SPECIES RECOGNITION IS DRIVING EVOLUTION OF THE ACOUSTIC MATING SYSTEM OF SHIELD BACK KATYDIDS (ORTHOPTERA: TETTIGONIIDAE: *AGLAOTHORAX*): BEHAVIORAL AND PHYLOGENETIC EVIDENCE

BY

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TABLE OF CONTENTS

ABSTRACT

Sexual selection and species recognition are two processes that may drive evolution of mating systems and contribute to speciation. Evidence from nature is rare. I asked whether song evolution in shield-back katydids (*Aglaothorax*) from southern California is consistent with either of these processes. Analysis of male song recordings identified four variable characters, and revealed a song cline in the Transverse Ranges. Of the variable song characters, I estimated female preferences for pulse number and interpulse interval, the former with choice tests on a Y-maze and the latter with no-choice tests on a locomotion compensator. Females of two coexisting Transverse Range species each discriminated against the interpulse intervals of the other. Phylogenetic relationships inferred from nuclear gene fragments were used to reconstruct ancestral distributions and song evolution. Significantly higher rates of song evolution were shown for the species coexisting in response to secondary contact, results consistent with species recognition.

Chapter 1. Diversity, taxonomic discrepancy, and clinal variation revealed through a multivariate analysis of calling song in shield-back katydids of the genus *Aglaothorax* (Orthoptera: Tettigoniidae: Tettigoniinae)

INTRODUCTION

The species-specific nature of insect songs has been widely documented (Otte, 1970; Percy et al., 2006; Wells and Henry, 1998), which makes song a useful trait for discovering and diagnosing morphologically cryptic species (e.g. Nityananda and Balakrishnan, 2006; Sueur and Puissant, 2007; Walker et al., 2003). Species specificity also suggests the involvement of song in the speciation process, as has been hypothesized with animal mating traits in general (West-Eberhard, 1983). Song diversity may arise through many evolutionary mechanisms, principally species recognition, i.e. reproductive character displacement or reinforcement after secondary contact (Butlin, 1987; Servedio and Noor, 2003), environmental constraints on signaling (Endler, 1992), genetic drift (Roff et al., 1999), and sexual selection, which has recently been the focus of considerable attention as an engine of speciation (reviewed by Coyne and Orr, 2004; Ritchie, 2007).

In order to gauge the relative importance of the hypotheses mentioned above, it is important to understand the song diversity of a wide variety of taxa. Numerous suitable systems are found within the Orthoptera, an insect order that harbors a large number of species and is well known for its diversity of acoustic reproductive behavior (Greenfield, 2002; Gwynne, 2001; Otte, 1970). Research on orthopteran systems has added greatly to our understanding of the relationship between acoustic mating system evolution and speciation. Examples include the Hawaiian cricket genus *Laupala*, in which songs are among the first phenotypes to diverge between ecologically equivalent sister species (Shaw and Herlihy, 2000; Shaw et al., 2007), and *Chorthippus* grasshoppers in the Pyrenees Mountains, where females discriminate against heterospecifics and hybrids using the male song (Bridle et al., 2006; Vedenina et al., 2007).

The shield-back katydids (Tettigoniinae, formerly Decticinae), despite being the most diverse subfamily of katydids in North America and representing nearly half the katydid fauna of the region (123 described species, Walker, 2000), have received little study. This is primarily due to a combination of the restricted ranges of most species in the mountainous western portion of the continent, secretive habits, sporadic emergences, and the taxonomic difficulty of the group. Here I focus on the genus *Aglaothorax*, a component of this considerable diversity found primarily in the biodiversity hotspot of southern California, U.S.A. *Aglaothorax*, together with *Neduba* and an undescribed genus (Rentz and Colles, 1990; Ueshima and Rentz, 1979) hereafter referred to as Jcp, form a distinct monophyletic group, the subfamiy Nedubini, which is unique among North American Tettigoniinae in having the paraprocts modified into clasping organs in lieu of the cerci (Rentz and Colles, 1990). *Aglaothorax* currently contains five species and 16 subspecies (Eades and Otte, 2002).

The following properties make *Aglaothorax* ideal for studying speciation. First, nedubine katydids are flightless and occur on mountain habitat islands, increasing isolation and limiting gene flow between populations. Second, both sympatric and allopatric taxa are known (Rentz and Weissman, 1981), allowing reproductive character displacement to be identified. Third, the existence of numerous subspecies based on subtle morphological variation and a fascinating biogeography (Rentz and Birchim, 1968; Rentz and Weissman, 1981) suggests incipient speciation may be occurring. For example, Rentz & Birchim (1968) hypothesized that the current distribution of *A. ovata* subspecies around the periphery of the Sonoran, Mojave, and Great

Basin deserts resulted from warming and drying after the Pleistocene. *A. ovata* inhabit Joshua tree and pinyon-juniper woodlands, plant communities that retreated from the desert floor into moderate elevations of surrounding mountain ranges since the Pleistocene (Jennings, 1995). Finally, all taxa possess the conspicuous acoustic mating system described below.

Like most katydids, male nedubines produce a calling song. No courtship song is known, but Weissman (2001) reported substrate vibration in *Neduba*, a behavior that may function in a courtship context. The calling songs of the Nedubini, like those of most tettigoniines, have received little study. Morris et al. (1975) analyzed the songs of two *Neduba* species, describing complex songs consisting of two to three pulse train types. Rentz and Birchim (1968) and Rentz and Weissman (1981) published several oscillograms of *Aglaothorax* as part of taxonomic descriptions, showing only one pulse type. Song structure therefore differs fundamentally between *Aglaothorax* and *Neduba*. Rentz and Weissman (1981) noted qualitative calling song features that may be useful in diagnosing taxa. For example, the long series of staccato pulses produced by *A. diminutiva* males was compared with the rapid delivery of closely spaced pulses in sympatric *A. morsei*, likened to a shuffling sound in the latter species. Some taxa were described as having distinctive songs, such as *A. longipennis* singing with “a brief series of two to five pulses” (Rentz and Weissman, 1981, p. 97), and at the subspecies level *A. morsei curtatus*, named for its short song compared to other subspecies. This data suggests that taxon-specific songs exist in *Aglaothorax*, and that diagnostic song features may be the pulse number, the spacing or interval between the pulses, and the pulse rate.

Here I analyze the calling songs of all *Aglaothorax* species and the majority of subspecies. I reveal diversity and clinal variation in song, and consider mechanisms by which the observed song diversity may have evolved.

METHODS

Specimen collection and rearing

All katydids in this study were collected in California and Nevada from 2004 to 2008. Fieldwork covered all major mountain ranges in southern California (Fig. 1a). I sampled many type localities in order to associate songs with described taxa, and collected heavily in the western Transverse Ranges, especially the Santa Monica Mountains (Fig. 1b), where *Aglaothorax* diversity is greatest (Rentz and Weissman, 1981). I measured the coordinates of each collecting locality with a handheld GPS receiver (Table 1). Over the course of the fieldwork, I recorded 190 males from 29 populations, representing all described species and most subspecies of *Aglaothorax* (Table 1, Supp. Table A, Fig. 1). To consider all genera of Nedubini, I also recorded one population of Jcp (Table 1, Supp. Table A, Fig. 1), and eight populations of *Neduba* representing six species (data not shown). Distribution maps (Fig. 1) were generated with Mesquite Cartographer (Maddison and Maddison, 2007). Localities mentioned in the text are followed by their site numbers in parentheses.

I captured most katydids as adults by tracking their calling songs, but also raised some from larvae. Katydids were maintained in the laboratory on a reverse 12/12 h light/dark schedule and received a diet modified from that of Rentz (1996), consisting of a mixture of cabbage, oatmeal, fish or cat food, and bee pollen. I preserved voucher specimens, both dry and in 95% ethanol, and identified them morphologically using keys in Rentz and Birchim (1968) and Rentz and Weissman (1981).

Recording

Because of the scope of this recording project and the fact most katydids were collected as adults of unknown age, I did not attempt to control the age of males when recording.

Nedubines are nocturnal singers, so I made laboratory recordings in a semi-anechoic chamber during the dark phase, illuminated by red light. I housed individual males in nylon net cages, and set up as many as 13 cages per recording session to allow males to stimulate each other to sing. The chamber temperature mean was 24.9±0.3^{o}C (range 23.5-25.5^{o}C). Laboratory recording equipment consisted of a 1/2 inch electret condenser microphone (model M51, Linear-X, Tualatin, OR) and a laptop computer running BatSound Pro v. 3.3 (Pettersson Elektronik AB, 2001). This equipment was used to make 1 min recordings at a sampling rate of 150 kHz. The laboratory recording apparatus captured frequencies up to 75 kHz, with a flat response from 10 Hz to 40 kHz.

I made field recordings with a DAT recorder (model DA-P1, TEAC America, Montebello, CA) and a condenser microphone (model BG-1, Shure Inc., Niles, IL), recording frequencies up to 18 kHz. DAT recordings were digitized with BatSound at a 44.1 kHz sampling rate. I generated song figures with Raven Lite v. 1.0 (Cornell Lab of Ornithology, 2007). Representative *Aglaothorax* songs from this study are posted on Singing Insects of North America (Walker, 2000, www.buzz.ifas.ufl.edu/index.htm).

Song measurements

Before analysis I applied a 1 kHz high pass filter to the recordings to eliminate background noise. This filter did not affect the frequency range of the songs, which did not extend below 3 kHz for any individual. I analyzed the recordings in BatSound using the pulse characteristics feature, which extracted times for the start, stop, duration, and peak frequency of each pulse. For each recording I calculated five mean parameters from the data extracted by BatSound: number of pulses per chirp (pn), interpulse interval (ipi), pulse length (pl), interchirp interval (ici), and peak frequency (pf). I then calculated two composite parameters: duty cycle per chirp (dc) and pulse rate (pr). The formulas used to calculate these composite parameters were $dc=\Sigma pl/(\Sigma pl+\Sigma ipi)$ and $pr=1/pp$, where pp is the pulse period measured from the start of one pulse to the start of the next. I measured minimum frequencies (mf) directly from spectrograms, using one terminal pulse in a randomly selected chirp. Spectrograms were generated in BatSound with a fast-fourier transform algorithm (length 512, Hanning window). I did not measure maximum frequency or frequency range due to limitations of the recording equipment above 40 kHz.

Statistical testing

I used Minitab v. 13 (Minitab Inc., 2000) for all statistical analyses. To meet variance and distribution assumptions of parametric tests, I log transformed ipi and square-root transformed all other song measurements. Statistical testing proceeded in the following sequence. First, I compared the behavior of individuals between field and laboratory environments, using a one-way anova to analyze pn, and least-squares regressions to test the relationship of temperature with ipi and pr, two parameters relating to rates of muscle contraction that are commonly temperature dependent in singing insects (Walker, 1975).

Second, I used linear discriminant functions to test species specificity of song and create a diagnostic index at the species level. I selected pn, ipi, dc, and pr as variables for discriminant analyses based on the qualitative song data in Rentz and Weissman (1981) and from an exploratory anova. Descriminant functions were derived from morphologically identified specimens of the five *Aglaothorax* species. The songs of four acoustically aberrant populations were classified as unknowns: Arroyo Seco (site 6), Millard Canyon (17), Kenneth Hahn (12), and Malaga Dune (15). The first two populations represent nominate *A. morsei morsei*, based on morphology and the proximity of the populations to the type locality of *A. morsei*, which is Mt.

Wilson in the western San Gabriel Mountains (Caudell, 1907). The second two are previously unknown populations occurring on isolated sand dunes in the Los Angeles Basin.

Third, I tested song variation within populations (subspecies) using one-way anovas. Before testing populations, I used *t*-tests to compare singletons with likely subspecies (method described in Sokal and Rohlf, 2000, pp. 227-229), combining those that did not differ significantly in both pn and ipi. Fourth, after observing a linear relationship in a plot of population ipi means across the Transverse Ranges, I tested for a cline by regressing song features against longitude.

RESULTS

Song structure of the nedubine genera

All *Aglaothorax* sing with chirps consisting of a varying number of pulses (Fig. 2a). Only one pulse type occurs in *Aglaothorax*, and each pulse corresponds to one closure of the tegmina (pers. obs.). All *Neduba* studied produce a continuous song consisting of more than one pulse type (Fig. 2b). *N. castanea* from Juniper Hills (10) produce the major pulse train during wing closing and the minor pulse train (sensu Morris et al., 1975) during wing opening (pers. obs.). Jcp produce single pulses (Fig. 2c).

Comparisons of field and lab recordings

I recorded a subset of individuals of *A. longipennis* (Topanga Canyon (21)), *A. morsei costalis* (Point Mugu (28)), and *A. morsei tectinota* (Point Dume (18)) in both the field and the laboratory. The pn in field recordings made at a variety of temperatures (10.3-16.7^{o}C) are not significantly different from those of laboratory recordings made at 24.9±0.3^{o}C (anova, $F_{1,68}$=0.07, p=0.798). Regressions of ipi and pr vs. temperature are significant for all three populations (all p<0.0001). Temperature shows a tighter relationship with pr (R^2=92.1-95.5%) than with ipi (R^2=69.8-88.2%): thus, temperature must also influence pulse length. All three populations have similar regression slopes for pr vs. temperature, with *A. morsei tectinota* having a slightly lower slope (0.59 $s^{-1o}C^{-1}$) than the other two taxa (0.69 $s^{-1o}C^{-1}$). The temperature regression results are preliminary: residuals are clumped and the statistics may therefore be influenced by leverage.

Species level song analysis

A bivariate plot of dc vs. pr (Fig. 3) identifies three song types. High pr and high dc occur in *A. longipennis* and *A. morsei* subspecies from the Santa Monica Mountains. More variation occurs along the pr axis, and Santa Monica Mountains *A. morsei* tend to have a faster pr than *A. longipennis*. Low pr and dc occur in *A. diminutiva* and *A. morsei morsei* from the San Gabriel Mountains. Variation exists along both axes, with *A. diminutiva* tending to have the lowest values of both parameters. Intermediate values of both dc and pr are found in *A. ovata*, *A. gurneyi*, and *A. morsei* populations from the Los Angeles Basin. Discriminant analysis using √dc and √pr as predictors is 81.6% effective in identifying song types (Table 2a), but *A. morsei* populations from the San Gabriel Mountains and the Los Angeles Basin must be excluded for the song types to agree with taxonomy.

A bivariate plot of pn vs. ipi (Fig. 4) more finely separates species than does the dc vs. pr plot, judging from a slightly higher percentage of correct classification (82.3%; Table 2b). Short ipi occur in *A. longipennis* and *A. morsei* subspecies from the Santa Monica Mountains. Of these two species, Santa Monica Mountains *A. morsei* tend to have the shortest ipi and higher pn than *A. longipennis*. *A. ovata* and *A. gurneyi* sing with few pulses and moderate ipi. High values and variability in pn and ipi are shown in both *A. diminutiva* and *A. morsei morsei* songs. Intermediate values for both pn and ipi occur in *A. morsei* from the Los Angeles Basin. Again, the recovered song types only agree with taxonomic classification when *A. morsei* populations from the San Gabriel Mountains and the Los Angeles Basin are excluded.

Combining √pn, log ipi, √dc, and √pr as predictors, discriminant analysis is 90.5% effective in identifying *Aglaothorax* species, but only if *A. morsei* populations from the San Gabriel Mountains and the Los Angeles Basin are excluded (Table 2c). The function identifies *A. diminutiva* and *A. gurneyi* with 100% accuracy, and *A. morsei* from the Santa Monica Mountains with 98.1% accuracy. Discrimination of *A. ovata* and *A. longipennis* is less accurate, with 81.3% and 69.6% correctly identified by the function, respectively.

Morphology identified all four unknown populations as *A. morsei*, but song did not place individuals from any of these populations with other *A. morsei* subspecies described from the Santa Monica Mountains (Table 2). Millard Canyon (17) and Arroyo Seco (6) populations are nominate *A. morsei morsei* from the San Gabriel Mountains, but songs of the former were identified exclusively as *A. diminutiva,* and those of the latter were categorized as *A. diminutiva* or *A. ovata*, species they do not resemble morphologically. Of the Kenneth Hahn (12) and Malaga Dune (15) populations, both Los Angeles Basin sand dune inhabitants, the former were classified as *A. ovata* and *A. longipennis*, and the latter as to *A ovata* or *A. diminutiva*.

Population level song analysis

A. diminutiva

I studied eight populations of *A. diminutiva* (Supp. Table A). A recording from junction SR23S and Mulholland Highway (19), the type locality of *A. d. dactyla*, is not significantly different from a large population at Point Mugu (28) (*t*-tests: pn t_{15}=0.686, p=0.504; ipi t_{15}=-0.246, p=0.810). Recordings from Nicholas Flat (14) were also not significantly different from Point Mugu (28) (*t*-tests: pn t_{16}=0.183, p=0.852; ipi t_{16}=-0.022, p=0.984), so I combined the three to represent *A. d. dactyla*. Populations near the coast (Coast Ranges and western Transverse Ranges, including the Santa Monica Mountains) tend to have longer ipi, lower dc, and lower pr than inland populations (anova, Table 3).

A. longipennis

I studied three populations of *A. longipennis* (Supp. Table A). A large population at Topanga Canyon (21) was not significantly different from a recording from the type locality at junction Big Rock Drive and SR1 (8) (*t*-tests: pn t_{14}=1.689, p=0.113; ipi t_{14}=-1.154, p=0.268).

Songs from Griffith Park males differed from the above populations only in pl and pr (anova, Table 3).

A. morsei

I studied 11 populations of *A. morsei* representing all mainland subspecies (Supp. Table A). A male from Charmlee Park (9) was not significantly different from a population at junction SR23S and Mulholland Highway (19), the type locality of *A. m. curtatus* (*t*-test: pn t_9=-0.975, p=0.356; ipi t_9=0.004, p=0.996), so I combined them. *A. m. morsei* from the San Gabriel Mountains sing with high pn and ipi values indistinguishable from *A. diminutiva*. In contrast, Santa Monica Mountains *A. morsei* have the shortest ipi of any *Aglaothorax*. Three pulse rates are found among Santa Monica Mountains *A. morsei*: *A. m. tectinota* (Point Dume (18)) has the slowest rate (15.4±1.6 s^{-1}), *A. m. costalis* (Point Mugu (28)) and Nicholas Flat (14) have intermediate rates (16.5±1.2 and 16.3±0.8 s^{-1}, respectively), and *A. m. curtatus* (junction SR23S and Mulholland Highway (19)) has the fastest rate (17.6±1.6 s^{-1}). *A. m. tectinota* from Point Dume (18) are distinctive, singing with a lower pn and tending to have a longer ipi than other subspecies (anova, Table 3). Los Angeles Basin *A. morsei* populations from Kenneth Hahn (12) and Malaga Dune (15) sing with ipi intermediate between Santa Monica Mountains and San Gabriel Mountains populations. I did not observe individuals from the type locality of *A. m. curtatus* to sing with a short chirp as described in Rentz & Weissman (1981), as the pn and pr were virtually identical with *A. m. costalis*, the subspecies with the longest chirp (i.e. highest mean pn).

A. ovata and *A. gurneyi*

I studied eight populations of *A. ovata* representing all subspecies, plus one population of *A. gurneyi* (Supp. Table A). The song of a male from McMurray Meadows (2) was not significantly different from the type population of *A. ovata gigantea* at Lone Pine Canyon (3) (*t*-tests: pn t_3=3.130, p=0.052, ipi t_3=-3.028, p=0.056) so I combined them. I did not combine Whitewater Canyon (24) with nearby Pinyon Flat (23) *A. o. tinkhamorum* because songs were significantly different in pn (*t*-test: t_8=-2.530, p=0.0352). *A. gurneyi* song is distinguished from that of *A. ovata* by its higher dc, lower pr, and higher mf, and in always having 1-2 pulses. Subspecies of *A. ovata* differed from each other in many features including pn, pl, pr, and mf (anova; Table 3) with no clear pattern among populations.

Transverse Range song cline

Regression analyses of 11 populations of *A. morsei* and *A. longipennis* sampled from the south slopes of the Transverse Ranges and the Los Angeles Basin of southern California revealed a song cline (Fig. 5). This cline organizes the discrepancy between song and taxonomy along an east-west spatial axis. The majority of populations along this cline are *A. morsei*, but *A. longipennis* is nested within the range of the former, between *A. morsei* subspecies in the western Santa Monica Mountains and nominate *A. morsei morsei* in the San Gabriel Mountains. Three song features are involved: from east to west, dc ($F_{1,9}$=7.63, p=0.022, R^2=45.9%) and pr ($F_{1,9}$=36.22, p<0.0001, R^2=80.1%) increase while ipi decreases ($F_{1,9}$=27.39, p=0.001, R^2=75.3%). Regression of pn vs. longitude was not significant ($F_{1,9}$=0.15, p=0.708), making pn the only diagnostic feature to not vary clinally.

DISCUSSION

Songs of the nedubine genera

In combination with cytological evidence (Ueshima and Rentz, 1979), the fundamentally different song structures of *Aglaothorax* and *Neduba* support their current status as separate genera (Rentz, 1988) despite morphological intermediates (Rentz and Birchim, 1968). The song of Jcp consists of one pulse type as in *Aglaothorax*. In their morphological phylogeny of world Tettigoniinae, Rentz and Colles (1990) recovered Jcp basal to an *Aglaothorax*-*Neduba* clade. If this hypothesis is true, the most parsimonious interpretation is that a single pulse type is the ancestral state for the North American Nedubini, with *Neduba* evolving additional pulse types.

Comparison of field and lab recordings

Song features involving rates of muscle contraction, exemplified here by ipi, pr, and by mathematical relationship pl, all show linear relationships with temperature as predicted by many studies (e.g. Walker, 1975). In contrast, pn is a temperature independent song feature, based on the lack of a significant difference between pn in field and lab recordings made of the same individuals. Given that pn is not significantly different between field and lab conditions, and that other song features are predicted by temperature, I conclude that the acoustic behavior measured in the laboratory is comparable to what occurs in the field.

Species level song analysis

From the qualitative song data in Rentz & Weissman (1981), I predicted the number, spacing, and rate of pulses were potentially useful for diagnosing species. As pn, ipi, and pr together with dc demarcate several song types, that prediction is borne out. However, there is discordance between the song types recovered and currently recognized *Aglaothorax* species. Two species, *A. morsei* and *A. longipennis*, are the main cause of the discrepancy between song type and taxonomy. As currently recognized morphologically, *A. morsei* does not conform to a single song type in the discriminant analyses. In fact, *A. morsei* encapsulates the extremes of variation in the genus: Santa Monica Mountains *A. morsei* sing with the shortest ipi of any *Aglaothorax* (range 20.4-43.6 ms), but nominate *A. morsei morsei*, 50 km to the east in the San Gabriel Mountains, sing with long ipi (72.4-195.6 ms). The ipi of the latter are statistically indistinguishable from *A. diminutiva* found at localities throughout southern California (Fig. 4; Table 2). Bridging the ipi extremes are *A. longipennis* from the eastern Santa Monica Mountains, and *A. morsei* from Kenneth Hahn (12) (48.1±3.5 ms) and Malaga Dune (15) (66.5±13.6 ms) in the Los Angeles Basin.

Population level song analysis

Some populations of *Aglaothorax* have distinctive songs that may be evidence of cryptic species diversity. For example, populations of *A. diminutiva* from localities near the coast (Coast Ranges and western Transverse Ranges) are divergent in ipi, dc, and pr from inland populations found in the eastern Transverse and Peninsular Ranges. Because the populations are allopatric, species boundaries may be challenging to establish. It is possible, even likely, that much of the song variation I demonstrated statistically between populations does not contribute to reproductive isolation. Female preference experiments can settle this question. In cases where females show no preference for varying song features, an appropriate conclusion under the biological species concept is that the allopatric song variants are conspecific.

Transverse Range ipi cline

Clines result from hybridization and selection (reviewed in Futuyma, 1998), two processes that are not mutually exclusive. Considering the former, the Transverse Range populations may not have evolved appreciable reproductive isolation, and gene flow may therefore cause isolation by distance. The stepping-stone model (Kimura, 1953; Kimura and Weiss, 1964) is perhaps most applicable to *Aglaothorax*. The katydids exist in discrete, local patches (Rentz & Weissman 1981; pers. obs.) and lack of flight capability will reduce migration and limit dispersal primarily to adjacent patches. The shallow slope of the cline suggests low gene flow (Barton and Gale, 1993). When laboratory crosses are made between orthopteran species with different songs, the songs of F_1 progeny are typically intermediate (Ritchie, 2000; Shaw, 2000), so the intermediate songs found along the Transverse Range cline can certainly be the result of gene flow and isolation by distance. If hybridization is occurring, a recent origin of the taxa is expected, given that the majority of hybrid zones are thought to have formed from secondary contact after the Pleistocene (Barton and Hewitt, 1985).

