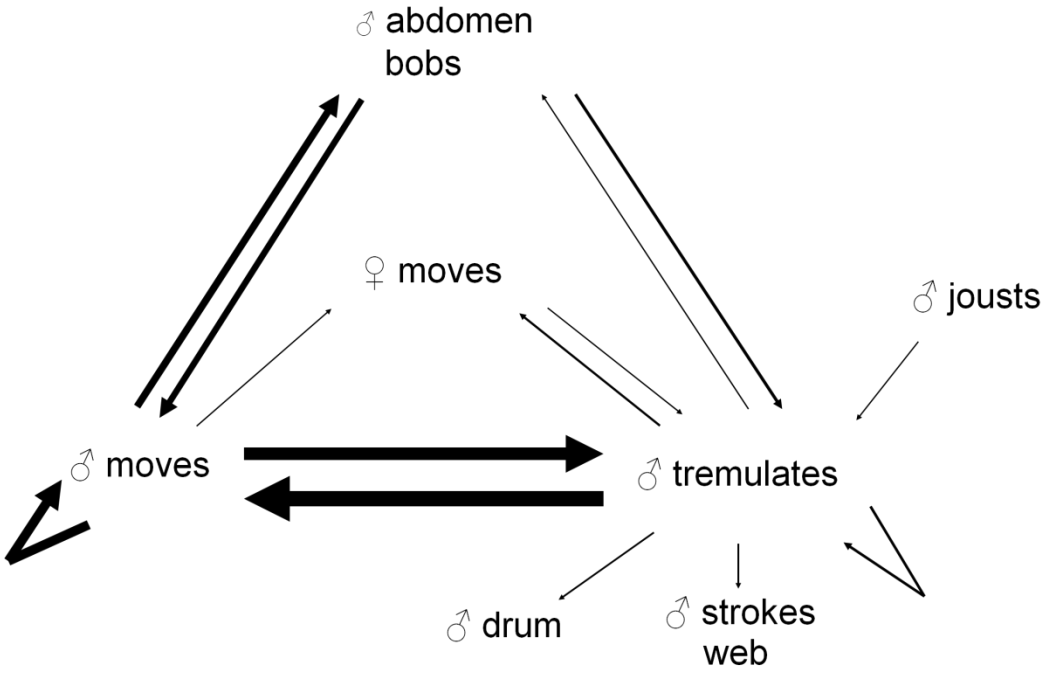
(L)


♂ abdomen bobs
♀ moves
♂ moves
♂ tremulates

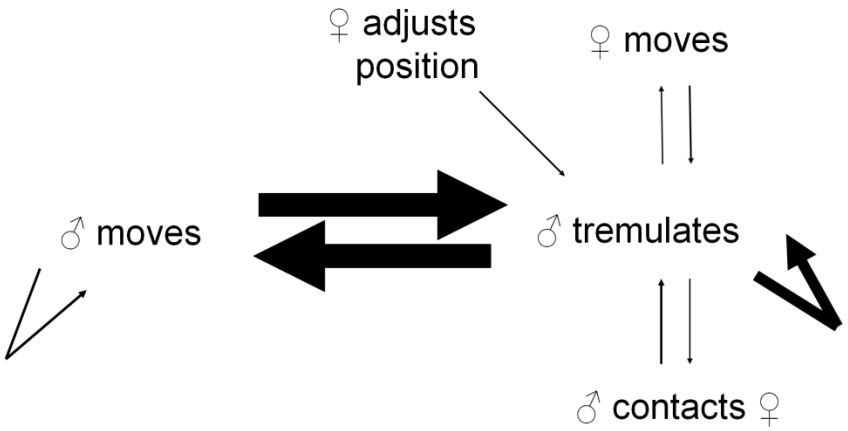
(M)


♂ move
♀ move
♀ retreat
♂ grooms
♀ flinch
♂ abdomen waggle
♂ bites web
♂ contacts ♀

(N)


♂ abdomen bobs
♀ moves
♂ jousts
♂ moves
♂ tremulates
♂ drum
♂ strokes web

(O)



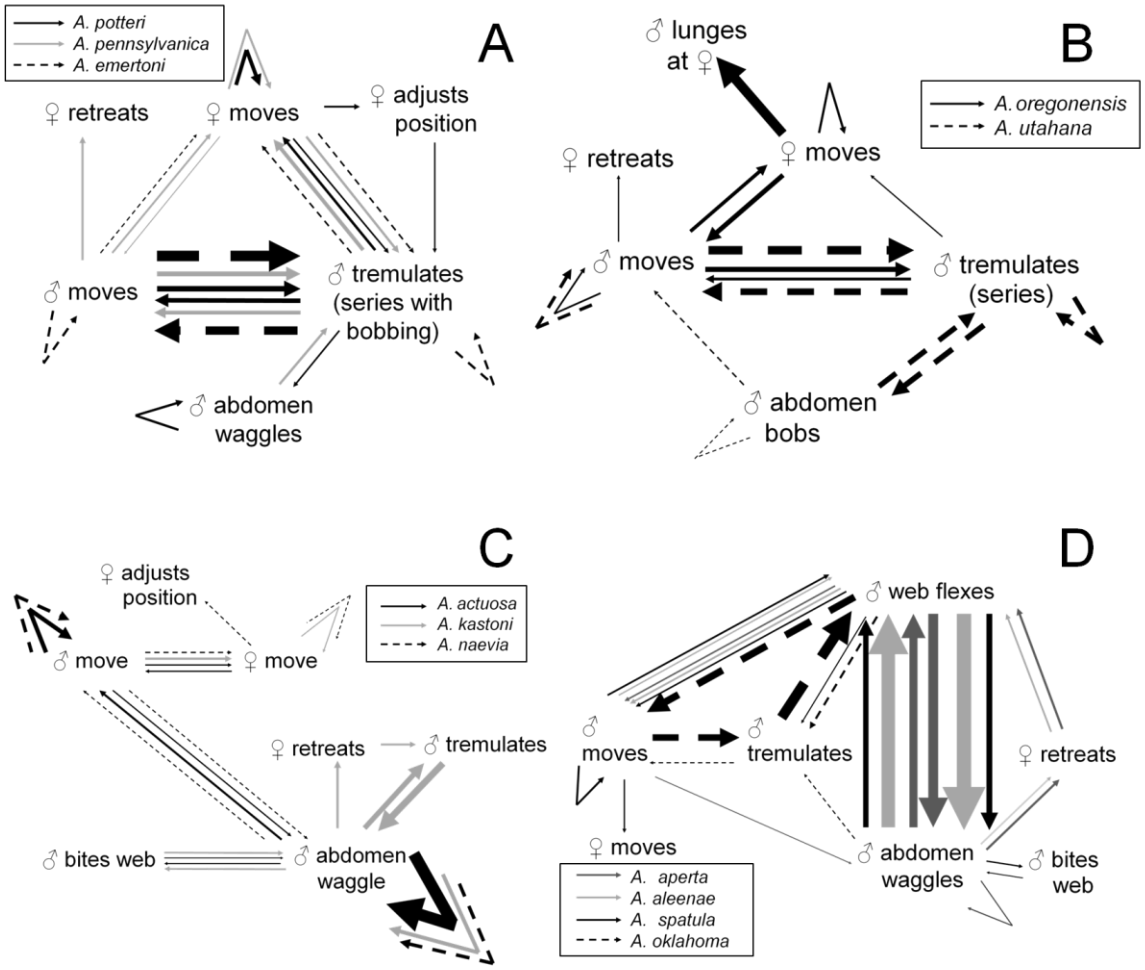
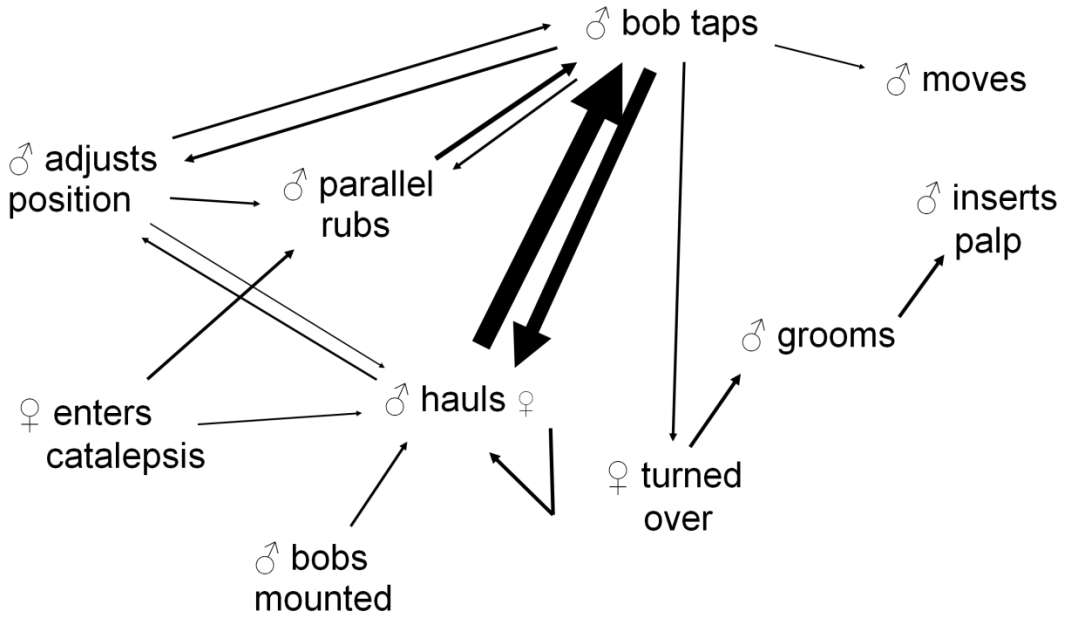


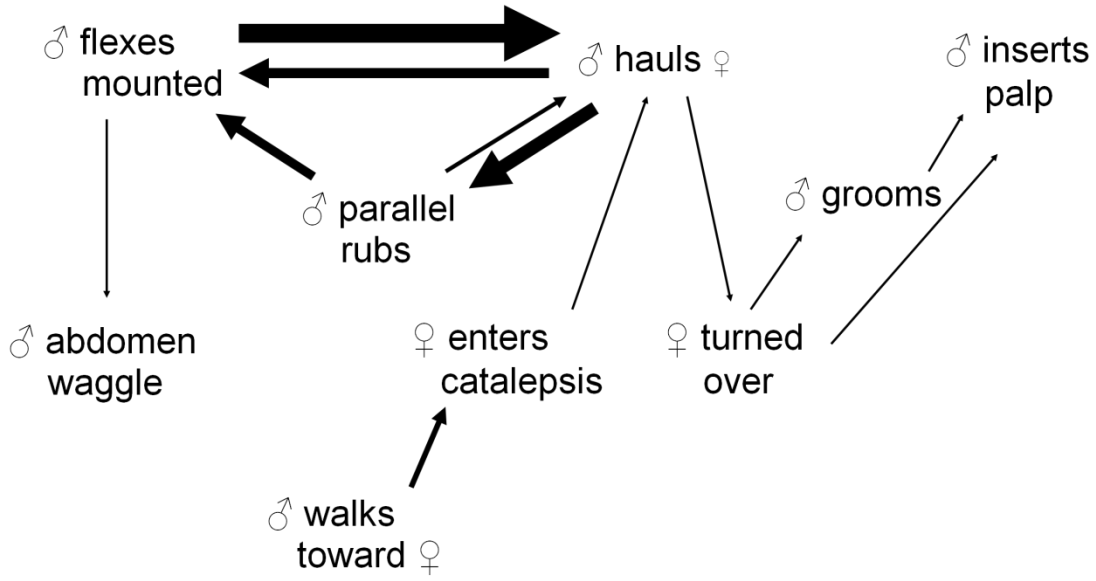
Figure I-4. Kinematic diagrams for web phase courtship illustrating clusters of similar *Agelenopsis* species. Transitions shown are the same as in Figure I-3, but transitions averaging less than 2% of all transitions were not included to improve clarity of presentation.

Figure I-5. Kinematic diagrams showing common behavioral transitions in courtship while the male is mounted on the female. The width of the arrow reflects the relative frequency of the transition. Transition frequencies were averaged across all trials in which the male mounted the female for the respective species: (A) *A. actuosa*, (B) *A. aleenae*, (C) *A. aperta*, (D) *A. emertoni*, (E) *A. kastoni*, (F) *A. naevia*, (G) *A. oklahoma*, (H) *A. oregonensis*, (I) *A. pennsylvanica*, (J) *A. potteri*, (K) *A. spatula*, (L) *A. utahana*, (M) *B. texana*, (N) *C. californica*, (O) *H. nedra*.

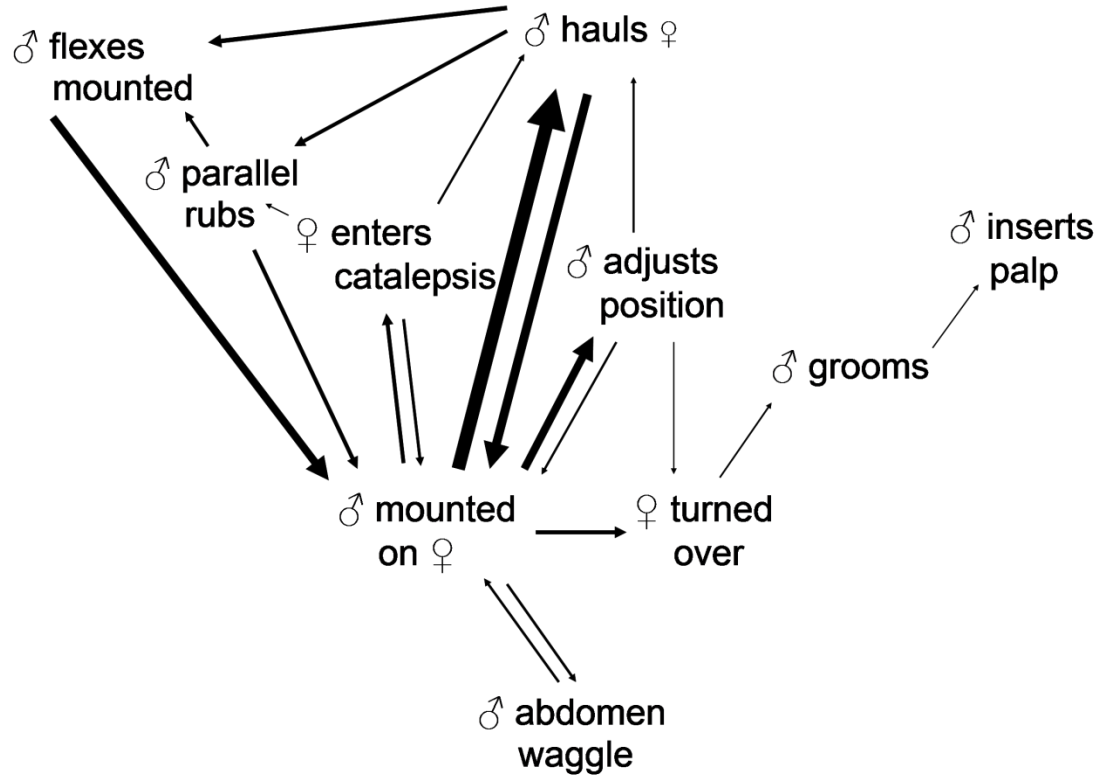
(A)


♂ bob taps
♂ moves
♂ adjusts position
♂ parallel rubs
♂ inserts palp
♂ grooms
♀ enters catalepsis
♂ hauls ♀
♀ turned over
♂ bobs mounted

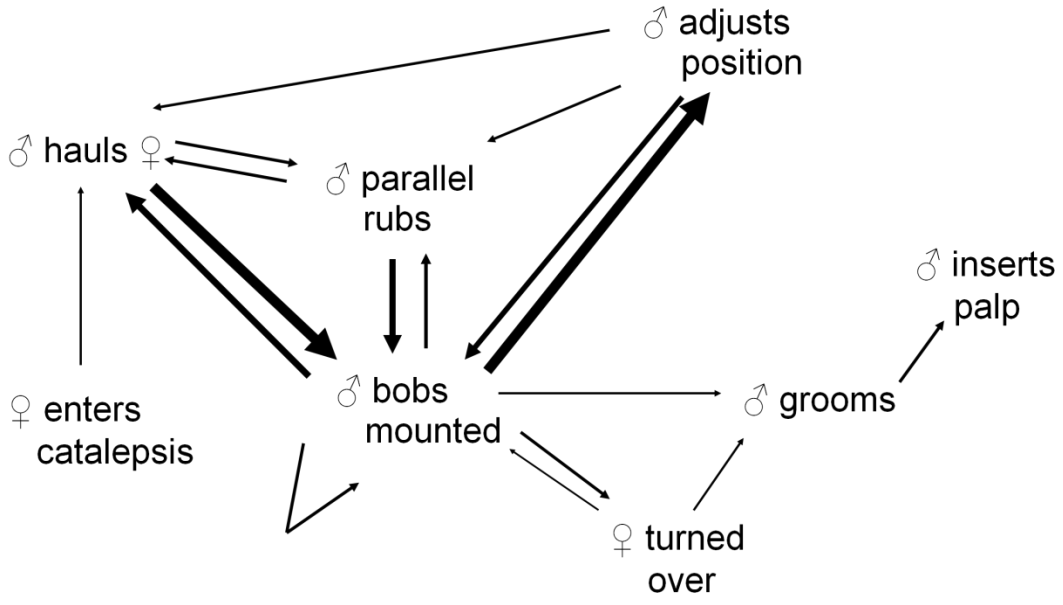
(B)


♂ flexes mounted
♂ hauls ♀
♂ inserts palp
♂ grooms
♂ parallel rubs
♂ abdomen waggle
♀ enters catalepsis
♀ turned over
♂ walks toward ♀

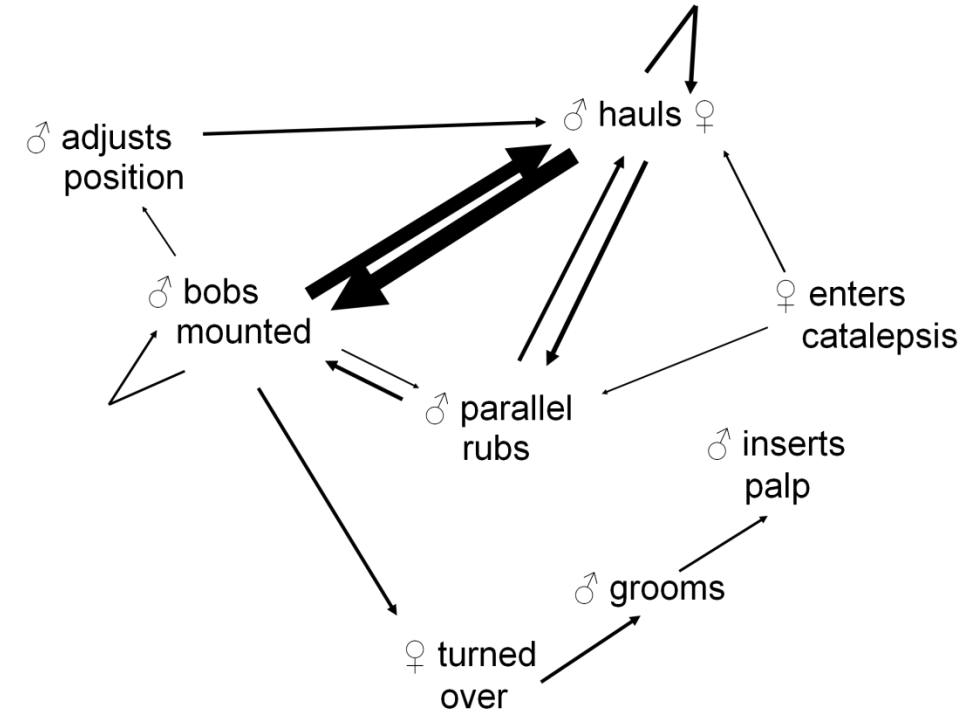
(C)


♂ flexes mounted
♂ hauls ♀
♂ parallel rubs
♀ enters catalepsis
♂ adjusts position
♂ inserts palp
♂ grooms
♂ mounted on ♀
♀ turned over
♂ abdomen waggle

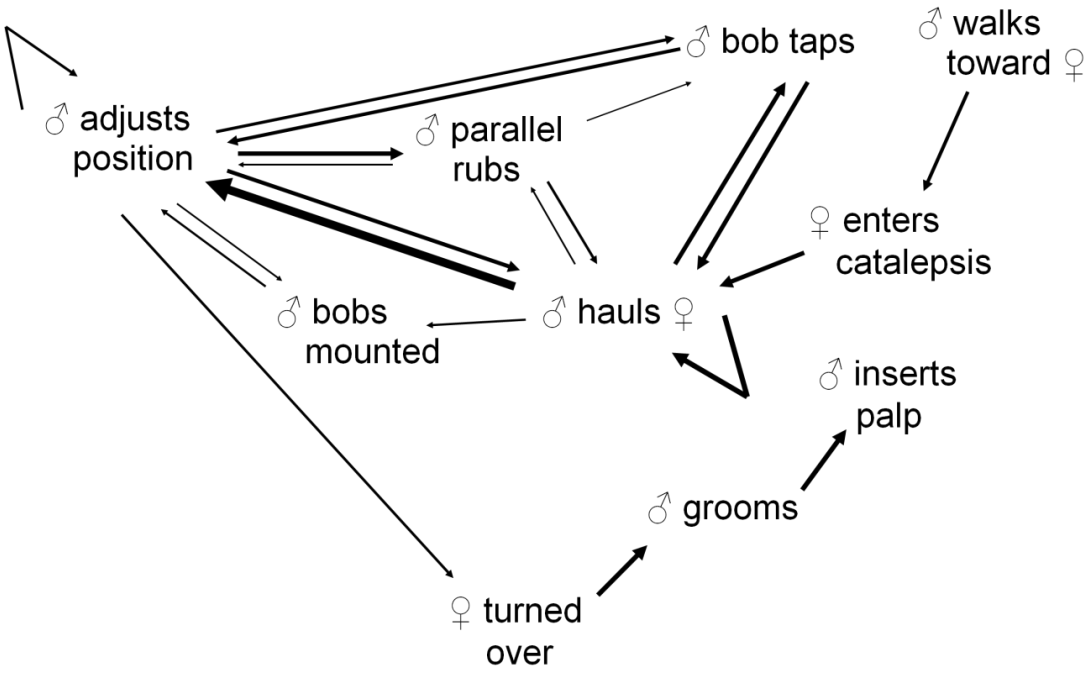
(D)


♂ adjusts position
♂ hauls ♀
♂ parallel rubs
♂ inserts palp
♀ enters catalepsis
♂ bobs mounted
♂ grooms
♀ turned over

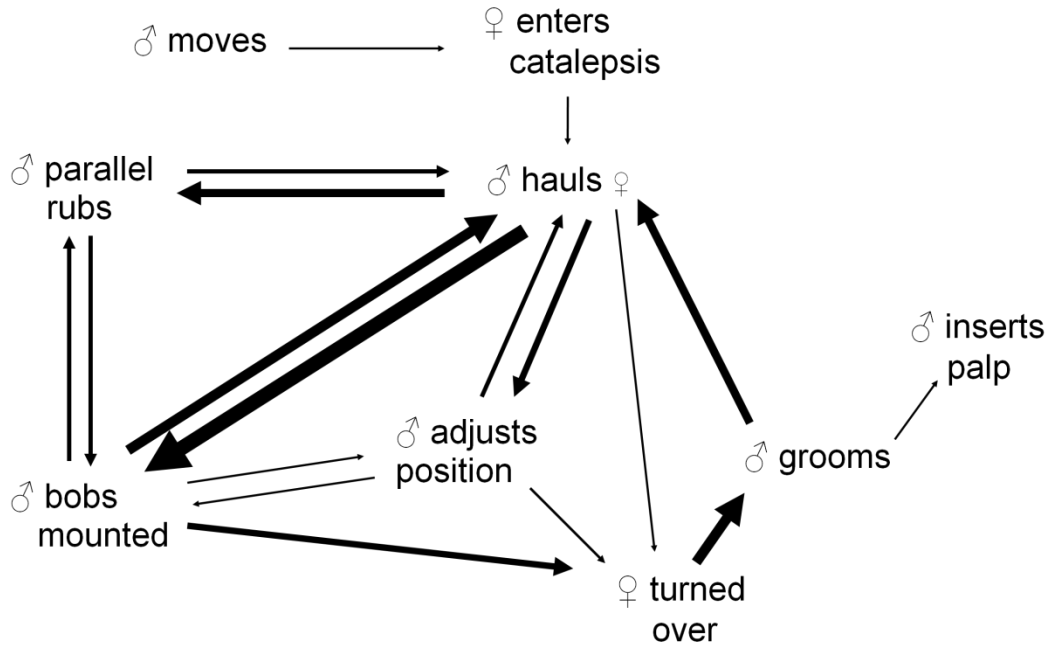
(E)


♂ adjusts position
♂ hauls ♀
♂ bobs mounted
♀ enters catalepsis
♂ parallel rubs
♂ inserts palp
♂ grooms
♀ turned over

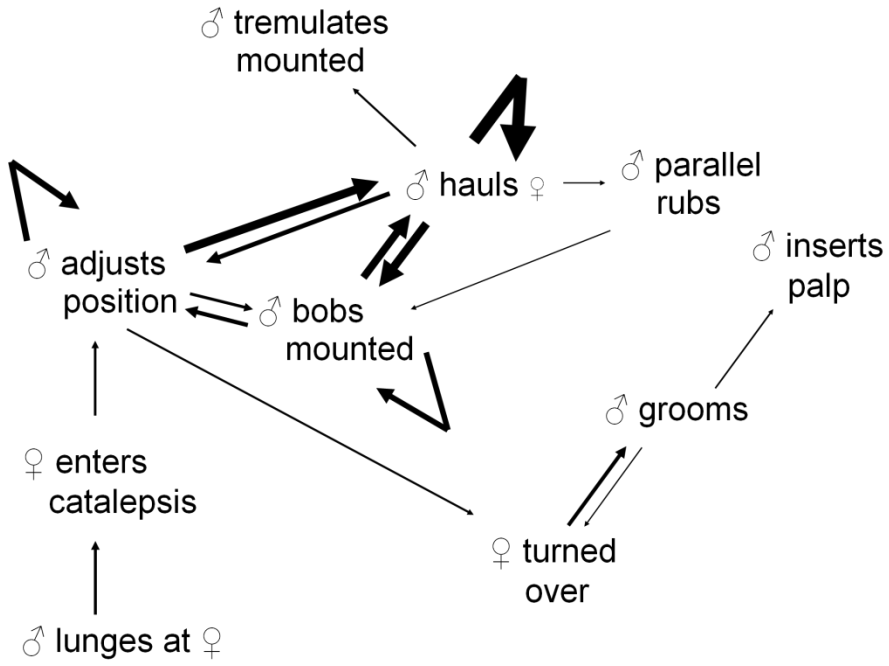
(F)