Hybridization sets the stage for natural selection for species recognition (Coyne and Orr, 2004; Servedio and Noor, 2003). Selection for species recognition is predicted to occur at the edges of species ranges and be facilitated by a stepping-stone spatial arrangement of populations (Liou and Price, 1994; Servedio and Noor, 2003). Three morphologically similar species of *Aglaothorax* inhabit the Santa Monica Mountains: *A. diminutiva, A. longipennis* and *A. morsei* (Rentz & Weissman 1981; pers. obs.). Of these three species, *A. diminutiva* and *A. morsei* are syntopic in the western Santa Monica Mountains at Point Mugu (28) and junction SR23S and Mulholland Highway (19) (Rentz & Weissman 1981; pers. obs.). The syntopic taxa occur at the edges of the respective species' ranges: the western Santa Monica Mountains lie at the southern limit of *A. diminutiva*, a Coast Range species, and at the western limit of *A. morsei*, a Transverse Range species. If the ancestors of *A. diminutiva* and *A. morsei* possessed similar mating signals, came into secondary contact, and interbreeding caused maladaptive hybridization or mating system interference, natural selection may have acted to displace mating signals. This process, termed reinforcement or reproductive character displacement depending on the presence or absence of hybridization, respectively (Butlin, 1987), may have formed the highly divergent songs of *A. diminutiva* and *A. morsei*, and explain why a high song diversity is confined to the limited geographic area of the Transverse Ranges. If selection for species recognition is occurring in the western Santa Monica Mountains, a narrow contact zone may exist where song traits and preferences have displaced. Reproductive isolation alleles, which may be selectively neutral outside the contact zone, may then diffuse through surrounding populations (Walker, 1974) via the stepping-stone process, generating the shallow cline to the east.

Sexual selection is capable of driving mating system evolution that leads to populations divergence and speciation. The classic runaway scenario that generates arbitrary divergence of male mating traits and female preferences (Lande, 1981), may operate in both mosaic (Gosden and Svensson, 2008) and clinal (Lande, 1982) geographic arrangements. Day (2000) showed that a moderate preference cost can cause mating traits and preferences to evolve in a manner resembling reproductive character displacement and reinforcement, even though maladaptive hybridization is not occurring. Day (2000) urged detailed study of intraspecific mating preference variation to understand the relative contributions of sexual selection and species recognition in generating clinal mating trait variation, which is forthcoming (Chapter 2).

Natural selection imposed by the physical structure of the environment may directly influence signal design, a process termed sensory drive (Endler, 1992). I find this an unlikely explanation for the evolution of *Aglaothorax* song diversity for the following reasons. First, although vegetation composition does change across the cline (for example, the low cover of coastal sage scrub at Point Dume (18) to the west compared with the dense riparian woodland at Arroyo Seco (6) in the east), all populations involved in the cline inhabit similar microhabitats, namely mesic canyon bottoms or coastal bluffs on south slopes, facing the ocean (pers. obs.). Second, habitat generally affects acoustic signaling only on a coarse level, i.e. fundamental song structure (Couldridge and van Staaden, 2004; Wiley, 1991). *Aglaothorax* species have identical song structures and show only one order of magnitude of variation in ipi means (27-164 ms). Finally, the two extremes of song variation occur syntopically (Point Mugu (28): *A. d. dactyla* ipi=167.2±41.1 ms and *A. m. costalis* ipi=26.5±2.1 ms).

Predation by parasitoid flies in the genus *Ormia* (Tachinidae) is yet another possible agent of natural selection shaping the cline. Females of these flies attack a variety of singing male orthopterans by phonotaxing to their calling songs (Walker, 1993). Larvae, deposited on or near the male, bore into and eventually kill the male (Adamo et al., 1995). Although primarily known as parasitoids of crickets, *Ormia* possess a remarkably flexible phonotactic behavior (Gray et al., 2007) and are known to infect katydids in another subfamily (Conocephalinae: Burk, 1982; Shapiro, 1995). *Ormia* flies are a powerful selective force on populations of singing Orthoptera. I reared a small number of *Ormia* flies from only two localities, Arroyo Seco (6) and Malaga Dune (15). Both are *A. morsei* populations that sing with intermediate ipi values. This calls to mind the situation in *Gryllus texensis*, where *Ormia* flies exert disruptive selection on the male crickets. Flies selectively eliminate males singing with mean pulse rates, while those producing extreme pulse rates survive even though extreme rates are less preferred by females (Gray and Cade, 1999). This hypothesis may be addressed by assessing the prevalence of parasitism across the cline.

Ongoing work is aimed at addressing the most plausible hypotheses, which are sexual selection and species recognition (Cole, in prep.). Sexual selection predicts female preferences and male mating traits are coevolving (reviewed in Greenfield, 2002), while species recognition predicts strong selection on females to discriminate against heterospecifics in sympatry (Partridge and Parker, 1999). These predictions were tested through analysis of female preference functions estimated for populations along the cline (Chapter 2). Sexual selection and species recognition are expected to leave different historical signatures that are testable using a phylogenetic methods (Panhuis et al., 2001). Character reconstruction (Chapter 3) was performed to test whether song evolution is associated with lineages experiencing secondary contact, as predicted by species recognition (e.g. Saetre et al., 1997), or is widely associated with recent cladogenesis (Barraclough and Nee, 2001) in single lineages, such as in the clinal model of Day (2000).

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Table 1. Localities and taxa sampled for recording. Points on the map (Fig. 1) use site numbers for abbreviation. Mention of localities in the text are referenced by site number.

Site #	State	County	Locality	Latitude	Longitude	Elev (m)	Taxa
1	CA	Inyo	Grandview Cpgr.	37.3332	-118.1923	2610	*A. ovata armiger*
2	CA	Inyo	McMurray Meadows Rd., 3 mi. S of Glacier Lodge Rd.	37.1023	-118.3196	1658	*A. ovata gigantea*
3	CA	Inyo	Whitney Portal Rd., 6 mi. W of Lone Pine	36.5949	-118.1563	1611	*A. ovata gigantea*
4	CA	Kern	Walker Pass Recreation Area	35.6646	-118.0370	1537	*A. ovata longicauda*
5	CA	Los Angeles	Acton, Sierra Hwy. 0.6 mi. E of Crown Valley Rd.	34.4936	-118.1834	929	*A. ovata ovata*
6	CA	Los Angeles	Arroyo Seco, Gabrielino Recreation Trail	34.2065	-118.1670	355	*A. morsei morsei*
7	CA	Los Angeles	Backbone Trail, 5 mi. N of SR1 on Malibu Cyn. Rd.	34.0799	-118.7037	156	*A. morsei*
8	CA	Los Angeles	jct. Big Rock Dr. and SR1	34.0377	-118.6088	5	*A. longipennis*
9	CA	Los Angeles	Charmlee Wilderness Park	34.0608	-118.8771	427	*A. morsei curtatus*
10	CA	Los Angeles	Devil's Punchbowl Rd. 2 mi. E of Longview Rd., Juniper Hills	34.4267	-117.8697	1316	*N. castanea*
11	CA	Los Angeles	Griffith Park, Mount Hollywood Trail	34.1240	-118.3009	370	*A. longipennis*
12	CA	Los Angeles	Kenneth Hahn SRA	34.0042	-118.3592	126	*A. morsei*
13	CA	Los Angeles	Ladera Linda Park	33.7383	-118.3479	149	*A. morsei*
14	CA	Los Angeles	Leo Carillo State Park, Nicholas Flat	34.0638	-118.9128	457	*A. morsei*
15	CA	Los Angeles	Malaga Dune, Rancho Palos Verdes	33.8004	-118.3858	61	*A. morsei*
16	CA	Los Angeles	Mescal Picnic Area	34.3901	-117.7206	1868	*A. gurneyi*
17	CA	Los Angeles	Millard Cyn. Cpgr.	34.2162	-118.1484	546	*A. morsei morsei*
18	CA	Los Angeles	Point Dume Headlands State Park	34.0046	-118.8055	44	*A. morsei tectinota*
19	CA	Los Angeles	SR23S & Mulholland Hwy.	34.0873	-118.8742	474	*A. diminutiva dactyla, A. morsei curtatus*
20	CA	Los Angeles	Sycamore Flat Cpgr.	34.4128	-117.8239	1309	*A. diminutiva*
21	CA	Los Angeles	Topanga State Park, Dead Horse Trail	34.0915	-118.5939	274	*A. longipennis*
22	CA	Riverside	Boulder Basin Cpgr.	33.8261	-116.7558	2498	Jcp
23	CA	Riverside	Pinyon Flat Cpgr.	33.5856	-116.4570	1219	*A. diminutiva, A. ovata tinkhamorum*
24	CA	Riverside	Whitewater Cyn.	33.9579	-116.6463	431	*A. ovata*
25	CA	San Diego	Boulder Oaks Cpgr.	32.7299	-116.4839	968	*A. diminutiva*
26	CA	San Luis Obispo	La Panza Cpgr.	35.3538	-120.2606	732	*A. diminutiva*
27	CA	Santa Barbara	Upper Oso Cpgr.	34.5540	-119.7680	376	*A. diminutiva*
28	CA	Ventura	Point Mugu State Park, Sycamore Cyn.	34.0728	-119.0145	4	*A. diminutiva dactyla, A. morsei costalis*
29	NV	Lincoln	Oak Spring Summit Picnic Area	37.5923	-114.6828	1901	*A. ovata segnis*

Table 2. Results of linear discriminant analyses. Song features used for classification were dc and pr (a), pn and ipi (b) and a combination of all four (c). Discriminant functions were derived from morphologically identified specimens of the five *Aglaothorax* species. The songs of four acoustically aberrant populations (Arroyo Seco (6), Millard Cyn., (17), Kenneth Hahn (12) and Malaga Dune(15)) were classified as unknowns.

Taxon/ population	Sample size	Percent classified as:				
		A. diminutiva	*A. gurneyi*	*A. longipennis*	*A. morsei*	*A. ovata*
	a. predicto	dc, pr				
A. diminutiva	35	97.14%				2.86%
A. gurneyi	5		100.00%			
A. longipennis	23			65.22%	26.09%	8.70%
A. morsei	52			15.38%	84.62%	
A. ovata	32	3.13%	28.13%			68.75%
Arroyo Seco	6	50.00%				50.00%
Millard	11	100.00%				
Kenneth Hahn	8			12.50%		87.50%
Malaga Dune	8	12.50%				87.50%
	b. predicto	pn, ipi				
A. diminutiva	35	100.00%				
A. gurneyi	5		100.00%			
A. longipennis	23		13.04%	65.22%	17.39%	4.35%
A. morsei	52			9.62%	90.38%	
A. ovata	32		28.13%	12.50%		59.38%
Arroyo Seco	6	66.67%				33.33%
Millard	11	100.00%				
Kenneth Hahn	8			25.00%		75.00%
Malaga Dune	8	12.50%				87.50%
	c. predicto	dc, pr, pn, ipi				
A. diminutiva	35	100.00%				
A. gurneyi	5		100.00%			
A. longipennis	23			69.57%	21.74%	8.70%
A. morsei	52			1.92%	98.08%	
A. ovata	32		18.75%			81.25%
Arroyo Seco	6	66.67%				33.33%
Millard	11	100.00%				
Kenneth Hahn	8			12.50%		87.50%
Malaga Dune	8	12.50%				87.50%

Table 3. Population-level song variation. One-way anovas were performed within species, using population (subspecies) as the factor and the song features as dependent variables. *F*-ratios and significance levels are given, and for song features that vary significantly among populations within a species, the significance level is show in bold.

Song feature	*F* ratio	p value	*F* ratio	p value	*F* ratio	p value	*F* ratio	p value
	A. diminutiva		*A. longipennis*		*A. morsei*		*A. ovata*	
pulse number	$F_{3,31}$=2.36	0.090	$F_{1,21}$=1.06	0.315	$F_{3,47}$=7.62	**<0.0001**	$F_{5,26}$=4.96	**0.003**
interpulse interval	$F_{3,31}$=5.82	**0.003**	$F_{1,21}$=1.61	0.211	$F_{3,47}$=3.12	**0.035**	$F_{5,26}$=2.55	0.053
pulse length	$F_{3,31}$=1.12	0.357	$F_{1,21}$=11.57	**0.003**	$F_{3,47}$=2.26	0.094	$F_{5,26}$=2.95	**0.031**
interchirp interval	$F_{3,28}$=4.66	**0.009**	$F_{1,21}$=2.26	0.149	$F_{3,47}$=1.07	0.372	$F_{5,26}$=0.85	0.526
peak frequency	$F_{3,31}$=5.83	**0.003**	$F_{1,21}$=1.49	0.237	$F_{3,47}$=0.78	0.508	$F_{5,26}$=1.72	0.165
minimum frequency	$F_{3,31}$=5.36	**0.004**	$F_{1,21}$=0.05	0.838	$F_{3,47}$=0.78	0.511	$F_{5,26}$=4.18	**0.006**
duty cycle	$F_{3,31}$=4.06	**0.015**	$F_{1,21}$=1.77	0.199	$F_{3,47}$=1.21	0.316	$F_{5,26}$=2.11	0.096
pulse rate	$F_{3,31}$=6.74	**0.001**	$F_{1,21}$=16.25	**0.001**	$F_{3,47}$=5.25	**0.003**	$F_{5,26}$=4.35	**0.005**

Supplementary Table A. Descriptive statistics of all populations recorded in the laboratory. Statistics are given as mean ± standard deviation, except for single recordings in which the raw value is shown. Site numbers refer to localities listed in Table 1.

Taxon	Site #	n	Pulse number (pn)			Interpulse interval (ipi, ms)			Pulse length (pl, ms)		
Aglaothorax diminutiva Coast Ranges, western Transverse Ranges (including Santa Monica Mountains)											
A. d. dactyla	19	1	50.0			156.8			41.6		
A. d. dactyla	28	16	38.5	±	16.2	167.2	±	41.1	37.1	±	4.6
A. d. dactyla	14	2	70.5	±	31.8	147.8	±	12.2	40.4	±	1.5
A. diminutiva	27	5	27.1	±	10.9	179.5	±	37.0	44.4	±	5.7
A. diminutiva	26	1	42.0			122.6			36.8		
Aglaothorax diminutiva inland localities											
A. diminutiva	23	5	28.5	±	8.6	129.1	±	35.5	42.2	±	10.4
A. diminutiva	20	5	24.3	±	3.7	110.9	±	16.0	42.3	±	11.9
A. diminutiva	25	2	21.8	±	12.4	157.9	±	102.7	48.5	±	13.0
Aglaothorax longipennis											
A. longipennis	8	1	6.6			27.4			32.8		
A. longipennis	11	7	3.2	±	1.0	39.9	±	4.7	45.4	±	6.1
A. longipennis	21	15	3.8	±	1.6	36.8	±	7.9	34.8	±	6.3
Aglaothorax morsei Santa Monica Mountains											
A. m. costalis	28	16	13.6	±	3.6	26.5	±	2.1	34.8	±	4.1
A. m. curtatus	19	10	13.5	±	3.8	26.7	±	5.5	30.8	±	2.7
A. m. curtatus	9	1	9.6			26.7			38.7		
A. m. tectinota	18	10	8.5	±	2.4	31.6	±	6.0	34.8	±	7.0
A. morsei	14	15	11.5	±	2.0	26.9	±	4.4	35.1	±	4.0
A. morsei	7	3	9.5	±	0.8	28.1	±	6.6	32.0	±	8.5
Aglaothorax morsei morsei San Gabriel Mountains											
A. m. morsei	6	6	17.5	±	4.0	99.6	±	23.7	51.2	±	6.3
A. m. morsei	17	11	9.8	±	2.8	164.1	±	50.5	48.6	±	5.9
Aglaothorax morsei Los Angeles Basin											
A. morsei	12	8	6.2	±	1.9	48.1	±	3.5	46.7	±	6.6
A. morsei	15	7	17.1	±	5.8	66.5	±	13.6	41.2	±	12.6
A. morsei	13	1	10.6			80.4			45.6		
Aglaothorax gurneyi											
A. gurneyi	16	5	2.0	±	0.0	57.3	±	4.5	57.9	±	5.4
Aglaothorax ovata											
A. o. armiger	1	4	3.3	±	0.9	71.6	±	13.7	37.4	±	2.1
A. o. gigantea	3	4	2.3	±	0.5	56.7	±	3.8	43.9	±	3.2
A. o. gigantea	2	1	4.1			44.0			56.8		
A. o. longicauda	4	6	5.7	±	2.1	62.5	±	27.8	52.9	±	16.1
A. o. ovata	5	6	4.4	±	0.7	58.5	±	12.6	55.1	±	16.7
A. o. segnis	29	3	2.4	±	0.9	86.0	±	19.5	28.9	±	3.1
A. o. tinkhamorum	23	8	3.3	±	1.0	51.6	±	9.1	45.8	±	8.1
A. ovata	24	2	2.0	±	0.0	67.3	±	16.5	53.6	±	5.1
Jcp	22	3	1.0	±	0.0	1934.5	±	545.6	17.3	±	6.7

5.7	23.7	6.2	0.213	5.0
10.2 ± 2.9	17.7 ± 1.9	6.5 ± 0.6	0.193 ± 0.040	5.0 ± 0.8
6.9 ± 0.6	17.8 ± 1.1	6.1 ± 1.1	0.218 ± 0.022	5.3 ± 0.3
7.7 ± 3.6	18.0 ± 1.4	7.4 ± 0.5	0.208 ± 0.026	4.6 ± 0.8
5.2	24.7	7.9	0.235	6.3
12.9 ± 3.1	18.6 ± 3.2	7.1 ± 0.7	0.263 ± 0.088	6.1 ± 0.6
14.7 ± 1.5	24.4 ± 4.9	6.9 ± 0.6	0.286 ± 0.083	6.6 ± 0.5
9.4	21.6 ± 0.1	8.5 ± 0.2	0.284 ± 0.181	5.3 ± 2.3
7.7	18.3	6.1	0.585	17.1
4.0 ± 0.8	18.9 ± 0.8	6.8 ± 0.6	0.629 ± 0.079	12.4 ± 1.0
4.7 ± 1.3	16.9 ± 3.8	7.0 ± 1.0	0.576 ± 0.084	14.9 ± 1.5
6.1 ± 4.5	17.7 ± 5.2	8.3 ± 0.9	0.586 ± 0.034	16.5 ± 1.2
6.0 ± 3.1	19.2 ± 3.1	8.3 ± 1.6	0.559 ± 0.056	17.6 ± 1.6
4.8	18.0	8.2	0.618	15.6
4.3 ± 0.4	19.6 ± 4.1	7.8 ± 0.8	0.558 ± 0.063	15.4 ± 1.6
4.5 ± 1.0	18.9 ± 2.5	7.9 ± 1.1	0.589 ± 0.061	16.3 ± 0.8
3.9 ± 1.7	19.5 ± 2.5	7.7 ± 0.5	0.556 ± 0.123	16.9 ± 0.8
10.7 ± 2.2	20.4 ± 3.7	5.9 ± 0.7	0.358 ± 0.048	6.8 ± 1.1
13.1 ± 5.5	17.9 ± 2.4	5.9 ± 1.2	0.252 ± 0.136	4.8 ± 2.2
3.3 ± 1.7	16.4 ± 4.0	7.0 ± 0.6	0.534 ± 0.042	10.5 ± 1.2
7.7 ± 2.4	18.1 ± 1.3	7.0 ± 0.6	0.399 ± 0.120	9.3 ± 0.4
5.8	16.8	9.1	0.385	8.0
1.5 ± 0.5	17.7 ± 1.2	6.1 ± 1.3	0.673 ± 0.027	8.4 ± 0.5
1.7 ± 0.7	16.4 ± 2.0	6.1 ± 1.0	0.444 ± 0.042	9.4 ± 1.1
2.3 ± 0.2	15.0 ± 2.5	4.1 ± 0.5	0.591 ± 0.052	10.0 ± 0.6
0.8	15.6	4.7	0.630	10.0
1.9 ± 0.7	16.3 ± 1.2	4.9 ± 0.8	0.523 ± 0.153	8.7 ± 0.8
1.6 ± 0.3	17.8 ± 3.1	4.7 ± 0.7	0.545 ± 0.132	8.7 ± 0.6
1.6 ± 0.6	14.2 ± 2.3	4.9 ± 0.5	0.387 ± 0.029	9.6 ± 0.8
2.1 ± 0.2	16.4 ± 1.3	4.3 ± 0.5	0.571 ± 0.104	10.1 ± 0.7
2.7 ± 1.2	21.0 ± 2.5	4.6 ± 0.0	0.616 ± 0.081	8.0 ± 0.5
	16.7 ± 0.7	7.9 ± 1.8	0.010 ± 0.008	0.5 ± 0.2

FIGURE CAPTIONS

Figure 1. Map of localities sampled in this study generated with Mesquite Cartographer (Maddison and Maddison, 2007). Numbers refer to localities indexed in Table 1. a, Plot of all localities. b, detail of Transverse Range song cline. The portion of the Transverse Ranges shown includes the Santa Monica and San Gabriel Mountains.

Figure 2. Song structures of the nedubine genera. Figures are 1.5 s oscillogram windows generated from laboratory recordings, made using a 1/2 inch condenser microphone and BatSound (Pettersson Elektronik AB, 2001) sampling at 150 kHz. a, *Aglaothorax ovata gigantea* McMurray Meadows (2), 25.0°C. b, *Neduba castanea* Juniper Hills (10), 26.1°C. c, Undescribed taxon Jcp, Boulder Basin (22). Note single pulse type in *Aglaothorax* and Jcp versus two pulse types in *Neduba*.

Figure 3. Bivariate plot of duty cycle (dc) vs. pulse rate (pr). Major groupings demarcated by these axes are outlined. A linear discriminant analysis using these axes was 81.6% effective in identifying song types, but each song type that was recovered contains more than one recognized taxon.

Figure 4. Bivariate plot of pulse number (pn) vs. interpulse interval (ipi). Major groupings demarcated by these axes are outlined. A linear discriminant analysis using these axes was 82.3% effective in separating song types. When all four axes were combined in a discriminant analysis, diagnosis was 90.5% effective. Again, song types that were delimited do not correspond to currently recognized taxa.

Figure 5. Transverse Range song cline, demonstrated by least squares regression of all song features varying significantly among populations against longitude. The cline spans the Santa Monica Mountains and the western San Gabriel Mountains. Location numbers, referring to Table 1 and Fig. 1, are shown above the data points in Fig. 6a. Eleven populations of *A. morsei* and *A. longipennis* are included. 6a, duty cycle (dc). 6b, pulse number (pn). 6c, interpulse interval (ipi). 6d, pulse rate (pr). Of these four song features, only pulse number (pn; 6b) does not show clinal variation.

Figure 1

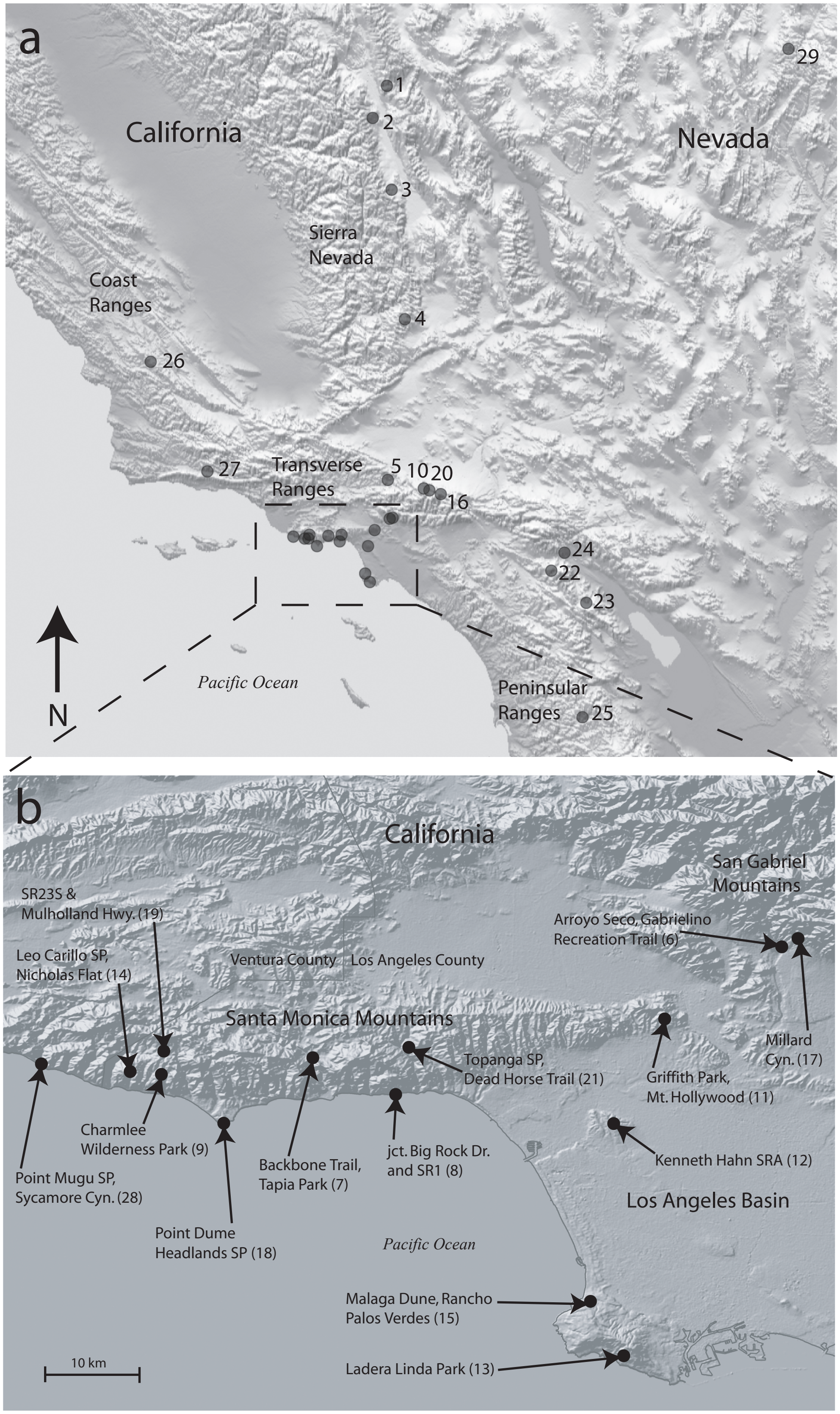

a
California
Nevada
Sierra Nevada
Coast Ranges
Transverse Ranges
Peninsular Ranges
Pacific Ocean
N
1
2
3
4
5
10
20
16
22
23
24
25
26
27
29
b
California
San Gabriel Mountains
SR23S & Mulholland Hwy. (19)
Leo Carillo SP, Nicholas Flat (14)
Ventura County
Los Angeles County
Arroyo Seco, Gabrielino Recreation Trail (6)
Santa Monica Mountains
Millard Cyn. (17)
Topanga SP, Dead Horse Trail (21)
Griffith Park, Mt. Hollywood (11)
Charmlee Wilderness Park (9)
Backbone Trail, Tapia Park (7)
jct. Big Rock Dr. and SR1 (8)
Kenneth Hahn SRA (12)
Point Mugu SP, Sycamore Cyn. (28)
Los Angeles Basin
Point Dume Headlands SP (18)
Pacific Ocean
Malaga Dune, Rancho Palos Verdes (15)
10 km
Ladera Linda Park (13)

Figure 2

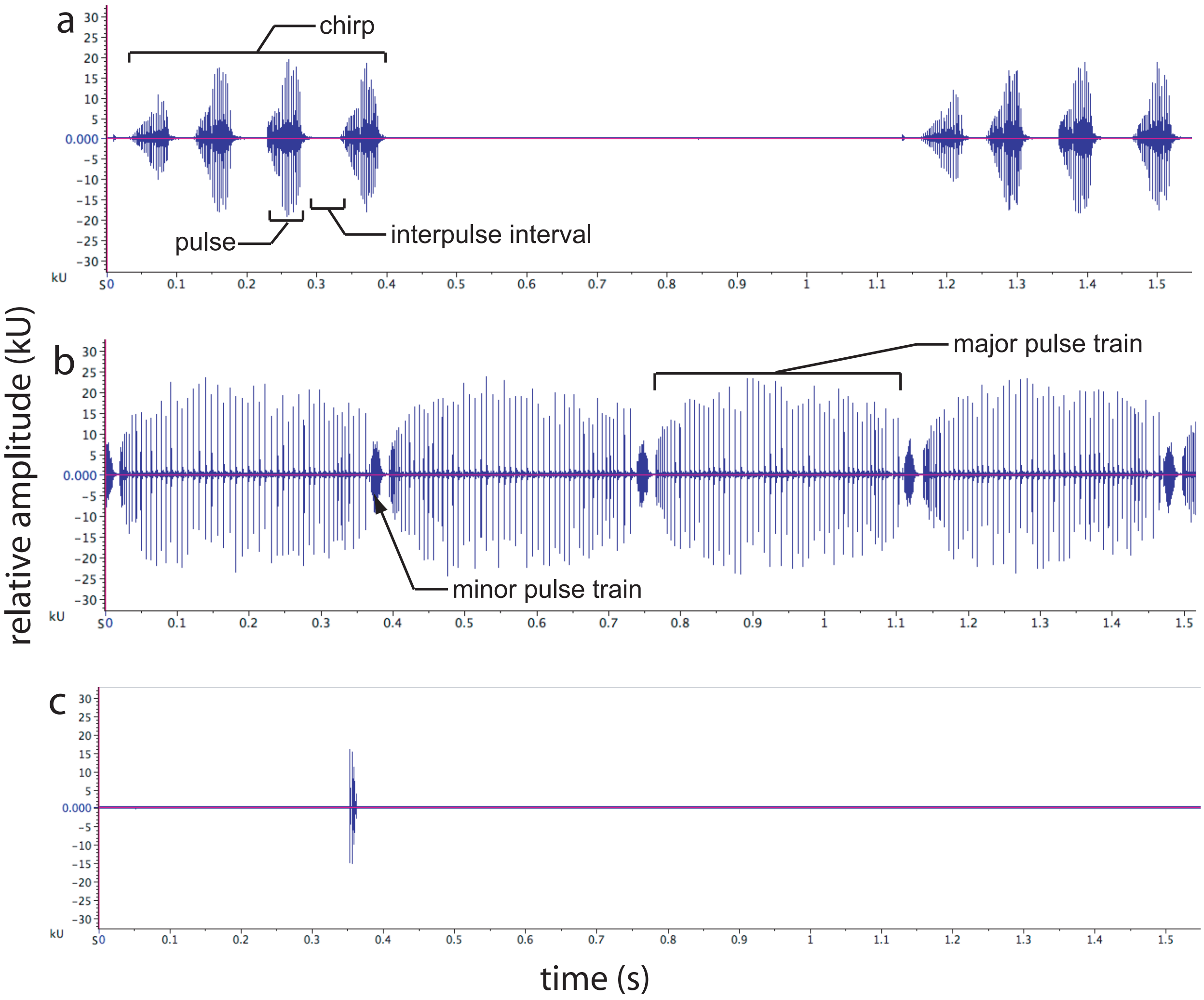

a
chirp
pulse
interpulse interval
b
major pulse train
minor pulse train
c
relative amplitude (kU)
time (s)
kU
30
25
20
15
10
5
0.000
-5
-10
-15
-20
-25
-30
S0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5

Figure 3

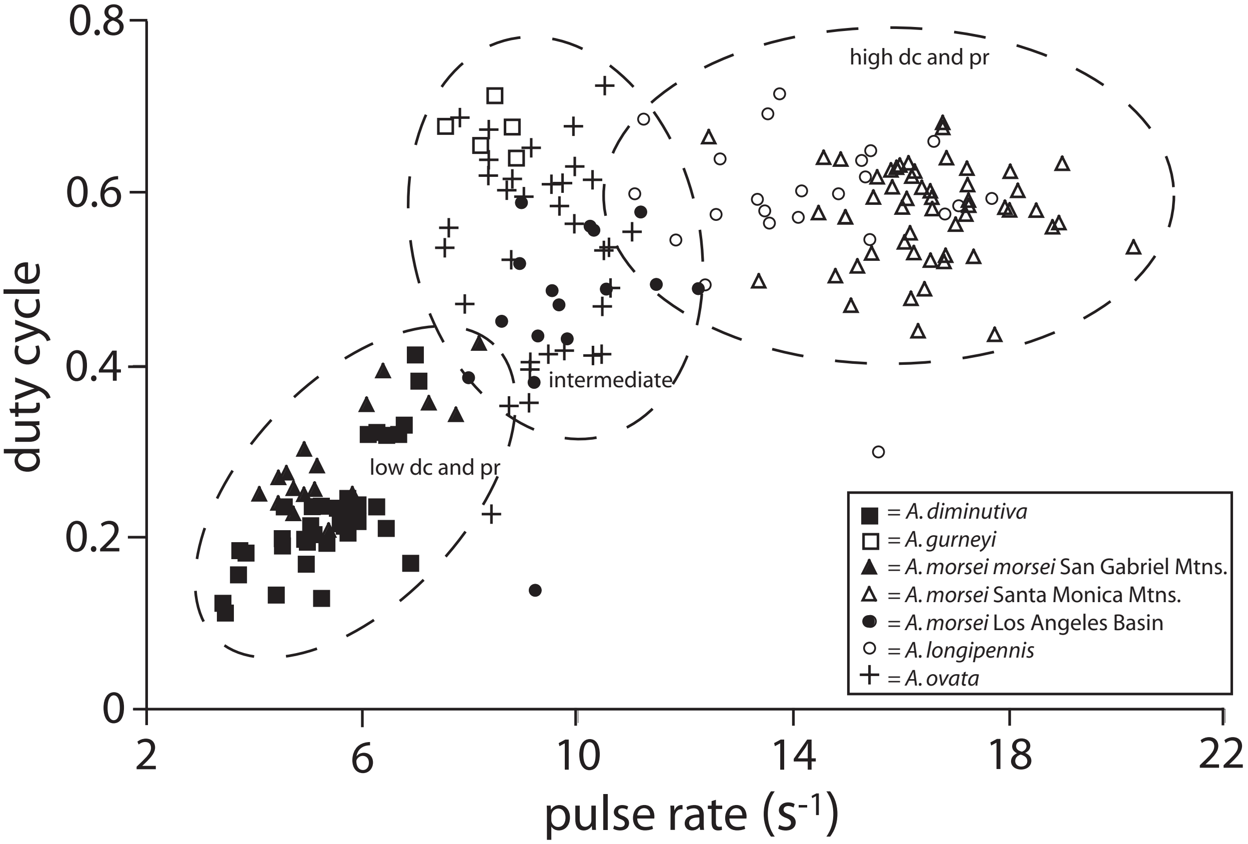

high dc and pr
intermediate
low dc and pr
duty cycle
0.8
0.6
0.4
0.2
0
pulse rate (s-1)
2
6
10
14
18
22
■ = A. diminutiva
□ = A. gurneyi
▲ = A. morsei morsei San Gabriel Mtns.
△ = A. morsei Santa Monica Mtns.
● = A. morsei Los Angeles Basin
○ = A. longipennis
+ = A. ovata

Figure 4

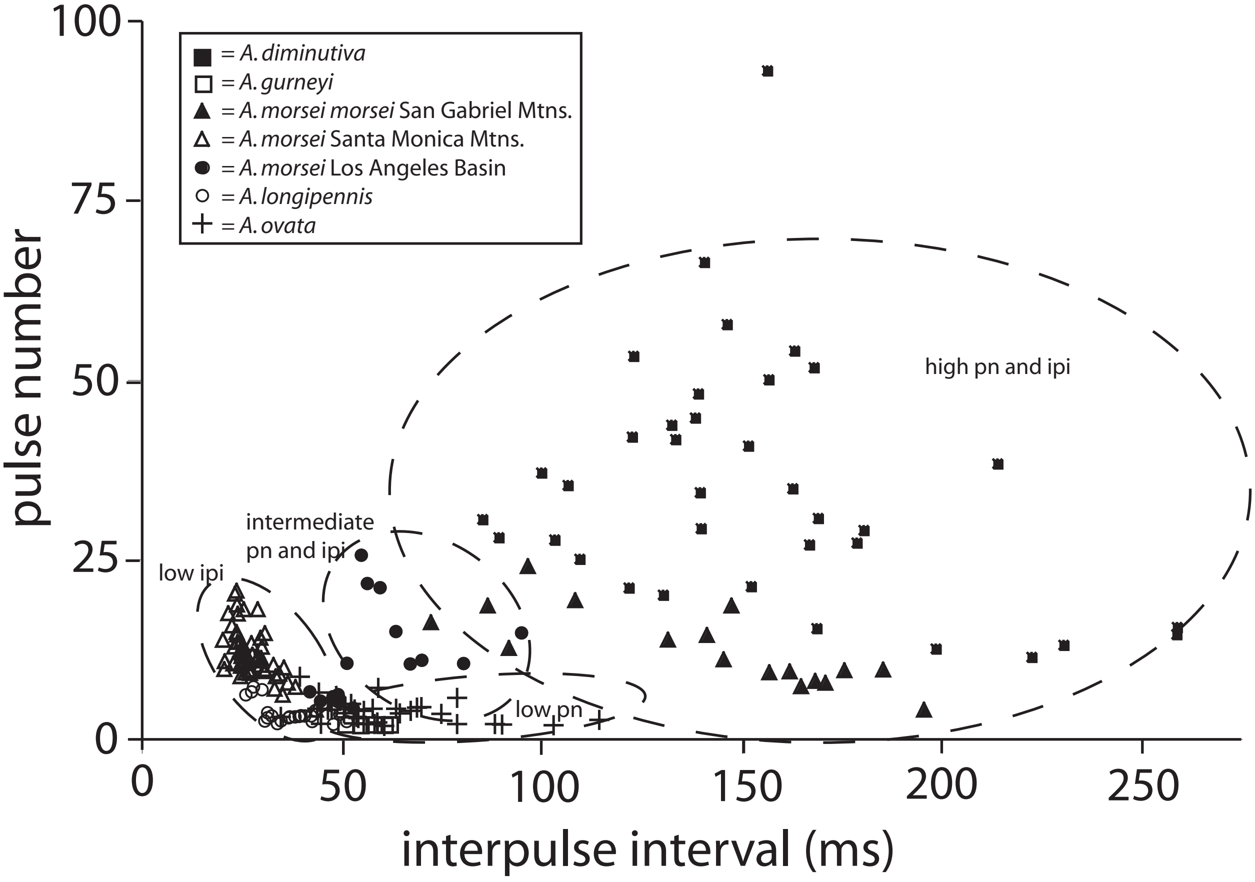
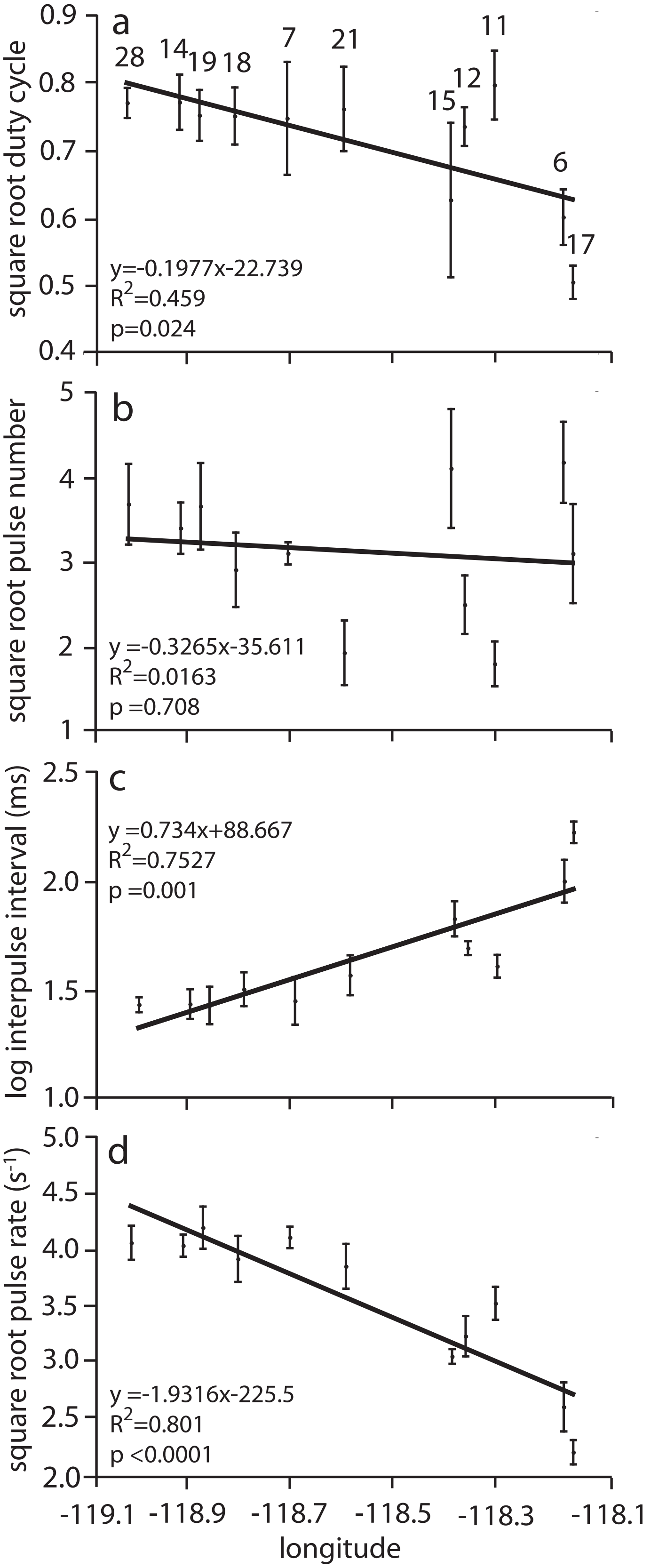

■ = *A. diminutiva*
□ = *A. gurneyi*
▲ = *A. morsei morsei* San Gabriel Mtns.
△ = *A. morsei* Santa Monica Mtns.
● = *A. morsei* Los Angeles Basin
○ = *A. longipennis*
+ = *A. ovata*
high pn and ipi
intermediate pn and ipi
low ipi
low pn
pulse number
interpulse interval (ms)
0
25
50
75
100
0
50
100
150
200
250

Figure 5


a
b
c
d
square root duty cycle
0.9
0.8
0.7
0.6
0.5
0.4
28
14
19
18
7
21
15
12
11
6
17
y=-0.1977x-22.739
R2=0.459
p=0.024
square root pulse number
5
4
3
2
1
y =-0.3265x-35.611
R2=0.0163
p =0.708
log interpulse interval (ms)
2.5
2.0
1.5
1.0
y =0.734x+88.667
R2=0.7527
p =0.001
square root pulse rate (s-1)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
y =-1.9316x-225.5
R2=0.801
p <0.0001
-119.1
-118.9
-118.7
-118.5
-118.3
-118.1
longitude

Chapter 2. Female preference functions supply evidence for species recognition in the acoustic mating system of shield-back katydids (Orthoptera: Tettigoniidae: *Aglaothorax*)

INTRODUCTION

Recently, sexual selection has attracted much attention as an engine of speciation (reviewed in Coyne and Orr, 2004; Ritchie, 2007). Sexual selection can lead to speciation through a coevolutionary process, whereby male mating signals diversify through coordinated evolution with divergent female preferences (Greenfield, 2002 p. 282; Lande, 1981; Uyeda et al., 2009), generating prezygotic isolation as a byproduct. Sexual selection may drive speciation primarily in allopatry, based on the lack of a correlation between sexual trait diversity and sympatric species diversity (Panhuis et al., 2001; Price, 1998). If different female preferences are incorporated into allopatric isolates of a formerly panmictic population, and male mating traits diverge to satisfy them, ecologically equivalent species may result that differ only in mating traits and preferences (Irwin and Price, 1999; Price, 1998).

Signal-receiver mating systems may also evolve to facilitate species recognition in sympatry after secondary contact (reviewed in Coyne and Orr, 2004). Natural selection to avoid wasteful or costly matings with heterospecifics causes signals to diverge and receiver preferences to narrow. If fully reproductively isolated species encounter one another, and hybrids are not formed, the strengthening of recognition is termed reproductive character displacement, and functions to minimize wasteful courtship attempts with heterospecifics (Butlin, 1987). If the taxa involved are capable of exchanging genes, the process is termed reinforcement. Here, enhanced prezygotic isolation evolves to reduce costly hybridization (Butlin, 1987; Servedio and Noor, 2003), thereby blocking gene flow and completing speciation.

Sexual selection and species recognition do not necessarily represent mutually exclusive hypotheses explaining signaler-receiver divergence. In the túngara frog (*Physalaemus pustulosus*), Ryan and Rand (1993) concluded that sexual selection and species recognition represent the extremes of a continuum, along which selection for an appropriate mate begins with selection of the correct species. Other research has concluded that sexual selection and species recognition occur as separate processes. For example, sexual selection and species recognition occur at different stages of the mating sequence in *Timema* stick insects (Arbuthnott and Crespi, 2009). In *Drosophils serrata* and *D. birchii*, Higgie and Blows (2008) showed that selection for species recognition displaced a male cuticular hydrocarbon phenotype away from the optimum preferred by females. Given that sexual selection and species recognition are complex processes, that the importance of each process may vary across taxa, and that both processes may interact, additional data is needed in order to better understand the importance of each process in speciation.

Signatures of sexual selection and species recognition are frequently looked for in male display traits, to which the dynamic and static criteria of Gerhardt (1991) are widely applied. As defined by Gerhardt (1991), dynamic traits are characterized by high coefficients of variation (CV, >12% within males and >15% between males). Within an individual, dynamic traits change dramatically throughout the course of signaling, even within a single signaling bout, and vary widely between individuals. Dynamic traits frequently pertain to the quantity of signaling and thus tend to represent indicator traits under sexual selection. Static traits, on the other hand, have low CV (<5% within males, <11% between males), change little within or between signaling bouts, and often involve features of the fundamental signal structure, such as pulse rate. Static traits are therefore likely to contain reliable species recognition cues.

Female preference data can provide a means of distinguishing the sexual selection and species recognition hypotheses, one that is potentially more powerful than an examination of male signals. Selection for species recognition may be stronger on female preferences than on male signals, because females, which invest more in gametes, suffer a greater fitness cost than males from choosing the incorrect species (Partridge and Parker, 1999). The former hypothesis predicts that, through coevolution, the distributions of female preferences and male mating signals will be correlated in a population (Boul et al., 2007; Rodriguez et al., 2006). The latter hypothesis predicts female preferences will discriminate against heterospecific signals and accept a narrower range of stimuli (von Helversen and von Helversen, 1995) in sympatric than in allopatric populations. However, preference and signal data alone cannot distinguish between reproductive character displacement and reinforcement, because both mechanisms are expected to produce the same pattern: a sharpening of the preference-signal recognition system in sympatry versus allopatry.

Female preferences are typically measured through one of two experimental procedures: choice tests in which females choose between pairs of stimuli, and no-choice tests in which stimuli are presented one at a time. Wagner (1998) discussed the advantages and disadvantages of each; Bush et al. (2002), comparing the results of both methods in *Hyla* treefrogs, found them to be equivalent. Closed-loop versus open-loop experimental design is another dichotomy in preference testing, one relating to the apparatus used. Closed-loop designs employ apparatus that allow females to move towards the stimulus source, for example open arenas (e.g. Bailey, 2008) and y- or t-mazes (e.g. Ritchie, 1995). If the position of the animal is fixed with respect to the stimulus source, such as with a locomotion compensator device (e.g. Greenfield et al., 2002; Wagner et al., 1995), the experiment has an open-loop design. Because many animals show preference for greater amplitude (e.g. Jang and Greenfield, 1996; Latimer and Sippel, 1987) a disadvantage of closed-loop designs is that, as females approach the sound source, non-preferred stimuli may be chosen because they are perceived as more intense than stimuli from a more distant sound source. One potential advantage closed-loop designs enjoy over open-loop designs is more efficient localization of the sound source. Certain groups of insects, for example crickets (Rheinlaender and Blätgen, 1982), have difficulty localizing sound and may not make appropriate steering movements in an open-loop scenario, although some katydids (Bailey and Stephen, 1984; Deily and Schul, 2006) are considerably more directional.

I tested the sexual selection and species recognition hypotheses in the acoustic mating system of shield-back katydids of the genus *Aglaothorax.* These katydids exhibit a pattern of calling song diversification that is consistent with both the sexual selection and species recognition hypotheses (Chapter 1). A diverse array of calling songs exist among closely related taxa. Four song features differ among populations: the number of pulses per chirp (pn), the interpulse interval (ipi), the duty cycle (dc), and the pulse rate (pr). The ipi, dc, and pr in two species, *A. morsei* and *A. longipennis*, show an east-west cline across the Transverse Ranges, with dc and pr increasing and ipi decreasing from east to west. All taxa are flightless and locally distributed, and perhaps only rarely exchange genes between neighboring populations in a stepping-stone fashion. Also, the taxa occur in similar montane coastal sage scrub and chaparral habitats in southern California, reducing the influence of ecological factors. Finally, both allopatric and sympatric populations are known (Rentz and Weissman, 1981), and if secondary contact is assumed as the cause of sympatry, selection for species recognition may have occurred.

I measured female preferences for pn and ipi, the former using choice tests on a y-maze and the latter using no-choice tests on a locomotion compensator device. Of the four varying song features, I tested pn and ipi because they are fundamental components of the song structure, whereas both dc and pr are composite measures. Pulse length and ipi both influence pr, but ipi is by far the more important component of pr as the pulse length tends not to vary between populations (Chapter 1). Choice tests are biologically realistic for testing pn, as females experience choruses of males varying in pn (pers. obs.). No-choice tests are likewise realistic for testing ipi; from the point of view of the female they represent a series of encounters with migrants from neighboring populations in the cline that differ in ipi.