♂ bob taps
♂ walks toward ♀
♂ adjusts position
♂ parallel rubs
♀ enters catalepsis
♂ bobs mounted
♂ hauls ♀
♂ inserts palp
♂ grooms
♀ turned over

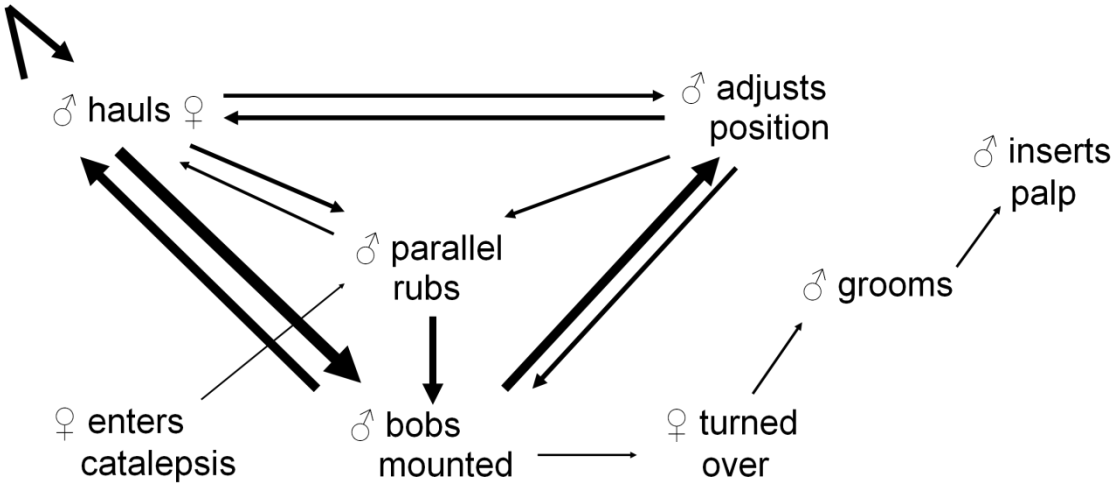
(G)


♂ moves
♀ enters catalepsis
♂ parallel rubs
♂ hauls ♀
♂ inserts palp
♂ adjusts position
♂ grooms
♂ bobs mounted
♀ turned over

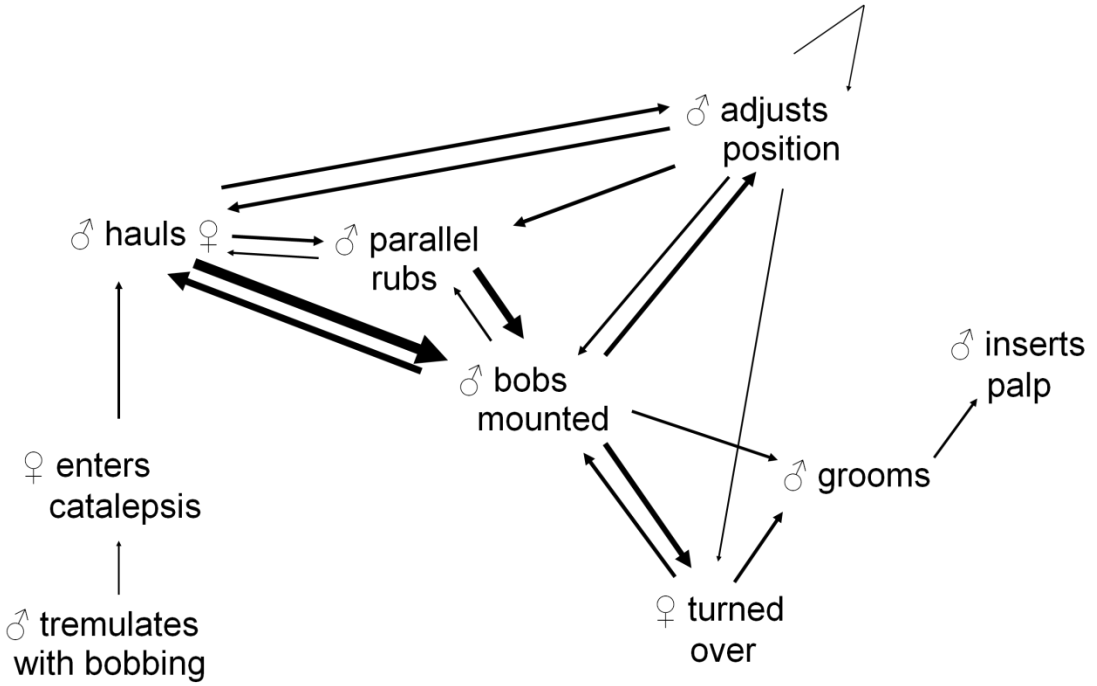
(H)


♂ tremulates mounted
♂ hauls ♀
♂ parallel rubs
♂ inserts palp
♂ adjusts position
♂ bobs mounted
♂ grooms
♀ enters catalepsis
♀ turned over
♂ lunges at ♀

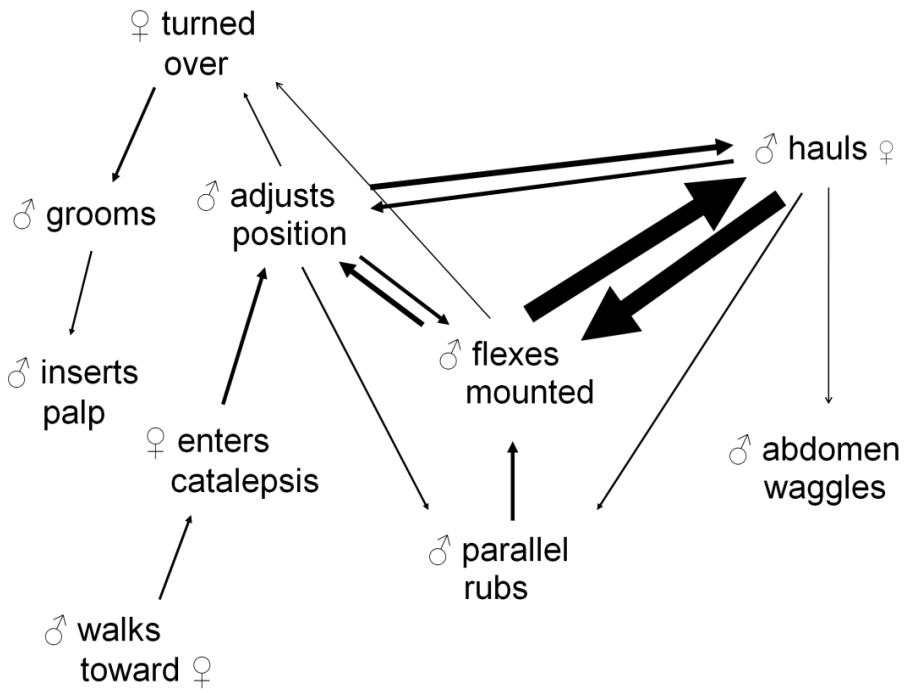
(I)


♂ hauls ♀
♂ adjusts position
♂ inserts palp
♂ parallel rubs
♂ grooms
♀ enters catalepsis
♂ bobs mounted
♀ turned over

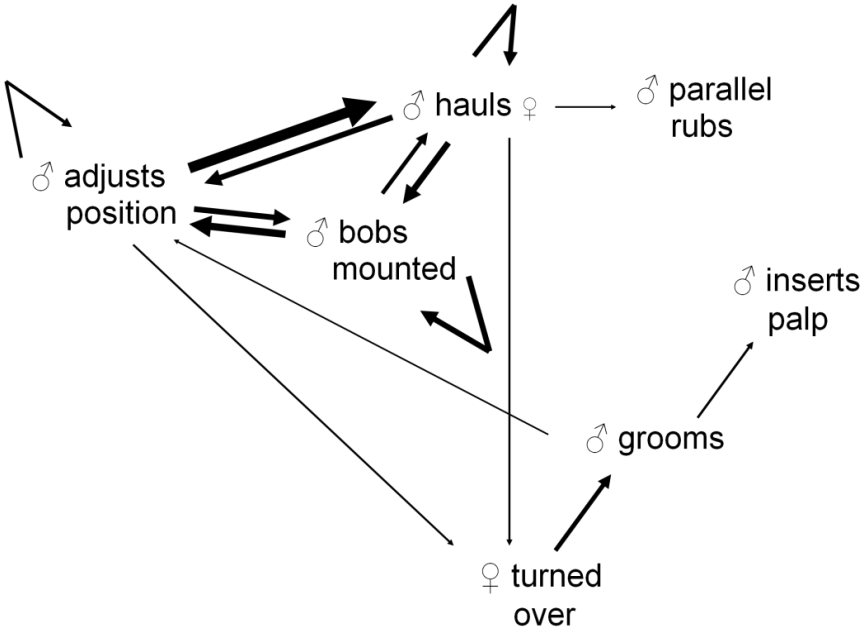
(J)


♂ adjusts position
♂ hauls ♀
♂ parallel rubs
♂ inserts palp
♂ bobs mounted
♂ grooms
♀ enters catalepsis
♂ tremulates with bobbing
♀ turned over

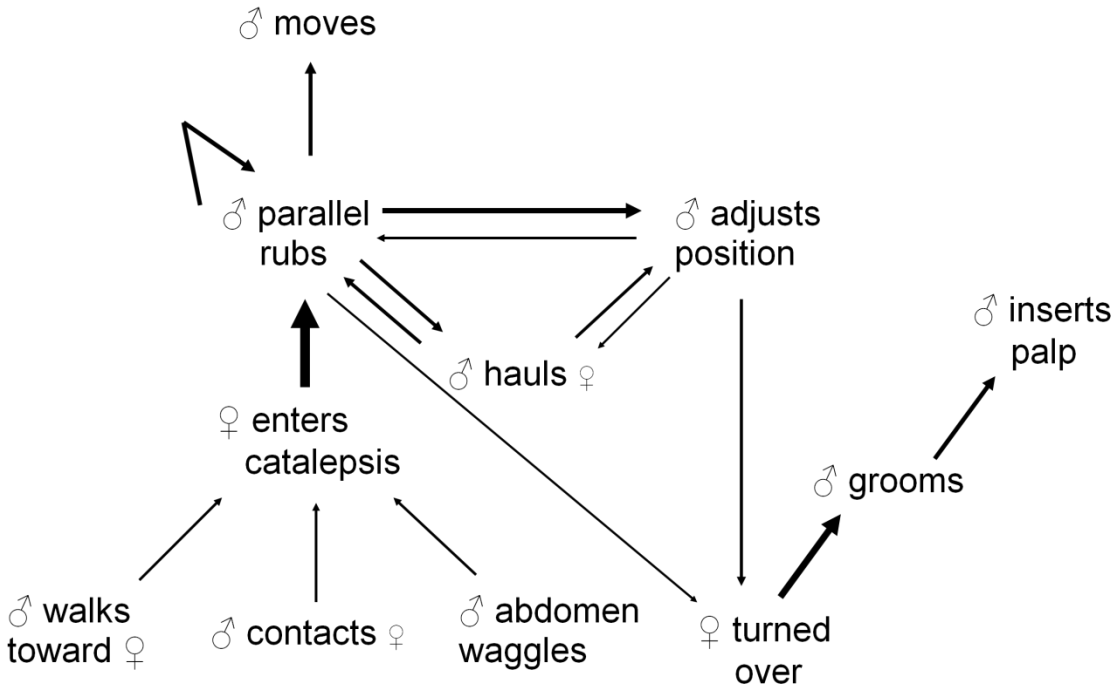
(K)


♀ turned over
♂ hauls ♀
♂ grooms
♂ adjusts position
♂ inserts palp
♂ flexes mounted
♀ enters catalepsis
♂ abdomen waggles
♂ parallel rubs
♂ walks toward ♀

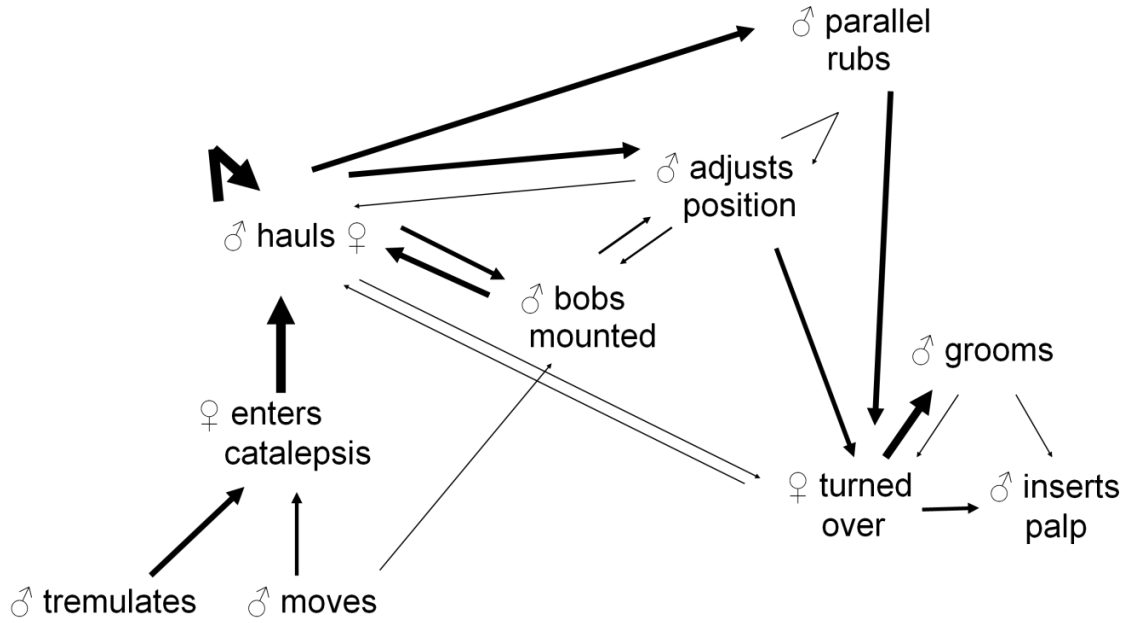
(L)


♂ hauls ♀
♂ parallel rubs
♂ adjusts position
♂ bobs mounted
♂ inserts palp
♂ grooms
♀ turned over

(M)


♂ moves
♂ parallel rubs
♂ adjusts position
♂ hauls ♀
♂ inserts palp
♀ enters catalepsis
♂ grooms
♂ walks toward ♀
♂ contacts ♀
♂ abdomen waggles
♀ turned over

(N)


♂ parallel rubs
♂ adjusts position
♂ hauls ♀
♂ bobs mounted
♂ grooms
♀ enters catalepsis
♀ turned over
♂ inserts palp
♂ tremulates
♂ moves

(O)

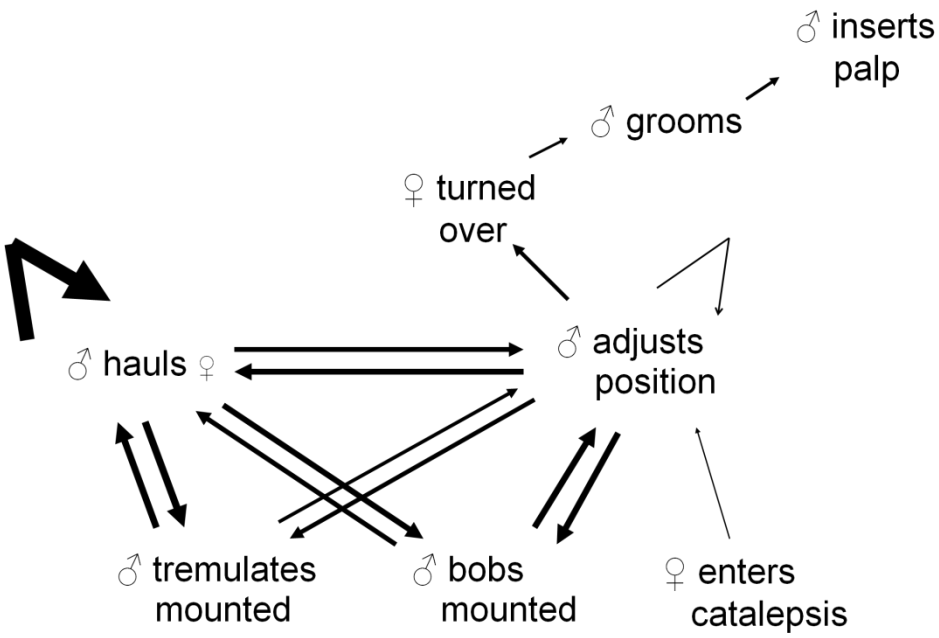
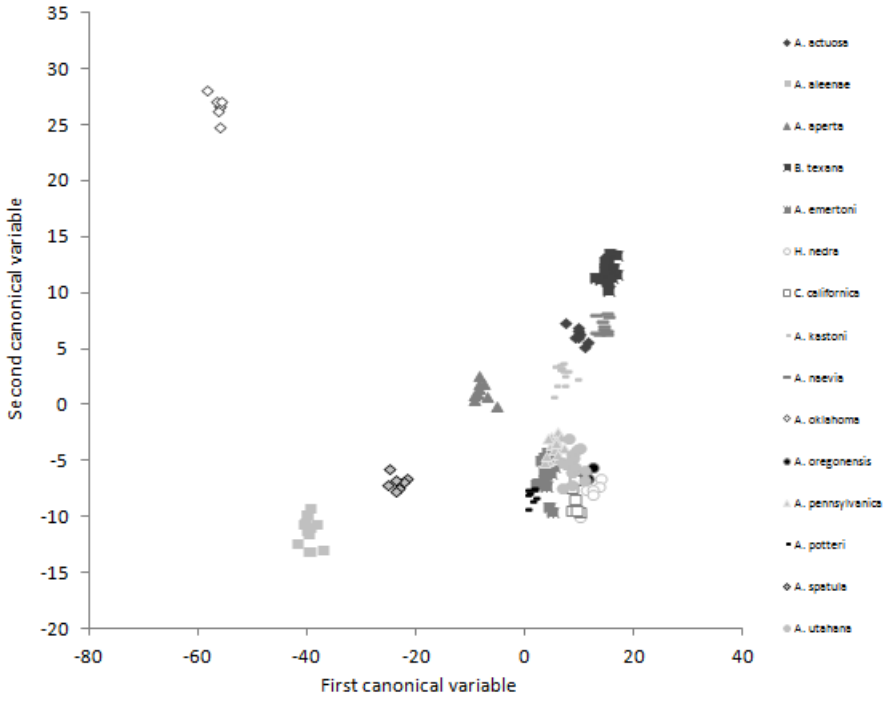
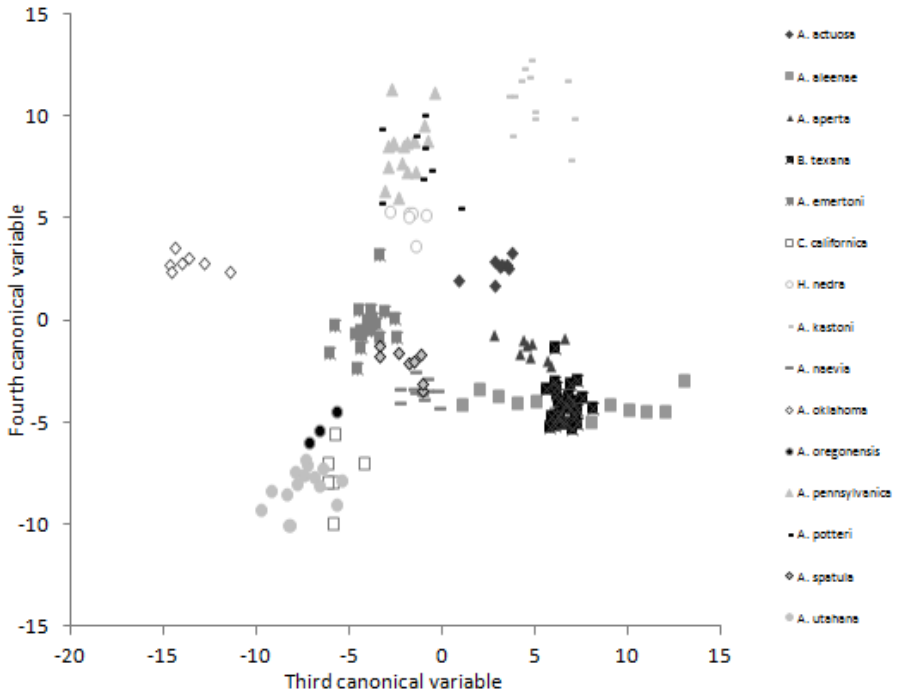

♂ inserts palp
♂ grooms
♀ turned over
♂ hauls ♀
♂ adjusts position
♂ tremulates mounted
♂ bobs mounted
♀ enters catalepsis

Figure I-6. Plots of canonical variables from canonical discriminant analysis of web phase. (A) Scatterplot of first two canonical variables. (B) Scatterplot of third and fourth canonical variables. (C) Scatterplot of fifth and sixth canonical variables.

(A)


Second canonical variable
First canonical variable
35
30
25
20
15
10
5
0
-5
-10
-15
-20
-80
-60
-40
-20
0
20
40
A. actuosa
A. aleenae
A. sperta
B. texana
A. emertoni
H. nedra
C. californica
A. kastoni
A. naevia
A. oklahoma
A. oregonensis
A. pennsylvanica
A. potteri
A. spatula
A. utahana

(B)


Fourth canonical variable
Third canonical variable
15
10
5
0
-5
-10
-15
-20
-15
-10
-5
0
5
10
15
A. actuosa
A. aleenae
A. sperta
B. texana
A. emertoni
C. californica
H. nedra
A. kastoni
A. naevia
A. oklahoma
A. oregonensis
A. pennsylvanica
A. potteri
A. spatula
A. utahana

(C)

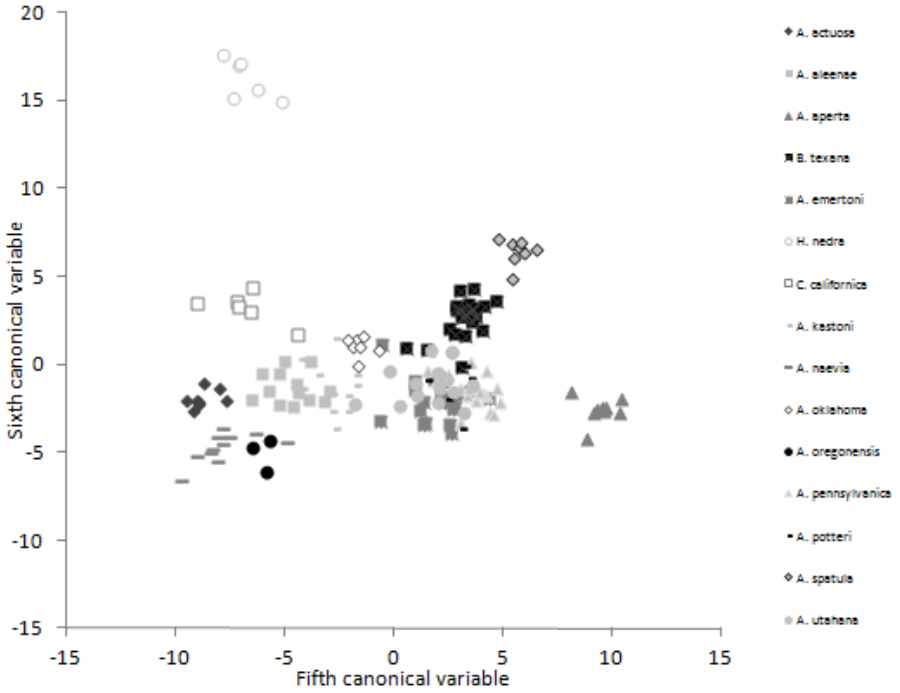
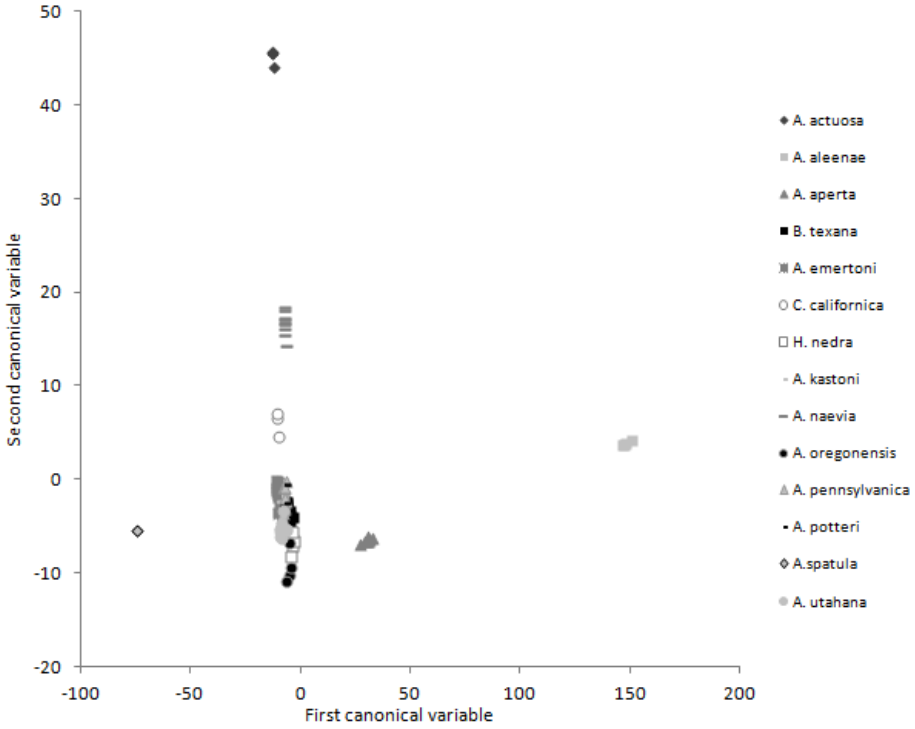
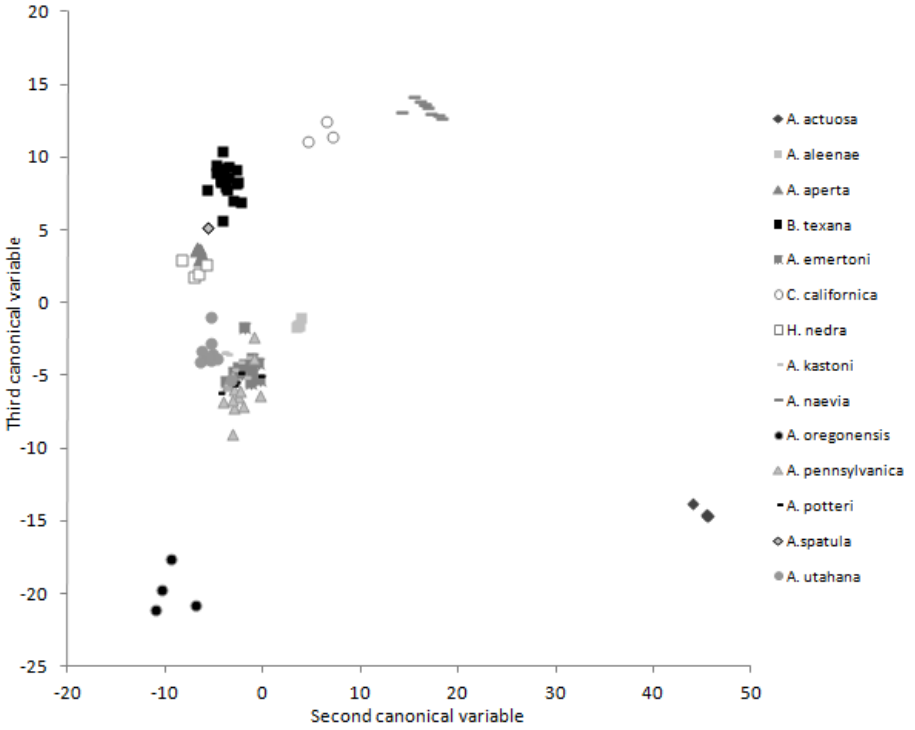

Sixth canonical variable
Fifth canonical variable
20
15
10
5
0
-5
-10
-15
-15
-10
-5
0
5
10
15
A. actuosa
A. aleense
A. sperta
B. texana
A. emertoni
H. nedra
C. californica
A. kastoni
A. naevia
A. oklahoma
A. oregonensis
A. pennsylvanica
A. potteri
A. spatula
A. utahana

Figure I-7. Plots of canonical variables from canonical discriminant analysis of mount phase. (A) Scatterplot of first two canonical variables. (B) Scatterplot of second and third canonical variables.