METHODS

Analysis of male songs

I calculated within and between male CV for each population from recordings used in a comprehensive song analysis of *Aglaothorax* (Chapter 1). Within-male CV values for pn and ipi were calculated from the means and standard deviations of all pulses counted per chirp, and all interpulse intervals measured within one recording, respectively (Table 1).

Collection and rearing of females

I collected females from six localities distributed along the Transverse Range song cline. I selected sites to cover both geographic and taxonomic diversity, including *A. longipennis* plus several recognized subspecies of *A. morsei* (Table 1, Fig. 1). Females for pn testing were collected from 2006 to 2008. Not all populations were sampled each year because abundance varied widely, most likely due to seasonal moisture variation (pers. obs.). Females for ipi testing were collected from four localities during 2008 (Table 1, Fig. 1). I excluded Arroyo Seco and Nicholas Flat from ipi testing because both of these populations are geographically proximal to other sampled localities (Millard and Point Mugu, respectively) and are perhaps redundant. I did, however, add females of a third species, *A. diminutiva dactyla* from Point Mugu, taking advantage of its abundance during 2008. *A. diminutiva dactyla* and *A. morsei costalis* are both sympatric and syntopic at Point Mugu.

All females were raised from larvae, thus controlling for mating history which may alter preference (e.g. Prosser et al., 1997). I visually located larvae on flowers and foliage of chaparral vegetation during evenings in spring and early summer (late May-late June). I separated sympatric species morphologically using the key in Rentz & Weissman (1981). Larvae were shipped to the University of Kansas, where I established them on a 12/12 h reverse light/dark schedule at 20°C. Katydids derived moisture from cut cabbage and received a diet adapted from recipes in Rentz (1996) consisting of oatmeal, bee pollen, and fish or cat food. Females were raised in acoustic isolation from males. To ensure sexual receptivity, I tested each female a minimum of 10 days after eclosion.

Choice testing of pulse number

Stimulus construction

For each taxon, I generated a set of 10 individual chirp stimuli, spanning a range of 1-28 pulses in 3 pulse increments. The set covered the entire range of pn variation (2-21) across all populations studied. For each population, I selected a single chirp from a bank of laboratory recordings made at 24.9±0.3°C and at a sampling rate of 150 kHz. Manipulation consisted of pasting and deleting terminal pulses with sound editing software (Cool Edit v. 1.53, Syntrillium Software, 1996). A length of silence corresponding to the minimum interchirp interval was added after the terminal pulse in each stimulus file. Female *Aglaothorax* require a chirp structure in order to respond, based on a lack of response to trials where the interchirp interval was diminished (pers. obs.). Other parameters were held constant, and fit within the distributions of their respective populations (Table 1; Chapter 1). I then created eight overlapping pairs out of the individual stimuli, combining those differing by six pulses into stereo files (Table 2). The members of the pair were arranged 180° out of phase to prevent masking and to eliminate precedence effects.

Testing protocol

Choice test design and analysis is based on that of Shaw (2000) and Shaw and Herlihy (2000). Testing took place in a semi-anechoic chamber under red light and at 25.5±2.5°C. Females were tested on a y-maze, made of 1.2 m dowels arranged at the vertices of an equilaterial triangle, positioned at an incline to stimulate upward movement of the females. The playback setup consisted of a laptop computer running BatSound Pro (Pettersson Elektronik AB, 2001) and two liquid-cooled super tweeters (model 40-1310A, RadioShack, Fort Worth, TX), equipment capable of reproducing the majority of song energy in the recordings (6-40 kHz). I adjusted broadcast amplitude to 90 dB SPL at the release point, using a precision sound level meter (model 1982, GenRad, Concord, MA) and a custom amplifier.

Stimulus presentation sessions began 1 h after the onset of the dark phase. Before testing a queue of females from a particular population, the eight stimulus pairs for that population were randomized. The first stimulus pair was played to a female, and 0 or 1 were scored for choice of the low or the high stimulus, respectively. Response was scored only if a female approached 2/3 the distance to a speaker within 2 min of broadcast; otherwise, a no response was scored. The 2 min time limit was developed after observing that females were unlikely to respond given more time. The female then received a second broadcast of the same stimulus pair with the presentation switched between speakers, necessitating a change of direction for the female to approach the preferred stimulus, thus controlling for side bias. I tested all females in the queue with the first stimulus pair as above, and then returned to the beginning of the queue for the second pair, proceeding in an identical fashion until all stimulus pairs had been tested for all females. Between exposure to different stimuli, females were acoustically isolated for 30-45 min to reduce habituation. Each female was tested again a week later in a second session, resulting in a total of four tests per stimulus pair per female. The repetitions were split into two sessions because females that received all four repetitions in the same session showed signs of habituation (pers. obs.).

Data analysis

I treated the binary choice data as counts in contingency tables and analyzed them with repeated-measures G tests (Sokal and Rohlf, 2000) calculated manually (Table 2). I tested all populations in this manner except Arroyo Seco, where a small sample size prevented complete statistical analysis. I addressed each stimulus pair in a separate table analysis because different numbers of females responded to each pair. The expected value for all G tests was a proportion of 0.5 to each stimulus in the pair, representing the expectation of random movement or no preference. The additive properties of G statistics allow partitioning of significance into individual, pooled, and heterogeneity components. For each stimulus pair, I first calculated $G_{\text{individual}}$ scores from each of the two test sessions the females experienced. The sums of the $G_{\text{individual}}$ scores are the G_{total} scores for that stimulus pair. I then calculated G_{pooled} from the column sums to derive overall measures of female response to each stimulus pair. Finally, I calculated $G_{\text{heterogeneity}}$ values by subtracting G_{pooled} from G_{total}. G scores were tested for significance against a χ^2 distribution, using 2 df for G_{total} values and 1 df for $G_{\text{individual}}$, G_{pooled}, and $G_{\text{heterogeneity}}$. Significant G_{pooled} values represent a deviation from the 0.5 expected response, and thus a significant preference for one of the two stimuli in the pair. Significant $G_{\text{heterogeneity}}$ values represent inconsistency of response between the two test sessions.

Preference functions of the pn choice data are plotted in two components (Fig. 2). The response bias (upper line graph) depicts the proportion of responses to the higher stimulus in the pair. Significant biases are those without confidence intervals intersecting 0.5, the null hypothesis of no preference (dashed line). One asterisk for each magnitude of significance of the G_{pooled} test components are indicated above the appropriate stimulus pairs. The response frequency (lower histogram, filled bars) shows the proportion of female responses to the stimulus pairs. Also depicted on the response frequency histograms are the proportion of male chirps with pulse numbers occurring in each stimulus pair category (lower histogram, open bars). Note that the plot of male pulse number is cumulative in these plots, and that proportions add to greater than one, because a single pulse number falls into multiple overlapping categories. Although I did not determine significance levels for Arroyo Seco because of a small sample size (Table 2), I plotted the data in order to compare the preference function shape.

I used the assumption that pn preferences are transitive in order to plot each pn stimulus tested against a reference stimulus, thereby converting the binary choice data into continuous preference functions (Fig. 3) that are comparable with the single stimulus testing performed for ipi. To illustrate transitive preference, consider a female that is presented with a pair of stimuli, a and b, of which she prefers stimulus b over stimulus a. The female is then given a second pair of stimuli, b and c, of which she prefers stimulus c. Assuming transitive preference, the female is expected to prefer stimulus c over stimulus a. Transitive preference is a widely studied phenomenon in humans and vertebrate animals (Vasconcelos, 2008), applied to such contexts as foraging (e.g. Bateson, 2002) and dominance hierarchies (e.g. Tufto et al., 1998), in which the transitive property is sometimes violated (e.g. Waite, 2001). Transitive preference has received little study in invertebrates. Experiments with honeybees (Bernard and Giurfa, 2004; Shafir, 1994) demonstrated intransitive preferences in a foraging application involving experience and cognition. The transitive assumption may however be valid in a non-cognitive, neuroethological application, such as the genetically determined female response to acoustic stimuli in the Orthoptera studied here.

To construct transitive preference functions (Fig. 3), I arranged the eight stimulus pairs into two non-overlapping series of four, i.e. the first series included the 1 vs. 7, 7 vs. 13, 13 vs.

19, and 19 vs. 25 pairs, and the second series included the 4 vs. 10, 10 vs. 16, 16 vs. 22 and 22 vs. 28 pairs. For each series, I then compared each higher stimulus pair to the lowest by multiplying the odds ratios obtained from the *G* tests (Table 3). The two series were then overlapped to create a continuous function. Finally, I created a standardized phonotaxis score by dividing each transitive response value by the maximum, thereby setting the maximum to 1 (Table 3).

No-choice testing of ipi

Stimulus construction

Stimuli for ipi testing were derived from natural song. For each population, I selected a single pulse of average length from a bank of laboratory recordings made at a sampling rate of 150 kHz. The starting point for all experimental stimulus ranges was the precise population-specific ipi means (Table 1). From this reference point, I adjusted the spacing of the pulses using sound editing software (CoolEdit v. 1.53, Syntrillium Software, 1996) at intervals appropriate to the population standard deviations (Table 4). I included silent intervals after the terminal pulses corresponding to the minimum interchirp intervals, and upsampled the sound files to 250 kHz. Although each population is tested widely I did not test across the entire possible ipi range, which spans an order of magnitude (range 24-259 ms). Because my goal was to test coevolutionary predictions, I chose stimulus increments that would allow the estimation of detailed preferences in the vicinity of each population mean. When females responded over a wider range of stimuli than expected, I compensated by adding stimuli.

Testing protocol

I performed no-choice tests with a Kramer locomotion compensator (kugel, servosphere). The testing protocol and data analysis are based on Schul (1998). An animal is positioned on top of the motor-driven sphere, which compensates for the movements of the animal to maintain its position at the zenith of the sphere. Computer tracking is accomplished via a detector that tracks a piece of reflective foil adhered to the pronotum of the katydid. The apparatus outputs the movement angle α and the elapsed time after every 1 cm of motion.

The treadmill was housed in a semi-anechoic test chamber maintained at 25-26^{o}C. Katydids were tested in the dark, and the trial was monitored in a separate room with an infrared camera. A custom computer program played the stimulus files at a 250 kHz sampling rate through a pair of leaf tweeters positioned 90^{o} apart, both aimed 1 cm above the zenith of the sphere. A custom attenuator adjusted playback to 80 dB SPL.

After an initial 2 min silent acclimation period, stimuli were delivered to females in the following sequence: control, 2-3 test stimuli, control. The control stimulus contains the population ipi mean and therefore approximates the most common song a female may encounter in nature. Two repetitions of each stimulus were offered in immediate succession, the first repetition played through the left speaker and the second through the right speaker. A responsive female changed direction 90^{o} as the presentation switched. All repetitions lasted 2 min, and the different stimuli in the program were separated by 2 min silent periods to reduce habituation. Up to three sequences of different stimuli were presented to a female in one session, with total session time lasting about 1 h. Half the animals tested received the stimuli in reverse order to control for presentation sequence effects.

Data Analysis

Test stimuli were analyzed only if surrounded by consistent, comparable controls. Failure to respond to controls meant females were no longer responsive, rendering the test data invalid. Left and right speakers were combined by adding 90^{o} to all α measured from the right speaker presentation. The data output from the sphere was then used to calculate three measures: 1) the vector length VL = $\sqrt{}$ [$(\Sigma\cos\alpha)^2 + (\Sigma\sin\alpha)^2$)]/n (n = number of α measurements), which measures the consistency of the female's course; 2) the walking speed; and 3) the vector angle $\gamma = \arctan(\Sigma \sin\alpha / \Sigma \cos\alpha)$, which measures the absolute direction of female movement. A phonotaxis score (PS) is then calculated for each test stimulus relative to the control stimulus with the following formula:

$$PS = VL \times (\text{speed test}/\text{speed ctrl}) \times (\cos |\gamma_{test} - \gamma_{ctrl}|)$$

The angle component now compares the test angle relative to the control and not to the actual speaker position, correcting for females that respond with an error angle (Schul, 1998) and do not walk in the absolute direction of the speaker. The phonotaxis score ranges from -1 (perfect negative phonotaxis) to +1 (perfect positive phonotaxis). A phonotaxis score of 0 indicates random orientation. Preference functions are plotted as the average phonotaxis score of all females tested for that stimulus with the corresponding standard errors (Fig. 4).

Hypothesis testing

To test the sexual selection hypothesis, I compared the female preference distributions with the male song distributions using nonparametric Spearman rank correlation (Sokal and Rohlf, 2000). The transitive pn functions and the ipi functions were all tested in this manner (Table 5). A significant Spearman statistic r_s indicates that the two distributions increase and decrease concomitantly, i.e. higher phonotaxis scores are correlated with a large proportion of males producing that stimulus in their songs, and low phonotaxis scores are likewise correlated with a low proportion of that stimulus in male songs. The null hypothesis is that the distributions of female preferences and male songs are uncorrelated.

To test the species recognition hypothesis, I asked if female response varied significantly across ipi stimuli, as the ranges of heterospecifics are approached, using Kruskall-Wallis tests (Sokal and Rohlf, 2000). A significant outcome rejects the null hypothesis that the response distributions to all ipi stimuli have the same median. The magnitudes and signs of the Z-scores identify stimulus categories responsible for the heterogeneity. Statistical analyses were performed with Minitab v. 13 (Minitab Inc., 2000).

RESULTS

Choice testing of pulse number

Females were highly phonotactic during y-maze testing, with average response rates ranging from 48% (Point Dume) to 64% (Point Mugu) (Fig. 2). Females responded consistently between the two test sessions: only two populations showed significantly different $G_{\text{heterogeneity}}$ values, each for only one stimulus pair (Table 2). Females from Point Mugu (Fig. 3a), Topanga Canyon (Fig. 3d), and Point Dume (Fig. 3c) have directional pn preferences, the former selecting for high pn, and the latter two selecting for low pn. Nicholas Flat (Fig. 3b) females have stabilizing preference on male songs with 7-10 pulses. Arroyo Seco (Fig. 3e) and Millard Canyon (Fig. 3f) females have bimodal preference functions: the Millard function is stabilizing at 4 pulses, and directional at the upper end, while Arroyo Seco shows two stabilizing peaks, one at 10 pulses and the other at 16-19 pulses.

No-choice testing of ipi

Females were not strongly phonotactic during servosphere testing, and had difficulty localizing the speakers. Many females phonotaxed in a spiral instead of a linear pattern, or moved little overall during testing. Individual variation in preference was also high. These factors produced generally low phonotaxis scores and wide confidence intervals.

Three populations show stabilizing preference for ipi (Fig. 4): Millard, Point Dume, and *A. diminutiva dactyla* from Point Mugu. The Millard Canyon function (Fig. 4e) shows little change in phonotaxis score across the entire range tested. The preference peak estimated at 84 ms is considerably lower than the male ipi mean of 164 ms. The Point Dume function (Fig. 4c) peaks at 47 ms, higher than the male ipi mean of 32 ms. The Point Mugu *A. diminutiva dactyla* function (Fig. 4a) is broadly stabilizing throughout the upper ipi range tested, with a peak estimated precisely at the male mean of 167 ms. The function drops sharply across the lower ipi range, with phonotaxis scores decreasing by nearly a factor of three between 67 and 27 ms.

Topanga *A. longipennis* and Point Mugu *A. morsei costalis* have directional preferences, selecting for the shortest ipi offered. The Topanga function (Fig. 4d) gradually declines with increasing ipi, and climbs again at the limit of the range tested. In Point Mugu *A. morsei costalis* (Fig. 4b) phonotaxis score generally decreases with increasing ipi, although abrupt change occurs at the upper end of the range, where a secondary peak exists at 87 ms.

Hypothesis testing

Female pulse number preference and male pulse number distributions were marginally significantly correlated in the Point Dume and Topanga Canyon populations (Table 5). A marginally significant correlation was also found in *A. m. costalis* from Point Mugu but this correlation is negative (r_s=-0.716), suggesting a mismatch between female preference and male pulse number. The Nicholas Flat correlation was highly significant. Millard Canyon and Arroyo Seco female preferences and male pulse number distributions were uncorrelated. With the single exception of *A. diminutiva dactyla* at Point Mugu, female ipi preference distributions and male ipi song distributions were uncorrelated (Table 5).

Of the five interpulse interval preference functions that were estimated, only the two sympatric species at Point Mugu showed significant heterogeneity. For *A. morsei costalis*, the heterogenity is caused by the highest two stimuli offered, 77 and 97 ms (Kruskal-Wallis test, H=22.14, Z_{77ms}=-2.54, Z_{97ms}=-2.98, p=0.008). For *A. d. dactyla*, the two lowest stimuli (27 and 47 ms) are responsible for the heterogeneity (Kruskal-Wallis test, H=32.14, Z_{27ms}=-2.31, Z_{47ms}=-2.98, p=0.0001). Functions from the three allopatric taxa were not significantly different across measurements.

DISCUSSION

The efficiency of female response depended on the testing protocol used. In particular, the ability of females to localize sound in an open area without landmarks, as occurred during the servosphere testing, is apparently limited. I do not find this surprising considering the thamnophilous habits of these katydids in the field. Phonotaxis on open ground is probably rare; instead, females likely follow routes along branches through the dense thicket of bushes, where numerous landmarks are available. The Y-maze simulates branches along which the female can move to a calling male. Response on the Y-maze was more efficient, emphasizing the need to select a biologically realistic testing protocol for female choice studies.

I hypothesize that sexual selection may be operating on pn. Marginally to highly significant correlations between female preference functions and male pn distributions were found in several populations (Table 3, Fig. 3b,c,d), agreeing with the predictions of coevolutionary sexual selection. Some preferences were open-ended (Fig. 3a,c,d), selecting for pn that exceed mean male values, a pattern consistent with pn being a dynamic trait under sexual selection. All within-male pn CV are greater than 10%, which likewise classify pn as dynamic (Table 1). Pn affects call duration, a sexually selected feature frequently associated with directional preference (reviewed in Greenfield, 2002). For example, Tuckerman et al. (1993), working with the phaneropterine katydid *Scudderia curvicauda*, showed a correlation between pn and body size, which is a male quality trait. Longer chirps are also expected to stimulate the female nervous system to a greater degree or provide more phonotactic information than shorter chirps.

Both intrasexual as well as intersexual selection may be operating on pn, and some pn variation may be explained by male-male competition. In the field, males aggregate closely, sometimes positioned centimeters from each other on the same stem, and field recordings showed pulses of neighboring males exactly overlaying each other (pers. obs.). I often had difficulty identifying calling males in these aggregated choruses. Males may compete to attract females by adding more pulses and overlapping neighbors' calls, exploiting the auditory precedence effect (Greenfield et al., 1997).

A. morsei costalis females at Point Mugu show preferences that are significantly negatively correlated with the male pn distribution (Fig. 3a). This strong exception to the coevolutionary expectation may relate to the presence of a sympatric and syntopic species, *A. diminutiva dactyla*. The male pn distributions of both of these species occupy separate regions in pn parameter space: *A. morsei costalis* songs have 13.6±3.6 pulses, and *A. diminutiva dactyla* songs have 38.5±16.2 pulses. Despite the fact that the male pn is displaced, female preference of *A. morsei costalis* has apparently not followed suit. Directional selection by *A. morsei costalis* females (Fig. 3a) on high pn should lead this species to choose *A. diminutiva dactyla* males, which sing with the higher pn mean. This discrepancy can be explained if *A. m. costalis* females override sexual selection for pn depending on whether males first meet a species recognition criterion found in the ipi (discussed below). I therefore speculate that sexual selection and species recognition in *A. morsei costalis* represent separate processes.

Based on the following lines of evidence, I hypothesize that ipi is evolving into a species recognition feature in *A. morsei costalis* as a consequence of secondary contact with *A. diminutiva dactyla* in the western Santa Monica Mountains. First, pr and ipi tend to be static traits involved in species recognition (Gerhardt, 1991; Shaw and Herlihy, 2000). However, the CV's classify ipi as a dynamic trait (Table 1), as they are much higher than the criteria established by Gerhardt (1991). The only CV measurement approaching the static criteria is *A.*

morsei costalis at Point Mugu, with a between-male CV of 8%. The ipi of *A. morsei costalis* is certainly less variable than the ipi of allopatric populations, and may represent a reduction in variability as it is evolving into a species recognition feature. The between-male CV of *A. diminutiva dactyla* ipi is 24.6%, one of the highest values measured, suggesting ipi displacement has occurred only in males of one member of the secondary contact event, i.e. *A. morsei costalis*. Saetre et al. (1997) demonstrated displacement of the male signal in only one member of a sympatric pair, in the breeding color in a European flycatcher hybrid zone caused by reinforcing selection.

Second, coevolutionary intersexual selection does not explain the evolution of the ipi distributions in any of the *A. morsei* or *A. longipennis* populations studied. To the contrary, females of allopatric populations had broad, flat, non-heterogeneous preferences (e.g. Fig. 4e) across the ipi ranges tested, and responded to a wide range of other stimuli in addition to their usually narrow population ipi distribution, without much reduction in phonotaxis score. Significant heterogeneity of female responses to ipi stimuli were found only for the sympatric species pair at Point Mugu (Fig. 4a,b), and the stimuli responsible for heterogeneity in each case were those approaching the ipi distribution of the heterospecific species. Therefore, ipi does not appear to function in species recognition outside the region of secondary contact, and so I conclude ipi secondarily evolved a species recognition function in sympatry.

A. diminutiva dactyla female preferences show both a significant correlation with male ipi means and a significant reduction in preference as the ipi range of sympatric *A. morsei costalis* is approached. Interpreted literally, this result indicates that ipi functions in both species recognition and sexual selection contexts in *A. diminutiva dactyla*. The female preference peak and the male ipi mean coincide very closely, suggesting little evolution of the male ipi distribution or the female preference for ipi. Instead, species recognition of ipi was "appended" to *A. diminutiva dactyla* preference, apparently facilitated by ipi displacement chiefly in *A. morsei costalis*, as speculated above. Males and females of each species have thus responded to selection for species recognition in fundamentally different ways. A more thorough understanding of how female preferences for different song characters act together to select males may be gained by examining attractiveness in combined, multidimensional framework, such as Jang and Greenfield (1998) applied to sexual selection in waxmoths (*Achroia grisella*).

Third, *Aglaothorax* species are predominantly allopatric throughout their ranges. The occurrence of two species in sympatry is infrequent, and Point Mugu is the only locality I studied where two sympatric species are widely syntopic (pers. obs.). At Point Mugu, *A. morsei costalis* and *A. diminutiva dactyla* can be found throughout the riparian and coastal sage scrub habitats during a favorable year, with males singing from the same plants. *A. morsei* is distributed throughout the western Transverse Ranges, and *A. diminutiva* has a wide distribution in the Coast Ranges (Rentz and Weissman, 1981), where its type locality is situated (Rentz and Birchim, 1968). The Coast and Transverse Ranges meet in the northwest corner of the Los Angeles Basin, a region of complex topography and biogeography marked by distinct lineage breaks (Chatzimanolis and Caterino, 2007). The Santa Monica Mountains (Fig. 1), one of the ranges at this intersection, harbors the greatest diversity of *Aglaothorax* taxa both in terms of song (Chapter 1) and morphology (Rentz and Weissman, 1981). Secondary contact between the *A. morsei* and *A. diminutiva* lineages, and subsequent selection for species recognition, may have generated this diversity.

The sympatric species pair is perhaps in an early stage of secondary contact, and displacement is evolving in two other components of their biology as well as mating system:

habitat and mating season. During most years I studied at Point Mugu, *A. morsei costalis* was common in the canyon bottoms in moist riparian habitats, while *A. diminutiva dactyla* occurred on the drier chaparral-covered slopes (pers. obs.). *A. diminutiva dactyla* also matures earlier than *A. morsei costalis*. In the former, acoustic activity was underway by late May (28-29 May), while in the latter, acoustic activity and female eclosion did not begin until mid to late June (10 and 19 June, respectively). Both patterns are obscured in favorable years, as occurred during this study in 2005 and 2008. During these years both species occurred abundantly throughout the riparian, coastal sage and chaparral habitats, and male calling overlapped broadly in June. The relatively weak female preferences found in this study render hybridization and gene flow across the Transverse Range populations a valid explanation for the song cline. A molecular phylogenetic analysis of *Aglaothorax* (Chapter 3) provides a historical framework for testing song evolution hypotheses. Additional studies should address ecology and the occurrence and costs, if any, of hybridization.

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Table 1. Taxa and localities studied. Populations are all situated along a song cline in the Transverse Ranges of southern California (Fig. 1) and are listed in geographical order from west to east. Male song data, derived from a comprehensive song analysis of *Aglaothorax* (Chapter 1), was used to calculate within male and between male coefficients of variation (CV).

				pulse number (pn)			interpulse interval (ipi)		
Taxon	Locality	Latitude	Longitude	mean±SD	within male CV	between male CV	mean±SD	within male CV	between male CV
A. diminutiva dactyla	Point Mugu State Park, Sycamore Canyon	34.0728	-119.0145	38.5±16.2	20.1%	42.1%	167.2±41.1	47.3%	24.6%
A. morsei costalis	Point Mugu State Park, Sycamore Canyon	34.0728	-119.0145	13.6±3.6	17.2%	26.2%	26.5±2.1	15.4%	8.0%
A. morsei	Leo Carillo State Park, Nicholas Flat Trail	34.0638	-118.9128	11.5±2.0	20.9%	17.8%	26.9±4.4	21.9%	16.2%
A. morsei tectinota	Point Dume Headlands State Park	34.0046	-118.8055	8.5±2,4	18.2%	28.2%	31.6±6.0	15.5%	18.9%
A. longipennis	Topanga State Park, Dead Horse Trail	34.0915	-118.5939	3.8±1.6	31.0%	42.8%	36.8±7.9	27.4%	21.5%
A. morsei morsei	Arroyo Seco, Gabrielino Recreation Trail	34.2065	-118.1670	17.5±4,0	16.2%	23.1%	99.6±23.7	52.6%	23.8%
A. morsei morsei	Millard Canyon Campground	34.2162	-118.1484	9.8±2.8	22.5%	28.6%	164.1±50.5	28.0%	30.8%

Table 2. Summary of repeated measures *G* test statistics calculated for pulse number choice tests, arranged by population. Significant tests are shown in bold. Significant $G_{\text{heterogeneity}}$ values represent inconsistency between the repeated measurements made on each female. Significant G_{pooled} statistics indicated a deviation from the null hypothesis of no preference (proportion of 0.5 to each stimulus in the pair).

stimulus pair (pulses)	n responses	G_{total}	df	p	G_{pooled}	df	p	$G_{\text{heterogeneity}}$	df	p
A. morsei costalis Point Mugu				n=17						
1 vs. 7	37	22.834	2	<0.00001	21.986	1	**<0.00001**	0.848	1	0.357
4 vs. 10	42	27.696	2	<0.00001	27.562	1	**0.000**	0.133	1	0.715
7 vs. 13	43	3.993	2	0.136	3.992	1	**0.046**	0.000	1	0.988
10 vs. 16	46	1.401	2	0.496	1.398	1	0.237	0.003	1	0.958
13 vs. 19	42	2.426	2	0.297	1.533	1	0.216	0.893	1	0.345
16 vs. 22	40	3.696	2	0.158	3.656	1	0.056	0.040	1	0.841
19 vs. 25	39	2.189	2	0.335	2.096	1	0.148	0.094	1	0.760
22 vs. 28	40	0.405	2	0.817	0.000	1	1.000	0.405	1	0.525
A. morsei Nicholas Flat				n=20						
1 vs. 7	33	21.726	2	<0.00001	21.372	1	**<0.00001**	0.354	1	0.552
4 vs. 10	36	17.466	2	0.000	17.466	1	**<0.00001**	0.000	1	1.000
7 vs. 13	45	19.727	2	<0.00001	17.347	1	**<0.00001**	2.380	1	0.123
10 vs. 16	41	2.117	2	0.347	1.201	1	0.273	0.916	1	0.339
13 vs. 19	37	0.440	2	0.803	0.027	1	0.869	0.413	1	0.521
16 vs. 22	33	1.974	2	0.373	0.761	1	0.383	1.213	1	0.271
19 vs. 25	32	0.876	2	0.645	0.501	1	0.479	0.375	1	0.540
22 vs. 28	34	3.176	2	0.204	2.985	1	0.084	0.190	1	0.663
A. morsei tectinota Point Dume				n=22						
1 vs. 7	55	30.652	2	<0.00001	30.624	1	**<0.00001**	0.028	1	0.867
4 vs. 10	56	11.351	2	0.003	10.626	1	**0.001**	0.725	1	0.395
7 vs. 13	48	0.510	2	0.775	0.334	1	0.563	0.176	1	0.674
10 vs. 16	45	18.190	2	0.000	12.330	1	**0.000**	5.860	1	**0.015**
13 vs. 19	40	16.326	2	0.000	15.420	1	**<0.00001**	0.907	1	0.341
16 vs. 22	40	2.554	2	0.279	2.527	1	0.112	0.027	1	0.870
19 vs. 25	28	2.040	2	0.361	1.296	1	0.255	0.745	1	0.388
22 vs. 28	28	2.417	2	0.299	1.296	1	0.255	1.121	1	0.290
A. longipennis Topanga Cyn.				n=28						
1 vs. 7	83	53.427	2	<0.00001	46.473	1	**<0.00001**	6.954	1	**0.008**
4 vs. 10	84	3.457	2	0.178	3.066	1	0.080	0.390	1	0.532
7 vs. 13	75	0.027	2	0.987	0.013	1	0.908	0.014	1	0.907
10 vs. 16	63	3.311	2	0.191	0.397	1	0.529	2.914	1	0.088
13 vs. 19	60	0.573	2	0.751	0.267	1	0.605	0.307	1	0.580
16 vs. 22	55	8.251	2	0.016	8.225	1	**0.004**	0.026	1	0.873
19 vs. 25	54	4.820	2	0.090	4.813	1	**0.028**	0.007	1	0.933
22 vs. 28	52	3.580	2	0.167	2.794	1	0.095	0.786	1	0.375
A. morsei morsei Arroyo Seco				n=8						
1 vs. 7	13									
4 vs. 10	11									
7 vs. 13	20									
10 vs. 16	11									
13 vs. 19	27									
16 vs. 22	27									
19 vs. 25	20									
22 vs. 28	15									
A. morsei morsei Millard Cyn.				n=13						
1 vs. 7	39	18.455	2	<0.00001	17.358	1	**<0.00001**	1.097	1	0.295
4 vs. 10	40	10.241	2	0.006	8.398	1	**0.004**	1.843	1	0.175
7 vs. 13	44	6.010	2	0.050	5.954	1	0.015	0.056	1	0.813
10 vs. 16	29	0.867	2	0.648	0.866	1	0.352	0.001	1	0.979
13 vs. 19	28	0.623	2	0.732	0.573	1	0.449	0.050	1	0.823
16 vs. 22	35	2.156	2	0.340	0.257	1	0.612	1.898	1	0.168
19 vs. 25	36	4.078	2	0.130	4.078	1	**0.043**	0.000	1	1.000
22 vs. 28	25	3.922	2	0.141	1.986	1	0.159	1.935	1	0.164

Table 3. Transitive pn preferences were calculated by multiplying the odds ratios obtained from the *G* tests across two separate, contiguous ranges (1-25, 4-28 pulses). For each series, a scaled phonotaxis score is created by dividing each transitive preference value by the maximum.

stimulus pair (pulses)	odds ratio	transitive stimulus	transitive preference	phonotaxis score (scaled)
A. morsei costalis Point Mugu				
1 vs. 7	6.400	1	6.400	0.228
7 vs. 13	1.867	7	11.949	0.425
13 vs. 19	1.471	13	17.577	0.625
19 vs. 25	1.600	19	28.123	1.000
4 vs. 10	7.400	4	7.400	0.379
10 vs. 16	1.421	10	10.515	0.539
16 vs. 22	1.857	16	19.527	1.000
22 vs. 28	1.000	22	19.527	1.000
A. morsei Nicholas Flat				
1 vs. 7	7.250	1	7.250	0.250
7 vs. 13	4.000	7	29.000	1.000
13 vs. 19	0.947	13	27.463	0.947
19 vs. 25	0.728	19	19.993	0.689
4 vs. 10	5.000	4	5.000	0.708
10 vs. 16	1.412	10	7.060	1.000
16 vs. 22	0.737	16	5.203	0.737
22 vs. 28	0.545	22	2.836	0.402
A. morsei tectinota Point Dume				
1 vs. 7	5.880	1	5.880	1.000
7 vs. 13	0.846	7	4.974	0.846
13 vs. 19	0.250	13	1.244	0.212
19 vs. 25	0.647	19	0.805	0.137
4 vs. 10	2.500	4	2.500	1.000
10 vs. 16	0.324	10	0.810	0.324
16 vs. 22	0.600	16	0.486	0.194
22 vs. 28	0.647	22	0.314	0.126
A. longipennis Topanga Cyn.				
1 vs. 7	5.917	1	5.917	0.974
7 vs. 13	1.027	7	6.077	1.000
13 vs. 19	0.815	13	4.953	0.815
19 vs. 25	0.543	19	2.689	0.443
4 vs. 10	1.471	4	1.471	0.853
10 vs. 16	1.172	10	1.724	1.000
16 vs. 22	0.447	16	0.771	0.447
22 vs. 28	0.625	22	0.482	0.279
A. morsei morsei Arroyo Seco				
1 vs. 7	5.500	1	5.500	0.404
7 vs. 13	1.857	7	10.214	0.750
13 vs. 19	0.889	13	9.080	0.667
19 vs. 25	1.500	19	13.620	1.000
4 vs. 10	4.500	4	4.500	0.375
10 vs. 16	2.667	10	12.002	1.000
16 vs. 22	0.889	16	10.669	0.889
22 vs. 28	0.875	22	9.336	0.778
A. morsei morsei Millard Cyn.				
1 vs. 7	4.570	1	4.570	0.281
7 vs. 13	2.140	7	9.780	0.602
13 vs. 19	0.830	13	8.117	0.500
19 vs. 25	2.000	19	16.234	1.000
4 vs. 10	2.640	4	2.640	1.000
10 vs. 16	0.700	10	1.848	0.700
16 vs. 22	1.190	16	2.199	0.833
22 vs. 28	1.180	22	2.595	0.983

Table 4. Test stimuli, sample sizes, and phonotaxis scores of no-choice interpulse interval testing. Phonotaxis scores for test stimuli are calculated relative to a control stimulus, the population ipi mean (shown in bold). Scaled scores are divided by the maximum.

ipi stimulus (ms)	n	phonotaxis score	scaled phonotaxis score (mean±SE)
A. diminutiva dactyla Point Mugu			
27	3	0.221	0.252 ± 0.210
47	5	0.294	0.335 ± 0.116
67	5	0.634	0.721 ± 0.092
87	7	0.611	0.695 ± 0.070
107	7	0.771	0.877 ± 0.056
127	7	0.786	0.894 ± 0.064
147	7	0.815	0.927 ± 0.042
167	7	0.879	1.000 ± 0.027
187	7	0.723	0.822 ± 0.047
207	7	0.764	0.870 ± 0.021
227	3	0.474	0.539 ± 0.065
A. morsei costalis Point Mugu			
7	7	0.753	1.000 ± 0.044
17	7	0.606	0.805 ± 0.089
27	7	0.650	0.862 ± 0.093
37	7	0.662	0.879 ± 0.112
47	7	0.532	0.707 ± 0.097
57	7	0.570	0.757 ± 0.099
67	7	0.446	0.592 ± 0.098
77	7	0.288	0.382 ± 0.083
87	7	0.664	0.881 ± 0.137
97	6	0.206	0.274 ± 0.116
A. morsei tectinota Point Dume			
17	6	0.597	0.726 ± 0.090
32	6	0.712	0.865 ± 0.086
47	6	0.823	1.000 ± 0.051
62	6	0.666	0.810 ± 0.086
77	6	0.687	0.835 ± 0.082
92	6	0.497	0.604 ± 0.074
A. longipennis Topanga Cyn.			
7	6	0.603	1.000 ± 0.064
37	6	0.593	0.983 ± 0.131
67	6	0.509	0.845 ± 0.159
97	6	0.351	0.582 ± 0.176
127	6	0.272	0.452 ± 0.127
157	6	0.406	0.673 ± 0.181
A. morsei morsei Millard Cyn.			
14	5	0.587	0.759 ± 0.102
44	5	0.637	0.824 ± 0.056
84	5	0.773	1.000 ± 0.026
124	5	0.720	0.931 ± 0.083
164	5	0.669	0.866 ± 0.076
204	5	0.541	0.699 ± 0.159

Table 5. Hypothesis testing of correlation between female preference and male trait distributions. Spearman rank correlation was used to test correlation between female preferences and male traits, which is predicted by sexual selection. The Spearman coefficients (r_s) show the magnitude and direction of correlation. Kruskall-Wallis tests were used to test for heterogeneity of ipi preference functions, as species recognition predicts narrowing of preference as females discriminate against heterospecifics. The magnitude of heterogeneity is reported by H. Significant tests are shown in bold.

	pulse number		interpulse interval			
Taxon and population	r_s	p	r_s	p	H	p
A. diminutiva dactyla Point Mugu	-	-	0.704	**0.016**	32.14	**0.0001**
A. morsei costalis Point Mugu	-0.716	**0.046**	0.174	0.631	22.14	**0.008**
A. morsei Nicholas Flat	0.916	**0.001**	-	-	-	-
A. morsei tectinota Point Dume	0.735	**0.038**	0.778	0.069	9.42	0.094
A. longipennis Topanga Cyn.	0.727	**0.041**	0.393	0.441	4.62	0.464
A. morsei morsei Arroyo Seco	0.506	0.201	-	-	-	-
A. morsei morsei Millard Cyn.	-0.018	0.966	-0.152	0.774	3.34	0.647

FIGURE CAPTIONS

Figure 1. Map of localities sampled for preference testing. Sites were chosen to sample both geographic breadth and taxonomic diversity along the song cline in the Transverse Ranges of southern California.

Figure 2. Pulse number preference functions. Upper line graphs are response biases generated from G_{pooled} statistics calculated from the binary choice test data. Each point on the response bias graph represents the odds ratio that the higher stimulus in the pair is chosen over the lower, with accompanying standard error. The X axis shows the number of pulses per chirp in the stimulus pairs tested. The Y axis on the right shows the proportion of females responding to the higher of the two stimuli in the pairs. The dashed line marks the null hypothesis of no preference, i.e. a proportion of 0.5 to each stimulus. Significance levels of tests in which the null hypothesis was rejected are marked with asterisks (*, $0.01<p<0.05$; **, $0.001<p<0.009$;***, $p<0.0001$). On the histograms below the response bias curves, the solid bars show the response frequencies, with the left y-axis showing proportion of trials in which females responded to either stimulus in the pair. The open bars show the proportion of male chirps falling into each stimulus category, measured from a bank of laboratory recordings (Chapter 1). Taxa and populations are presented from west to east, and these are: a. *A. morsei costalis* Point Mugu; b. *A. morsei* Nicholas Flat; c. *A. morsei tectinota* Point Dume; d. *A. longipennis* Topanga Cyn.; e. *A. morsei morsei* Arroyo Seco; f. *A. morsei morsei* Millard Cyn.

Figure 3. Continuous pn preference functions, constructed assuming female preference obeys a transitive property. Transitive phonotaxis scores are calculated by multiplying odds ratios, obtained in repeated measures *G* tests, across the series of stimulus pairs. Histograms show the frequency of male chirps falling into the stimulus category, binned by increments of three pulses. Taxa and populations shown are: a. *A. morsei costalis* Point Mugu; b. *A. morsei* Nicholas Flat; c. *A. morsei tectinota* Point Dume; d. *A. longipennis* Topanga Canyon.; e. *A. morsei morsei* Arroyo Seco; f. *A. morsei morsei* Millard Canyon.

Figure 4. Interpulse interval (ipi) preference functions, generated from no-choice testing on a Kramer locomotion compensator device. The line graphs are the interpulse interval preference functions with standard errors. The Y axis shows phonotaxis scores computed from the Kramer treadmill output of the vector length, walking speed, and walking angle, and subsequently scaled by dividing each by the maximum score. Histograms show the frequency of male chirps containing ipi falling into each stimulus category.

Figure 1

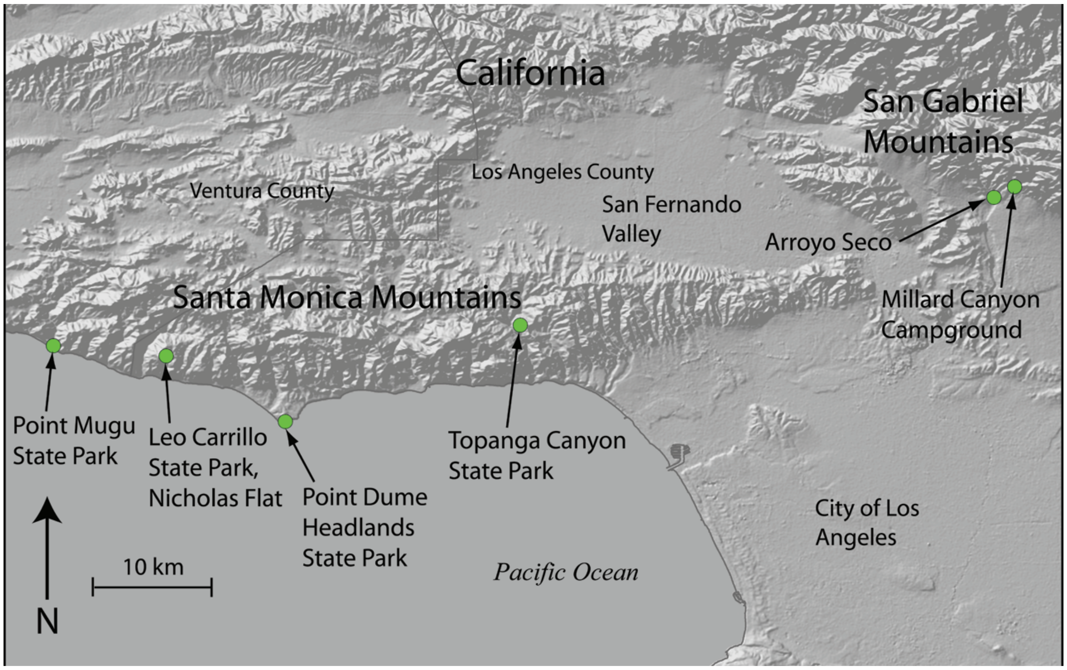

California
San Gabriel Mountains
Ventura County
Los Angeles County
San Fernando Valley
Arroyo Seco
Millard Canyon Campground
Santa Monica Mountains
Point Mugu State Park
Leo Carrillo State Park, Nicholas Flat
Point Dume Headlands State Park
Topanga Canyon State Park
City of Los Angeles
Pacific Ocean
10 km
N

Figure 2

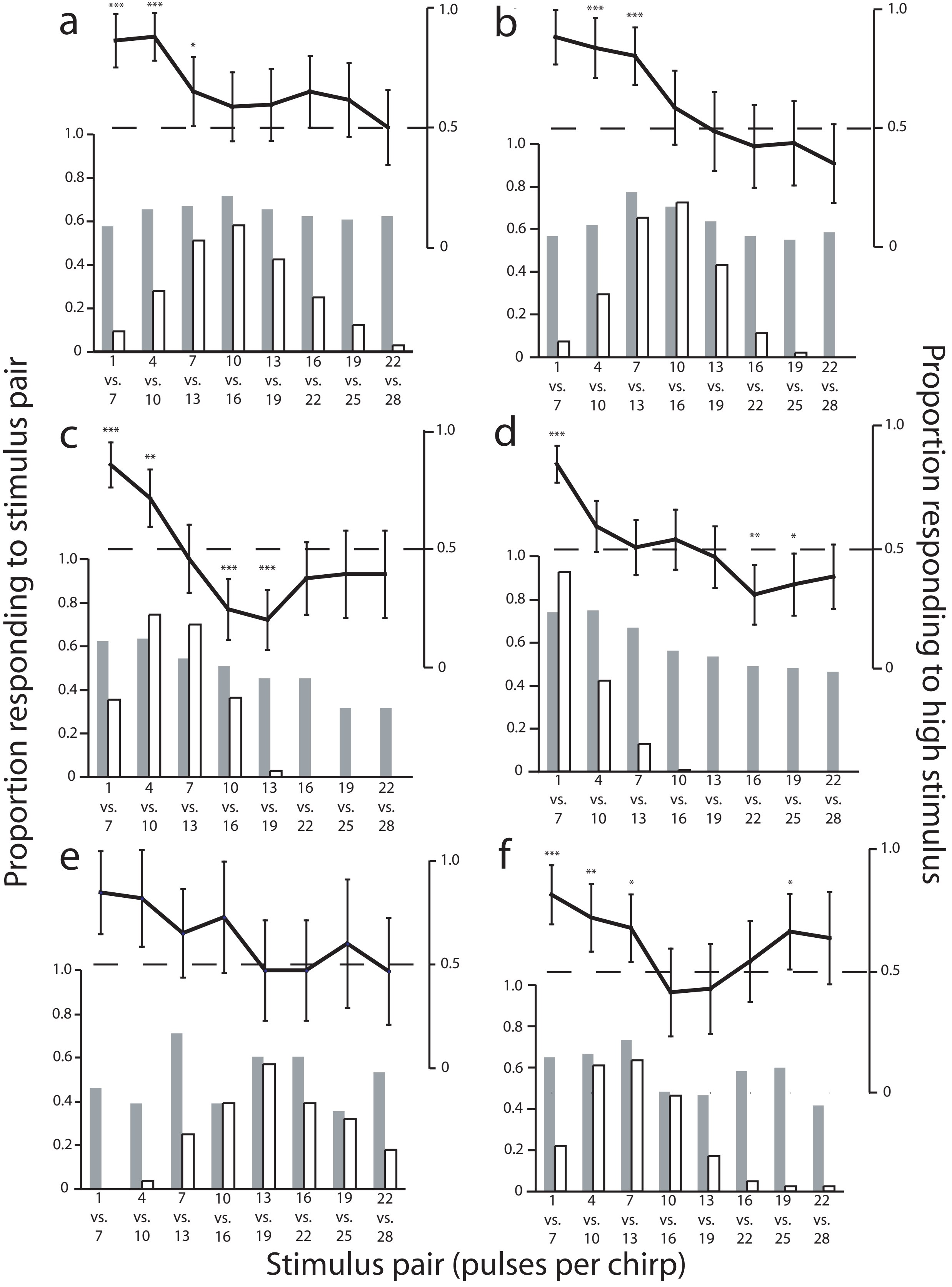

a
b
c
d
e
f
***
**
*
1.0
0.8
0.6
0.4
0.2
0
0.5
1 vs. 7
4 vs. 10
7 vs. 13
10 vs. 16
13 vs. 19
16 vs. 22
19 vs. 25
22 vs. 28
Proportion responding to stimulus pair
Proportion responding to high stimulus
Stimulus pair (pulses per chirp)