(A)


Second canonical variable
First canonical variable
A. actuosa
A. aleenae
A. aperta
B. texana
A. emertoni
C. californica
H. nedra
A. kastoni
A. naevia
A. oregonensis
A. pennsylvanica
A. potteri
A.spatula
A. utahana

(B)


Third canonical variable
Second canonical variable
A. actuosa
A. aleenae
A. aperta
B. texana
A. emertoni
C. californica
H. nedra
A. kastoni
A. naevia
A. oregonensis
A. pennsylvanica
A. potteri
A.spatula
A. utahana

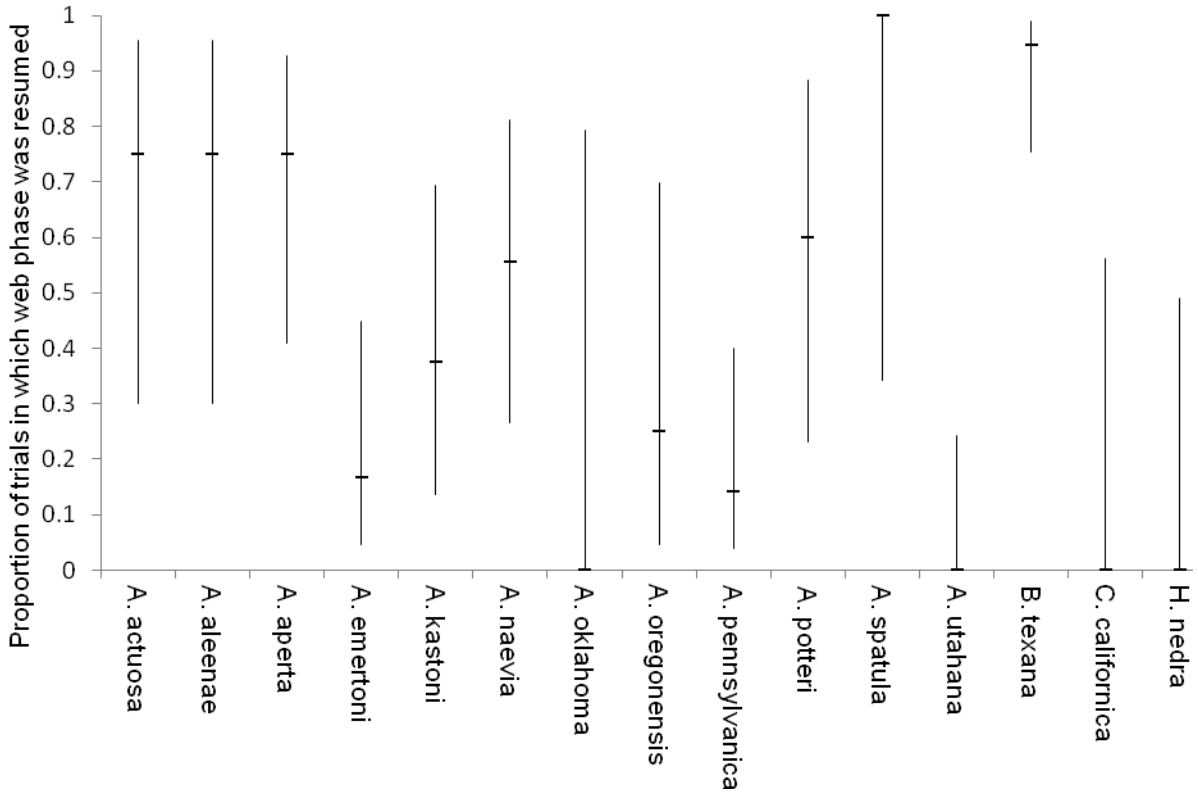


Figure I-8. Prevalence of male resumption of web phase after mounting a female in courtship trials by species. Horizontal bars represent actual proportion and vertical bars represent 95% Wilson (score) binomial confidence limits.

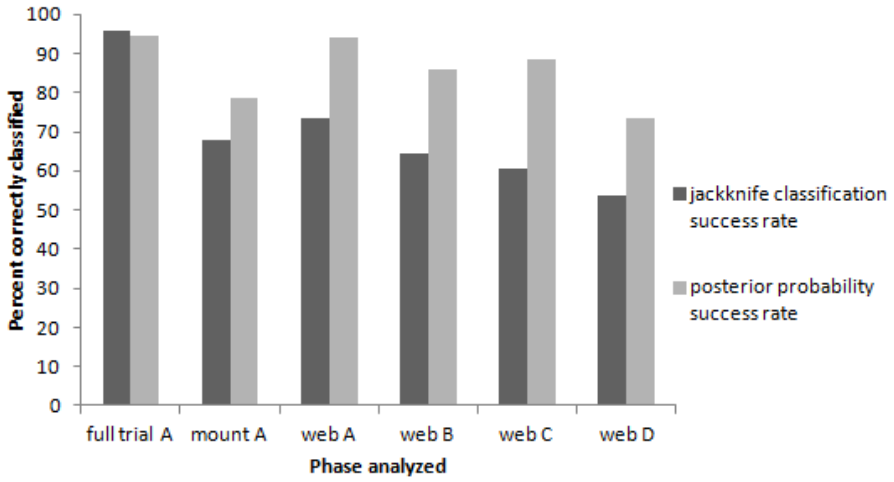
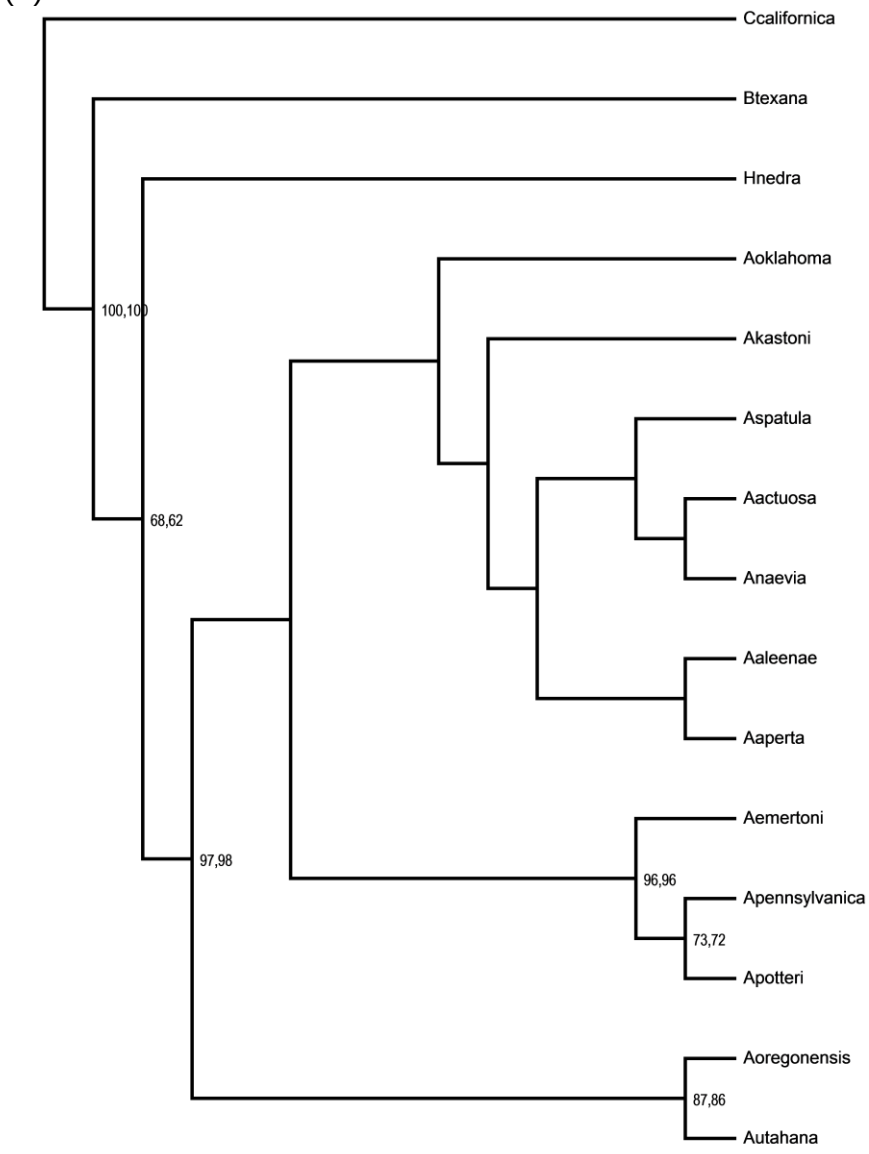


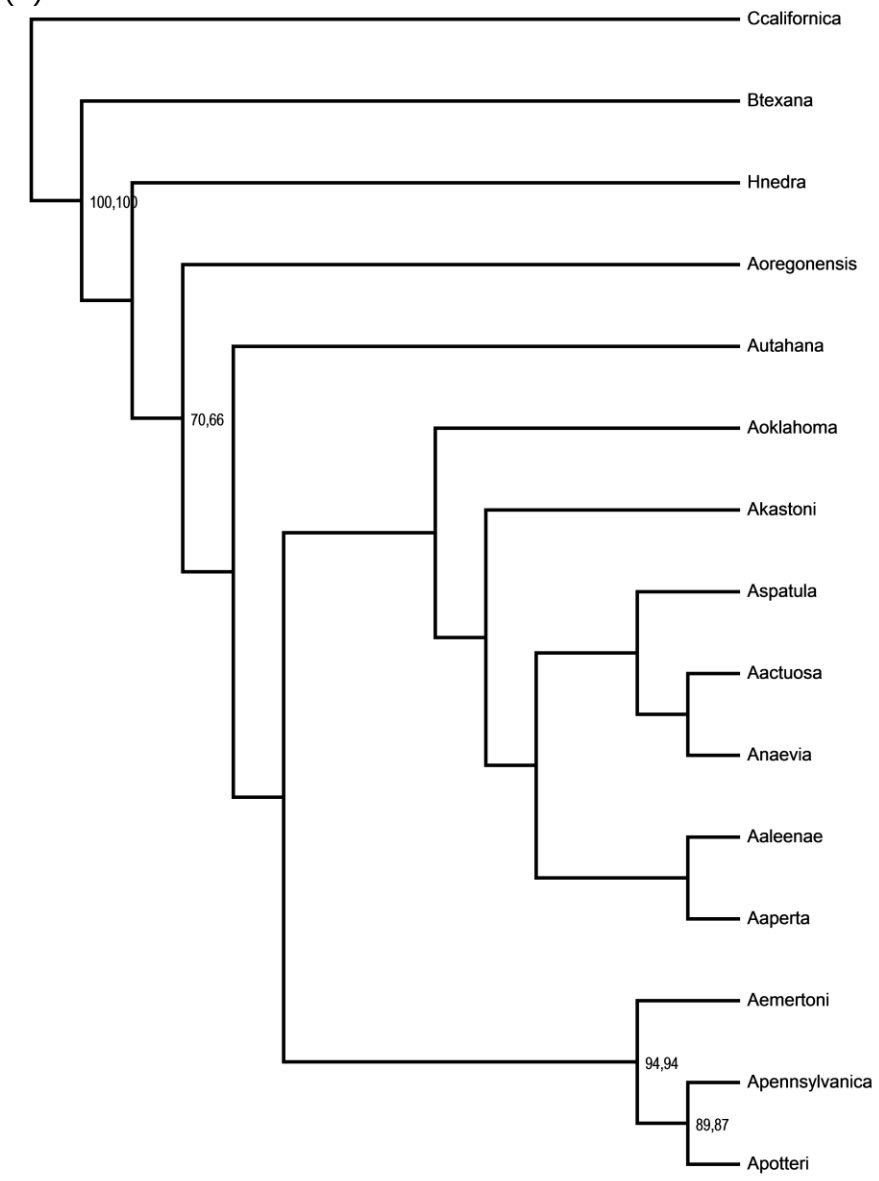
Figure I-9. Cross-validation classification success rates for discriminant analyses, labeled according to which courtship phase was analyzed. Success rate based on both jackknife and jackknife posterior probability error rates are shown. The trials included in the analyses are indicated by letter: (A) trials with both courtship phases, not including *A. oregonensis* and *A. oklahoma*, (B) trials with at least web phase, not including *A. oregonensis* and *A. oklahoma*, (C) all trials with at least web phase, all species and (D) all trials with at least web phase, broken down by population.

Figure I-10. Most parsimonious tree at species level based on: (A) all courtship characters, RI : 0.50, CI : 0.62, Score: 4.57; (B) web phase characters, RI : 0.51, CI : 0.62, Score: 3.92 ; and (C) mount phase characters. RI : 0.57, CI : 0.70, Score: 0.46. Symmetric resampling and bootstrap values indicated for nodes with greater than 50% support. Additionally, symmetric resampling provided some support (52%) for an *A. apertaA spatula* node not found in the most parsimonious tree based on all courtship characters. Resampling of web phase characters also found some support (55-59%) for an *A. oregonensisA. utahana* node not found in the most parsimonious tree.

(A)


Ccalifornica
Btexana
Hnedra
Aoklahoma
100,100
Akastoni
Aspatula
Aactuosa
68,62
Anaevia
Aaleenae
Aaperta
Aemertoni
97,98
96,96
Apennsylvanica
73,72
Apotteri
Aoregonensis
87,86
Autahana

(B)


Ccalifornica
Btexana
Hnedra
100,100
Aoregonensis
Autahana
70,66
Aoklahoma
Akastoni
Aspatula
Aactuosa
Anaevia
Aaleenae
Aaperta
Aemertoni
94,94
Apennsylvanica
89,87
Apotteri

(C)

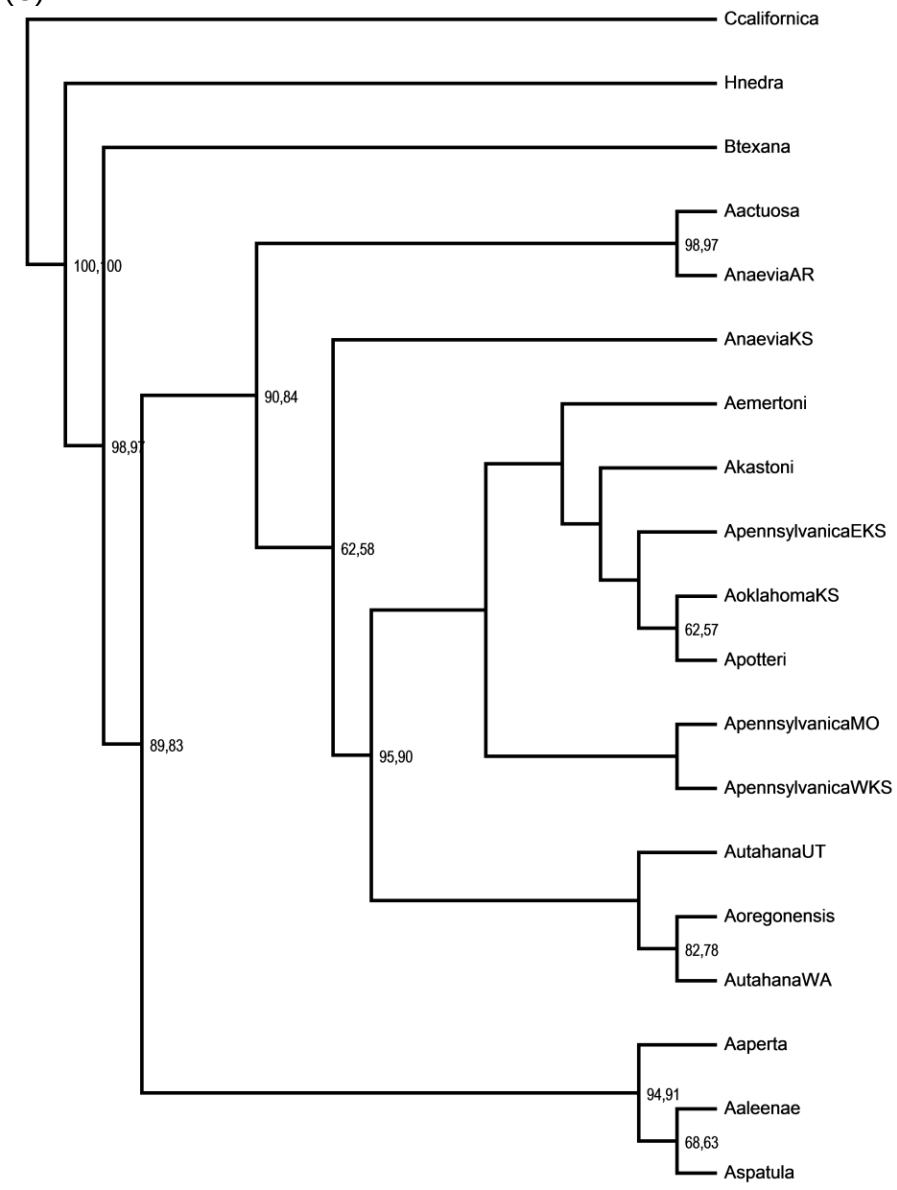
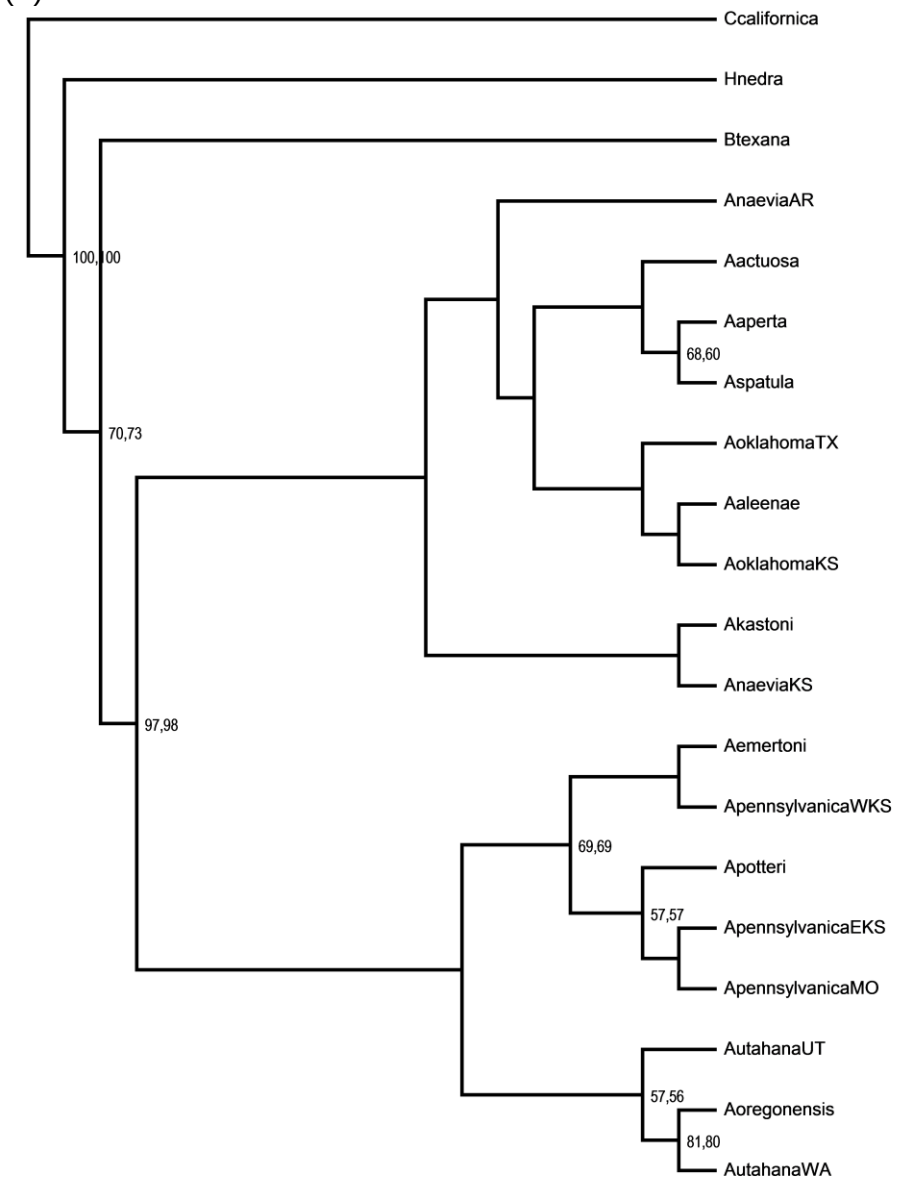
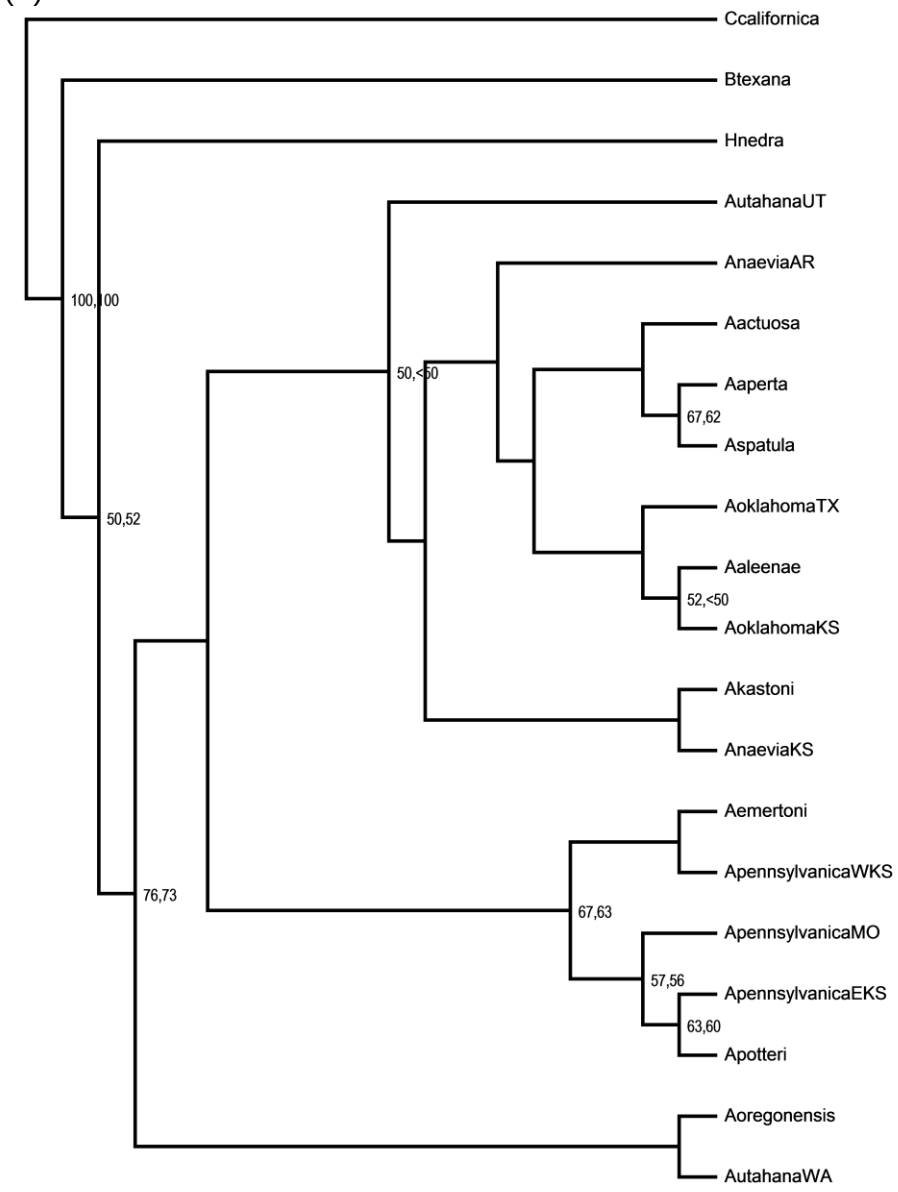

Ccalifornica
Hnedra
Btexana
Aactuosa
98,97
AnaeviaAR
AnaeviaKS
Aemertoni
Akastoni
ApennsylvanicaEKS
AoklahomaKS
62,57
Apotteri
ApennsylvanicaMO
ApennsylvanicaWKS
AutahanaUT
Aoregonensis
82,78
AutahanaWA
Aaperta
94,91
Aaleenae
68,63
Aspatula
100,100
98,97
90,84
62,58
89,83
95,90

Figure I-11. Most parsimonious tree at population level based on: (A) all courtship characters: RI : 0.62, CI : 0.57, Score: 6.57; (B) web phase characters: RI : 0.62, CI : 0.56, Score: 5.59; and (C) mount phase characters. RI : 0.57, CI : 0.64, Score: 0.62. Symmetric resampling and bootstrap values indicated for nodes with greater than 50% support.