Figure 3

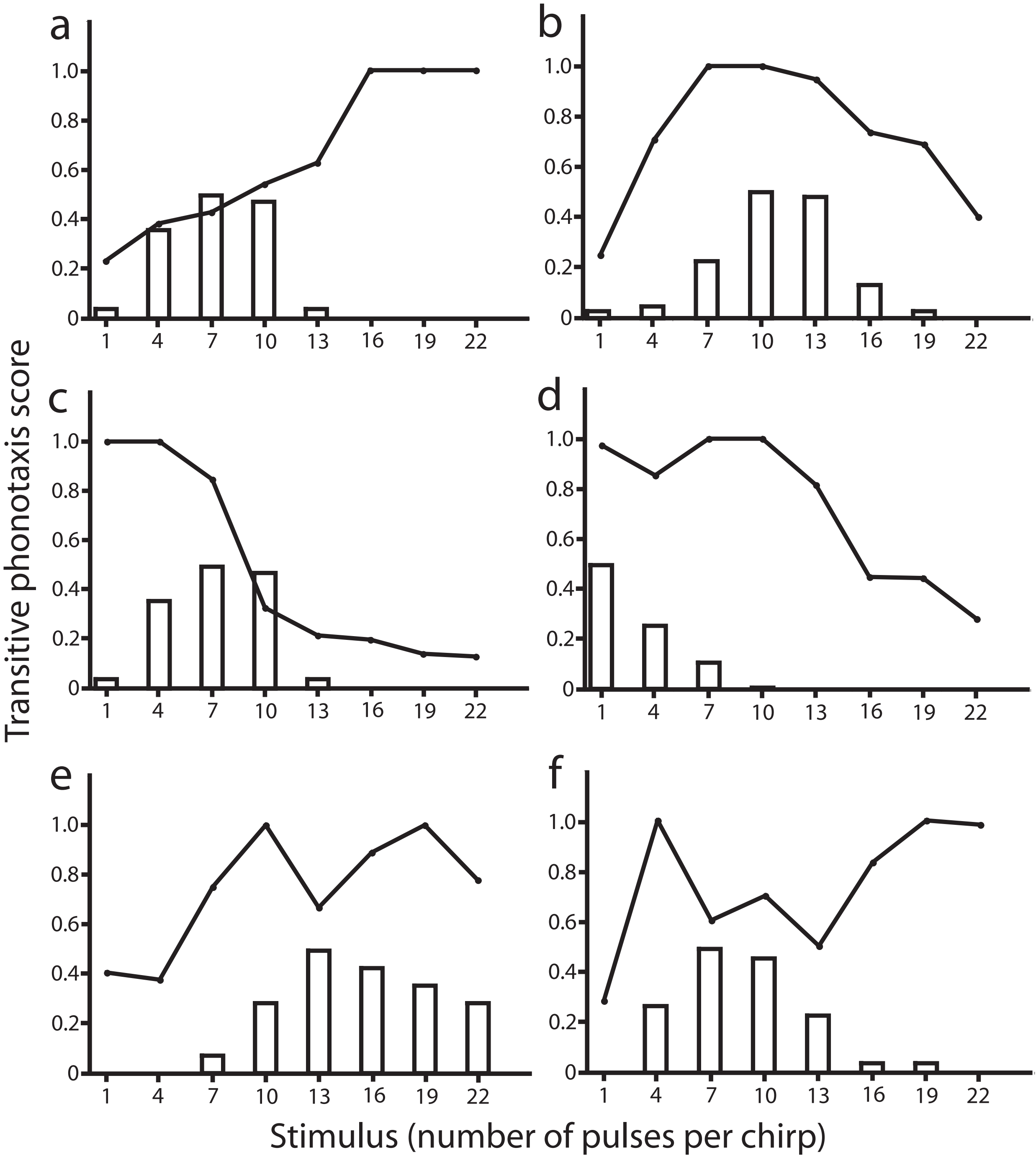
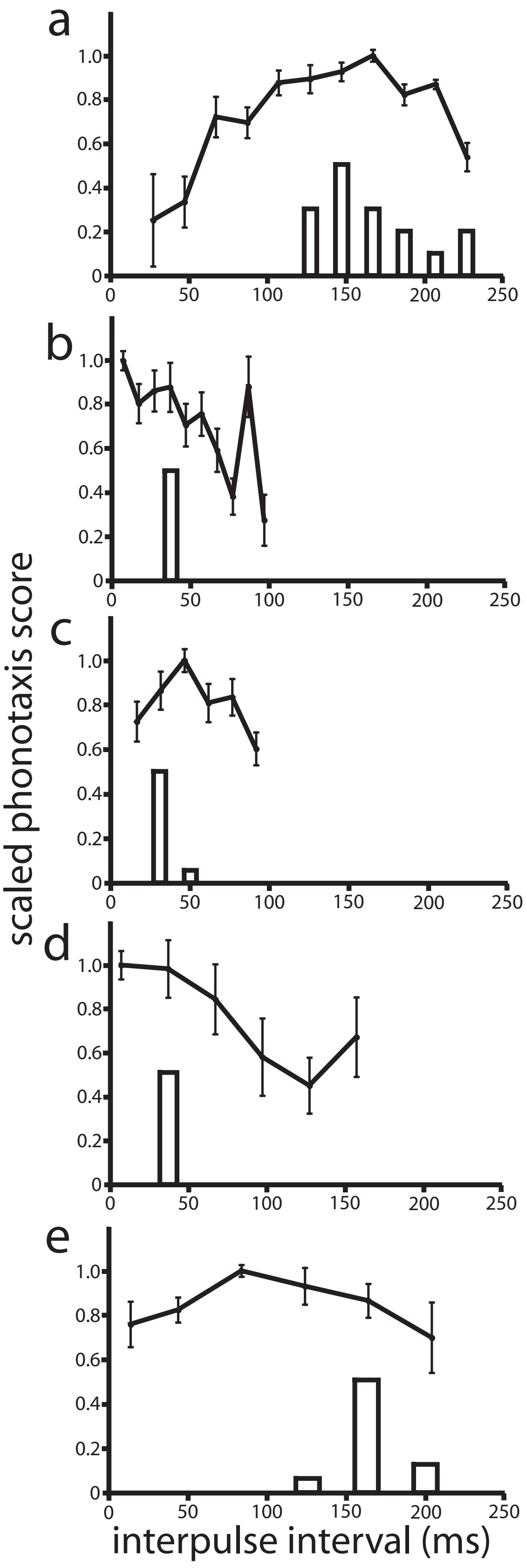

a
b
c
d
e
f
1.0
0.8
0.6
0.4
0.2
0
1
4
7
10
13
16
19
22
Transitive phonotaxis score
Stimulus (number of pulses per chirp)

Figure 4


a
b
c
d
e
1.0
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
scaled phonotaxis score
interpulse interval (ms)

Chapter 3. Phylogenetic evidence for species recognition driving evolution of the acoustic mating system of shield-back katydids (Orthoptera: Tettigoniidae: *Aglaothorax*).

INTRODUCTION

Sexual selection and species recognition are two behavioral processes that may drive the evolution of reproductive isolation by altering mating systems (reviewed in Coyne and Orr, 2004). Sexual selection may set up divergent coevolutionary trajectories of male mating trait and female preference evolution among populations (Greenfield, 2002; Ritchie, 2007). Species recognition, which is partitioned into reproductive character displacement and reinforcement based on the absence or presence of gene flow, respectively (Butlin, 1987; Coyne and Orr, 2004), is the strengthening of mate recognition systems under natural selection to avoid mating with heterospecifics. Sexual selection is theorized to drive diversification most readily in allopatry, where drifting preferences alter mating systems (Uyeda et al., 2009). Although species recognition for efficient mate localization may evolve within allopatric species (see Coyne and Orr, 2004, p. 220), the context of reproductive character displacement and reinforcement is more typical, in which divergent mating traits evolve as consequence of sympatry after secondary contact (Butlin, 1987; Noor, 1999).

Because hypotheses of behavioral isolation forge a direct connection between mating behavior and speciation, and this connection has a strong historical component, the comparative method is an essential research strategy (Darwin, 1871; Emlen et al., 2005; Ryan and Rand, 1995; West-Eberhard, 1983). Sexual selection and species recognition are predicted to leave diagnostic signatures in phylogenetic trees. If a character is hypothesized to be driving speciation in a lineage, evolution in that character is expected to be associated with recent cladogenesis, and character state changes will thus appear clustered at the terminal nodes of the phylogeny (Barraclough and Nee, 2001). Sexual selection is predicted to rapidly drive mating trait evolution that generates new, reproductively isolated species (Boul et al., 2007; Lande, 1981; Panhuis et al., 2001), so the evolution of a mating trait under sexual selection is expected to generate this pattern of terminal clustering. An alternative hypothesis is that character state change is associated with long term persistence of the lineage, which predicts changes to be clustered at the base of the tree (Barraclough and Nee, 2001). The null hypothesis is that character state changes occur at random, roughly in proportion to the time available for them to occur (Barraclough et al., 1999; Barraclough and Nee, 2001). The null hypothesis thus predicts changes will be distributed in proportion to branch lengths and will not be clustered at the tips or the base of the tree.

Selection for species recognition may be supported by two lines of evidence. First, species recognition results after secondary contact between separate lineages (Noor, 1999). If terminal taxa inhabiting a single geographic region resolve in separate clades on a phylogenetic tree, secondary contact is suggested (Futuyma, 1998). Secondary contact is further supported if reconstructions of ancestral distributions show the recent ancestors of co-occurring taxa inhabited different geographical areas (Barraclough and Vogler, 2000). Second, with species recognition, reinforcement or reproductive character displacement drives the evolution of mating traits after secondary contact. Therefore, species recognition predicts elevated amounts of mating system character state change in the lineages experiencing secondary contact compared to allopatric lineages. The null hypothesis is that secondary contact occurs without selection for species recognition. For example, ecological factors may be responsible for partitioning incipient species among niches during secondary contact (Nosil et al., 2009). The null hypothesis predicts mating traits of sympatric and allopatric lineages will exhibit amounts of character state change that are not significantly different from one another.

Previously, I established the western North American shield-back katydid genus *Aglaothorax* as a system in which to study mating system evolution and speciation (Chapter 1). The shield-back katydids (subfamily Tettigoniinae, formerly Decticinae) are the most species-rich katydid group in North America (Walker, 2000), so an understanding of their diversification may make a valuable contribution to speciation research. *Aglaothorax* belongs to the tribe Nedubini, distinguished from all other North American shield-back katydids by having the paraprocts modified into clasping organs instead of the cerci (Rentz, 1988), and thus the Nedubini are likely to represent a monophyletic group. Two other North American genera are also classified in the Nedubini: *Neduba* with eight extant species (plus one extinct species, see Rentz, 1977), and an undescribed genus and species referred to here as Jcp (Rentz and Colles, 1990; Ueshima and Rentz, 1979). Although Jcp possesses a distinctive habitus (short hind femora, reddish dome-shaped pronotum in the male) and is readily distinguished, *Aglaothorax* and *Neduba* are morphologically uniform, and intermediate forms have created confusion as to the rank of the names (genera or subgenera) and as to the assignment of species to each name (see Caudell, 1907; Rentz, 1988; Rentz and Birchim, 1968). Currently *Aglaothorax*, *Neduba*, and Jcp are treated as separate genera (Eades and Otte, 2002). A phylogeny of the Tettigoniinae based on morphological characters recovered Jcp basal to an *Aglaothorax+Neduba* clade (Rentz and Colles, 1990). The Nedubini perhaps represent the incursion of a Gondwanan lineage into North America and show morphological relationship with South American rather than other North American tettigoniine genera (Rentz and Colles, 1990; Rentz and Gurney, 1985).

Five species are currently included in *Aglaothorax*. Two are large species (body length 20-30 mm): *A. gurneyi*, a brown coniferous forest species restricted to the San Gabriel and San Bernardino Mountains of southern California, and *A. ovata*, with six subspecies, a striking, robust green and white katydid of southern California and Nevada desert mountains (Rentz and Birchim, 1968). The other three are small (body length 15-20 mm) typically brown or gray species known from southern California coastal sage scrub, chaparral, and riparian woodlands: *A. diminutiva*, *A. morsei*, and *A. longipennis*. The small species are difficult to separate morphologically, and variation within the first two species led Rentz & Weissman (1981) to describe numerous subspecies. *A. diminutiva* has the mesal tooth on the paraproct situated apically; in the latter two species, the tooth is subapical. The protrusion of the male tegmina from beyond the apex of the pronotum distinguishes *A. longipennis* from *A. morsei*. Females in some cases may be separated by the shape of the subgenital plate. The small *Aglaothorax* species are largely allopatrically distributed, but all three small species are found in the Santa Monica Mountains of southern California, where *A. diminutiva* and *A. morsei* are sympatric and syntopic (Rentz & Weissman 1981; Chapter 1.).

Calling song is the male mating trait generally responsible for pair formation in katydids (Gwynne, 2001; Spooner, 1968). I analyzed the songs of the Nedubini and found them to vary significantly among genera, species, and some subspecies (Chapter 1). Specific song features that delineated taxa were the pulse number (pn), interpulse interval (ipi), duty cycle (dc), and pulse rate (pr). Song differences did not always agree with taxa as currently defined by morphology, however, a discrepancy caused primarily by the overlapping song character distributions of *A. morsei* and *A. longipennis*. Together, the songs of these taxa form a cline across the Transverse Ranges of southern California, including the Santa Monica and San

Gabriel Mountains. Across the cline, *A. morsei* exhibit the extremes of song variation found in the genus. Female preferences for calling song ipi were consistent with the species recognition hypothesis (Chapter 2). Allopatric *A. morsei* populations showed non-heterogeneous ipi preference functions, but members of a pair of sympatric taxa from Point Mugu State Park (site 28) in the Santa Monica Mountains (*A. diminutiva dactyla* and *A. morsei costalis*; Table 1, Fig. 1) each showed a significant reduction in the probability of approaching songs with the heterospecific interval. Here I provide phylogenetic evidence to further support the species recognition hypothesis.

METHODS

Specimens and DNA Extraction

The ingroup was thoroughly sampled, including all species and the majority of subspecies (Table 1). Exemplars from numerous published localities, including type localities (Rentz and Birchim, 1968; Rentz and Weissman, 1981) were included in this study. In addition, many new localities were sampled in an effort to cover the major geographic regions of southern California (Fig. 1). Because of the uncertainty of relationships among tettigoniine genera, I used the other North American nedubine genera as outgroups, including six of the eight extant *Neduba* species and Jcp. Taxa missing from this analysis are California Channel Islands endemics (*A. morsei islandica*, *A. morsei santacruzae*, *N. propsti*), *N. steindachneri* from Puget Sound, Washington, and *N. extincta*, known only from museum specimens (Rentz, 1977). Collecting in California and Nevada occurred from 2002 to 2008. Specimens were identified morphologically with the keys in Rentz & Birchim (1968) and Rentz & Weissman (1981), and the vouchers are at this time retained in the collection of J. Cole.

Specimens for DNA extraction were preserved directly in 95% ethanol or frozen at -20°C. Muscle tissue was dissected from a hind femur, from which genomic DNA was extracted using an isopropanol precipitation protocol equivalent to the Puregene extraction kit (Gentra Systems, Inc.), resuspended in dH_20, and stored at -20°C.

Gene selection, fragment amplification, and sequencing

The morphological uniformity of nedubines and the likelihood of encountering cryptic taxa led me to choose DNA sequence data as the source of phylogenetic characters. The goal was to infer the relationships of an insect group with no existing phylogenetic hypotheses, spanning taxonomic rank from population to tribe. To meet this goal, I selected rapidly evolving genes previously characterized for phylogenetic utility.

Mitochondrial DNA (mtDNA) is widely used in species-level phylogenetics (e.g. Crespi and Sandoval, 2000; Saux et al., 2003) and population genetics (e.g. Pinto-Juma et al., 2009; Zemlak et al., 2008), and from the mitochondrial genome I used cytochrome oxidase subunit II (COII), which amplified successfully using primers in Simon et al. (1994). Nuclear genes (nDNA) remain non-trivial to obtain for species and population-level work (Broughton and Harrison, 2003; Wild and Maddison, 2008). I chose the internal transcribed spacer (ITS2), a rapidly evolving region of noncoding ribosomal nDNA used widely in species and population-level studies acoss kingdoms (Coleman, 2009; Foley et al., 2007; Mort et al., 2007). ITS2 primers used in Blattodea (Slaney and Blair, 2000) amplified well in the Nedubini. While the rapid evolution of ITS2 is a desirable quality, alignment of distantly related taxa may be problematic due to numerous insertion-deletion events. The alignment of protein coding genes by amino acid sequence is unambiguous, so to compensate for the deficiency of ITS2 at deeper phylogenetic levels, I added a protein-coding nuclear gene, *wingless*, which evolves more slowly and tends to be informative from the subgeneric to the subfamily level (Campbell et al., 2000). Primers in Brower and DeSalle (1998) amplified successfully (Table 2).

Fragments of the three genes were amplified with the polymerase chain reaction (PCR). Ten or 25 μL PCR reactions contained 0.32 mM dNTP's, 1.5 mM $MgCl_2$, 0.3 pmole/μL each of the forward and reverse primers (Table 2), 0.02 U/μL Taq polymerase (iTaq, Bio-Rad, Hercules, CA), 1X reaction buffer, and 5 ng to 1 μg template DNA. Thermocyclers (MyCycler, Bio-Rad, Hercules, CA) were programmed for a 5 min initial denaturation at 95^{o}C, 30-40 cycles of the following: 15-60 s denaturation at 94°C, 15-60 s at the primer-specific annealing temperatures (Table 2), and 1 min extension at 72°C, followed by a final 5 min extension at 72°C.

PCR reactions were purified with ExoSAP-*it* (USB Corporation, Cleveland, OH) and sequenced in both directions using PCR primers on an automated capillary sequencer (model 3730, ABI, Foster City, CA) at the Natural History Museum and Biodiversity Research Center, University of Kansas. Forward and reverse sequences were assembled into contigs and edited with Sequencher v. 4.7 (Gene Codes, 2006). All protein coding genes were aligned manually by amino acid sequence in SeAl v. 2.0 (Rambaut, 2002). Noncoding ITS2 sequences were aligned initially with the Clustal W algorithm implemented in MegAlign (DNASTAR, 2006). Clustal was set to a gap opening penalty of 10, a gap extension penalty of 2, and delay divergent sequences to 40%. These settings allow gaps to occur more liberally than the Clustal defaults meant for protein coding sequences, and were decided upon after visual inspection of alignments using a variety of parameters. The Clustal output was then adjusted manually in SeAl.

Alignments were brought into Mesquite v. 2.5 (Maddison and Maddison, 2007) for phylogenetic matrix construction. Matrices were made for individual genes as well as a concatenated dataset of nDNA. The concatenated dataset did not include mtDNA because of apparent gene tree incongruence (see Results). All exemplars were included in the individual gene matrices, including multiple specimens from the majority of localities, in order to gauge within-taxon variability and estimate the boundaries of taxa. The combined nDNA analysis contained only one representative from each population to speed up analysis.

Phylogenetic analyses

Parsimony analyses were performed using PAUP* v. 4.0b10 (Swofford, 2002), in which heuristic tree searches were conducted using a simple addition sequence and tree-bisectionreconnection (TBR) branch rearrangement. *N. carinata, N. convexa,* and *N. diabolica* were used as outgroup taxa. Support for the resulting topology was estimated from 100 bootstrap replicates.

For model-based approaches, models of sequence evolution were selected using ModelTest v. 3.8 (Posada and Crandall, 1998) implemented over the ModelTest server (http://darwin.uvigo.es/software/modeltest_server.html). For ITS2 and *wingless*, likelihood ratio tests, the Akaike Information Criterion (AIC), and the Bayesian Information Criterion chose various 2-rate models (HKY+Γ, K80+Γ), which have separate rate parameters for transitions and transversions. I implemented a general 2-rate model with gamma-distributed rate parameters in both model-based inference methods below, allowing specific parameter values to be estimated by the analyses. Likelihood ratio tests and AIC both chose GTR+Γ+I for COII, a 6-rate model with separate rates for all substitution types.

Maximum likelihood analyses were performed using GARLI v. 0.951 (Zwickl, 2006). Heuristic searches ran for 10,000 generations past the last significant topology improvement (a likelihood increase of 0.010 or greater), and 100 bootstrap replicates estimated topological support. Bayesian analyses were performed with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Bayesian analyses used default Dirichlet priors and considered all starting topologies equally likely. For the concatenated nDNA dataset, ITS2 and *wingless* were partitioned separately with all parameters unlinked across partitions. MCMCMC searches, consisting of three heated chains (heating parameter 0.2) and one cold chain, explored posterior probability landscapes until convergence was indicated by the standard deviation of split frequencies dropping to less than 0.02. ITS2, *wingless*, and COII Bayesian analyses ran for $1x10^6$, $3x10^6$, and $1x10^6$ generations, respectively, and the combined nuclear dataset ran for

$5x10^6$ generations. The first 25% of each run was discarded as burnin and was not used in calculation of Bayesian posterior probabilities.

I used topological constraints and Shimodaira-Hasegawa (S-H) tests to test for gene tree incongruence between mtDNA and nDNA topologies. I implemented S-H tests in PAUP*, testing whether the likelihoods of the constraint topologies were significantly different from those of a population of 100 unconstrained bootstrap replicates. The null hypothesis is that all topologies explain the data equally well.

Hypothesis testing: geographic distribution

I coded nine distributional character states representing major geographical regions inhabited by Nedubini (Table 3). The Transverse Ranges are a region of complex biogeography (Chatzimanolis and Caterino, 2007), where the highest diversity of *Aglaothorax* is found (Rentz and Weissman, 1981). To obtain a detailed reconstruction, I divided the Transverse Ranges into eastern (San Gabriel and San Bernardino Mountains) and western (Santa Monica Mountains) regional components. I further subdivided the eastern Transverse Ranges and the Sierra Nevada into cismontane (facing the ocean) and transmontane (facing inland) slopes: the cismontane slopes are the western Sierra Nevada and southern Transverse Ranges, and the transmontane slopes are the eastern Sierra Nevada and northern Transverse Ranges. This is an important ecological distinction (Schoenherr, 1992): moisture traveling inland from the Pacific is blocked by the Transverse Ranges and Sierra Nevada, and falls on the cismontane slopes. Rainshadows exist on the transmontane sides, forming major deserts in the lowlands beyond (Mojave and Great Basin Deserts, respectively).

Distributional character states were coded into an unordered, multistate discrete character matrix (Table 3) and interpolated onto the terminals of the concatenated nDNA phylogeny. Two character states I used break with convention. First, the alluvium in the Los Angeles Basin is geologically part of the Peninsular Ranges (Schoenherr, 1992), but I coded it separately to clearly delimit its taxa. Second, The Santa Ynez Mountains are geologically part of the Transverse Ranges (Schoenherr, 1992), but I coded Santa Ynez Mountains taxa from Upper Oso (27) as part of the Coast Ranges on the basis of shared floristic and faunistic elements (pers. obs.) and geographic proximity. The most parsimonious ancestral distributions were reconstructed using the Trace Character History function of Mesquite. The reconstructions thus minimize the number of range shifts required to explain the distributions of extant taxa.

Hypothesis testing: song evolution

I reconstructed the evolution of four continuous song characters shown to vary significantly among taxa in a previous song analysis (Chapter 1): pn, ipi, dc, and pr. Population song statistics were coded into a matrix of continuous characters (Table 3). Before interpolating song characters onto the terminals, the outgroup *Neduba*, Jcp, and a small number of *Aglaothorax* populations lacking behavioral data were pruned from the phylogeny. Phylogenetic independent contrasts (Felsenstein, 1985) were then calculated using the Phenotypic Diversity Analysis Program (PDAP module) in Mesquite (Midford et al., 2005). Rather than combine terminals to eliminate polytomies, I kept polytomies and subtracted one degree of freedom for each polytomy from statistical tests (Garland et al., 1992). Contrasts were standardized by their standard deviations, i.e. the square roots of the sums of their branch lengths, creating statistically independent measures of character change that are also independent of time, of which branch length is a proxy (Garland et al., 1992). Adequacy of standardizations were assessed with least squared regressions of absolute contrast values vs. their standard deviations (Garland et al., 1992;

Midford et al., 2005). If contrasts are properly standardized, significant regressions are not expected.

To evaluate the sexual selection hypothesis, I tested for a concentration of song character change at tip nodes, using phylogenetic statistical methodology described by Barraclough et al. (1999). I tested *Aglaothorax* as a unit, using the well-supported topology and branch lengths estimated from the consensus tree generated by the concatenated nDNA Bayesian analysis, which contained one exemplar from every population sampled. For the observed topology, I calculated a test statistic measuring the amount of change across all nodes $\sum|C_i|H_i$ (Barraclough et al., 1999). This statistic sums over all nodes the absolute contrast value at each node C_i, a measure of the amount of change, multiplied by the relative node height H_i, an integer that identifies the position and relative age of each node. Node heights in the concatenated nDNA phylogey tested here range from 1 (tip nodes) to 7 (root node). This node height system is equivalent to setting all branch lengths equal to 1.

The observed statistic was evaluated against a null distribution of statistics derived from 1000 randomizations of the contrasts among node heights, a procedure accomplished in Minitab v. 13 (Minitab Inc., 2000; Slade, 1999). The significance of the observed value was evaluated with a two-tailed test under $\alpha=0.05$ (0.025 per tail). A non-significant result is a failure to reject the null hypothesis, which states that song evolution in *Aglaothorax* does not differ from a random association of standardized contrasts with branches. A significant result at the low tail of the distribution represents contrasts clustering at the tips of the tree, supporting song evolution in association with recent cladogenesis as predicted by sexual selection. A significant result at the high tail indicates clustering at the basal nodes, supporting an alternative hypothesis that song evolution is associated with long term persistence.

To evaluate the species recognition hypothesis, I tested for significantly higher rates of song character change across the major clades of *Aglaothorax*. Standardized independent contrasts represent amounts of character change independent of time (Garland et al., 1992). To compare evolutionary rates among clades, nonparametric statistics can be used to test for heterogeneity among the standardized contrast distributions of the clades (Garland, 1992). I used the Kruskal-Wallis test to compare the standardized contrast medians of the major clades. As above, pn, ipi, dc, and pr were all tested in this manner. A significant result rejects the null hypothesis that there is no difference between the medians of the contrast distributions among clades. Significant results were considered in light of the reconstruction of ancestral distribution.

RESULTS

Phylogenetic analyses

Parsimony, likelihood and Bayesian analyses show *Neduba* to be monophyletic and separated by a long branch from *Aglaothorax* +Jcp (Figs. 2, 3). The position of Jcp outside *Aglaothorax* is weakly supported. The combined nDNA analysis (Fig. 3b) recovered less support for a monophyletic Jcp than individual ITS2 and COII gene trees, because Jcp formed a polytomy with *Aglaothorax* or nested with the latter genus in *wingless* gene trees. *Aglaothorax* split into two major clades, one containing the large species *A. gurneyi*+*A. ovata*, in which *A. gurneyi* was always basal, and the other containing the small species *A. diminutiva*, *A. longipennis*, and *A. morsei* (Figs. 2b, 3).