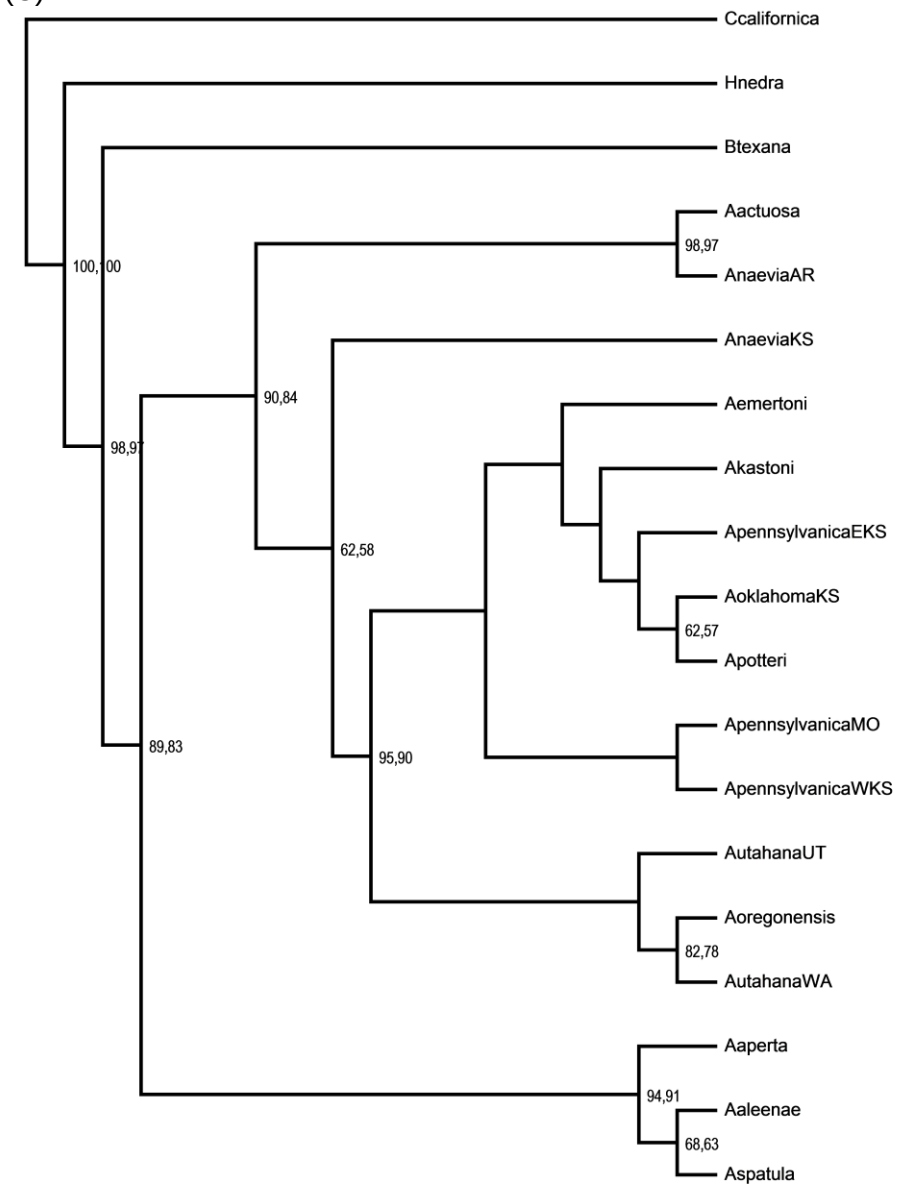
(A)


Ccalifornica
Hnedra
Btexana
AnaeviaAR
Aactuosa
Aaperta
Aspatula
AoklahomaTX
Aaleenae
AoklahomaKS
Akastoni
AnaeviaKS
Aemertoni
ApennsylvanicaWKS
Apotteri
ApennsylvanicaEKS
ApennsylvanicaMO
AutahanaUT
Aoregonensis
AutahanaWA
100,100
70,73
97,98
68,60
69,69
57,57
57,56
81,80

(B)


Ccalifornica
Btexana
Hnedra
AutahanaUT
AnaeviaAR
Aactuosa
Aaperta
Aspatula
AoklahomaTX
Aaleenae
AoklahomaKS
Akastoni
AnaeviaKS
Aemertoni
ApennsylvanicaWKS
ApennsylvanicaMO
ApennsylvanicaEKS
Apotteri
Aoregonensis
AutahanaWA
100,100
50,<50
67,62
50,52
52,<50
76,73
67,63
57,56
63,60

(C)


Ccalifornica
Hnedra
Btexana
Aactuosa
98,97
AnaeviaAR
AnaeviaKS
Aemertoni
Akastoni
ApennsylvanicaEKS
AoklahomaKS
62,57
Apotteri
ApennsylvanicaMO
ApennsylvanicaWKS
AutahanaUT
Aoregonensis
82,78
AutahanaWA
Aaperta
94,91
Aaleenae
68,63
Aspatula
100,100
98,97
90,84
62,58
89,83
95,90

Description of courtship in each spider species

***Agelenopsis actuosa* courtship**. Prior to mounting the female, the most common male behaviors are abdomen wagging and movement and the most common behavioral transition is abdomen wagging followed by more abdomen wagging (Table I-5 & Figure I-3A). Often, movement and abdomen wagging were interspersed with biting the web. A little over half of all courtships included little to no stationary signaling behaviors, though a few included some brief tremulations. One courtship included many slow, single abdomen bobs after bouts of abdomen wagging, but that pairing was unsuccessful, so this unusual pattern may not be typical courtship behavior for *A. actuosa*. *Agelenopsis actuosa* mount phase contained a substantial amount of the behavior bob tap, and in this species the abdomen bobbing and leg tapping components were highly synchronized (Figure I-4A, Table I-7).

***Agelenopsis aleenae* courtship.** *Agelenopsis aleenae* males spent the large majority of their active web phase cycling back and forth between abdomen wagging and web flexing, similar to *A. aperta* males (Figure I-3B-C,Table I-5). Typically a bout of abdomen wagging was followed by a bout of web flexing, then a long rest. Both females and males spent a relatively large amount of time resting (Tables I-5 & I-6). Web flexes in *A. aleenae* tended to be longer than web flexes in *A. aperta*. During mount phase, the males often alternated between flexing while mounted, hauling and parallel rubbing (Figure I-4B, Table I-7).

***Agelenopsis emertoni* courtship.** In *A. emertoni*, males made a rather slow approach toward the female, interspersing their movements with tremulation (Figure I-3D). When they were very near the female, they typically would make physical contact with the female for a relatively long time, occasionally tremulating, until mounting (Figure I-3D, Table I-5). Each tremulation was accompanied by a single bob of the abdomen, and these tremulations typically occurred singly when they were between bouts of movement, but occurred in a series of several syllables when they occur at the end of a series of movements (Table I-4). During mount phase, males spent a substantial amount of time bobbing while mounted, and transitioned often between this behavior and hauling, adjusting position and parallel rubbing (Figure I-4D, Table I-7).

***Agelenopsis kastoni* courtship.** Web phase in *A. kastoni* was dominated by abdomen wagging, and bouts of abdomen wagging often ended with single tremulations (Figure I-3E, Table I-5). Very often, abdomen wagging was interspersed with biting the web. In one *A. kastoni* pairing, the male mounted the female almost immediately, with no abdomen wagging or web flexing. Mount phase involved a large percentage of bobbing while mounted, alternated with hauling (Figure I-4E, Table I-7).

***Agelenopsis naevia* courtship.** Prior to mounting the female, the most common male *A. naevia* behavior was contacting the female, with abdomen wagging and movement next most common (Table I-4). The most common behavioral transitions were abdomen wagging followed by more abdomen wagging and movement followed by more movement, with some transitions between the two behaviors (Figure I-3F). About half of all courtships included tremulation or abdomen bobbing, though they did not occur very often, typically only when the male was near the female (Table I-4). Tremulations were brief and consisted of a single syllable. During mount phase, there were no particular transitions that were very common, and several different signaling behaviors were occasionally observed, including bob tap, although this behavior was not nearly as synchronized as in *A. actuosa* (Figure I-4F, Table I-7).

***Agelenopsis oklahoma* courtship.** Web phase in *A. oklahoma* featured a more complex sequence of movement (sometimes abdomen wagging) followed by a long, intense single bout of tremulation, followed by a relatively low-intensity but long web flex (Figure I-3G). Often, males would tremulate while moving or abdomen wagging as well, especially at the start of the movement. Biting the web was also fairly common. Only one *A. oklahoma* courtship was successful, making it difficult to generalize about their mount phase. In the successful courtship, bobbing while mounted and hauling and the transitions between them were quite common (Table I-7, Figure I-4G). Also, a very large fraction of mount

phase was spent grooming, and the mount phase period lasted over twenty minutes, which suggests the male may have experienced mechanical difficulty inserting his palp (Table I-7, Figure I-2).

***Agelenopsis oregonensis* courtship.** During web phase move and tremulate were the most common male activities, and transitions between these behaviors were relatively common (Table I-5, Figure I-3H). Tremulations often occurred in singly, interspersed between movements, but they sometimes occurred in a series (Table I-4). Abdomen bobbing was also a fairly common signaling behavior in this species. Females were rather active, spending an average of 10.2% of the time moving (Table I-6). The male lunged at and mounted the female without performing any web phase at all in three out of six pairings, an unusually high fraction when compared to the other species. Males spent about a quarter of mount phase positioning the female, but also did spend some time bobbing while mounted, tremulating while mounted and parallel rubbing (Table I-7). As the males often mounted females on the sheet portion of the web, additional hauling and adjusting may have been required to properly position females in the funnel.

***Agelenopsis pennsylvanica* courtship.** As they approached the female, *A. pennsylvanica* males alternated tremulation with either abdomen wagging or moving with relatively long pauses between bouts of activity (Table I-5, Figure I-

3I). As in *A. emertoni*, each tremulation is accompanied by a single bob of the abdomen, and these tremulations typically occur singly when they are between bouts of movement, but occur in a series of several syllables when they occur at the end of a series of movements (Table I-4). Females averaged a relatively high percent of time spent moving, which was often in response to male activity (Table I-6). Mount phase involved a high proportion of bobbing while mounted, which was often alternated with hauling, adjusting position and parallel rubbing (Table I-7, Figure I-4I).

***Agelenopsis potteri* courtship.** During web phase, *A. potteri* males alternated tremulation with either abdomen wagging or movement with relatively long rests between bouts of activity (Table I-5, Figure I-3J). Abdomen wagging also occurred in a series of repeated bouts without any tremulation. As in *A. emertoni and A. pennsylvanica*, each tremulation is accompanied by a single bob of the abdomen, and these tremulations typically occur singly when they are between bouts of movement, but occur in a series of several syllables when they occur at the end of a series of movements (Table I-4). In two out of fourteen pairings, the male mounted the female very quickly without typical web phase. Mount phase involves a high proportion of bobbing while mounted, which is often alternated with hauling, adjusting position and parallel rubbing (Table I-7, Figure I-3J). Males also spent a large fraction of time mounted on the female grooming their palps.

***Agelenopsis spatula* courtship**. Male web phase consisted primarily of web flexing alternated with either abdomen wagging or movement (Table I-5, Figure I-3K). Often, movement and abdomen wagging were interspersed with biting the web. Sometimes web flexing and abdomen wagging were followed by brief single tremulations. Like *A. aleenae* and *A. oklahoma* web flexes*, A. spatula* web flexes tended to be longer than those of *A. aperta*. Mount phase included many transitions between haul and flex while mounted (Figure I-4K). As in *A. aperta* and *A. aleenae*, no bobbing while mounted or tremulating while mounted was observed (Table I-7).

***Agelenopsis utahana* courtship.** *Agelenopsis utahana* males spent relatively little of their courtship resting, and spent a relatively large proportion tremulating and abdomen bobbing (Table I-5). Males primarily alternated between these two behaviors and movement (Figure I-3L). The abdomen bobbing was typically a fast but low amplitude movement of the abdomen and was sometimes hard to detect. The tremulations typically occurred singly, alternated with movement or bobbing, but sometimes they occurred in uninterrupted series (Table I-4). In one out of twelve pairings, the male mounted the female immediately, without web phase. Mount phase included a substantial proportion of bobbing while mounted, hauling and adjusting position, and transitions between those behaviors were common (Table I-7, Figure I-4L).

***Barronopsis texana* courtship.** *Barronopsis texana* males spent a large fraction of web phase abdomen wagging repeatedly; contacting the female, grooming and biting the web were also common (Table I-5, Figure I-3M). Males did not engage in any tremulation or web flexing, and abdomen bobbing was uncommon. However, abdomen wagging often took place without any movement across the web, and so could function as a stationary signaling behavior in this species. Females often moved, retreated or flinched in response to male activity. Mount phase involved a high proportion of parallel rubbing, which was typically quite vigorous, but there were no other signaling-type behaviors during mount phase (Table I-7).

***Calilena californica* courtship.** Males spent most of their web phase alternating between movement, tremulation and abdomen bobbing (Table I-5, Figure I-2N). Abdomen bobbing was typically brief, fast, and low-intensity; tremulations were moderately intense and occurred singly (Table I-4). Mount phase was rather brief and consisted of a high proportion of haul and turned over (Table I-7). Bobbing while mounted also occurred.

***Hololena nedra* courtship.** *Hololena nedra* male web phase involved repeated transitions between movement and tremulation (Figure I-2O). Abdomen bobbing was also observed, but it was only very common in one pairing which was not

successful, so a high frequency of this behavior may not be typical (Table I-5). Tremulations were single when interspersed with movements, but occurred in short series when at the end of a series of movements (Table I-4). Mount phase was brief and included frequent transitions between bobbing while mounted tremulating while mounted, hauling and adjusting position (Figure I-4O).

CHAPTER II : VIBRATORY SIGNALING IN FUNNEL-WEB SPIDERS

This chapter is a modified version of an original research article to be submitted for publication by A. B. Galasso, S. E. Riechert, G. W. Uetz and S. Gordon.

In the following chapter, the word ‘our’ refers to my co-authors and me. My contributions to this paper include 1) formulation of the original research idea, 2) collection of almost all subjects and data, 3) analysis of all data, 4) construction of figures and tables and 5) most of the writing.

Abstract

Substrate-borne vibration is a critical sensory modality for many taxa, especially spiders. In *Agelenopsis* (Araneae, Agelenidae) funnel-web spiders, long sequences of vibratory courtship typically precede mating. To determine the potential for these courtship signals to maintain reproductive isolation among funnel-web spider species, male courtship vibrations were recorded from thirteen agelenid species. Several species were found to produce longer, multi-syllable signals, and these showed clear temporal and amplitudinal patterning. The vibratory signals produced by similar courtship behavior patterns were compared across species to determine their species-specificity. While some significant differences were found in the temporal structure of male courtship signals, many species’ signals were indistinguishable. Reproductive isolation between most *Agelenopsis* species does not appear to be maintained by differences in the structure of their vibratory signals.

Introduction

Substrate-borne vibration is an important and often overlooked sensory modality for many groups, including reptiles, anurans and mammals, but it is best known in arthropods (Hill 2001). For small animals, transmitting vibrations through a substrate can provide less attenuation and greater range than transmitting sound through the air, though vibration is still a relatively close-range modality (Bennett-Clark 1998). One potential disadvantage to substrate-borne vibration is the need for the signaler and the receiver to be on a contiguous substrate (Bennett-Clark 1998). However, one group of arthropods produces their own effective and relatively private substrate: web-building spiders.

As a group, the Araneae rely heavily on vibratory signal communication transmitted not only through webs but other substrates such as leaf litter, water, soil and live plants (Barth 1982). Even jumping spiders, a group known for their reliance on visual signals, have recently been found to utilize a variety of vibratory signals during courtship (Elias et al. 2003). Spiders' bodies are covered in mechanoreceptors, including tactile hairs, trichobothria (fine, highly sensitive hairs) and slit sense organs (strain receptors in exoskeleton). Web building spiders possess relatively more slit sense organs, probably to better detect vibrations of the web, while ground spiders possess relatively more trichobothria, which are more sensitive to airborne vibrations (Foelix 2011). Vibratory signals may be utilized in prey capture (Masters, Markl & Moffat 1986, Virant-Doberlet et

al. 2011), social interactions (Buskirk 1975, Nørgaard 1956) and courtship (Table II-1) in this group.

In spiders, three mechanisms have been identified for the production of vibratory signals: tremulation, percussion and stridulation (reviewed in Uhl & Elais 2011). Tremulation involves oscillations of body parts that are transmitted to the substrate through the legs, yielding low frequency signals. Percussion is produced by tapping the substrate with a body part or body parts against one another, creating broad frequency signals. Stridulation produces high frequency signals from the rubbing of two stiff body parts against each other. All three signal production mechanisms have been found to be used in spider courtship (Table II-1). Vibratory courtship has been hypothesized to serve several functions in spiders, including species recognition, mate quality assessment and the stimulation of females in the induction of mating (Uhl and Elias 2011, Table II-1).

Species recognition has been well-studied in *Schizocosa* wolf spiders and *Cupiennius* wandering spiders. In one of the very early studies of vibratory communication in the genus *Schizocosa*, Uetz and Denterlein (1979) noted that males of two morphologically similar species, *Schizocosa ocreata* and *Schizocosa rovneri*, will court heterospecific females, but females will discriminate males of the two species based on their courtship, which includes stridulation, tremulation and percussion. Both vibratory and visual courtship signals are used in this genus, and the relative importance of these signals varies among species, possibly because of variation in the signaling environment

(Hebets 2008, Hebets & Uetz 1999, Rundus, Santer & Hebets 2010, Uetz & Roberts 2002). Male *Cupiennius* wandering spiders produce vibratory courtship by tremulation and percussion on plants such as bromeliads (Schüch & Barth 1985). Interspecific crosses between three species found that vibratory signals were more important in species discrimination than female silk trails and differed among species in several temporal and amplitudinal characteristics (Barth 1993, Barth & Schmitt 1991).

The use of male vibratory signals as a mate quality signal has been suggested for several species and well-documented for the wolf spider *Hygrolycosa rubrofasciata* (Lycosidae). Females prefer males that abdomen drum at a higher rate; these males have greater viability and offspring that are more likely to survive (Alatalo et al. 1998, Kotiaho et al. 1996, Mappes et al. 1996). Similarly, mated *Leucauge mariana* females discriminate among conspecific males based on the duration of some aspects of male vibratory courtship, which is transmitted through the female's orb web. (Aisenberg 2009).

Several studies provide evidence for a female stimulating effect of male vibratory signaling, which leads to reduced female aggression towards the male and increased willingness to mate. For example, in *Stegodyphus lineatus,* male tremulation on the female's web is associated with shorter latencies to copulation with virgin females (Maklakov, Bilde & Lubin 2003). Male bowl-and-doily spiders, *Frontinella pyramitela,* exhibit a long, complex vibratory courtship on the web of

the female which appears to reduce female aggression, but does not affect the probability of copulation (Suter & Renkes 1984).

Form and function of vibratory signaling in the funnel-web spider genus Agelenopsis *(Araneae, Agelenidae)*

Males in the funnel-web spider genus *Agelenopsis* typically exhibit a long vibratory courtship sequence when placed on females' webs, both before and after mounting occurs (Chapter I herein, Singer et al. 2000). It is unlikely that female stimulation or aggression reduction is a very important function of courtship in these spiders because sexual cannibalism is not very common and males sometimes mount females without any preliminary courtship at all (Chapter I herein, Singer et al. 2000). Mate quality assessment is a possible function in *Agelenopsis*, as successful and unsuccessful male *Agelenopsis aperta* do differ in their vibratory courtship (Singer et al. 2000). Another function that seems likely to be important is species recognition. Many species in this genus have overlapping habitat requirements and geographic ranges, and as many as five species have been found syntopically (Paison 1997, personal observation by Riechert). Breeding seasons of the species also overlap, especially among closely related groups (Ayoub et al. 2005, Paison 1997). Thus, species-specific courtship signals may be necessary to prevent interspecific mating attempts. However, a comparative study of the genus found that closely related species used the same behavior patterns in courtship (Chapter I herein).

These behavior patterns included flexing the web with the legs, tremulating with whole body vibrations, bobbing the abdomen up and down, and wagging the abdomen side to side.

A hypothesis we explore in this study is that while male *Agelenopsis* courtship signals visually appear similar, they may transmit vibratory signals across the sheet web that are sufficiently different for species isolation. We thus examine the seismic patterns the males of various *Agelenopsis* species produce during vibratory courtship. Much effort has been spent on recording vibrations produced by non-web building spiders from various substrates (Elias et al. 2006, Gordon & Uetz 2011, Elias, Mason & Hoy 2004, Hebets et al. 2008, Quirici & Costa 2007, Schüch & Barth 1985, Sivalinghem et al. 2010), but surprisingly fewer attempts have been made to record vibratory signals from webs. Those studies that have looked at web-borne vibrations have found them to be of low frequency, with peak frequencies usually less than 100 Hz (Krafft 1978, Singer et al. 2000, Suter & Renkes 1984). Krafft (1978) examined courtship web vibrations of four species of amaurobiid spiders and one agelenid species and found differences among the species, even for similar behavior patterns (e.g. drumming the palps). Singer et al. (2000) recorded web vibrations produced by courting *A. aperta* males. Web vibrations included web flex signals composed of repeated syllables (individual bouts of raising and lowering the body), which decreased in amplitude over the course of a signal. Abdomen waggle signals, which did not have such clear temporal and amplitudinal structure, were also very common;

these showed a decrease in frequency during the course of the courtship sequence.

In this study, we examine the web vibratory signals for thirteen agelenid species. Species that share courtship behavior patterns are compared to determine if the vibrations produced by these behavior patterns differ in their temporal patterning, amplitude patterning or frequency characteristics. Since these behavior patterns are shared by species that vary in their phylogenetic relatedness and degree of sympatry (Figure II-1), the relationship between the species-specificity of signals and these two factors will be assessed.

Methods

Species collections and maintenance

Individuals of eleven *Agelenopsis* species, *Barronopsis texana*, and *Hololena nedra* were collected from sites throughout the continental United States (Table II-2). For most species, individuals were collected in the field as juveniles, reared in the lab, and then mated a few weeks after reaching maturity. *Agelenopsis kastoni* was an exception as field collections ended up producing sexually mature females, most of which had been mated in the field. Because mated females are known to be less attractive to and less receptive to potential mates in *Agelenopsis* (Riechert & Singer 1995, Singer & Riechert, 1995), offspring of the field-collected *A. kastoni* were reared in the lab for use in the vibration recordings. Also, half of the vibration recordings for *H. nedra* were

performed with the offspring of field-collected individuals, to increase sample sizes. *Barronopsis texana* and *H. nedra* were included in this study for two reasons. First, both species are sympatric with several *Agelenopsis* species, indicating a potential need for reproductive isolation. Second, *Agelenopsis*, *Barronopsis* and *Hololena* are part of the agelenid subfamily Ageleninae, and thus have been considered closely related due to morphological similarity by many workers (Bennett & Ubick 2005). Recent molecular work also supports a sister relationship between *Agelenopsis* and *Barronopsis* (Ayoub et al. 2005, Miller et al. 2010). Thus, these two species are suitable outgroups with which to compare *Agelenopsis* vibratory signals.

Small spiders were housed individually in round plastic cups (3.0 cm height x 4.2–5.6 cm diameter), while larger (> 100 mg, approximately) spiders were housed individually in round plastic dishes (3.0 cm height x 11.0 cm diameter). Once per week, juveniles' containers were misted with water and they were fed a satiation diet of termites, crickets, and adult *Drosophila*. Adults were fed crickets, also once per week. Spiders were maintained on a light-dark cycle of approximately 12L:12D, at temperatures of 20-25ºC.

Vibration recordings

Web vibrations were recorded as part of a larger study on courtship behavior (Chapter I herein). Eighty-one trials were conducted in total (Table II-2). Trials were recorded with a digital camcorder (Sony HDR-HC3 HDV 1080i

Handycam®, New York, NY) and later coded using an event-recording system (Noldus Observer 5.0, Wageningen, Netherlands).

Prior to the introduction of a male, each female was released into a transparent plastic arena that consisted of a clear plastic cylinder (10.0 cm x 3.0 cm diameter) attached to a round clear plastic box (6.5 cm x 15.5 cm diameter). This arena provides the structure necessary to building a web funnel and capture sheet equivalent to what an adult female *Agelenopsis sp.* would construct in nature. At the start of a courtship trial, the lid was removed from the arena, and the arena was placed in a larger plastic tub, so that the male or female could leave the arena without escaping into the laboratory. The male was introduced onto the center of the sheet portion of the web at trial initiation.

Vibrations were recorded from webs produced by captive females of the respective species with a Polytec LDV-100 laser doppler vibrometer. To accomplish this, a small square of retro-reflective tape (approximately 0.2 mm x 0.2 mm) was attached to the sheet portion of the web in all trials. The web was positioned so that this reflective spot was directly below the laser. The vibration signals were processed through an Oros vibration and noise analyzer (Model OR24, Dulles, VA USA) which quantified amplitude relative to an internal reference calibration (0 dB = 1 V) and saved the recording as a .wav file. Since courtship trials were generally rather long (over one hour), vibrations were recorded intermittently throughout each trial in five to ten minute segments.

General vibration analysis

Segments of the vibration recordings with high quality signals from specific behavior patterns (defined in Table II-3) were identified using the software program Cool Edit (Syntrillium Software Corporation, Phoenix, AZ). Avisoft SASLab (Avisoft Bioacoustics, Berlin, Germany) was used to analyze these segments. For all signals, peak frequencies were determined by examining amplitude spectra. To avoid pseudoreplication, measurements for each trial were averaged prior to the completion of the statistical tests.

Analysis of particular signal types

Multi-syllable signals. For signals that consisted of a series of repeated syllables (i.e. tremulation series and web flexes), the duration, the number of syllables, the maximum amplitude of each syllable and the duration of the intervals between the syllables' peaks (points of maximum amplitude) were determined. Since these signals showed changes in amplitude and interval duration over time, we examined the pattern of these changes in more detail. To quantify changes in amplitude over the course of a signal, two measures were calculated for each signal (Figure II-2). First, the syllables with the highest and lowest peaks were identified and the ratio of their amplitudes was calculated (highest divided by lowest). Second, the relative position within the series of the syllable with the highest peak was determined. To do this, syllable amplitudes and syllable numbers were converted into percentiles, as in Singer et al. (2000).

This conversion compensates for differences among signals in syllable number and overall amplitude. The highest amplitude peak was given a percentile of 1, while the lowest amplitude peak was given a percentile of 0. Similarly, the first syllable in a series was given a percentile of 0, while the last syllable in a series was given a percentile of 1. To quantify changes in the temporal patterning of syllables over the course of a signal, two measures were calculated for each signal, similar to those calculated for amplitude (Figure II-2). First, the peak-to-peak intervals with the highest and lowest durations were identified and the ratio of their durations was calculated (highest divided by lowest). Second, the relative position within the series of the interval with the longest duration was determined. To do this, interval durations and interval numbers were converted into percentiles, in the same manner as syllable amplitudes and syllable numbers were. Species exhibiting the same signal type were compared with a MANOVA for the above variables. If significant differences were found overall, species were compared for each variable individually using an ANOVA, and significant ANOVAs were followed up with Tukey-Kramer tests for post hoc comparisons. The GLM procedure in the Statistical Analysis System software (SAS 9.2, SAS Institute Inc., Cary, NC) was used for all analyses of variance. In cases of nonnormality, variables were log-transformed for ANOVA. When variables were heteroscedastic, Welch's ANOVA was performed, as it is robust to departures from homoscedasticity.

Single syllable tremulation. Several species produced single syllables of tremulation that were not part of longer series. These single syllables were described with two variables, duration and peak frequency. Species exhibiting these signals were compared with a MANOVA, followed by ANOVAs and Tukey-Kramer post-hoc tests as was done for species exhibiting tremulation series.

Pauses between abdomen waggles. In several species, abdomen waggles tended to be repeated over and over with pauses of varying lengths, and the durations of these pauses were measured. Since males often switched back and forth between abdomen wagging and simple movement, pauses between movements were also included. The distribution of pauses was compared across species using a X^2 test using one second increments. This resulted in many cells having expected values below five, a potential problem for the X^2 test. However, grouping pauses together into longer increments to avoid this problem did not change the significance level of the test. The FREQ procedure in SAS was used for this analysis. Longer pauses (≥ 16 seconds) were uncommon and probably indicated breaks between separate bouts of activity. These pauses were not included in the X^2 test. For species exhibiting abdomen waggles, peak frequencies were compared with an ANOVA.

Alternation between tremulations, tremulation series and abdomen bobbing. Signaling in *A. utahana* was complex, involving alternation between tremulations, which were sometimes in series, and abdomen bobbing. Tremulations tended to be spaced fairly evenly in time, producing a fairly

continuous sequence of behavior, in contrast to the other species using tremulations, which typically occurred in discrete bouts of behavior. To describe signaling in *A. utahana*, the durations of intervals between single tremulations and the durations of intervals between tremulation series were measured. Also, the duration of syllables within a series was measured from the start of one syllable to the start of the next. This method differed from that used for other species as the peak-to-peak durations could not be measured for many recordings because the amplitude of the tremulations exceeded the range of the laser Doppler vibrometer. The combination of low intensity abdomen bobbing and high intensity tremulation made it difficult to record both signals simultaneously.

Results

Multi-syllable series

Males of *A. aleenae*, *A. emertoni*, *A. oklahoma*, *A. pennsylvanica*, *A. potteri*, *A. spatula* and *H. nedra* produced web vibrations by repeating the same behavior pattern to form a series of similar syllables. Within a series, amplitude typically peaked early on and the latter syllables were the weakest (Figure II-3). This trend was least clear in *A. emertoni* and *H. nedra*, and these species also exhibited the shortest series (Table II-4). Also, within a series, the duration of the interval between amplitude peaks tended to increase (Figure II-3). This trend was clear in the three *Agelenopsis* species, but *H. nedra* syllables were more evenly spaced throughout a series.

Males of four species, *A. emertoni*, *A. pennsylvanica*, *A. potteri* and *H. nedra*, primarily produced tremulation series (Table II-3, Figure II-5). The three *Agelenopsis* species bobbed their abdomens at the start of each tremulation syllable, while *H. nedra* males did not. A MANOVA comparing several signaling variables in these four species found that they differed significantly (Wilks' $\lambda = 0.0028$, $p < 0.0001$). ANOVAs found significant differences in three of seven individual variables (Table II-4). *Agelenopsis pennsylvanica* and *A. potteri* series had longer durations than *A. emertoni* and *H. nedra* series. There were also significant differences between species in the number of syllables per series, with *A. pennsylvanica* exhibiting the most syllables and *A. emertoni* exhibiting the fewest. Finally, the three *Agelenopsis* species differed from *H. nedra* in that the longest peak-to-peak interval typically occurred at the end of an *Agelenopsis* series, while *H. nedra* signals did not show any clear temporal patterning.

No significant differences were found in peak frequency, but it is worth noting that *H. nedra* signals typically showed two frequency peaks, one at a mean of 15.10 Hz and another at a mean of 20.59 Hz. The other species' tremulation series usually only had one clear frequency peak, and only the highest peak was included in the statistical analyses. Tremulation series were also observed in *A. utahana* (discussed in more detail below), but there were not enough data collected for this species to include in these analyses. Only two of the six *A. utahana* males observed produced tremulation series; three males did not court at all.

Males of three species, *A. aleenae*, *A. oklahoma*, and *A. spatula*, produced web flex signals (Table II-3, Figure II-6). In *A. oklahoma*, web flexes were immediately preceded by single bouts of tremulation that were much more intense than the web flexes. Individual web flex syllables were somewhat less distinct in *A. oklahoma* when compared to the other two species. Web flexes tended to consist of many individual flexes, but the exact number was quite variable (Table II-5). The two *A. oklahoma* males from Kansas had longer web flexes than the two *A. oklahoma* males from Texas, suggesting possible population-level variation, but sample sizes were too small to test for this because of limited subject availability. A MANOVA comparing eight variables describing web flexes in these three species found no significant difference overall (Wilks' $\lambda = 0.064$, $p = 0.29$).

Single tremulation syllables

In all species included in this study except *B. texana*, males are at least occasionally observed producing single syllables of tremulation. However, in some species this behavior is relatively uncommon and was not recorded frequently enough for statistical analyses. Recordings of this behavior pattern were obtained from at least three males in six species (Table II-6). A MANOVA comparing these species for the duration and peak frequencies of their signals found that they differed significantly (Wilks' $\lambda = 0.048$, $p < 0.0001$). An ANOVA comparing duration across these species found that they differed significantly (*F*

(5, 16) = 30.95; $p < 0.0001$) and post hoc tests revealed that *A. oklahoma* tremulations were longer than those seen in other species. In the other species, males usually start off a tremulation syllable intensely and then taper off rather quickly. In *A. oklahoma*, males maintain intense tremulation for several seconds and then either taper off into a web flex or move across the web. Peak frequency also differed across species (Welch's ANOVA, F (5, 6.42) = 17.89, $p = 0.0007$), but post-hoc tests did not reveal any significant pairwise differences between species.

Abdomen waggles

Males of *A. actuosa*, *A. kastoni*, *A. naevia* and *B. texana* often abdomen waggle and/or move across the web for extended periods of time, interrupted by occasional, brief pauses (Figure II-7). The distribution of these pauses differed significantly among species ($X^2 = 91.12$, $df = 45$, $p < 0.0001$). *Agelenopsis kastoni* males took relatively few very brief pauses (< 2 seconds) while *B. texana* males took relatively many longer (> 10 seconds) pauses. In addition to the previously mentioned four species, *A. aleenae* and *A. spatula* males frequently abdomen waggle, usually alternated with web flexing. An ANOVA comparing the peak frequency of abdomen waggles in these six species (Table II-7) found no significant differences among them (Welch's ANOVA; F (5, 8.91) = 0.99; $p = 0.48$).

Signals and patterns in A. utahana and A. oregonensis

Agelenopsis utahana males commonly signal by producing tremulations, either singly or in short series (mean = 4.6, range = 2-8). These tremulations will often be interspersed with abdomen bobbing in a comparatively complex sequence (Figure II-9). Abdomen bobbing produced signals of much lower amplitude than tremulation did. Abdomen bobbing was so subtle, it was sometimes difficult to detect in video recordings and even during live coding. Despite being much lower in amplitude than tremulation, abdomen bobbing produced signals of similar form and peak frequency (tremulation: 18.91 Hz, abdomen bobbing: 19.10 Hz). Tremulation series were only recorded from two males, making generalization difficult, but they did not clearly follow the general *Agelenopsis* trends of decreasing amplitude and increasing interval duration during a series. These series averaged 1.6 seconds per syllable, often involved movement across the web at the start, and occurred on average 52.8 seconds apart. Single tremulation syllables occurred between series and very rarely involved movement across the web. They were 9.5 seconds apart on average.

Agelenopsis oregonensis males often mounted females without any web vibration, and only one good quality recording of *A. oregonensis* signals was obtained (Figure II-9). This sequence of tremulations was similar to the spacing of *A. utahana* tremulations, but abdomen bobbing between tremulations was not evident. Note that this behavior did occur in other trials from which vibrations were not recorded. There was one tremulation series of two consecutive

syllables. The tremulation syllables averaged 0.8 seconds in duration, similar to the durations seen in most other species (Table II-6). These tremulations exhibited two peak frequencies, averaging 11.18 Hz and 36.02 Hz.

Discussion

In the genus *Agelenopsis*, there are several behavior patterns used by males to produce vibratory signals that are transmitted through the webs of females they are courting. Several of these common behavior patterns are exhibited by males of multiple species. This study found that the vibratory signals produced by some of these behavior patterns differ across species, but others were not found to differ significantly.

The effect of sympatry and phylogenetic relatedness on vibratory signaling

Sympatric species often have more divergent courtship signals than allopatric species (Höbel & Gerhardt 2003, Seddon 2005, Yang & Gerhardt 2006) Also, courtship behavior is often phylogenetically conserved, resulting in closely related species exhibiting more similar courtship signals than distantly related species (de Kort & ten Cate 2001, Price & Lanyon 2002, Slikas 1998). However, there was no clear relationship between sympatry or phylogenetic relatedness and the species-specificity of signals in this study.

Within the closely related trio of eastern species, the signals of two species, *A. pennsylvanica* and *A. potteri*, differed from the third, *A. emertoni*, but

not each other. All three species are sympatric show very little genetic divergence (Figure II-1, Ayoub et al. 2005). Thus, it would seem that all three species would require distinct signals. *Agelenopsis pennsylvanica* and *A. potteri* also exhibit more similar courtship sequences overall, perhaps indicating that they are more closely related to one another than to *A. emertoni* (Chapter I herein). Alternatively, the lack of differences in *A. potteri* and *A. pennsylvanica* signals may reflect the fact that *A. pennsylvanica* was collected in a part of its range that overlaps with *A. emertoni* but not *A. potteri*.

The three species that produced web flexes in this study did not differ from one another, though there is a qualitative difference in the courtship of *A. oklahoma*, as this species produces intense tremulations prior to web flexing. Also, web flexes in *A. aperta* courtship have fewer syllables than web flexes from the three species in this study (Singer et al. 2000). All four *Agelenopsis* species known to produce web flexes have ranges that seem to overlap in the southwest, but the two species with courtship most similar to one another, *A. aleenae* and *A. spatula*, have not been collected syntopically. These two species are also the most closely related to one another (Figure II-1). Thus, either relatedness or a lack of selection for reproductive isolation could be responsible for the similarity in their signals.

Abdomen wagging and single tremulations were phylogenetically and geographically widespread behavior patterns, yet the differences noted in these signals appear to be attributable to single species. Thus, they appear to be highly

conserved behavior patterns, perhaps reflecting their simplicity in comparison to the temporally and amplitudinally patterned multi-syllable signals.

The two outgroup species included in this study did not differ dramatically from *Agelenopsis* in the form of their vibratory signals. *Barronopsis texana* abdomen waggle signals were quite similar to those produced by *Agelenopsis* species, though it is worth noting that their overall courtship sequences are distinctive due to high activity levels and no tremulation. The tremulation series of *H. nedra* were somewhat distinctive because they lacked the pattern of increasing intervals between syllables that appear to be characteristic of *Agelenopsis*. Nevertheless, they were quite similar to *A. emertoni* in other respects. Overall, species seem to differ more in the behavior patterns used and the sequence of these behavior patterns (Chapter I herein) than in the fine details of the signals produced.

The form of vibratory signaling in funnel-web spiders

Overall, most interspecific differences in vibratory signals were found in temporal characteristics, and temporal and amplitude patterning was found in most repeated signals. Temporal and amplitude patterning of substrate-borne vibrations are important in species recognition in many taxa (lacewings: Henry & Wells 2010, Wells & Henry 1992; leafhoppers and planthoppers: Claridge 1985; stink bugs: Vibrant-Doberlet & Čokl 2004, wandering spiders: Barth 1993, Barth & Schmitt 1991, Schmitt, Schuster & Barth 1994), perhaps because vibrations at

different frequencies transmit at different speeds, thus degrading any frequency modulation (Bennett-Clark 1998). In contrast, airborne signals produced by vertebrates often have species-specific frequency characteristics (Braune, Schmidt & Zimmermann 2008, Rand, Ryan & Wilczynski 1992, Seddon 2005)

Frequency seems to be relatively unimportant in distinguishing funnel-web spider species. The magnitude and even the number of frequency peaks varied quite a bit between and within trials, though peak frequencies for all signals were typically below 50 Hz. The most probable explanation for this variation is that frequency transmission depends on the location of both sender and receiver on the web, as well as the structure of the web. For example, preliminary recordings of signals made from the funnel retreat portion of the web had higher peak frequencies (40-60 Hz) than recordings from the sheet portion of the web. Resonance is also important in funnel-webs, with the fundamental frequency in the range of 5-10 Hz, with many overtones under 200 Hz (Naftilan 1999). These frequencies depended on several attributes of the web, including its radius and thickness, which varied among webs in this study. Also, the placement of the reflective square from which vibrations were detected varied from web to web, as a relatively flat surface was necessary for maximum reflection and accuracy. This variability in transmission from web to web and spot to spot suggests that small frequency differences are unlikely to be very important in species recognition. This variability was not noted in the previous study of *A. aperta* signals, possibly

because the webs used were more homogenous and all recordings were made from the very center of the sheet.

The function of vibratory courtship in funnel-web spiders

While some species signaling with the same behavior pattern differed in the characteristics of the vibrations produced, many did not differ. This suggests that species recognition is not the primary function of these signals in all species. Two closely related pairs of species (*A. pennsylvanica*/*A. potteri* and *A. aleenae*/*A. spatula*) produce indistinguishable, but long, repeated series of signals. The long duration of courtship in most species studied combined with the fact that males of many species will continue courtship even after female acceptance suggests courtship is under sexual selection, perhaps indicating mate quality in some way. Courtship may also serve a physiological purpose, stimulating one or both partners to copulate, though it is not always necessary (Chapter I herein). Further work to determine whether and how closely related species distinguish conspecifics and heterospecifics is needed.

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Appendix II

Table II-1 Examples of vibratory courtship diversity in spiders Mechanisms of production include percussion (P), tremulation (T) and stridulation (S). These are indicated along with the part of the body used. Proposed functions of courtship signals include species recognition (SR), mate quality assessment (MQ), female stimulation/aggression reduction (FS) or unknown (UK).

Spider taxa	Mechanism	Substrate	Function	Reference
Ctenidae				
Cupiennius spp.	T (abdomen) P (palps)	Plants	SR	Barth & Schmitt 1991
Dipluridae				
Thelechoris karschi	T (legs, palps, whole body), P (legs)	Web	MQ, FS	Coyle & O'Shields 1990
Eresidae				
Stegodyphus lineatus	T (legs, abdomen)	Web	FS	Maklakov, Bilde & Lubin 2003
Linyphiidae				
Frontinella pyramitela	T (abdomen)	Web	FS	Suter & Renkes 1984
Lycosidae				
Hygrolycosa rubrofasciata	P (palps)	Dry leaves	MQ	Alatalo et al. 1998, Kotiaho et al. 1996, Mappes et al. 1996
Rabidosa rabida	S (palp)	Grassland plants	MQ	Wilgers & Hebets 2011
Schizocosa spp.	S (palp), P (legs) T (abdomen, body)	Leaf litter	SR MQ	Uetz & Denterlein 1979 Gibson & Uetz 2008
Mecicobothriidae				
Mecicobothium thorelli	T (whole body), P (legs, palps)	Web	FS	Costa & Pérez-Miles 1998
Pholcidae				
Pholcus phalangioides	T (abdomen, legs), P (legs)	Web	FS	Bartos 1998
Salticidae				
Habronattus dossenus	T (abdomen), P (legs), S (cephalothorax-abdomen)	Leaf litter, rocks, sand	UK	Elias et al. 2003
Phidippus clarus	T (abdomen)		MQ	Sivalinghem et al. 2010
Sparassidae				
Heteropoda venatoria	T (abdomen, legs), P (body)	Dry leaves	UK	Rovner 1980
Tetragnathidae				
Leucauge mariana	T (abdomen, whole body), P (legs, palps)	Web	MQ or FS	Eberhard & Huber 1998
Theraphosidae				
Eupalaestrus weijenberghi & *Acanthoscurria suina*	T (whole body, males), P (legs, females)	Soil	SR	Quirici & Costa 2007

Table II-2. Species, localities, and sample sizes for respective *Agelenopsis* species. See Table II-3 for description of behavior patterns.

Species	Collecting locality	Trials attempted	Behavior pattern	# of trials behavior pattern recorded	# of signals recorded per trial
A. actuosa	Rochester, WA	7	Abdomen waggles*	5	1-3
A. aleenae	Las Vegas, NM	8	Web flexes	6	1-22
			Abdomen waggles*	5	2-15
A. emertoni	Woodward, OK Russellville, AR	2 2	Tremulation series with bobbing	4	1-5
			Single tremulations	4	1-2
A. kastoni	Andersonville, TN	5	Abdomen waggles*	5	3-18
			Single tremulations	5	3-10
A. naevia	Morrilton, AR Lawrence, KS	5 5	Abdomen waggles*	5	1-6
A. oklahoma	Wichita Falls, TX Meade, KS	2 2	Web flexes Single tremulations	4 4	3-9 4-10
A. oregonensis	Silver Creek, WA	5	Single tremulations	1	3
A. pennsylvanica	Meade, KS Knob Noster, MO Topeka, KS	2 5 3	Tremulation series with bobbing	8	1-4
A. potteri	Dane County, WI	7	Tremulation series with bobbing	4	3-5
			Single tremulations	3	1-2
A. spatula	Quitaque, TX Canyon, TX	3 1	Web flexes Abdomen waggles*	4 3	1-12 4-6

Table II-2 (continued)

Species	Collecting locality	Trials attempted	Behavior pattern	# of trials behavior pattern recorded	# of signals recorded per trial
A. utahana	Cle Elum, WA	6	Single tremulations	3	6-8
			Tremulation series	2	4-9
			Abdomen bobbing	2	12-23
B. texana	Knoxville, TN	5	Abdomen waggles*	5	2-12
H. nedra	Mabton, WA	6	Tremulation series	4	3-6
			Single tremulations	3	3

*Abdomen waggle sample sizes are for peak frequency measurements.

Table II-3. Descriptions of male courtship behavior patterns analyzed in this study. Descriptions of web phase behavior patterns are modified from Singer et al. (2000) and Chapter I herein.

Behavior	Description
Abdomen waggle	Spider waves abdomen parallel to the web while walking across the surface of the web. It may be associated with laying silk and may also include brief stops of less than three seconds.
Bob abdomen	Spider alternately raises and lowers abdomen. It varies from a slow, distinct raising and lowering to fast, indistinct, low amplitude up and down movement.
Tremulate	Spider vibrates entire body, sometimes violently. It may drum pedipalps briefly at start. Can be a series of tremulation bouts.
Web flex	Male flexes web with walking legs, while raising his body; then retracts his legs while lowering his body. Each web flex (slow bounce) is comprised of several syllables. Usually the male also vibrates his legs and body during the course of the bounce.

Table II-4. Descriptive statistics and ANOVAs for differences in tremulation series characteristics between four funnel-web spider species. Highest amplitude syllable refers to the syllable within the series (converted to a percentile) that had the highest maximum amplitude. Amplitude ratio refers to the ratios of the amplitudes of the syllable with the highest maximum amplitude to the syllable with the lowest maximum amplitude within a given series. Longest interval refers to the longest peak-to-peak interval within a series (converted to percentile). Interval ratio refers to the ratio of the durations of the longest interval within a series to the shortest interval within a series. Different letters indicate significant differences in Tukey-Kramer post-hoc tests.

Variable	Species	Mean	Range of male means	Range of all signals	*F*-ratio*	*p*
Duration (s)	*A. emertoni*^{B}	7.6	5.9-8.2	4.2-11.4	21.98	<0.0001
	A. pennsylvanica^{A}	23.0	17.5-33.5	6.3-60.7		
	A. potteri^{A}	23.9	17.9-30.8	15-50.1		
	H. nedra^{B}	6.4	5.2-7.4	3.5-8.3		
Number of syllables	*A. emertoni*^{C}	4.9	4.5-5.3	3-7	11.10	0.0003
	A. pennsylvanica^{A}	11.4	5.0-15.0	3-17		
	A. potteri^{AB}	9.7	8.0-11.3	4-15		
	H. nedra^{BC}	5.9	4.5-7.3	4-9		
Highest amplitude syllable	*A. emertoni*	0.30	0.17-0.42	0.00-0.50	1.29	NS
	A. pennsylvanica	0.26	0.00-1.00	0.00-1.00		
	A. potteri	0.11	0.00-0.41	0.00-0.90		
	H. nedra	0.24	0.10-0.37	0.00-0.67		
Amplitude ratio	*A. emertoni*	1.75	1.42-1.99	1.13-3.46	0.41	NS
	A. pennsylvanica	3.42	1.73-5.34	1.24-8.36		
	A. potteri	3.91	1.69-8.00	1.10-16.81		
	H. nedra	3.64	2.28-6.64	1.03-20.55		
Longest interval	*A. emertoni*^{A}	1.00	1.00	1.00	15.55	<0.0001
	A. pennsylvanica^{A}	0.90	0.47-1.00	0.00-1.00		
	A. potteri^{A}	0.95	0.83-1.00	0.50-1.00		
	H. nedra^{B}	0.41	0.28-0.51	0.00-1.00		
Interval ratio	*A. emertoni*	1.77	1.30-2.53	1.07-3.37	2.04	NS
	A. pennsylvanica	3.53	2.21-8.43	1.09-15.30		
	A. potteri	3.76	2.38-6.36	1.03-15.18		
	H. nedra	1.82	1.44-2.31	1.11-3.40		
Highest frequency peak (Hz)	*A. emertoni*	23.44	18.82-33.25	10.54-33.25	3.06	NS
	A. pennsylvanica	15.60	10.44-18.96	4.12-54.46		
	A. potteri	17.35	11.50-21.91	4.50-25.02		
	H. nedra	16.00	14.81-20.92	11.32-57.24		

*ANOVA, df = 3, 16.

Table II-5. Descriptive statistics for web flex series characteristics in four funnel-web spider species. Highest amplitude syllable refers to the syllable within the series (converted to a percentile) that had the highest maximum amplitude. Amplitude ratio refers to the ratios of the amplitudes of the syllable with the highest maximum amplitude to the syllable with the lowest maximum amplitude within a given series. Longest interval refers to the longest peak-to-peak interval within a series (converted to percentile). Interval ratio refers to the ratio of the durations of the longest interval within a series to the shortest interval within a series.

Variable	Species	Mean	Range of male means	Range of all signals
Duration (s)	*A. aleenae*	16.1	10.8-25.0	4.6-44.0
	A. oklahoma	10.9	5.1-15.2	3.8-22.0
	A. spatula	11.5	6.1-17.8	5.7-30.1
Number of syllables	*A. aleenae*	23.2	13.4-39.4	11-66
	A. oklahoma	14.2	4.3-25.5	4-42
	A. spatula	17.2	10.0-24.5	10-40
Highest amplitude syllable	*A. aleenae*	0.17	0.10-0.34	0.00-0.88
	A. oklahoma	0.16	0.00-0.25	0.00-1.00
	A. spatula	0.15	0.03-0.30	0.00-0.88
Amplitude ratio	*A. aleenae*	16.06	6.13-24.95	2.03-62.59
	A. oklahoma	8.65	2.65-14.24	2.15-46.78
	A. spatula	11.37	8.13-13.47	3.47-32.09
Longest interval	*A. aleenae*	0.94	0.80-1.00	0.37-1.00
	A. oklahoma	0.85	0.75-1.00	0.65-8.46
	A. spatula	0.92	0.88-0.98	0.29-1.00
Interval ratio	*A. aleenae*	6.81	5.95-8.33	2.41-15.26
	A. oklahoma	5.89	1.89-9.25	1.16-16.31
	A. spatula	6.78	1.85-14.43	1.85-24.54
Low frequency peak (Hz)	*A. aleenae*	3.38	2.25-5.29	1.35-6.90
	A. oklahoma	2.23	1.08-3.85	0.65-8.46
	A. spatula	2.86	1.43-4.91	1.09-8.17
High frequency peak (Hz)	*A. aleenae*	17.41	12.41-22.64	9.56-31.17
	A. oklahoma	16.88	12.18-24.25	5.81-31.69
	A. spatula	15.10	12.30-17.99	9.39-21.03

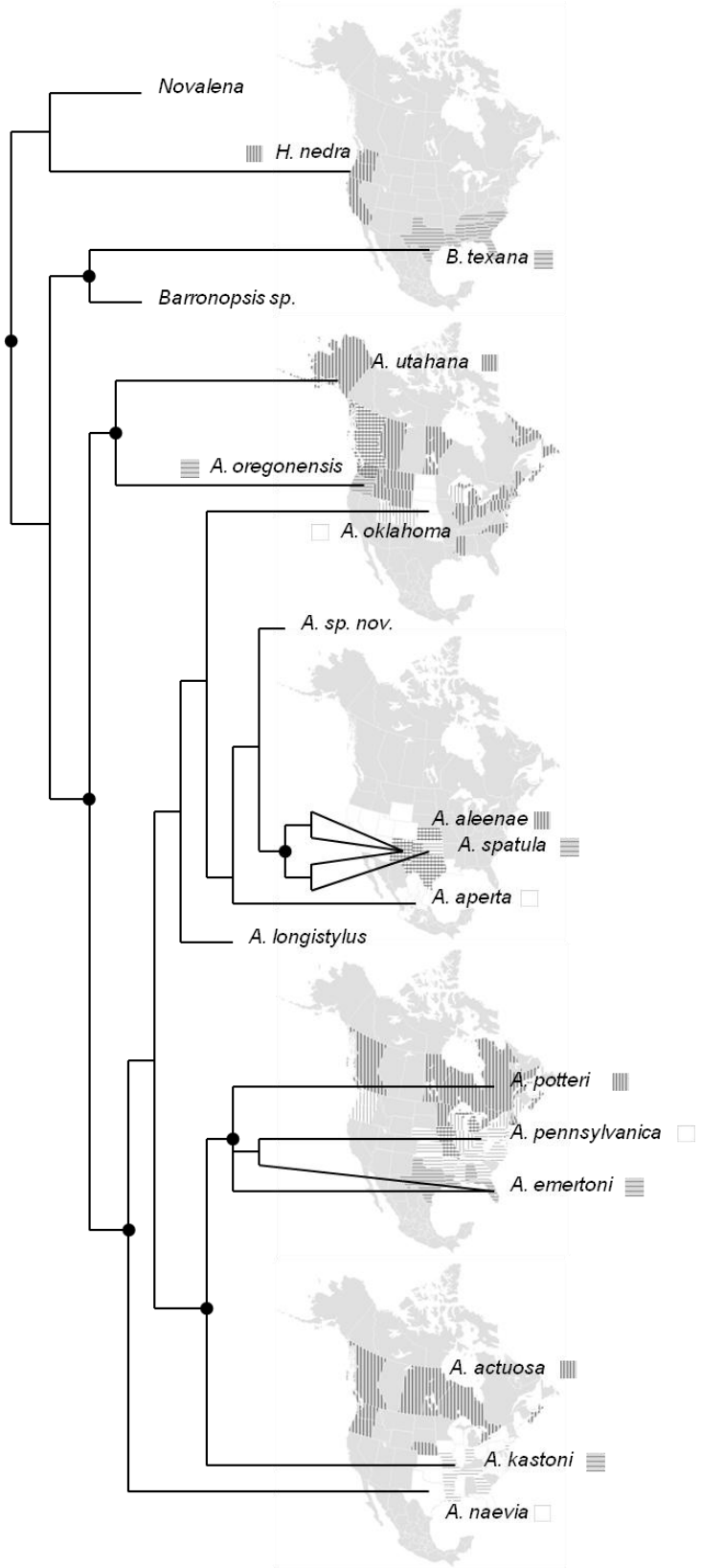
Table II-6. Descriptive statistics for single tremulation syllables in six funnel web spider species. Different letters indicate significant differences in Tukey-Kramer post-hoc tests.