Relationships within the clade of small *Aglaothorax* were well resolved and supported by nDNA (Fig. 3b). *A. diminutiva* split into two clades. The first clade, which I refer to as the Coastal Clade, grouped populations from the southern Coast Ranges, including the type locality (Mt. Diablo), the northeastern and western Transverse Ranges, and the Santa Monica Mountains. The other clade, which I refer to as the Inland Clade, consists of inland populations in the eastern Transverse and Peninsular Ranges. The two are sister clades in the combined nDNA analysis (Fig. 3b) and likelihood ITS2 gene tree, but in the Bayesian and parsimony ITS2 gene trees (Fig. 3a) the inland *A. diminutiva* clade forms a paraphyletic grade with *A. longipennis*+*A. morsei*. In the *A. longipennis*+*A. morsei* clade, *A. morsei morsei* from the type locality in the San Gabriel Mountains were always basal. Santa Monica Mountains *A. morsei* subspecies and *A. longipennis* were not resolved separately in any gene tree or in the combined nDNA analysis.

Except for a basal clade of central Coast Range *A. diminutiva*, COII gene trees (Fig. 2b) placed all small *Aglaothorax* in a poorly supported assemblage of populations from the Coast, Transverse, and Peninsular Ranges. *A. d. dactyla*, which are sympatric with *A. morsei* in the Santa Monica Mountains, grouped into this assemblage, contrary to their placement near central Coast Range *A. diminutiva* in nDNA analyses.

To test for gene tree incongruence, I performed S-H tests of two constraint topologies. First, I ran a COII analysis with the constraint that all *A. d. dactyla* exemplars must group with the *A. diminutiva* Coastal Clade, as occurred in the ITS2 analysis, rather than forming a polytomy with *A. longipennis+A.morsei* as in the unconstrained COII analysis (Fig. 2b). The constraint analysis of the COII dataset was not significantly better at explaining the data than the population of unconstrained trees (S-H test, –ln L difference=71.201, p=0.596). Second, I analyzed the concatenated nDNA dataset, constraining *A. d. dactyla* to group with *A. longipennis+A. morsei* as occurred in the COII analysis. This second analysis rejected the constraint topology (S-H test, –ln L difference=122.167, p=0.018).

Hypothesis testing: geographic distribution

A parsimony reconstruction of ancestral distribution estimated two equally parsimonious states for the ancestor of the Nedubini: the Peninsular Ranges and the western Sierra Nevada, both of which are inland (Fig. 4). The ancestors of Jcp+*Aglaothorax* and *Aglaothorax* are reconstructed as inhabiting the Peninsular Ranges. Sympatric taxa inhabiting the Santa Monica Mountains arose from two separate, reciprocally monophyletic sister clades. The first of these clades is the *A. morsei+longipennis* clade, arising from an ancestor inhabiting the southern (cismontane) slope of the eastern Transverse Ranges. The second is a branch of the Coastal *A. diminutiva* clade containing western and northeastern Transverse Range as well as Santa Monica Mountains populations.

Hypothesis testing: song evolution

Linear regressions of absolute contrast values of song characters vs. their standard deviations showed that the independent contrasts for three song features were adequately standardized (pn: R^2=0.011, p=0.598; ipi: R^2=0.016, p=0.521; dc: R^2=0.014, p=0.552). The pr regression was not adequately standardized, evidenced by a marginally significant (R^2=0.134, p=0.055) negative correlation between contrasts and their standard deviations. Natural log and exponential transformation of branch lengths did not improve the standardization. As the regression was marginally significant, I proceeded to analyze the pr contrasts. Failure of contrasts to standardize to their branch lengths means the contrasts may not be equally weighted, which may affect the outcome of statistical tests relying on parametric distributions (Garland et al., 1992). Because I estimated distributions with randomization and conducted nonparametric tests, the effects of this violation may be mitigated; I nevertheless interpreted the results of the pr test with caution. All clustering tests of contrasts were not significant (2-tailed p values: pn, p=0.780; ipi, p=0.138; dc, p=0.132; pr, p=0.966). There was a tendency for ipi and dc contrasts to cluster towards basal nodes.

Distributions of standardized contrasts of pn (H=6.42, p=0.040) and pr (H=11.34, p=0.003) showed significant heterogeneity among the three major *Aglaothorax* clades (*A. ovata, A. diminutiva,* and *A. morsei+longipennis*; Fig. 5a, d). Heterogeneity of dc (Fig. 5c) was marginally significant (H=5.84, p=0.054). Although no significant heterogeneity occurred among clades in ipi (H=1.76, p=0.415), two outlying contrasts are found within the *A. morsei+longipennis* clade (Fig. 5b). Significant heterogeneity in pn was caused by contrasts in the *A. diminutiva* coastal clade (Z =1.76; Fig. 6). Heterogeneity in pr and dc were caused by contrasts in the *A. morsei+longipennis* clade (Z=3.19 and 1.59, respectively). These contrasts occur where distributional shifts to Santa Monica Mountains are reconstructed (Fig. 4).

DISCUSSION

Phylogenetic analyses

Nuclear gene sequences support most currently recognized genera and species of the Nedubini. At the generic level, *Neduba* and *Aglaothorax* were recovered as monophyletic and are separated by a long branch, supporting the elevation of these names from subgenera to genera by Rentz (1988). The tendency for the undescribed genus Jcp to group with *Aglaothorax* is unexpected given its strongly divergent morphology, but this relationship is predicted by calling song: Jcp sing with one pulse type as in *Aglaothorax* (Chapter 1), while *Neduba* songs have more than one type (Morris et al., 1975). The relationships among the nedubine genera and among the shield-back katydids in general require further systematic investigation. With regard to the North American Nedubini, analyses must include a thorough sampling of the southern hemisphere taxa hypothesized to be their relatives (Rentz, 1988; Rentz and Colles, 1990; Rentz and Gurney, 1985).

Gene tree conflict occurred between the mitochondrial and nuclear loci used in this study, a phenomenon typically explained by incomplete lineage sorting or hybridization (Maddison, 1997). These phenomena are not mutually exclusive (Meng and Kubatko, 2009), and both are expected in recently diverged lineages, where ancestral polymorphism remains and reproductive barriers are not yet complete (Hey, 2006). Hybridization is an intriguing hypothesis for the gene tree conflict, because additional lines of evidence suggest *Aglaothorax* may hybridize. First, a song cline was revealed across the Transverse Ranges (Chapter 1) involving both *A. morsei* and *A. longipennis*, and clines can form from hybridization (reviewed in Futuyma, 1998). Second, although sympatric females of *A. d. dactyla* and *A. m. costalis* at Point Mugu discriminated against the ipi of heterospecific calling songs, preferences were variable and a proportion of females approached the incorrect song (Chapter 2). Thus, incomplete reproductive isolation may exist between sympatric species, allowing gene flow across species boundaries. Mitochondrial DNA is known to leap species boundaries readily (e.g. Ballard et al., 2002; Bossu and Near, 2009; Gompert et al., 2008; Shaw, 2002), a phenomenon known as mitochondrial capture. Tests aimed at partitioning the incongruence into hybridization and lineage sorting components (Buckley et al., 2006; Meng and Kubatko, 2009) may be a fruitful future direction, in which female preference data (Chapter 2) may be incorporated as prior information regarding the likelihood of hybridization. For the purposes of this study, I recognize that gene tree conflict exists, and I proceeded with hypothesis testing using the concatenated nDNA analysis, which recovers monophyletic units that agree with morphological, geographical, and behavioral data.

Molecular data support the distinction between coastal and inland *A. diminutiva* (Fig. 3a, b), previously revealed by a song analysis of the genus (Chapter 1). The Coastal lineage contains the type population at Mt. Diablo (30), and therefore represents true *A. diminutiva*. Genitalic morphology varies among the inland populations (pers. obs.). The paraprocts of males from Boulder Oaks (25) and Sycamore Flat (20), for example, have the mesal tooth situated apically, as in nominate *A. d. diminutiva* (Rentz and Weissman, 1981). Populations from Pinyon Flat (23) and Whitewater Canyon (24), however, resemble *A. morsei* in that the mesal tooth is subapical, although it is located much further towards the base in these populations than in typical *A. morsei*. The combination of behavioral, molecular, and morphological evidence leads me to conclude that the inland *A. diminutiva* populations represent a cryptic lineage. Currently, no inland populations are taxonomically recognized.

Geographic distribution

The topology of the nDNA analysis contains strong geographic signal. Most clades occur allopatrically in separate geographic regions (Fig. 4). Also, the geologic history of California is reflected in the reconstructed ancestral distributions. The Sierra Nevada, an inland mountain range, attained sufficient elevation 10-12 million years ago to create a rainshadow to the east, while the South Coast Ranges are perhaps the youngest mountain range in California, having only emerged above sea level within the last 1-2 million years (Schoenherr, 1992). Concordantly, Nedubini are hypothesized to have an inland origin, while Coast Range inhabitants (the *N. carinata* complex and the Coastal Clade of *A. diminutiva*) are among the youngest in their lineages (Fig. 4). The tumultuous geologic history and the diverse, fragmented geography of California are powerful forces that have shaped the evolution of many taxa (e.g. Kuchta and Tan, 2006; Law and Crespi, 2002; Starrett and Hedin, 2007). I see no reason to doubt allopatric divergence as the major force behind speciation in *Aglaothorax*.

Sympatric species in the Santa Monica Mountains represent secondary contact between two genetically distinct, formerly allopatric lineages (Fig. 4). The first of these is the Coastal Clade of *A. diminutiva*, in which the Santa Monica Mountains subspecies *A. d. dactyla* is recently derived from ancestors inhabiting the South Coast Ranges. The second of these is the *A. morsei+longipennis* lineage, which colonized the Santa Monica Mountains from the San Gabriel Mountains. Nominate *A. morsei morsei* in the San Gabriel Mountains appear to represent the source population; individuals of this subspecies resolved basal to a polytomy consisting of the other *A. morsei* subspecies together with *A. longipennis*, the only taxon of species rank that did not resolve. Song also does not consistently separate *A. longipennis* (Chapter 1), leading me to question its validity as a species. *A. longipennis*, along with populations in the Los Angeles

Basin (*A. morsei* from Kenneth Hahn (12) and Malaga Dune (15)), represent intermediates along a song cline (Chapter 1). All *A. morsei-longipennis* populations may be non-reproductively isolated populations that are currently exchanging or have recently exchanged genes.

Song evolution

Recent cladogenesis occurred without a general trend of correlated song evolution, arguing against sexual selection being the primary cause of speciation. Instead, high rates of song evolution map to two clades that are sympatric after secondary contact: the first derived from the *A. diminutiva* Coastal Clade, and the second from the Transverse Range *A.morsei-longipennis* clade (Figs. 4, 6). Previous behavioral data found a correlation between male pn distributions and female preference for pn in several *A. morsei* and *A. longipennis* populations, consistent with the expectations of coevolutionary sexual selection (Chapter 2). Male ipi and female responses to ipi were not correlated in allopatry; however, females of sympatric *A. diminutiva dactyla* and *A. morsei costalis* discriminated against heterospecific male ipi, and I concluded ipi evolved as a novel species recognition feature. To explain the retention of preference for high pn in *A. morsei costalis*, which would apparently lead females of this species to incorrectly choose *A. diminutiva dactyla* males, I speculated that female evaluation of ipi overrides sexual selection for pn. Secondary contact thus appears to have had a strong influence on both male songs and female preferences, in which the context of species recognition is of paramount importance. Furthermore, this study may represent the first evidence for reproductive character displacement or reinforcement in the songs of North American Orthoptera. Several studies on Orthoptera have considered these hypotheses but found no evidence (Izzo and Gray, 2004; Marshall et al., 2002; Walker, 1974), and studies reporting this phenomenon are rare in general (e.g. Boughman, 2001; Cooley et al., 2001; Nosil et al., 2003; Pfennig and Simovich, 2002).

The character reconstructions suggest secondary contact spurred evolution of different suites of song characters in the two clades that are involved. Showing high rates of change in the *A. morsei-longipennis* clade are pr, dc and ipi (Fig. 5b,c,d), all of which are related song features that depend on rates of muscle contraction. In the *A. diminutiva* Coastal Clade, pn evolved rapidly (Fig. 5a). Although pn preference of *A. diminutiva dactyla* females has not been measured, this study suggests pn may be selected by females in the Coastal Clade of *A. diminutiva*. Future behavioral work should target this character, which may have increased due to displacement away from the pn distribution of *A. morsei costalis*. Also noteworthy is the absence of high song evolution rates in both the *A. ovata* clade and *A. diminutiva* Inland Clade, in which all members are completely allopatric.

Species recognition was articulated as the primary explanation for species-specific mating trait diversity in early speciation theory (Dobzhansky, 1937; Mayr, 1942), The popularity of species recognition as an explanation of mating trait diversity escalated to the point Dobzhansky (1940) argued that reinforcement is necessary to complete speciation. Recent focus, however, has shifted to sexual selection (West-Eberhard, 1983). A great deal of evidence linking sexual selection and speciation comes from comparative studies (e.g. Arnqvist et al., 2000; Barraclough et al., 1995). However, some of this evidence is also consistent with species recognition (see Barraclough et al., 1995; Ritchie, 2007). In addition, several studies have concluded that premating isolation evolves more rapidly in sympatry (Coyne and Orr, 1989; Funk et al., 2006; Gavrilets and Boake, 1998) suggesting species recognition is a common evolutionary context for mating behavior, and perhaps it is time to reassess its importance. Further investigation of species recognition hypotheses, such as reinforcement, are needed.

Important approaches include comparative and ecological studies (Ortiz-Barrientos et al., 2009), for which *Aglaothorax* is eminently suited.

The consequences of the evolution of species recognition in sympatry may extend far from the zone of contact. For example, gene flow may allow reproductive isolation alleles to diffuse from the zone of contact into surrounding allopatric populations (Lande, 1982), where they may persist if selectively neutral. I hypothesize that this latter effect is responsible for the Transverse Ranges song cline (Chapter 1). I propose the following scenario, using pulse rate as an example of a song character showing clinal variation. Initially, the pulse rates in the ancestral, allopatric *A. diminutiva* and *A. morsei-longipennis* lineages were similar, approximately 5 pulses/s at 25^{o}C. As the *A. diminutiva* lineage spread south through the South Coast Ranges, and the *A. morsei-longipennis* lineage spread west across the Transverse Ranges, secondary contact occurred in the western Santa Monica Mountains. Selection for species recognition then displaced the pulse rate of the *A. morsei-longipennis* lineage upward to 16.5 pulses/s at the zone of contact. Low levels of gene flow between the zone of contact and a stepping-stone network of Transverse Range populations to the east diffused fast pulse rate alleles across the Santa Monica and San Gabriel Mountains, forming the shallow cline. The result is a pulse rate that regularly decreases over distance from west to east, until eventually, at the eastern extreme, the ancestral pulse rate persists.

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Table 1. Collecting localities of specimens used in molecular phylogenetic analyses. Points on the map (Fig. 1) use site numbers for abbreviation.

Site #	State	County	Locality	Latitude	Longitude	Elev (m)	Taxa
1	CA	Inyo	Grandview Cpgr.	37.3332	-118.1923	2610	*A. ovata armiger*
2	CA	Inyo	McMurray Meadows Rd., 3 mi. S of Glacier Lodge Rd.	37.1023	-118.3196	1658	*A. ovata gigantea*
3	CA	Inyo	Whitney Portal Rd., 6 mi. W of Lone Pine	36.5949	-118.1563	1611	*A. ovata gigantea*
4	CA	Kern	Walker Pass Recreation Area	35.6646	-118.0370	1537	*A. ovata longicauda, N. castanea*
5	CA	Los Angeles	Acton, Sierra Hwy. 0.6 mi. E of Crown Valley Rd.	34.4936	-118.1834	929	*A. ovata ovata*
6	CA	Los Angeles	Arroyo Seco, Gabrielino Recreation Trail	34.2065	-118.1670	355	*A. morsei morsei*
7	CA	Los Angeles	Backbone Trail, 5 mi. N of SR1 on Malibu Cyn. Rd.	34.0799	-118.7037	156	*A. morsei*
8	CA	Los Angeles	jct. Big Rock Dr. and SR1	34.0377	-118.6088	5	*A. longipennis*
9	CA	Los Angeles	Charmlee Wilderness Pk.	34.0608	-118.8771	427	*A. morsei curtatus*
10	CA	Los Angeles	Devil's Punchbowl Rd. 2 mi. E of Longview Rd., Juniper Hills	34.4267	-117.8697	1316	*N. castanea*
11	CA	Los Angeles	Griffith Park, Mount Hollywood Trail	34.1240	-118.3009	370	*A. longipennis*
12	CA	Los Angeles	Kenneth Hahn SRA	34.0042	-118.3592	126	*A. morsei*
13	CA	Los Angeles	Ladera Linda Park	33.7383	-118.3479	149	*A. morsei*
14	CA	Los Angeles	Leo Carillo State Park, Nicholas Flat	34.0638	-118.9128	457	*A. morsei*
15	CA	Los Angeles	Malaga Dune, Rancho Palos Verdes	33.8004	-118.3858	61	*A. morsei*
16	CA	Los Angeles	Mescal Picnic Area	34.3901	-117.7206	1868	*A. gurneyi*
17	CA	Los Angeles	Millard Cyn. Cpgr.	34.2162	-118.1484	546	*A. morsei morsei*
18	CA	Los Angeles	Point Dume Headlands State Park	34.0046	-118.8055	44	*A. morsei tectinota*
19	CA	Los Angeles	SR23S & Mulholland Hwy.	34.0873	-118.8742	474	*A. diminutiva dactyla, A. morsei curtatus*
20	CA	Los Angeles	Sycamore Flat Cpgr.	34.4128	-117.8239	1309	*A. diminutiva*
21	CA	Los Angeles	Topanga State Park, Dead Horse Trail	34.0915	-118.5939	274	*A. longipennis*
22	CA	Riverside	Boulder Basin Cpgr.	33.8261	-116.7558	2498	Jcp
23	CA	Riverside	Pinyon Flat Cpgr.	33.5856	-116.4570	1219	*A. diminutiva, A. ovata tinkhamorum*
24	CA	Riverside	Whitewater Cyn.	33.9579	-116.6463	431	*A. ovata*
25	CA	San Diego	Boulder Oaks Cpgr.	32.7299	-116.4839	968	*A. diminutiva*
26	CA	San Luis Obispo	La Panza Cpgr.	35.3538	-120.2606	732	*A. diminutiva*
27	CA	Santa Barbara	Upper Oso Cpgr.	34.5540	-119.7680	376	*A. diminutiva*
28	CA	Ventura	Point Mugu State Park, Sycamore Cyn.	34.0728	-119.0145	4	*A. diminutiva dactyla, A. morsei costalis*
29	NV	Lincoln	Oak Spring Summit Picnic Area	37.5923	-114.6828	1901	*A. ovata segnis*
30	CA	Contra Costa	Mt. Diablo State Park, Juniper Camp	37.8785	-121.9339	886	*A. diminutiva diminutiva, N. diabolica*
31	CA	El Dorado	China Flat Cpgr.	38.7535	-120.2671	1470	*N. convexa*
32	CA	Inyo	Sage Flat Camp	37.1279	-118.4037	2243	*N. macneilli*
33	CA	Kern	Hobo Cpgr. overflow area	35.5752	-118.5305	700	*N. sierranus*
34	CA	Los Angeles	Bob's Gap	34.4550	-117.8139	1300	*A. ovata ovata*
35	CA	Los Angeles	County Rd. N4, 3 mi. E of Big Rock Springs	34.4264	-117.8078	1407	*A. diminutiva*
36	CA	Plumas	Hallsted Cpgr.	40.0174	-121.0745	864	*N. convexa*
37	CA	San Benito	Fremont Peak State Park, Valley View Cpgr.	36.7597	-121.5050	829	*N. carinata*
38	CA	Santa Clara	Uvas Canyon County Park	37.0855	-121.7954	349	*N. diabolica*

Table 2. PCR primers and protocols used to amplify each gene fragment. Primers are written left to right 5'-3'.

Gene	Primer name	Direction	Sequence	Anneal temp (ºC)	Anneal time (s)	Cycles	Reference
COII	C2-J-3279	fwd	GGACAAACAATTGAGTTAATTGGAAC	47	30-60	30	Simon et al., 1994
	TD-N-3862	rev	TTTAGATTGACATTCTAATGTTAT				Simon et al., 1994
ITS2	3S	fwd	GGTACCGGTGGATCACTGGGCTCGTG	53	15	35	Slaney and Blair, 2000
	BD2	rev	TATGCTTAAATTCAGCGGGT				Slaney and Blair, 2000
wingless	LEP WG1	fwd	GARTGYAARTGYCAYGGYATG	59	15	35	Brower and DeSalle, 1998
	LEP WG2	rev	ACTICGCARCACCARTGGAAT				Brower and DeSalle, 1998

Table 3. Geographic and song character matrix. Distribution was coded as a multistate discrete character. Ten states classified distribution by mountain range, and in some cases mountain ranges were subdivided into regions and slopes to account for ecological differences. North and west (cismontane) slopes are more mesic than east and south (transmontane) slopes. Song characters were coded directly from Chapter 1 as continuous data.

			Song character states			
Population	Distribution	Distribution character state	pn	ipi (ms)	dc	pr (s-1)
A. d. dactyla 23S&Mulholland	Santa Monica Mountains	0	50.0	156.8	0.213	5.0
A. d. dactyla Nicholas Flat	Santa Monica Mountains	0	70.5	147.8	0.218	5.3
A. d. dactyla Point Mugu	Santa Monica Mountains	0	38.5	167.2	0.193	5.0
A. d. diminutiva Mt. Diablo	Coast Ranges S	2	-	-	-	-
A. diminutiva Boulder Oaks	Peninsular Ranges	3	21.8	157.9	0.284	5.3
A. diminutiva Harris Grade	Coast Ranges S	2	-	-	-	-
A. diminutiva La Panza	Coast Ranges S	2	42.0	122.6	0.235	6.3
A. diminutiva N4	Transverse Ranges NE	6	-	-	-	-
A. diminutiva Pinyon Flat	Peninsular Ranges	3	28.5	129.1	0.263	6.1
A. diminutiva Sycamore Flat	Transverse Ranges NE	6	24.3	110.9	0.286	6.6
A. diminutiva Troy Cyn.	Peninsular Ranges	3	-	-	-	-
A. diminutiva Upper Oso	Transverse Ranges W	2	27.1	179.5	0.208	4.6
A. diminutiva Whitewater	Transverse Ranges SE	7	-	-	-	-
A. gurneyi Mescal	Transverse Ranges NE	6	2.0	57.3	0.673	8.4
A. longipennis Big Rock	Santa Monica Mountains	0	6.6	27.4	0.585	17.1
A. longipennis Fryman Cyn.	Santa Monica Mountains	0	-	-	-	-
A. longipennis Griffith Park	Santa Monica Mountains	0	3.2	39.9	0.629	12.4
A. longipennis Topanga Cyn.	Santa Monica Mountains	0	3.8	36.8	0.576	14.9
A. m. costalis Point Mugu	Santa Monica Mountains	0	13.6	26.5	0.586	16.5
A. m. curtatus 23S&Mulholland	Santa Monica Mountains	0	13.5	26.7	0.559	17.6
A. m. curtatus Charmlee	Santa Monica Mountains	0	9.6	26.7	0.618	15.6
A. m. morsei Arroyo Seco	Transverse Ranges SE	7	17.5	99.6	0.358	6.8
A. m. morsei Millard Cyn.	Transverse Ranges SE	7	9.8	164.1	0.252	4.8
A. m. tectinota Point Dume	Santa Monica Mountains	0	8.5	31.6	0.558	15.4
A. morsei Backbone Tr.	Santa Monica Mountains	0	9.5	28.1	0.556	16.9
A. morsei Kenneth Hahn	Los Angeles Basin	1	6.2	48.1	0.534	10.5
A. morsei Malaga Dune	Los Angeles Basin	1	17.1	66.5	0.399	9.3
A. morsei Nicholas Flat	Santa Monica Mountains	0	11.5	26.9	0.589	16.3
A. o. armiger Grandview	Great Basin Ranges	8	3.3	71.6	0.444	9.4
A. o. gigantea Lone Pine	Sierra Nevada E (transmontane)	5	2.3	56.7	0.591	10.0
A. o. longicauda Walker Pass	Sierra Nevada E (transmontane)	5	5.7	62.5	0.523	8.7
A. o. ovata Acton	Transverse Ranges NE	6	4.4	58.5	0.545	8.7
A. o. segnis Oak Spring	Great Basin Ranges	8	2.4	86	0.387	9.6
A. o. tinkhamorum Pinyon Flat	Peninsular Ranges	3	3.3	51.6	0.571	10.1
A. ovata Whitewater	Transverse Ranges SE	7	2.0	67.3	0.616	8.0
Jcp Boulder Basin	Peninsular Ranges	3	-	-	-	-
N. carinata Fremont Peak	Coast Ranges S	2	-	-	-	-
N. castanea Juniper Hills	Transverse Ranges NE	6	-	-	-	-
N. castanea Walker Pass	Sierra Nevada E	5	-	-	-	-
N. convexa China Flat	Sierra Nevada W (cismontane)	4	-	-	-	-
N. convexa Hallsted Cpgr.	Sierra Nevada W (cismontane)	4	-	-	-	-
N. diabolica Mt. Diablo	Coast Ranges S	2	-	-	-	-
N. diabolica Uvas Cyn.	Coast Ranges S	2	-	-	-	-
N. sierranus Hobo Cpgr.	Sierra Nevada W (cismontane)	4	-	-	-	-

FIGURE CAPTIONS

Fig. 1. Map showing location of collecting sites. Site numbers match the localities listed in Table 1. Major geographical regions referred to in this paper are outlined. a. Overview of study area. b. Detail of intensified collecting in the Transverse Ranges of southern California, where the highest diversity of *Aglaothorax* is found and clinal song variation exists.