Variable	Species	Mean	Range of male means	Range of all signals
Duration (s)	*A. emertoni*^{B}	1.0	0.7-1.2	0.6-1.2
	A. kastoni^{B}	1.3	0.8-1.8	0.6-2.8
	A. oklahoma^{A}	3.4	3.0-4.1	0.7-5.2
	A. potteri^{B}	1.5	1.2-1.8	1.2-1.9
	A. utahana^{B}	1.1	0.9-1.3	0.5-1.6
	H. nedra^{B}	0.7	0.6-0.8	0.5-0.9
Peak frequency (Hz)	*A. emertoni*	21.51	15.32-26.68	14.18-26.68
	A. kastoni	16.39	13.18-17.56	9.39-19.94
	A. oklahoma	18.09	14.85-20.04	12.52-26.81
	A. potteri	13.86	5.92-20.82	3.97-20.82
	A. utahana	18.91	16.29-21.24	8.65-43.77
	H. nedra	12.37	11.86-12.84	10.28-14.96

Table II-7. Descriptive statistics for abdomen waggles in six funnel-web spider species.

Variable	Species	Mean	Range of male means	Range of all signals
Peak frequency (Hz)	*A. actuosa*	15.27	10.59-18.59	5.79-18.59
	A. aleenae	17.23	13.78-20.72	2.96-25.57
	A. kastoni	16.09	12.37-17.33	9.20-23.22
	A. naevia	26.16	14.71-43.63	3.92-47.72
	A. spatula	14.51	9.76-21.75	2.06-45.85
	B. texana	17.22	15.00-20.08	13.38-23.22

Figure II-1. Phylogenetic relationships and geographic ranges for the funnel-web spiders in this study. Phylogenetic relationships are based on the maximum likelihood tree of COI and 16S mitochondrial DNA in Ayoub et al. (2005). Some species included in the phylogeny were not part of this study (*Novalena sp.*, *Barronopsis sp.* and *A. longistylus*), and one species in this study (*A. actuosa*) was not included in the molecular phylogeny. Nodes marked with a black circle had high (>80%) parsimony bootstrap support. Branch lengths are not informative. States included in geographic ranges are based on recorded collection localities (see Paison 1997, Stocks 1999 and Chamberlin & Ivie 1942) and our collections. This map is a modified version of a blank map of North America with subdivisions created by NuclearVacuum and distributed through Wikimedia Commons.


Novalena
H. nedra
B. texana
Barronopsis sp.
A. utahana
A. oregonensis
A. oklahoma
A. sp. nov.
A. aleenae
A. spatula
A. aperta
A. longistylus
A. potteri
A. pennsylvanica
A. emertoni
A. actuosa
A. kastoni
A. naevia

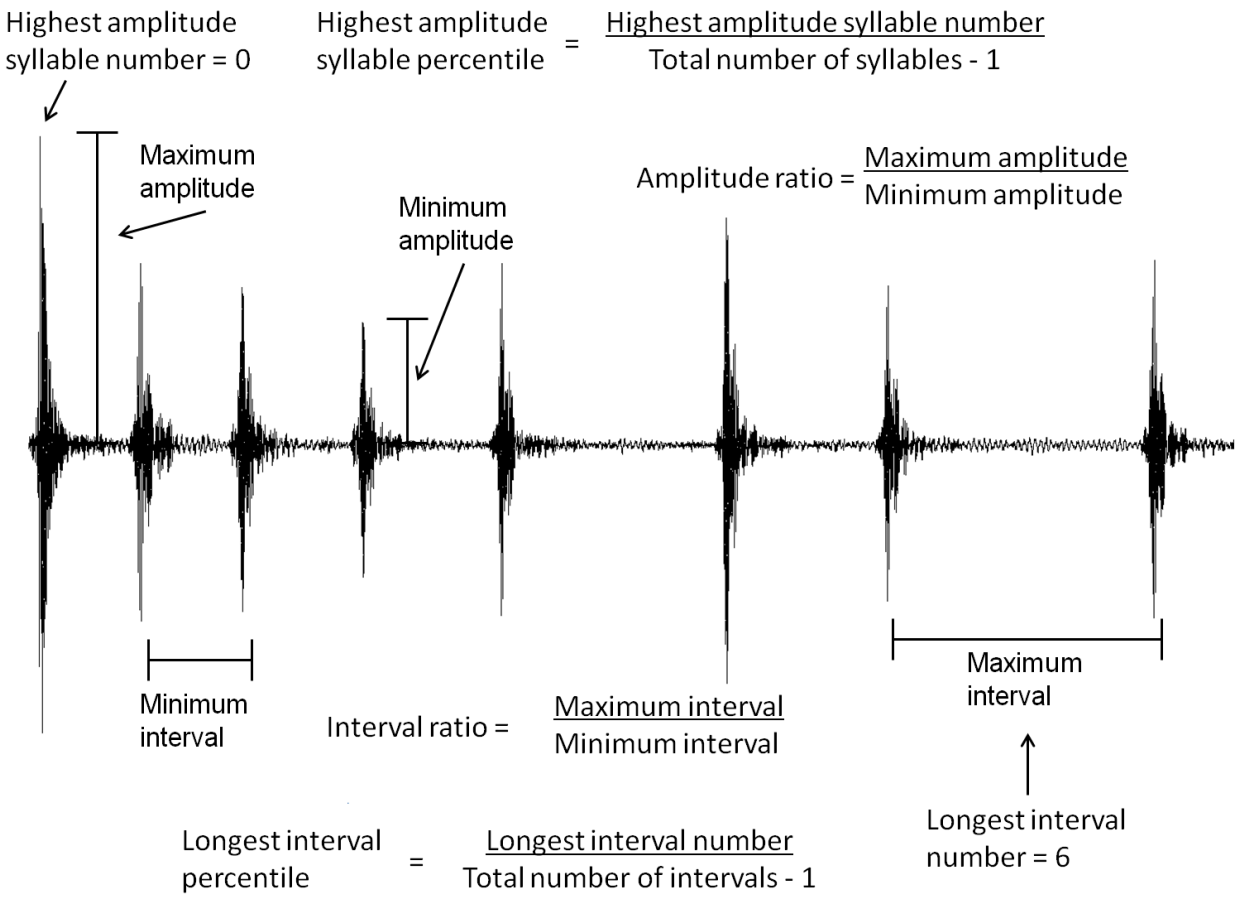


Figure II-2. Vibration signal measurements for multi-syllable signals.
Syllable and interval numbering begins with zero.

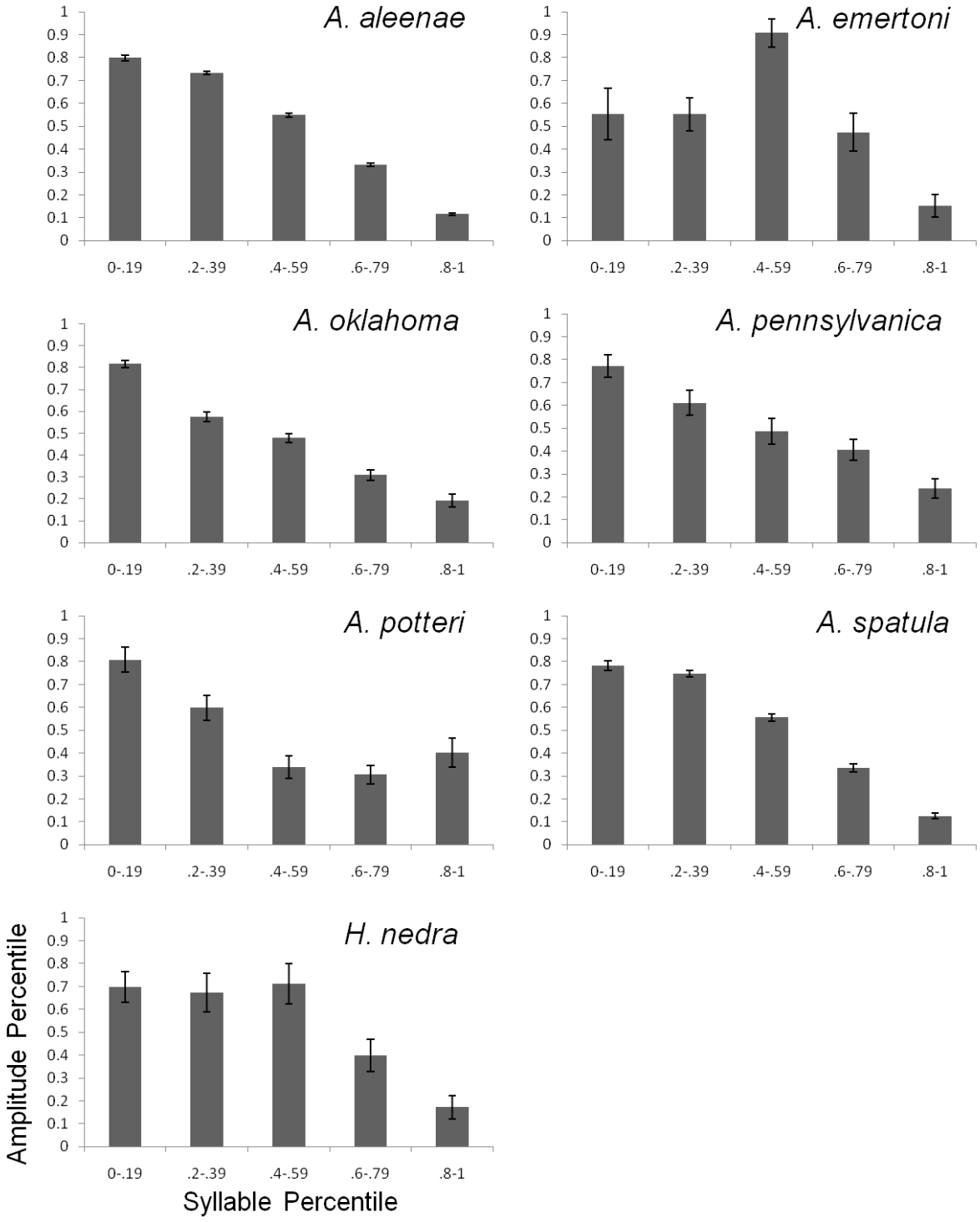


Figure II-3. Maximum amplitude (converted into percentiles, mean ± SE) in relation to syllable number (converted into percentiles) for seven funnel-web spider species that produce series of repeated syllables.

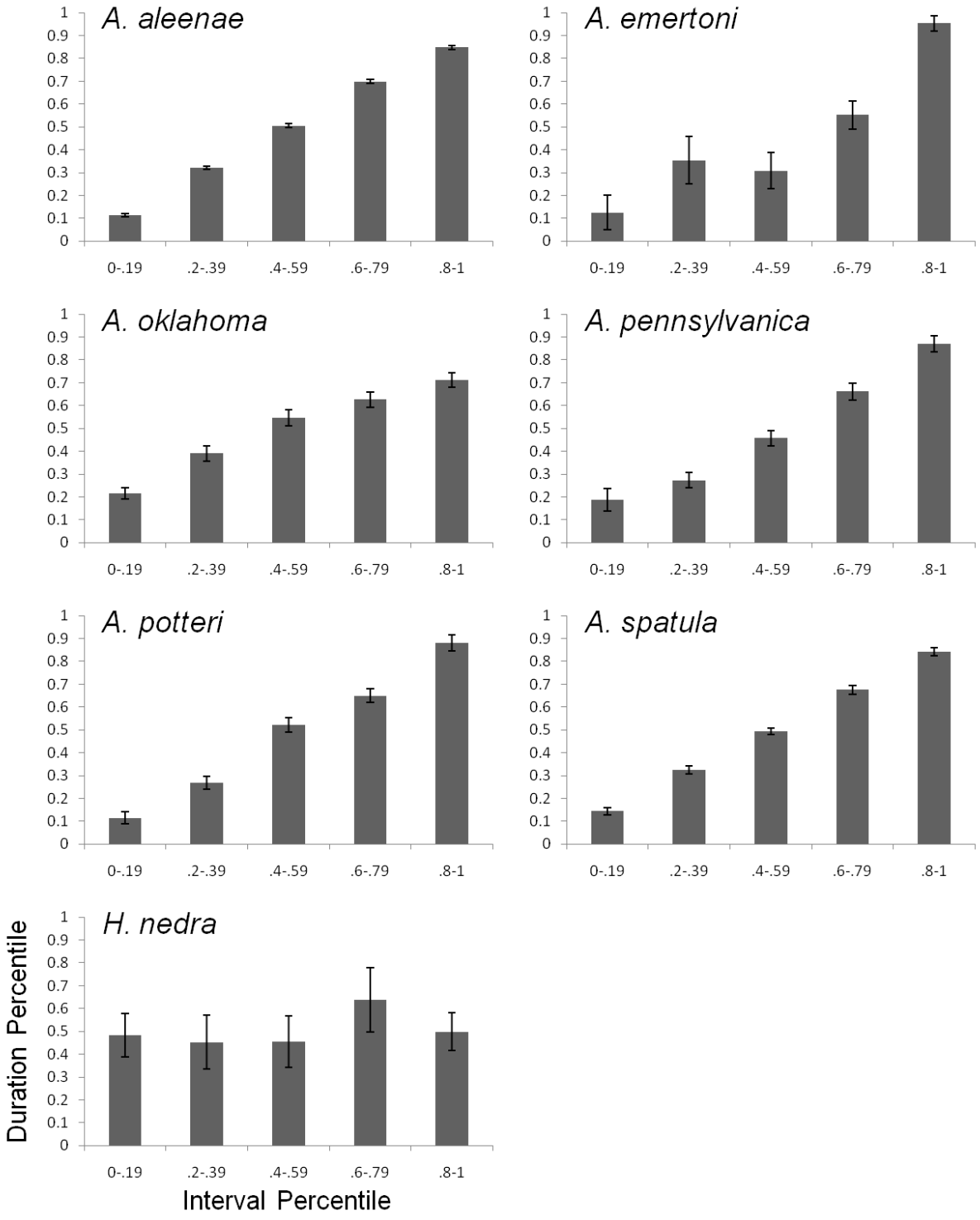


Figure II-4. Peak-to-peak interval duration (converted into percentiles, mean ± SE) in relation to syllable number (converted into percentiles) for seven funnel-web spider species that produce series of repeated syllables.

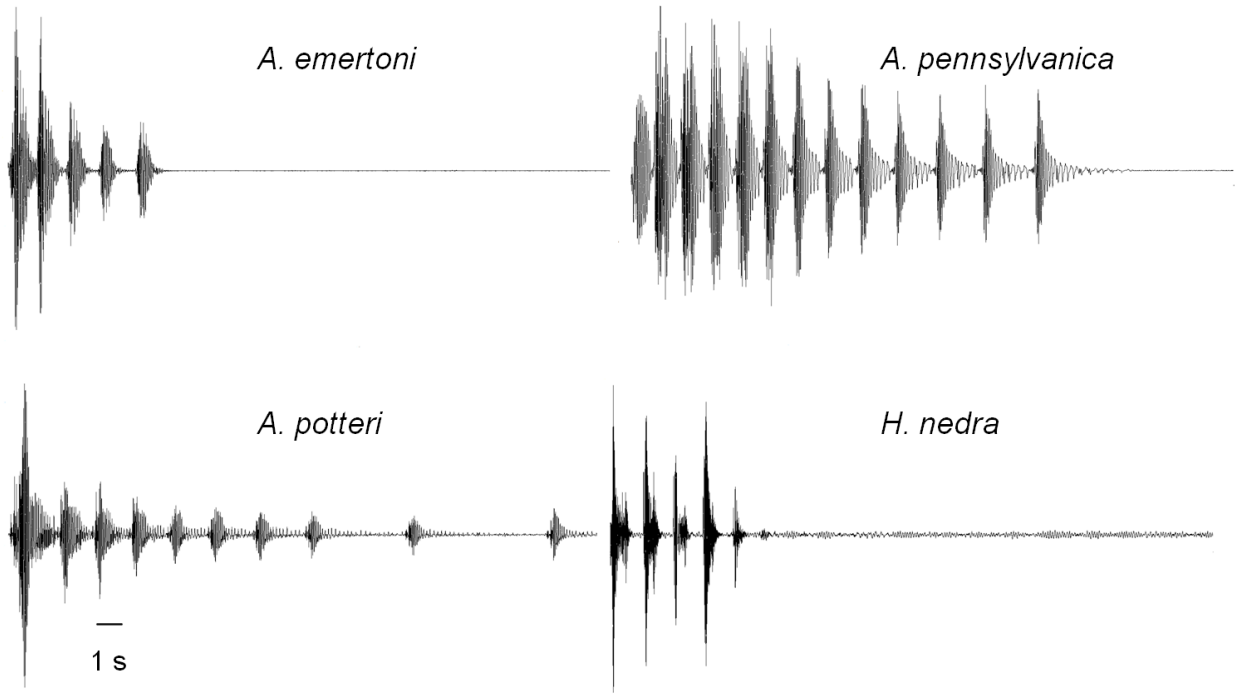


Figure II-5. Oscillograms of typical tremulation series for four funnel-web spider species. *Agelenopsis emertoni*, *A. pennsylvanica* and *A. potteri* males bob their abdomens at the start of each syllable; *H. nedra* males do not.

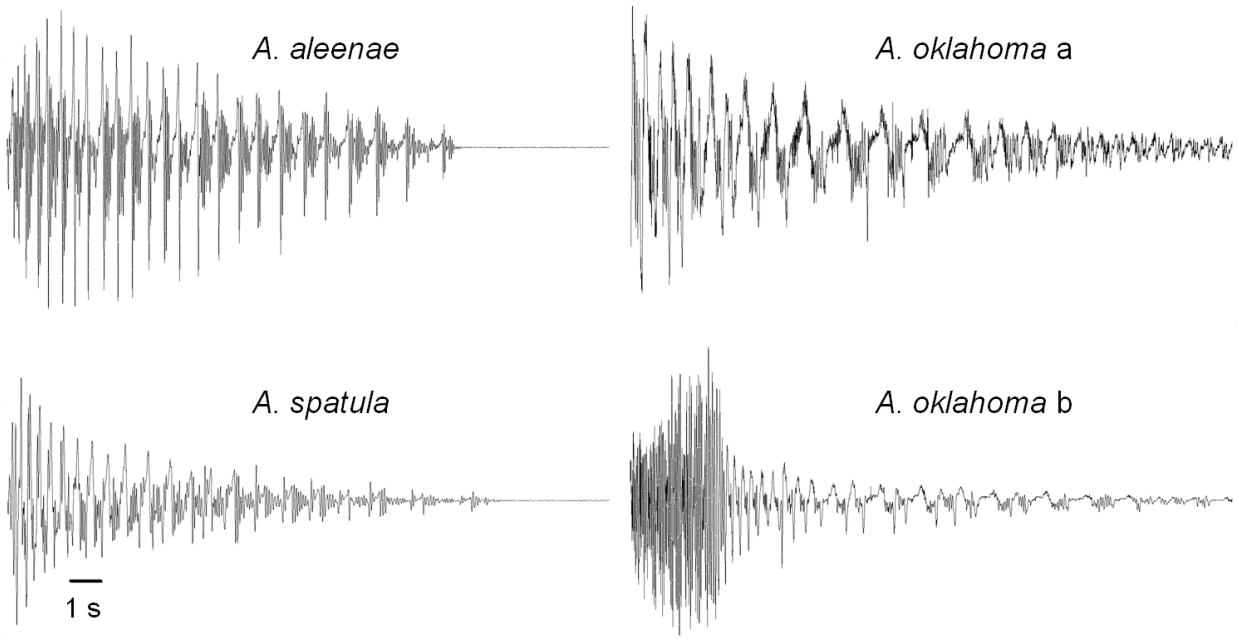


Figure II-6. Oscillograms of typical web flexes for three funnel-web spider species. An *A. oklahoma* web flex is pictured alone (a) and preceded by an intense bout of tremulation (b).

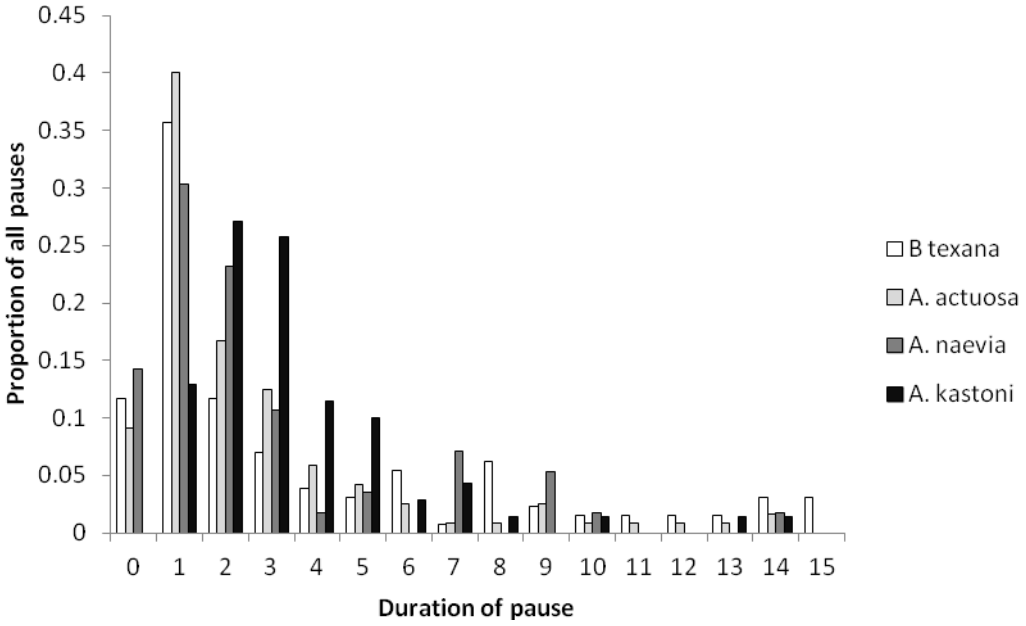


Figure II-7. Distribution of pause durations in abdomen waggle/movement sequences. Durations are grouped into one second increments, such that a duration of '0' indicates 0 ≤ duration < 1.

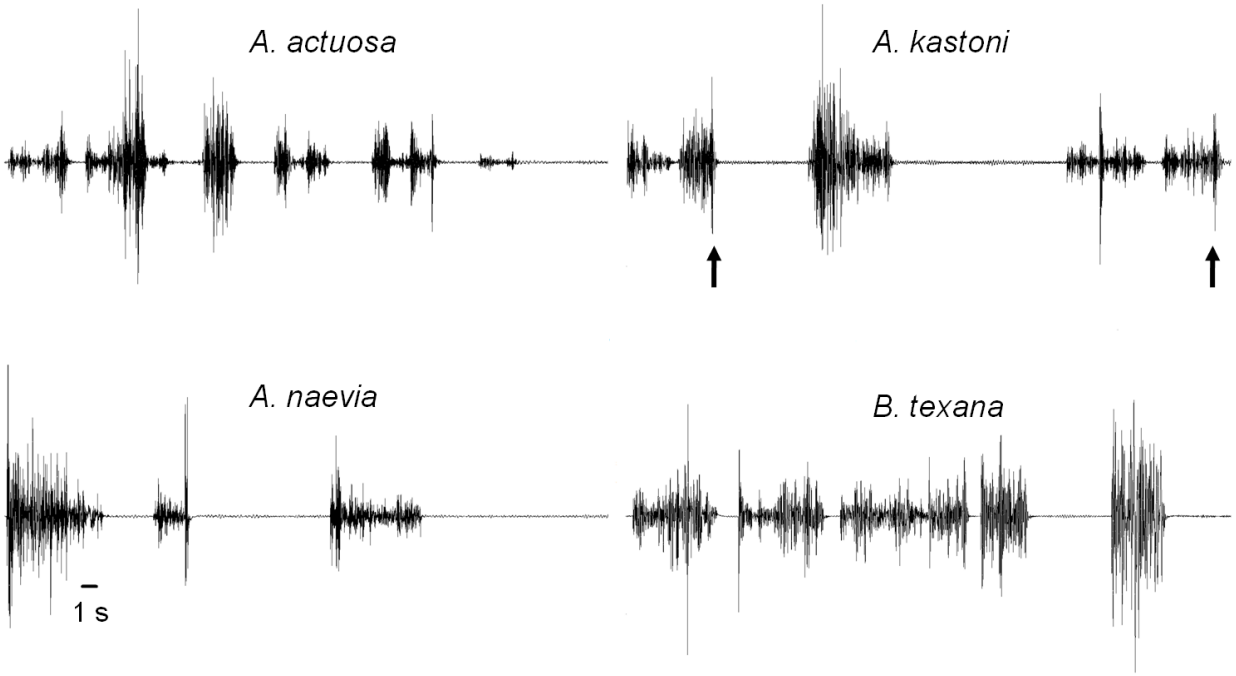


Figure II-8. Oscillograms of typical abdomen waggles for four funnel-web spider species. The arrows point at the single tremulation syllables often produced at the end of a bout of abdomen wagging in *A. kastoni*.

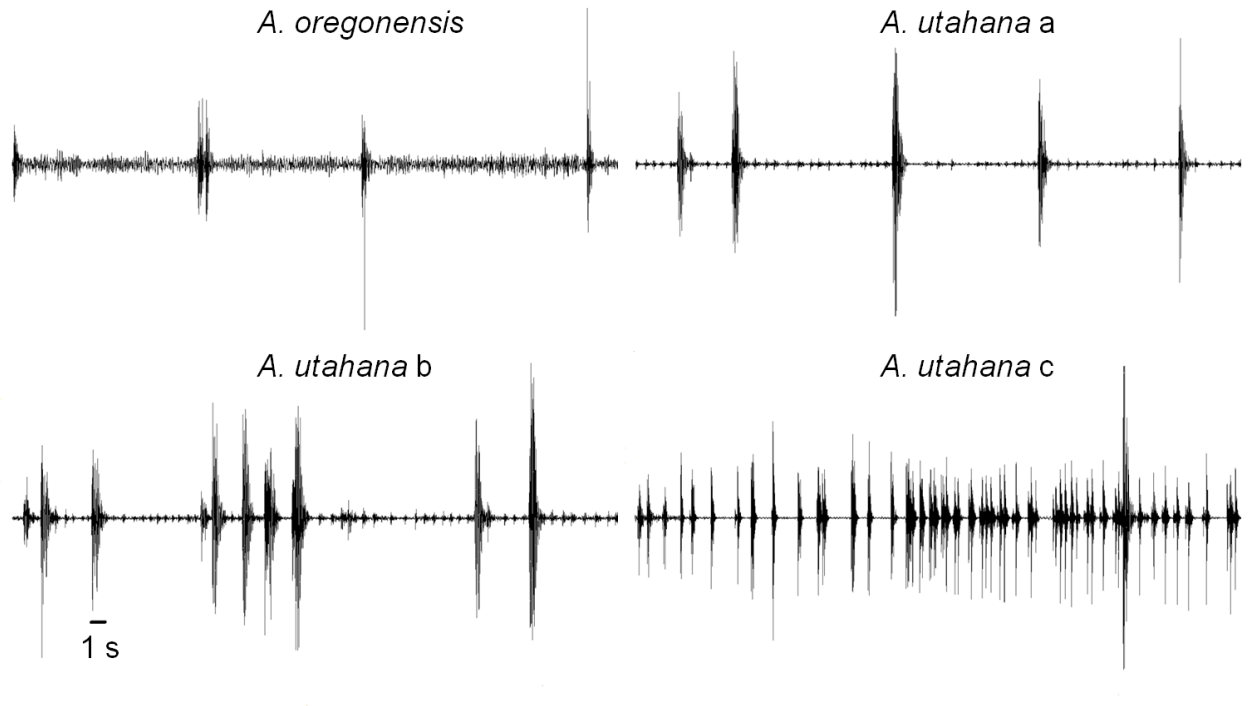


Figure II-9. Oscillograms of *A. oregonensis* and *A. utahana* vibratory signals. Single and double syllables of tremulation are shown for *A. oregonensis.* Three recordings are shown for *A. utahana*: (a) several single tremulation syllables interspersed with abdomen bobbing, (b) a tremulation series, with some movement, and single tremulation syllables interspersed with abdomen bobbing and (c) abdomen bobbing with just one tremulation syllable, recorded while the male was very close to the reflective dot. The single tremulation syllable in (c) exceeded the maximum amplitude range of the LDV, so its amplitude was actually greater than what is depicted here.

CHAPTER III : REPRODUCTIVE ISOLATION AMONG CLOSELY RELATED FUNNEL-WEB SPIDERS

This chapter is a modified version of an original research article to be submitted for publication by A. B. Galasso and S. E. Riechert.

My contributions to this paper include 1) formulation of the original research idea, 2) collection of almost all subjects and all data, 3) analysis of all data, 4) construction of figures and tables and 5) most of the writing.

Abstract

The evolution of reproductive isolation is vital in the creation and maintenance of biodiversity. Many species in the funnel-web spider genus *Agelenopsis* are known to co-occur and molecular evidence suggests this genus has undergone recent speciation. This study examined potential mechanisms of reproductive isolation in two closely related species groups that would permit species to coexist, while maintaining their respective identities. Web attraction trials were performed in which males were placed on empty webs recently constructed by conspecific females, heterospecific females, or conspecific juveniles. In two of four species studied, male courtship activity varied significantly based on the web source, and in all but one species, males courted the most on conspecific female webs. Mate choice trials were staged within and between species for two species pairs: *A. emertoni*/*A. pennsylvanica* (eastern) and *A. aleenae*/*A. spatula* (western). Males in the eastern pair exhibited significantly less courtship when

offered heterospecific females and no interspecific matings occurred. Thus, it appears that male choice based on chemical cues maintains reproductive isolation in these two sympatric and syntopic species. In contrast, no differences existed in the frequency of courtship exhibited between mixed species and within species pairings in the case of the western species. Though interspecific matings did take place as a result of the courting of heterospecific females in the case of the western species, these males appeared to encounter mechanical difficulties in copulating. This lack of reproductive isolation prior to copulation may explain the apparently peripatric distribution of the western species.

Introduction

Reproductive isolation from other species is the defining characteristic of 'biological species' (Mayr 1963). Reinforcement or the evolution of barriers to maintain the integrity of a species over time is thus a critical issue in the origins of biodiversity. Reinforcement occurs when populations that have diverged during a period of isolation come back into contact. If they are not fully differentiated genetically, they may produce less fit hybrid offspring than would be produced by within population matings. Reinforcement thus involves mechanisms that inhibit mating between individuals from different origin populations in incipient species (Dobzhansky 1937, Mayr 1963). Reinforcement favoring reproductive isolation occurs prominently in both parapatric and sympatric speciation, but will not be strongly selected for in allopatric speciation.

Thus stronger prezygotic reproductive isolating mechanisms should be observed between related species in areas of sympatry than in areas of allopatry. Further, this pattern may be observed even when the interacting taxa are fully genetically differentiated (not capable of producing hybrids) because misdirected courtship and copulations waste energy and gametes (Noor 1999). Note, however, that species may become reproductively isolated without interacting with one another, as a byproduct of other processes such as sexual selection (West-Eberhard 1983), ecological divergence (Schluter 1998) or drift.

This study investigates reproductive isolation among two species pairs of the funnel-web spider genus *Agelenopsis* (Araneae: Agelenidae). The degree of reproductive isolation between each group is unknown, but no evidence of hybridization has been found. From a molecular taxonomic study of the genus completed by Ayoub et al., (2005), we do know that the species within both pairs are closely related to each other. They also exhibit similar courtship sequences (Chapter I herein). Males of one species pair, *Agelenopsis aleenae* and *Agelenopsis spatula*, even produce indistinguishable vibratory signals from the same behavior patterns (Chapter II herein). These two species appear to have peripatric ranges and have not been collected syntopically (Ayoub et al. 2005, ABG & SER personal collections). In contrast males of the other species pair, *Agelenopsis emertoni* and *Agelenopsis pennsylvanica*, do differ in the structure of their vibratory signals and these species are sympatric and occasionally syntopic (Ayoub et al. 2005, ABG & SER personal collections, Chapter II herein).

Barriers to interbreeding between each of the two species pairs selected for study could act at several points in the mating process. In these spiders, it is the male that searches for potential matings, though a pheromone released by female *A. aperta* has been shown to both attract male *A. aperta* to the female's web and to elicit courtship behavior (Papke, Riechert & Schulz 2001). Female pheromones may well be species-specific, preventing interbreeding before courtship even occurs as males may not be attracted to females other than those of the same species. Males of several other spider species have been found to distinguish females based on chemical cues. For instance, male western black widow (*Latrodectus hesperus*) spiders not only distinguish between conspecific female webs and webs built by heterospecifics, but also between conspecifics from the same local population and those from distant populations (Kasumovic & Andrade 2004). Also males of the wolf spider *Schizocosa ocreata* (Lycosidae) court more intensely in response to the silk of conspecifics and more closely related species than in response to those of more distantly related species (Roberts & Uetz 2004).

Male courtship in the genus *Agelenopsis* entails the production of vibratory signals on the female's web. While significant differences exist in the courtship sequences among the 12 *Agelenopsis* species so far studied, males within these two species pairs utilize the same behavior patterns in courtship (Chapter I herein). Males of one species pair, *A. aleenae* and *A. spatula*, even produce indistinguishable vibratory signals from the same behavior patterns (Chapter II

herein). In contrast males of the other species pair, *A. emertoni* and *A. pennsylvanica*, do differ in the structure of their vibratory signals (Chapter II herein). Typically, females usually do not exhibit vibratory courtship of their own, though they may move around the web. Once any male has entered a web and initiated courtship, females may reject it by retreating or by exhibiting aggressive acts towards it. In the wandering spider family (Ctenidae), females have been shown to reject the vibratory courtship of most heterospecific males (Barth & Schmitt 1991). Females in pairs of closely related wolf spiders, *Schizocosa ocreata* and *Schizocosa rovneri*, also have been found to distinguish males based on their courtship behavior (Uetz & Denterlein 1979).

In the genus *Agelenopsis*, the successful male induces a quiescent or 'cataleptic' state in the female. For *A. aperta* at least, this is accomplished through the close range release of what is believed to be a heavy molecular weight pheromone (Becker et al., 2005). (The species-specificity of either the female or male produced pheromones is as yet unknown.) The male subsequently mounts the female and spends hours in copulation with her. If heterospecific males reach this stage of mating, they may still be unable to transfer sperm due to genitalic differences. The 'lock and key' hypothesis, which posits the evolution of genitalic differences to prevent interbreeding, was once widely held, but recently cryptic female choice on male genitalia and antagonistic coevolution of male and female genitalia have become more widely supported explanations of genitalic divergence (Eberhard 2010). Also, a detailed study of

agelenid genitalia and copulatory mechanics by Gering (1953) found that it was unlikely that genitalic differences could mechanically prevent interbreeding between most *Agelenopsis* species.

These three phases of the mating process will be examined in this study to determine the extent of reproductive isolation that might be attributed to potential courtship and mating barriers within each of the two species pairs examined. First, the response of males to webs built by conspecific and heterospecific females within a group will be examined to determine the species-specificity of female silk cues. Results from the two groups can be compared to determine whether sympatry has a strong effect on the species-specificity of these cues. Also, the courtship of intraspecific and interspecific pairs will be observed and its success will be determined for the *A. aleenae*/*A. spatula* and *A. emertoni*/*A. pennsylvanica* species pairs. Lastly, copulations will be observed to determine if there are any mechanical difficulties in interspecific matings.

Methods

The species

Agelenopsis aleenae and *A. spatula* are two of three *Agelenopsis* species that occupy both arid and riparian habitats in the desert southwest United States (Paison 1997). *Agelenopsis aleenae* is paraphyletic with respect to *A. spatula* and *A. aperta.* This last species is the likely sister group to the *A. aleenae*/*A. spatula* clade (Ayoub et al. 2005). *Agelenopsis aperta* has been found to co-

occur with the other two species, but *A. aleenae* and *A. spatula* appear to be geographically isolated with peripatric ranges (Ayoub et al. 2005, Riechert & Galasso, personal observation). Other than range differences, *A. aleenae* and *A. spatula* differ only in their genitalic morphology, and these differences are subtle. On the basis of genitalic similarity, *A. aleenae* was once hypothesized to be a hybrid between *A. aperta* and *A. spatula*, but this has been disproven (Paison 1997, Ayoub et al. 2005). In comparison with the other two species, *A. aperta* tends to be larger, matures a few weeks earlier in the season, and has a much larger geographic range (Paison 1997).

We also chose two of three closely related Agelenopsis species, *A. emertoni* and *A. pennsylvanica* that occupy temperate deciduous forest habitats primarily in the eastern half of the United States for study here. The ranges of these two species and the third, *Agelenopsis potteri*, overlap in the Midwestern US, with *A. potteri* having the northernmost distribution. *Agelenopsis pennsylvanica* and *A. potteri* have apparently disjunct ranges, as both species have also been collected in the northwestern US. These three species have overlapping habitat requirements, including wooded areas and areas around buildings, though *A. potteri* and *A. pennsylvanica* may require greater humidity. Each species has a distinctive genitalic morphology, but otherwise they are morphologically similar (Paison 1997). All three species breed in mid to late summer. Ayoub et al. (2005) found very little mitochondrial DNA sequence divergence between these species, and they hypothesized the three might form

one polymorphic species. *Agelenopsis emertoni* and *A. pennsylvanica* were selected for this study because both species could be collected from the same geographic area (Knoxville, TN) where they are known to breed simultaneously. Thus, there should be selection for reproductive isolation in these populations if they are indeed separate species.

Collections and rearing

The spiders used in this study were primarily field-collected as juveniles and reared to maturity in the lab; available 2nd generation lab-reared individuals were also used. *Agelenopsis aperta* individuals were collected as heterospecific test species for the web attraction trials. Most individuals of this species were collected in Canyon, Texas, but some individuals also came from Carrizozo, New Mexico. All of these sites are within the range of either *A. aleenae* or *A. spatula*. All *A. aleenae* individuals used were either collected in Las Vegas, New Mexico or were the offspring of spiders collected there. All *A. spatula* subjects were either collected in Quitaque, Texas or were the offspring of spiders collected there. *Agelenopsis emertoni* and *A. pennsylvanica* were all field-collected in Knoxville, Tennessee. Because juvenile males were less common than juvenile females, some *A. emertoni* and *A. pennsylvanica* males were collected after maturing in the field. All females were collected as juveniles to ensure their virginity. Finally, *A. potteri* subjects used as heterospecific females in the web

attraction trials were collected in Dane County, Wisconsin, and a few lab-reared individuals from that population were also used.

Spiders were kept in the lab individually in round plastic condiment cups (3.0 cm height x 4.2–5.6 cm diameter) until they reached approximately 100 mg when they were transferred to round plastic dishes (3.0 cm height x 11.0 cm diameter). We fed and misted all individuals with water weekly. The diet consisted primarily of crickets; young instar juveniles additionally received termites and adult *Drosophila*. All individuals were kept on a light-dark cycle of approximately 12L:12D, at temperatures of 20-25ºC.

Web attraction trials

Web attraction trials were conducted to determine whether or not males distinguish among the web-borne chemical cues produced by females of different species. For each species pair, males were tested on webs built by conspecific adult females, conspecific juveniles and on webs built by heterospecific females. The heterospecific females used were those from the other member of the pair as well as an additional, closely related heterospecific (*A. aperta* for the western species pair and *A. potteri* for the eastern species pair).

Males were introduced onto webs built by conspecific females, heterospecific females and conspecific juveniles. Each male was exposed to all possible web types in a repeated measures design. Thus, each male was tested in four trials, and these trials were at least 24 hours apart. The order of

presentation was randomly determined for each male. After introduction, male behavior was observed for 15 minutes and recorded using an event-recording system (Noldus Observer 5.0, Wageningen, Netherlands). Behavioral categories used were the same as those used in Chapter I of this dissertation. Juvenile webs were used as a control for the mechanical cues provided by the web.

To obtain the webs used in these trials, spiders of the various classes needed were allowed to build webs in round plastic boxes (6.5 cm deep x 15.5 cm diameter) for at least 24 hours prior to testing. Mature females were removed from their webs immediately prior to male introduction to guarantee any web-borne chemical cues would not have time to degrade. Juveniles were removed in advance of the trials, typically several weeks prior to male introduction. (Since spiders of a given species typically mature at about the same time, juvenile webs had to be constructed before mature females and males were available.) All trials were conducted no more than three days after a females' last meal on a particular web. This was done because male spiders of other species have been found to detect female hunger status from silk cues and to avoid courting hungry females (Johnson et al. 2011).

Male courtship duration was compared across web types with the Friedman test, as the data were not normally distributed. These and all other statistical analyses were conducted with the SPSS statistics software package (version 16.0, IBM, Armonk, NY). Male courtship duration was defined as the total time spent on three key courtship behaviors: abdomen wagging (moving

abdomen side-to-side while walking across the web), tremulating (vibrating whole body intensely) and web flexing (moving body up and down by extending and retracting legs). In the three eastern species, males tremulate repeatedly, in a series, and each tremulation is accompanied by a bob of the abdomen. Web flexing is only exhibited by the three western species.

Mating trials

Mating trials were performed by placing males on females' webs while the females were present and recording male and female behavior. We terminated a trial when either a) two hours of male activity had passed without catalepsis being induced, b) the male did not exhibit courtship behavior within the first hour, c) the male failed to move for one hour, d) the male did not begin copulation within one hour of inducing catalepsis or e) one spider left the web. One trial was also terminated when the female attacked the male. For these trials, females were allowed to build webs in plastic arenas consisting of the round containers used in the web attraction trials with plastic cylindrical retreats (10.0 cm x 3.0 cm diameter) attached to one side. These arenas approximate the dimensions of a typical female web in nature. The actions of both male and female individuals were coded as in the web attraction trials.

Trials were performed within and between species for two species pairs. Males and females were assigned to interspecific or intraspecific trials at random, with the goal of ten trials per species/sex combination, though sample size was

sometimes limited by the availability of subjects. Trials were performed within *A. aleenae* (n = 10) and *A. spatula* (n = 10), between *A. spatula* males and *A. aleenae* females (n = 11) and between *A. aleenae* males and *A. spatula* females (n = 6). For the eastern species pair, trials were performed within *A. emertoni* (n = 11) and *A. pennsylvanica* (n = 9), between *A. emertoni* males and *A. pennsylvanica* females (n = 10) and between *A. pennsylvanica* males and *A. emertoni* females (n = 10).

Several aspects of courtship were quantified and compared by means of Mann Whitney U tests (the data did meet the assumptions of normality) to compare courtship behavior between interspecific and intraspecific trials. The overall activity level was determined for males and females by dividing the total time spent on any type of movement by the duration of the trial, not including any time the male spent mounted on the female. To determine if there were more aggressive interactions in interspecific trials, the percent of time spent on agonistic behaviors was divided by the duration of the trial for both males and females. Agonistic behaviors included raising the front pair of legs, striking at a partner with the front pair of legs and chasing or retreating from a partner. Finally, the amount of courtship-specific behavior exhibited by males was determined by calculating the amount of time spent on the three courtship behaviors described for the web attraction trials. The percent of total courtship duration spent on exhibiting these behaviors was calculated. Also, the amount of time spent on these behaviors during the first fifteen minutes of a trial was determined. This

measure should be less affected by any attempts by the female to reject the male than courtship from the entire trial. Additionally, it allows for comparisons between male behavior with a female present and male behavior during the web attraction trials. Finally, the relationship between trial success (i.e. ending in copulation) and trial type (intraspecific vs. interspecific) was assessed for both species pairs by means of a X^2 test.

Copulation success determination

The first thirty minutes of copulation were observed for the successful courtships: male behavior was coded as before. Additional observations of copulation were performed by pairing individuals that did not mate during the courtship trials. Copulation was defined as the insertion of part of the male sperm-transfer organ, the palpus or palp, inside the female's genital opening, the epigynum. For the purposes of this study, male copulatory behavior was divided into three behavior patterns: grooming the palps, engaging the embolus (intromittant region of the palp which contains the opening to the ejaculatory duct) and embolus insertion. These behavior categories were adapted from Gering's (1953) description of copulation in agelenids. Prior to each palpal insertion, the male grooms his pedipalps by moving them between his chelicerae to clean and moisten them. After grooming, the male attempts to engage the embolus in the epigynum of the female by moving it back and forth across her abdomen. Once the tip of the embolus has engaged the male twists the entire

embolus into the female's reproductive tract until it locks into place and then sperm transfer commences. After sperm transfer, the embolus is removed and the entire process is usually repeated many times with each pedipalp inserted into the respective right and left receptacles of the female genital opening (epigynum).

To determine whether interspecific copulations encountered any mechanical difficulties due to differences in genitalic morphology, T-tests were performed on the total amount of time spent in each of the three copulatory behavior patterns between interspecific and intraspecific copulations. The adjusted degrees of freedom are reported for t-tests, for comparisons in which the variances were not equal. Total copulation duration of interspecific and intraspecific trials was also compared using a t-test. Finally, the relationship between egg sac production (laid at least one egg case vs. none) and trial type (interspecific vs. intraspecific) was assessed with Fisher's exact test. The X^2 test was inappropriate since most cell counts were below five.

Results

Web attraction trials

Agelenopsis pennsylvanica males exhibited high levels of courtship on webs built by females of conspecific females and the heterospecific *A. potteri*, but not on conspecific juveniles and *A. emertoni* female webs (Figure III-1). A Friedman's test found that male courtship duration differed significantly among

web types in *A. pennsylvanica* (X^2 = 16.16, *df* = 3, *p* < 0.001). *Agelenopsis pennsylvanica* males courted significantly more on webs built by conspecific females than on webs built by conspecific juveniles (*Z* = -2.70, *p* = 0.004) and webs built by *A. emertoni* (*Z* = -2.67, *p* = 0.004). Males also courted more on *A. potteri* female webs than on *A. emertoni* female webs (*Z* = -2.37, *p* = 0.008). Male *A. emertoni* courted the most on conspecific female webs. However, differences in male courtship duration on different web types only bordered on significance (X^2 =6.49, *df* = 3, *p* = 0.080), and *A. emertoni* males were less active overall than *A. pennsylvanica* males (Figure III-1).

A. aleenae males exhibited high levels of courtship on conspecific female webs and low levels on other types of webs (Figure III-2). In contrast, *A. spatula* males were not very active on any web type and they courted nearly as much on *A. aperta* webs as they did on conspecific female webs. Differences in courtship duration across web type were significant in *A. aleenae* (X^2 = 16.16, *df* = 3, *p* < 0.001), but not *A. spatula* (X^2 = 1.17, *df* = 3, *p* = 0.78). Pairwise comparisons indicated *A. aleenae* males courted significantly more on *A. aleenae* female webs than juvenile webs (*Z* = -2.37, *p* = 0.008); other pairwise comparisons did not reach significance at the Bonferroni-adjusted alpha level.

Mating trials

Male *A. emertoni* and *A. pennsylvanica* exhibited a higher incidence of courtship behavior in intraspecific trials than interspecific trials, both during the

first 15 minutes of a trial (Z = -2.56, p = 0.010) and during the entire trial (Z = -3.57, p < 0.001, Table III-1). Male and female agonistic behavior and total activity did not differ between interspecific and intraspecific trials. No interspecific courtship trials ended in mating, while 9 out of 20 intraspecific trials ended in mating. This difference was significant (X^2 = 11.61, p = 0.001, Figure III-3).

In *A. aleenae* and *A. spatula*, intraspecific and interspecific courtship trials did not differ frequency of courtship trials that ended in mating (X^2 = 0.47, p = 0.72, Figure III-3). The only difference found in male or female courtship behavior was a decrease in female activity in interspecific trials (Z = -1.98, p = 0.048, Table III-1).

Copulation success

Copulation was observed in six intraspecific pairs (four *A. aleenae* pairs and two *A. spatula* pairs) and six interspecific pairs (two *A. aleenae* male/*A. spatula* female pairs and four *A. spatula* male/*A. aleenae* female pairs). Intraspecific copulations appeared to be more successful than interspecific copulations. During the first 30 minutes of copulation, males in intraspecific pairs spent significantly more time with their emboli inserted into the females' epigyna than males in interspecific pairs (t = 3.56, df = 5.20, p = 0.015; Figure III-4). The amount of time the male's embolus was inserted in the female during the first 30 minutes correlated negatively with the number of insertions during that period (r = -0.62, p = 0.031). Males in intraspecific pairs also spent more time grooming their

palps and engaging the embolus, but these differences were not significant (grooming: t = -1.73, p = 0.12, engaging embolus: t = -1.21, df = 5.56, p = 0.28). Only intraspecific copulation was observed in *A. emertoni* (n = 7) and *A. pennsylvanica* (n = 5), and the time spent on copulation behavior patterns in these species was similar to what was observed in *A. aleenae* and *A. spatula* intraspecific copulations.

Total copulation duration was quite variable, and did not differ between interspecific and intraspecific *A. aleenae*/*A. spatula* trials (t = 1.64, p = 0.13). Intraspecific trials averaged 6.8 hours of copulation (SE = 1.4 hours) while interspecific trials averaged 3.7 hours (SE = 1.3 hours). Intraspecific copulations in *A. emertoni* and *A. pennsylvanica* averaged 5.1 hours (SE = 0.6 hours).

All western females that were intraspecifically mated laid egg cases, but only one interspecifically mated female laid an egg case, and this difference was significant (Fisher's exact test, p = 0.015). The one egg case from an interspecific mating did not hatch, while 37.5% of the intraspecific mating egg cases did.

Discussion

Reproductive isolation through chemical signaling

The males of one species from each region varied their courtship activity significantly among the different web types, while males of the other species from the respective regions did not. Inspection of the web attraction results suggests that males are not stimulated to court by juvenile webs, but they are stimulated to

court by the web-borne chemical cues of conspecific females and those of some heterospecific females. Also, when females were present, eastern males clearly discriminated conspecifics and heterospecifics. These results indicate a potential for pheromonal differences to maintain reproductive isolation among funnel-web spider species. A number of studies have looked at species recognition via pheromones in spiders and the results have been mixed (reviewed in Gaskett 2007). Some closely related species appear to share long-range attraction pheromones but utilize species-specific close-range pheromones. This study found that chemical cues from webs alone were about equally species-specific in the eastern and western species groups, but when females were present on their webs, eastern males distinguished between conspecific and heterospecific females, while western males did not. This difference may reflect selection for discrimination of female cuticular pheromones in the sympatric *A. emertoni*/*A. pennsylvanica* pair, but not in the peripatric *A. aleenae*/*A. spatula* pair.

Another possible example of geographic distance resulting in a lack of reproductive isolation is the apparent inability of *A. pennsylvanica* males to distinguish females of *A. potteri* and *A. pennsylvanica*. Though these two species do have overlapping ranges, the *A. pennsylvanica* used in this study were collected from an area south of *A. potteri*'s range, where there would be no selective pressure for divergent chemical signaling. To determine whether this is truly a case of reproductive character displacement, *A. pennsylvanica* populations sympatric with *A. potteri* should be examined. There were not

enough *A. potteri* subjects to perform extensive crosses between these two species, but one pairing between an *A. potteri* male and an *A. pennsylvanica* female was attempted and informally observed. The male courted and copulated, but he seemed to encounter mechanical difficulties similar to those seen in *A. aleenae*/*A. spatula* pairings (discussed below). This suggests that *A. potteri* and *A. pennsylvanica* are not one polymorphic species, as suggested by Ayoub et al. (2005), but more data are needed on reproductive isolation between them.

The two species that did not show significant differences in courtship response to different web types, *A. spatula* and *A. emertoni*, exhibited comparatively little courtship on any web type. It is intriguing males of these two species also exhibited very little abdomen wagging in the web attraction trials. This absence is particularly surprising in *A. spatula* males, as they typically abdomen waggle frequently during courtship (Chapter I herein). Perhaps web-borne pheromones elicit different aspects of courtship behavior in different species. The airborne pheromone identified for *A. aperta* elicits normal levels of abdomen wagging but low levels of web flexing in comparison to courtship produced in the presence of a live female (Papke, Riechert & Schulz 2001). *A. spatula* males may only abdomen waggle when the additional stimulation provided by a live female is present. It may be that the lack of abdomen wagging lowered overall courtship activity to the point where it was not possible to detect significant differences with these sample sizes. Alternatively, the lack of abdomen wagging and courtship activity in general could indicate that females of

these two species produce very little web-borne pheromone. It could also be that males and/or females of these species were simply not sexually receptive in the lab setting. However, a lack of receptivity was not noted in the mating trials with these species.

One puzzling result from the web attraction trials was that *A. spatula* males exhibited about as much courtship on *A. aperta* female webs as they did on conspecific webs. Since these two species are sympatric and syntopic, this result was unexpected. The overall low level of courtship exhibited by *A. spatula* in these web attraction trials suggests this may be a spurious result of low sample sizes.

Reproductive isolation through vibratory courtship

Females are typically thought to be the more discriminating sex because of their greater reproductive investment in offspring (Trivers 1972). Female spiders have been found to discriminate between conspecific and heterospecific males based on their vibratory courtship (reviewed in Uhl & Elias 2011). However, this study found little evidence of female choice based on vibratory courtship. Females did not exhibit more agonistic behavior with heterospecific males. The only significant difference in female behavior was a decrease in activity in cross species trials in the case of the western species pair. The lesser activity, however, did not reduce the overall success rate of those courtships. Female funnel-web spiders do not exhibit any clear acceptance signals other

than allowing males to mount them and becoming cataleptic. The lack of interspecific matings in the eastern species pair did not result from females resisting males' efforts to mount them. Rather, males did not attempt to mount heterospecific females at all and they exhibited significantly less courtship behavior toward them. This was true even during the first fifteen minutes of the trials. Thus, it seems that male choice, not female choice is primarily responsible for preventing interspecific copulations.

The somewhat surprising importance of male choice may be due to the predatory, solitary nature of spiders. Males that approach unreceptive females may be attacked or killed, providing a significant fitness advantage for males to identify appropriate, receptive females before initiating courtship. Sexual cannibalism is rare in these spiders (but see Riechert et al., 2001 for an exception [genetic mixing as a result of directional gene flow]): only one female in this study attacked her male partner (an *A. pennsylvanica* intraspecific trial). Another possible explanation for male selectivity is that males may not often have the opportunity to mate multiply. In a field study of mating in *A. aperta*, Singer and Riechert (1995) found that 91% of males mated only once. Courtship in *Agelenopsis* spiders is typically quite long, and the energetic cost of courting an inappropriate mate could be high.

Reproductive isolation through mechanical incompatibility

Interspecific copulations were observed in several *A. aleenae*/*A. spatula* trials, and these copulations appeared to encounter mechanical difficulties. Males in interspecific pairings averaged only half as much time with their emboli inserted as males in intraspecific pairings. This difference could significantly limit a male's opportunity to transfer sperm. It appeared that males in interspecific pairings had difficulty fully inserting and expanding their pedipalps. Males that made many insertions during the first thirty minutes tended to spend less time inserted overall, suggesting that these insertions were short and unsuccessful. However, some long, apparently successful insertions were observed in interspecific pairings, indicating that the mechanical difficulties were not always insurmountable. Copulation in *Agelenopsis* species lasts many hours and includes many insertions, and it is not clear how much of that time is necessary for successful insemination.

A study of copulation in the wolf spider *Pardosa agrestis* found that although copulation typically lasted more than two hours, experimentally shortening it to just 40 minutes had no effect on fertilization success or number of spiderlings produced (Szirányi, Kiss & Samu 2005). Even copulations lasting only 10 minutes often resulted in fertilized egg cases producing normal numbers of spiderlings. A survey of copulation patterns across dozens of species of wolf spiders found copulation durations varied from less than a minute to several hours (Stratton et al. 1996). In this study, intraspecific copulations in *Agelenopsis*

varied from three to 12.5 hours. It is possible that only a few successful insertions are actually needed to inseminate *Agelenopsis* females, and additional insertions serve only to influence cryptic female choice or limit other males' access to the female.

It is unclear whether or not *A. aleenae* and *A. spatula* are actually capable of producing fertile hybrids even if sperm transfer were successful. Only one interspecifically mated female laid an egg case, and it did not hatch. Even this one egg case may not indicate a successful insemination, as even unmated females will sometimes lay unfertilized egg cases. It appears that males are not often able to induce egg case production in heterospecific females. This raises the possibility that interspecifically mated females may remain sexually receptive to additional mates, as was seen in *Schizocosa malitiosa* wolf spider females that experienced copulations without sperm transfer (Aisenberg & Costa 2005).

The mechanical difficulties observed in *A. aleenae*/*A. spatula* crosses are rather surprising given the rather minor differences genitalic morphology between the two species (Paison 1997) and Gering's (1953) determination that most species of *Agelenopsis* should be physically capable of copulating with one another. This result is consistent with the lock-and-key hypothesis of spider genitalia. However, only allopatric populations have been observed copulating interspecifically in the lab. This suggests that naturally co-occurring species do not rely on mechanical incompatibility to maintain reproductive isolation. Rather, mechanical incompatibility may result in selection for the evolution of isolating

mechanisms earlier in the mating process. To further explore this issue, populations of *A. aleenae* and *A. spatula* closer to the boundary between their ranges should be examined. No *A. emertoni*/*A. pennsylvanica* pairs attempted interspecific copulation, but given the substantial differences in genitalic morphology between those species, mechanical difficulties would be very likely in such a pairing.

Conclusions

The results of this study indicate that chemical signaling plays an important role in maintaining reproductive isolation in sympatric species, but vibratory courtship does not. In the *A. aleenae*/*A. spatula* species pair, neither of these signaling types is sufficient to prevent interspecific matings. However, morphological differences in genitalia were found to have a negative effect on interspecific copulations when they occurred and these copulations were less likely to result in egg case production.

An examination of reproductive isolation can provide insight into the speciation process in funnel-web spiders. The results of this study suggest that genitalic divergence can cause mechanical incompatibilities between previously allopatric taxa. If such taxa remain in contact over many generations, reproductive isolation based on chemical signaling is likely to evolve. Since these results indicate a relationship between sympatry and degree of reproductive

isolation, future work should focus on comparing allopatric and sympatric populations within these species groups.

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Appendix III

Table III-1. Descriptive statistics and Mann-Whitney U test results for courtship trials within and between two western species, *A. aleenae* and *A. spatula*, and two eastern species, *A. emertoni* and *A. pennsylvanica*.

	Intraspecific		Interspecific		
Variable	Mean	Standard deviation	Mean	Standard deviation	*Z*
Female agonistic					
Eastern	2.8%	11.6%	0.4%	1.2%	-1.32
Western	0.1%	0.2%	0.1%	0.1%	-0.22
Male agonistic					
Eastern	0.4%	1.7%	0.2%	0.3%	-1.76
Western	0.3%	0.7%	0.1%	0.3%	-0.55
Female activity					
Eastern	4.7%	11.5%	5.9%	16.9%	-0.43
Western	3.2%	4.7%	1.0%	1.5%	-1.98*
Male activity					
Eastern	6.5%	5.6%	6.2%	6.4%	-0.78
Western	8.2%	8.4%	6.1%	6.8%	-0.95
Male courtship (first 15 minutes)					
Eastern	17.6 s	17.7 s	12.5 s	40.6 s	-2.56*
Western	23.2 s	44.7 s	22.9 s	50.5 s	-1.21
Male courtship (whole trial)					
Eastern	3.3%	3.4%	0.8%	1.9%	-3.57*
Western	6.2%	6.4%	5.3%	6.8%	-0.91

*$p < 0.05$

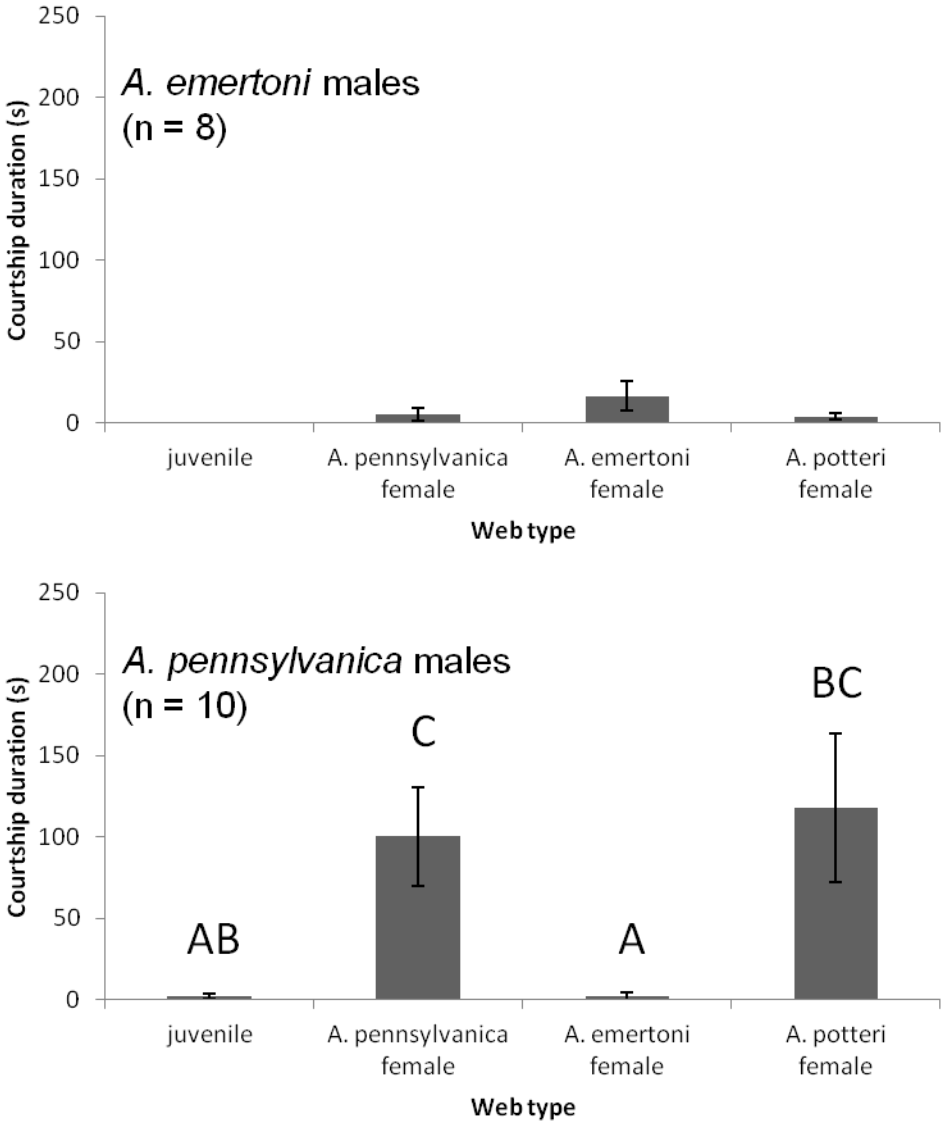

Figure III-1. Web attraction trial results for two eastern funnel-web spider species, *A. emertoni* and *A. pennsylvanica*. For each species, mean (± standard error) courtship duration exhibited by males during 15 minute trials is shown for all web types.

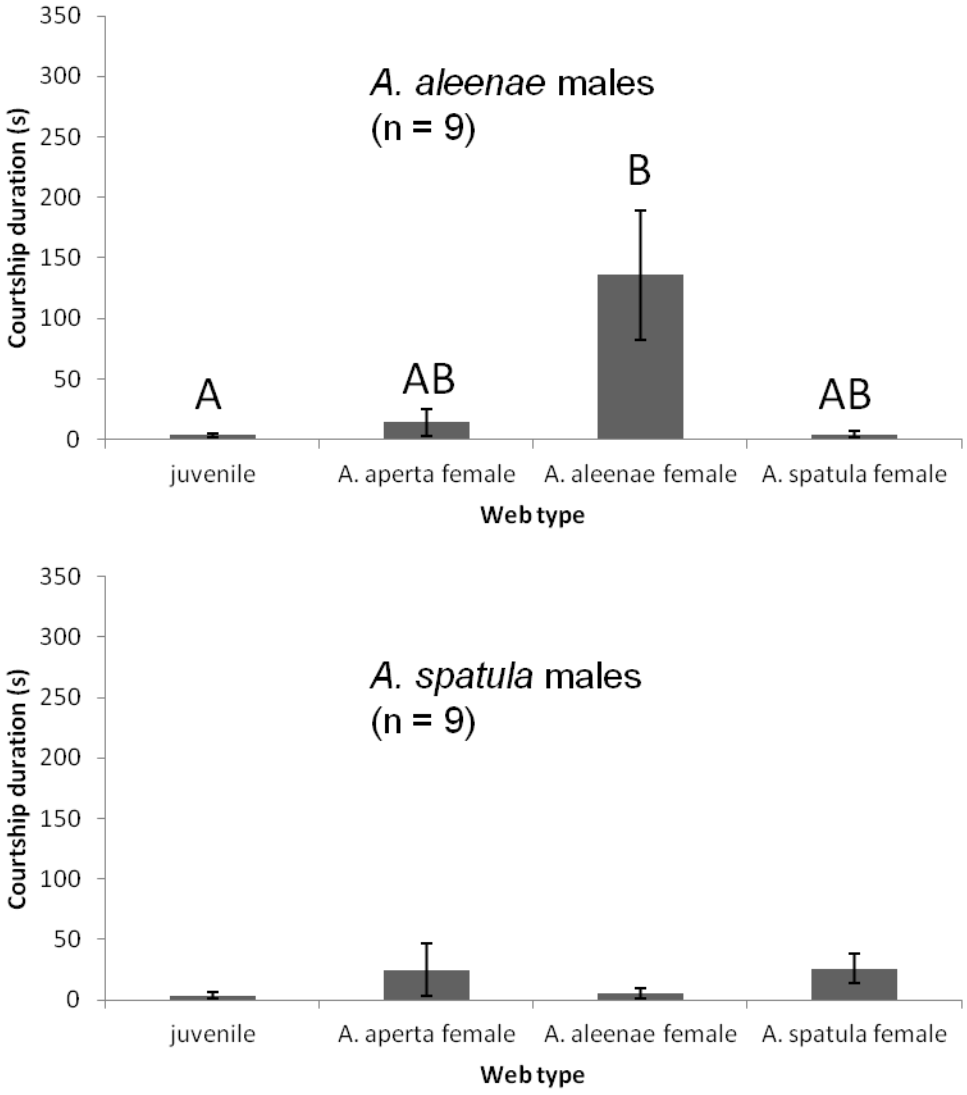

Figure III-2. Web attraction trial results for two western funnel-web spider species, *A. spatula* and *A. aleenae*. For each species, mean (± standard error) courtship duration exhibited by males during 15 minute trials is shown for all web types.

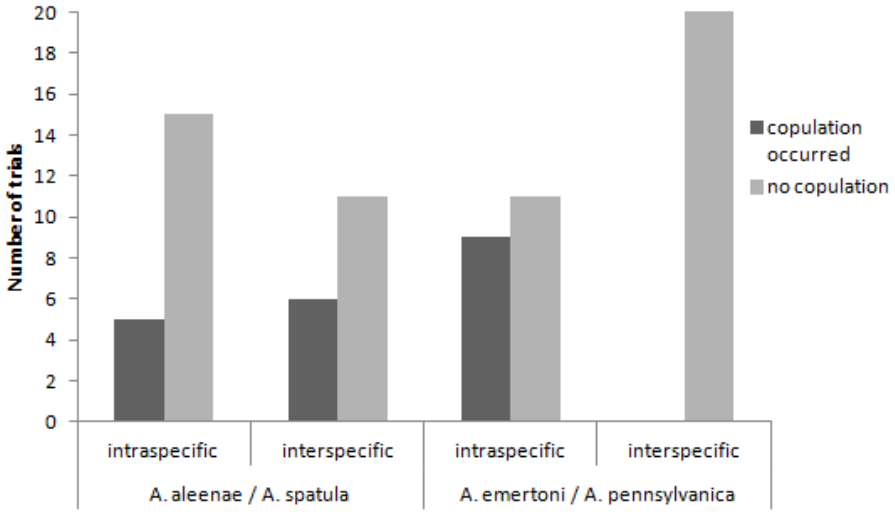

Figure III-3 Outcome of courtship trials by species pair and type (interspecific versus intraspecific).

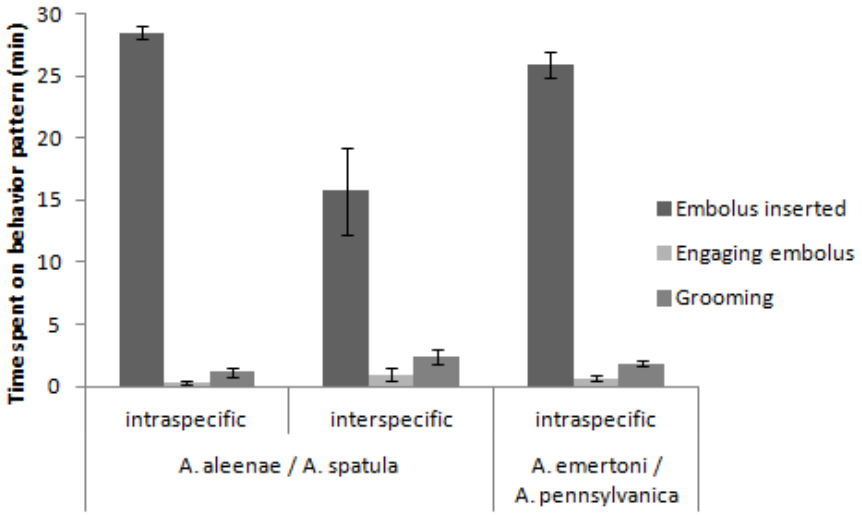

Figure III-4 Time spent on copulation behavior patterns (mean ± SE) by species pair and type (interspecific versus intraspecific).

CONCLUSION

Summary

In Chapters I & II, vibratory courtship behavior patterns, behavioral sequences and web-borne signal characteristics were compared among funnel-web spider species in the genus *Agelenopsis*, as well as species from related genera. These aspects of courtship did vary significantly among species, but were not completely species-specific. Closely related species tended to exhibit similar courtship, but some courtship behavior patterns were taxonomically widespread. Thus, courtship behavior was only somewhat phylogenetically informative. Courtship in *Agelenopsis* is long and includes a relatively long mount phase after female acceptance. Males of several species will unmount females prior to mating and resume web phase courtship. In contrast, courtship was shorter with no resumption of web phase in males from the two more distantly related outgroup species, *H. nedra* and *C. californica*.

In Chapter III, the roles of chemical cues, vibratory courtship and mechanical incompatibility in maintaining reproductive isolation within two species pairs were examined. In the western, peripatric species pair reproductive isolation appears to be incomplete. Interspecific pairings of *A. aleenae* and *A. spatula* were just as likely to result in copulations as intraspecific pairings. However, there were differences in copulatory behavior and egg case production between the two types of pairings that suggest interspecific pairings encounter mechanical difficulties due to genitalic differences. In contrast, no interspecific matings or mating attempts were observed between the two sympatric, eastern

species, *A. emertoni* and *A. pennsylvanica*. In that species pair, males exhibited very little courtship toward heterospecific females, probably because of pheromonal differences between the two species. This high degree of reproductive isolation may be what allows these two species to coexist.

Taken together, the results of this dissertation do not support the initial hypothesis of reproductive isolation via vibratory courtship in funnel-web spiders. Instead of reinforcement acting on vibratory courtship, there may be reinforcement acting on chemical cues, which act earlier in the mating process. Species that lack pheromonal differences may not be able to coexist due to the high costs of wasted courtship effort and unsuccessful copulation attempts (Gröning & Hochkirch 2008). Though it does not appear to be important in species recognition, vibratory courtship in *Agelenopsis* is long and males often continue to court after female acceptance, suggesting it plays an important role in these spiders. Sexual selection is the most likely explanation for courtship effort and diversity in these funnel-web spiders.

Future work

The results of this dissertation suggest a number of areas for future research. The long, involved mating process in *Agelenopsis* offers opportunities to study the functions and evolution of courtship. The prevalence of sympatry, breeding season overlap and habitat overlap indicate that species isolating mechanisms do exist in this group, despite the fact that vibratory communication

is not that mechanism. Papke et al. (2001) found male *A. aperta* to be strongly attracted to unoccupied *A. aperta* webs if the webs were treated with a drop of the methylated ketone pheromone identified from abdomen swipes and from headspace air sample collections from sexually mature females. The presence of the pheromone in the absence of a female also elicited courtship behavior by males. These results taken together with the results of Chapter III suggest that pheromone communication is the likely species isolation mechanism in the group. The situation in funnel-web spiders may parallel that found in moths. For example, male moths find their mates via volatile pheromones, as male *A. aperta* do, and these pheromone blends often differ from those used by related, sympatric species (Lofstedt 1993, Monti et al. 1995, Tumlinson et al. 1994, Yang, Han & Boo 2009). Males of some small ermine moth species have receptors for the pheromones of congeners, and they are much less attracted to pheromone blends containing these compounds (Lofstedt et al. 1990). Perhaps males of sympatric *Agelenopsis* species have similarly evolved an aversion to the chemical cues produced by heterospecific females.

Another open question is the function of prolonged interactions after female acceptance, such as mount phase courtship, resumed web courtship and long copulations. Courtship duration varies widely among spiders (Schneider & Andrade 2011) as does copulation duration (Stratton et al. 1996). *Agelenopsis* males exhibit rather long durations for both. One possible explanation for these trends is that males are attempting to reduce the likelihood of female remating. In

A. aperta, females become less sexually receptive after mating (Singer & Riechert 1995) and they stop producing the male attractant pheromone (Papke, Riechert & Schulz 2001). It is not known which aspects of the mating process induce this reduction in receptivity. Stimulation from courtship and copulation are both possibilities. To investigate these possibilities, the effect of natural variation in courtship duration or experimental manipulation of copulation duration on female receptivity could be assessed. Alternatively, the presence of sperm in the reproductive tract could trigger cessation of receptivity and onset of egg case production, as the results of Chapter III suggest. In that case, males may extend copulation as a form of mate guarding until pheromone production ceases. Some males remain on their mates' webs for some time after copulation, further supporting this possibility. Future work could address the exact timing of the reduction in female receptivity after mating. Finally, the puzzling resumption of web phase courtship after mounting could be explained by the behavior pattern often exhibited at this time, abdomen wagging. First, it is possible that males resume courtship after mounting to deter other males by laying silk as an anti-aphrodisiac. Extracts of male *Schizocosa ocreata* silk have been found to inhibit courtship of other males (Ayyagari & Tietjen 1986). The effect of male courtship activity on the attractiveness of female webs could be assessed to address this possibility. Second, it is possible that the drumming that usually accompanies abdomen wagging serves to stimulate the male for mating. This could be examined by photographing the male genitalia after varying durations of

courtship, to look for evidence of palpal expansion. Also, the effect of recent male courtship on the incidence of web phase resumption could be examined. Males could be allowed to court one female for some time, and then be transferred to the web of another female. If courtship serves to prepare the male for mating, these males should be well-prepared and thus would be less likely to resume web phase with their new partners.

A question about the evolution of courtship behavior patterns was also raised by this study. The behavior patterns used in *A. aperta* web phase courtship are also seen in *A. aperta* agonistic encounters, along with behavior patterns seen in other species' courtship (Riechert 1978). A comparative study of agonistic behavior in *Agelenopsis* could reveal whether there is a tendency to use the same behavior patterns in both agonistic encounters and sexual encounters. Perhaps behavior in the two contexts is linked. For example, web flexing appears to be limited to only four species – is it found the agonistic encounters of all four species? Alternatively, there might be greater variability in the behavior patterns seen in agonistic encounters than in sexual encounters, as courtship tends to involve cycling between just a few common behavior patterns. Perhaps these courtship behavior patterns are drawn from a larger pool of agonistic signaling behavior common to most species.

Finally, the results of Chapter III suggest that sympatry is an important determinant of males' species recognition capacity. The sympatric species pair studied exhibited clear male mate choice while the two peripatric species did not

exhibit any effective pre-copulatory isolating mechanisms. Additionally, *A. pennsylvanica* did not appear to be able to distinguish conspecific females from *A. potteri* females, possibly because these subjects were collected from part of their range not sympatric with *A. potteri*. Future work should examine geographic variation in chemical cues and reproductive isolation within the *A. potteri*/*A. pennsylvanica*/*A. emertoni* group. It would also be interesting to determine if *A. spatula* and *A. aleenae* populations in closer geographic proximity show the same degree of isolation as found in the two populations in Chapter III. The results of Chapter III suggest that *A. spatula* and *A. aleenae* are not capable of producing hybrids; further work on this is needed.

Although the results of this dissertation did not find vibratory courtship in *Agelenopsis* to be important in species recognition, it does seem to hold great potential as a subject of sexual selection research. Furthermore, this research found that mechanical incompatibility due to genitalic differences may play a more important role in the speciation process than previously thought. Future work on speciation in *Agelenopsis* should examine the roles of genitalic and pheromonal divergence in the evolution of reproductive isolation.

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