Fig. 2. Representative gene trees: a. *wingless* parsimony tree, b. COII parsimony tree. Clade support values from 100 bootstrap replicates are shown above branches. Both trees were rooted using *Neduba* as an outgroup.

Fig. 3. Representative trees: a. ITS2 Bayesian gene tree, b. Concatenated Bayesian topology from combined analysis of the nuclear genes *wingless* and ITS2. Both trees were rooted using *Neduba* as an outgroup. Bayesian posterior probabilities are shown at nodes. Clades referred to in the text are indicated.

Fig. 4. Parsimony reconstruction of the geographic distribution of the Nedubini. The concatenated nDNA topology (Fig. 3b) is used for this reconstruction. Distributions of terminal taxa were coded as a multistate discrete character (Table 3). White lineages inhabit the Santa Monica Mountains; symatric taxa are derived from two separate clades, the *A. diminutiva* Coastal Clade and the *A. morsei-longipennis* clade.

Fig. 5. Comparison of song character evolution rates among major clades. Heterogeneity among clades was tested with nonparametric Kruskal-Wallis tests. Contrasts for pn and pr show significant heterogeneity (p=0.040 and p=0.003, respectively). Contrasts for dc show marginally significant heterogeneity (p=0.054).

Fig. 6. Table of raw and standardized independent contrasts of song features. The topology and branch lengths of the concatenated nDNA phylogeny were used for these computations. The designation p before a node number denotes a polytomy, One degree of freedom was subtracted for each polytomy in statistical analyses. Standardized contrasts shown in bold represent high rates of song evolution. Node numbers refer to the tree below, on which lineages with high rates of evolution are shown in red. High song evolution rates are found only in lineages experiencing secondary contact.

Figure 1

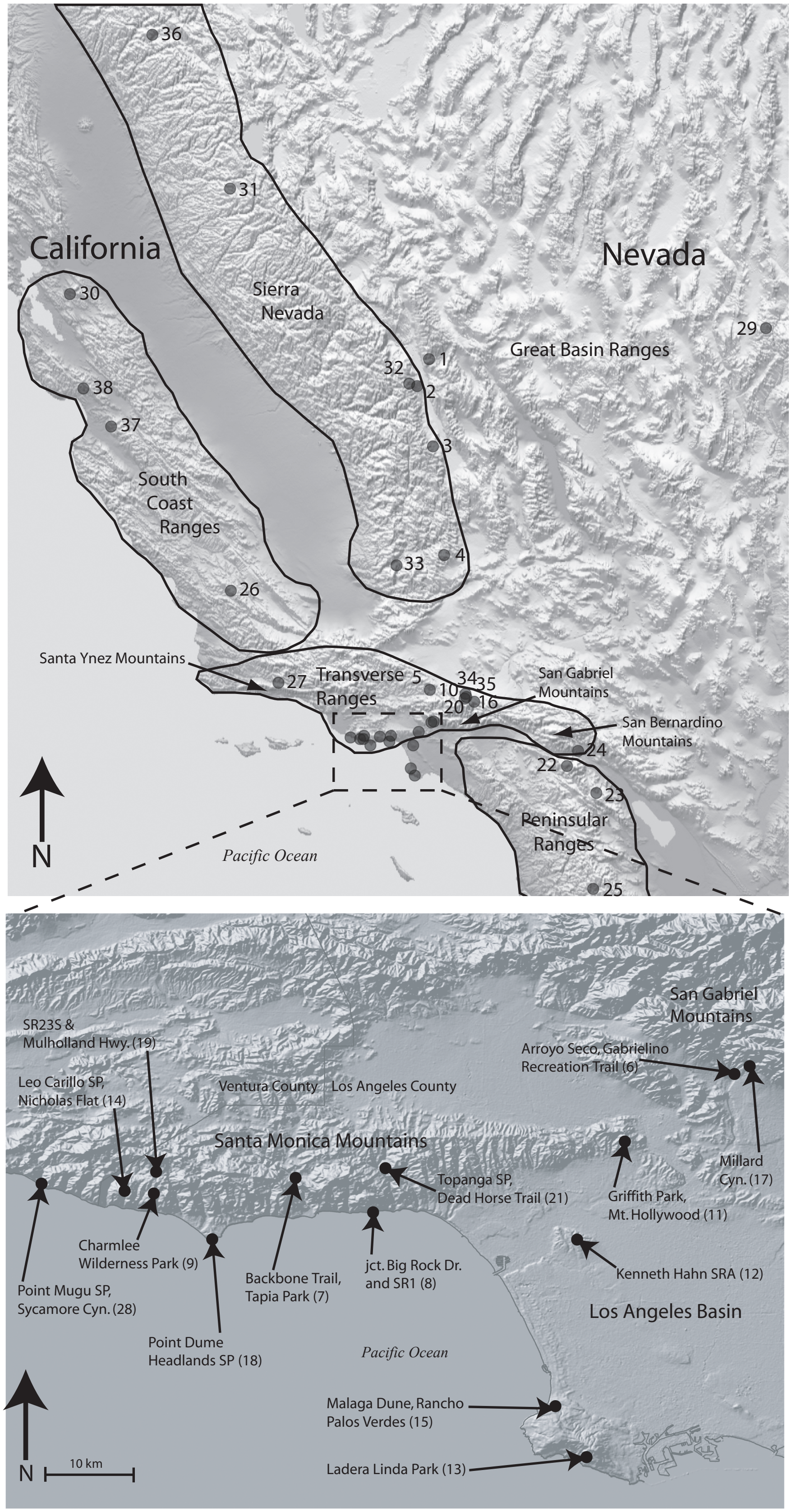

36
31
California
Nevada
Sierra Nevada
Great Basin Ranges
29
30
1
32
2
38
37
3
South Coast Ranges
4
33
26
Santa Ynez Mountains
27
Transverse Ranges
5
34
35
10
16
20
San Gabriel Mountains
San Bernardino Mountains
24
22
23
Peninsular Ranges
25
N
Pacific Ocean
San Gabriel Mountains
SR23S & Mulholland Hwy. (19)
Arroyo Seco, Gabrielino Recreation Trail (6)
Leo Carillo SP, Nicholas Flat (14)
Ventura County
Los Angeles County
Santa Monica Mountains
Millard Cyn. (17)
Topanga SP, Dead Horse Trail (21)
Griffith Park, Mt. Hollywood (11)
Charmlee Wilderness Park (9)
Backbone Trail, Tapia Park (7)
jct. Big Rock Dr. and SR1 (8)
Kenneth Hahn SRA (12)
Point Mugu SP, Sycamore Cyn. (28)
Los Angeles Basin
Point Dume Headlands SP (18)
Pacific Ocean
Malaga Dune, Rancho Palos Verdes (15)
N
10 km
Ladera Linda Park (13)

Figure 2

a

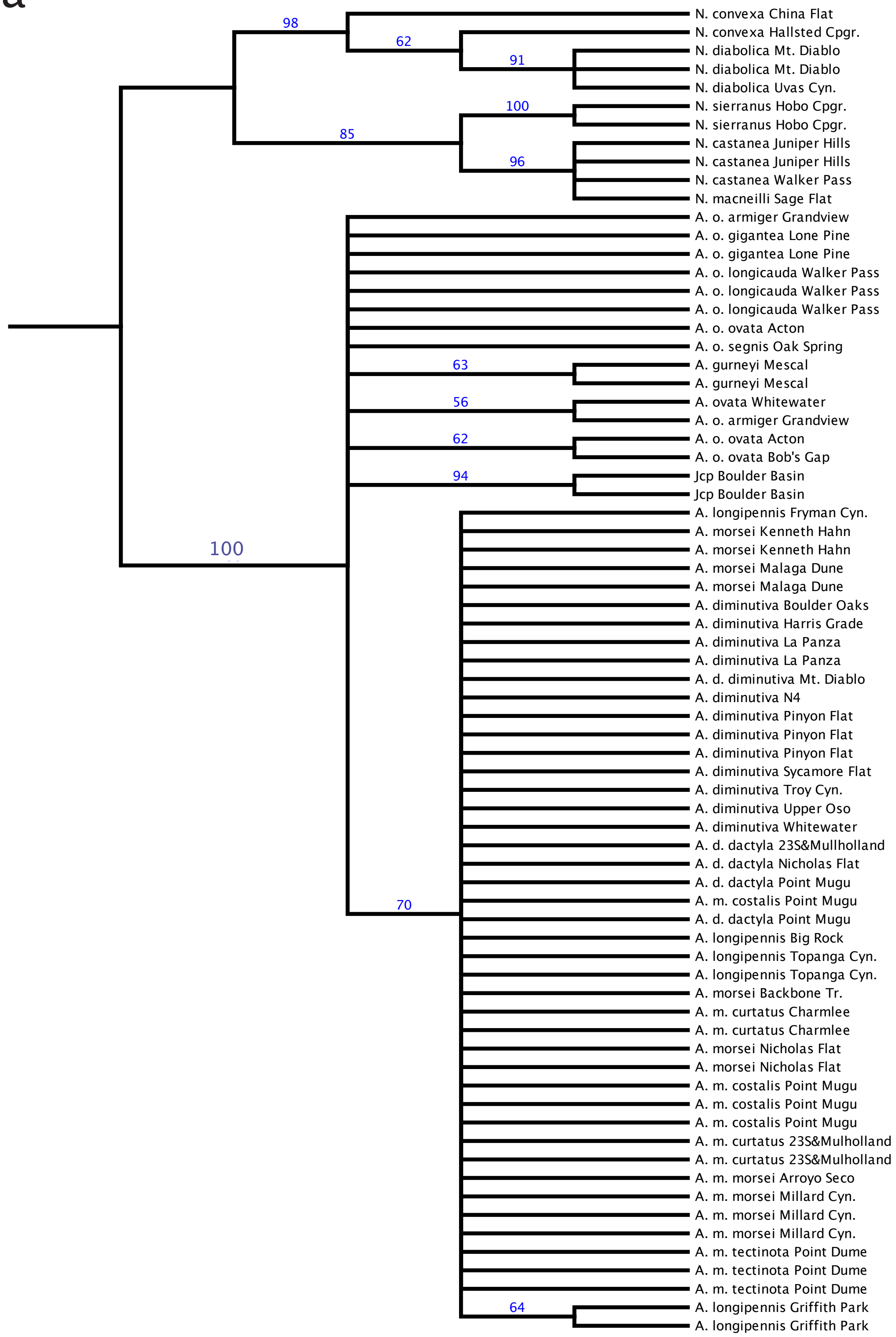
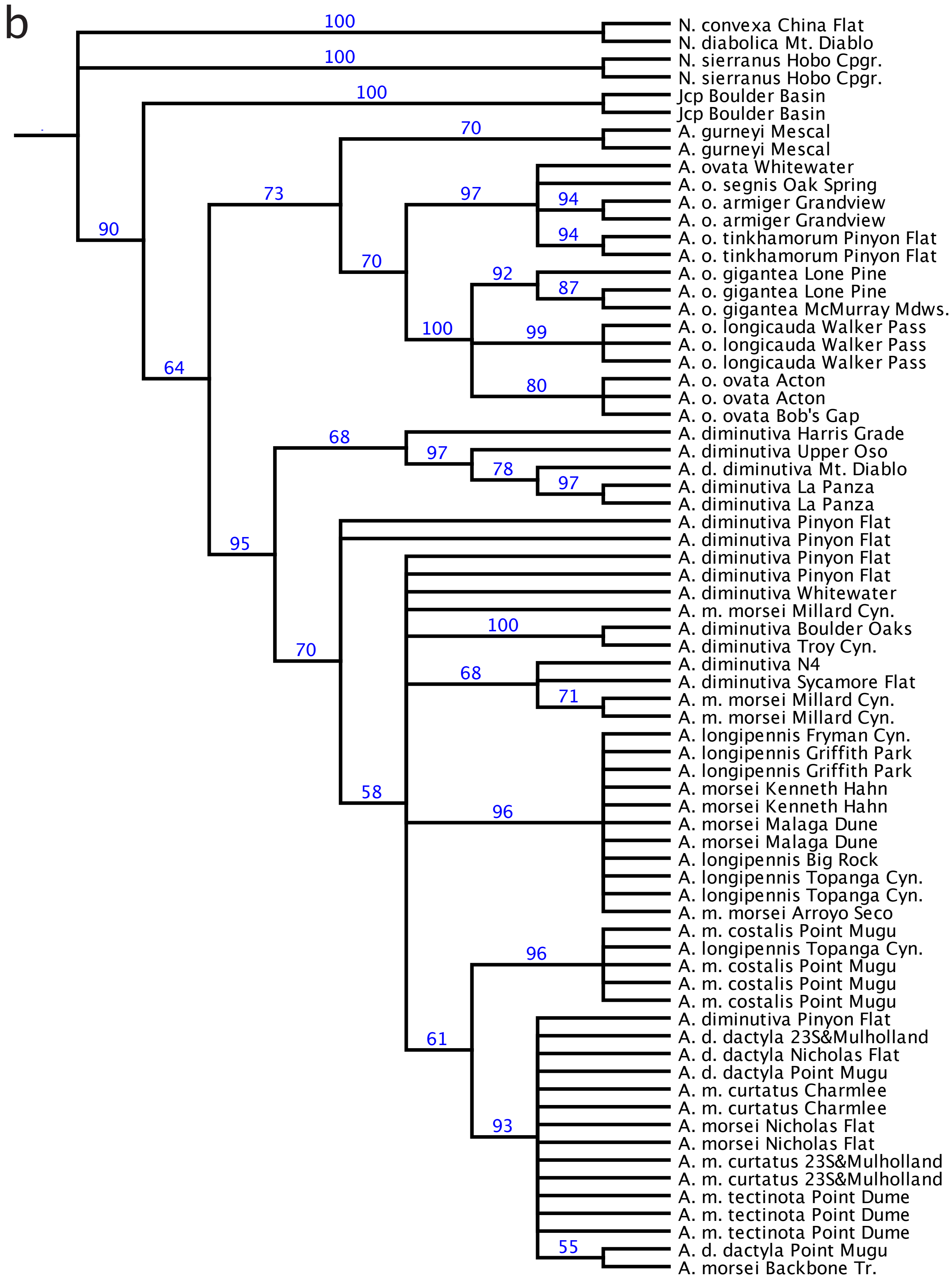

98
N. convexa China Flat
62
N. convexa Hallsted Cpgr.
91
N. diabolica Mt. Diablo
N. diabolica Mt. Diablo
N. diabolica Uvas Cyn.
100
N. sierranus Hobo Cpgr.
N. sierranus Hobo Cpgr.
85
N. castanea Juniper Hills
96
N. castanea Juniper Hills
N. castanea Walker Pass
N. macneilli Sage Flat
A. o. armiger Grandview
A. o. gigantea Lone Pine
A. o. gigantea Lone Pine
A. o. longicauda Walker Pass
A. o. longicauda Walker Pass
A. o. longicauda Walker Pass
A. o. ovata Acton
A. o. segnis Oak Spring
63
A. gurneyi Mescal
A. gurneyi Mescal
56
A. ovata Whitewater
A. o. armiger Grandview
62
A. o. ovata Acton
A. o. ovata Bob's Gap
94
Jcp Boulder Basin
Jcp Boulder Basin
A. longipennis Fryman Cyn.
A. morsei Kenneth Hahn
A. morsei Kenneth Hahn
100
A. morsei Malaga Dune
A. morsei Malaga Dune
A. diminutiva Boulder Oaks
A. diminutiva Harris Grade
A. diminutiva La Panza
A. diminutiva La Panza
A. d. diminutiva Mt. Diablo
A. diminutiva N4
A. diminutiva Pinyon Flat
A. diminutiva Pinyon Flat
A. diminutiva Pinyon Flat
A. diminutiva Sycamore Flat
A. diminutiva Troy Cyn.
A. diminutiva Upper Oso
A. diminutiva Whitewater
A. d. dactyla 23S&Mullholland
A. d. dactyla Nicholas Flat
A. d. dactyla Point Mugu
70
A. m. costalis Point Mugu
A. d. dactyla Point Mugu
A. longipennis Big Rock
A. longipennis Topanga Cyn.
A. longipennis Topanga Cyn.
A. morsei Backbone Tr.
A. m. curtatus Charmlee
A. m. curtatus Charmlee
A. morsei Nicholas Flat
A. morsei Nicholas Flat
A. m. costalis Point Mugu
A. m. costalis Point Mugu
A. m. costalis Point Mugu
A. m. curtatus 23S&Mulholland
A. m. curtatus 23S&Mulholland
A. m. morsei Arroyo Seco
A. m. morsei Millard Cyn.
A. m. morsei Millard Cyn.
A. m. morsei Millard Cyn.
A. m. tectinota Point Dume
A. m. tectinota Point Dume
A. m. tectinota Point Dume
64
A. longipennis Griffith Park
A. longipennis Griffith Park

Figure 2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80
64
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78
97
95
100
70
68
71
58
96
96
61
93
55
N. convexa China Flat
N. diabolica Mt. Diablo
N. sierranus Hobo Cpgr.
N. sierranus Hobo Cpgr.
Jcp Boulder Basin
Jcp Boulder Basin
A. gurneyi Mescal
A. gurneyi Mescal
A. ovata Whitewater
A. o. segnis Oak Spring
A. o. armiger Grandview
A. o. armiger Grandview
A. o. tinkhamorum Pinyon Flat
A. o. tinkhamorum Pinyon Flat
A. o. gigantea Lone Pine
A. o. gigantea Lone Pine
A. o. gigantea McMurray Mdws.
A. o. longicauda Walker Pass
A. o. longicauda Walker Pass
A. o. longicauda Walker Pass
A. o. ovata Acton
A. o. ovata Acton
A. o. ovata Bob's Gap
A. diminutiva Harris Grade
A. diminutiva Upper Oso
A. d. diminutiva Mt. Diablo
A. diminutiva La Panza
A. diminutiva La Panza
A. diminutiva Pinyon Flat
A. diminutiva Pinyon Flat
A. diminutiva Pinyon Flat
A. diminutiva Pinyon Flat
A. diminutiva Whitewater
A. m. morsei Millard Cyn.
A. diminutiva Boulder Oaks
A. diminutiva Troy Cyn.
A. diminutiva N4
A. diminutiva Sycamore Flat
A. m. morsei Millard Cyn.
A. m. morsei Millard Cyn.
A. longipennis Fryman Cyn.
A. longipennis Griffith Park
A. longipennis Griffith Park
A. morsei Kenneth Hahn
A. morsei Kenneth Hahn
A. morsei Malaga Dune
A. morsei Malaga Dune
A. longipennis Big Rock
A. longipennis Topanga Cyn.
A. longipennis Topanga Cyn.
A. m. morsei Arroyo Seco
A. m. costalis Point Mugu
A. longipennis Topanga Cyn.
A. m. costalis Point Mugu
A. m. costalis Point Mugu
A. m. costalis Point Mugu
A. diminutiva Pinyon Flat
A. d. dactyla 23S&Mulholland
A. d. dactyla Nicholas Flat
A. d. dactyla Point Mugu
A. m. curtatus Charmlee
A. m. curtatus Charmlee
A. morsei Nicholas Flat
A. morsei Nicholas Flat
A. m. curtatus 23S&Mulholland
A. m. curtatus 23S&Mulholland
A. m. tectinota Point Dume
A. m. tectinota Point Dume
A. m. tectinota Point Dume
A. d. dactyla Point Mugu
A. morsei Backbone Tr.

Figure 3

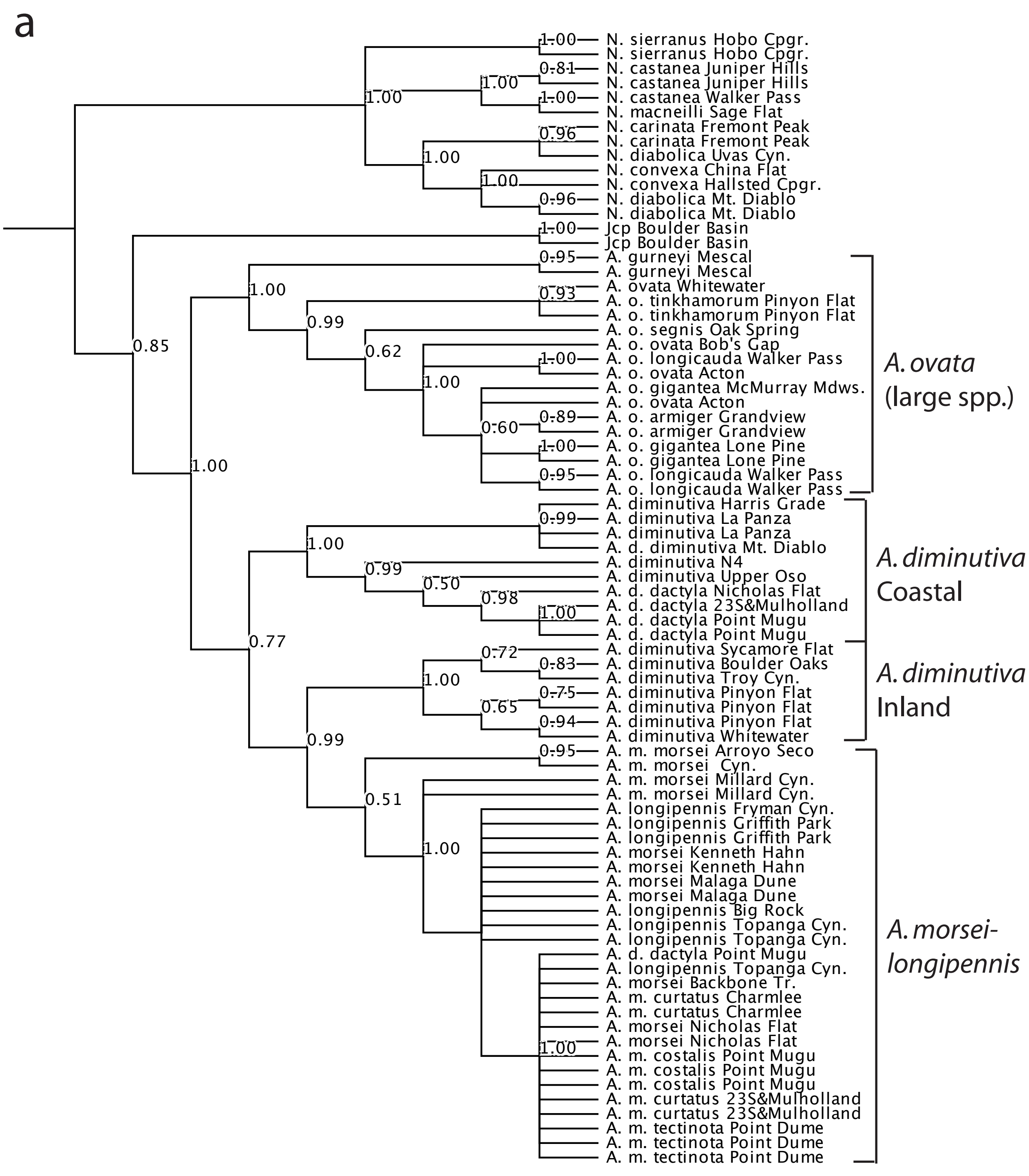

a
1.00
N. sierranus Hobo Cpgr.
N. sierranus Hobo Cpgr.
0.81
N. castanea Juniper Hills
1.00
N. castanea Juniper Hills
1.00
1.00
N. castanea Walker Pass
N. macneilli Sage Flat
N. carinata Fremont Peak
0.96
N. carinata Fremont Peak
1.00
N. diabolica Uvas Cyn.
N. convexa China Flat
1.00
N. convexa Hallsted Cpgr.
0.96
N. diabolica Mt. Diablo
N. diabolica Mt. Diablo
1.00
Jcp Boulder Basin
Jcp Boulder Basin
0.95
A. gurneyi Mescal
A. gurneyi Mescal
1.00
A. ovata Whitewater
0.93
A. o. tinkhamorum Pinyon Flat
A. o. tinkhamorum Pinyon Flat
0.99
A. o. segnis Oak Spring
A. o. ovata Bob's Gap
0.85
0.62
1.00
A. o. longicauda Walker Pass
A. o. ovata Acton
1.00
A. o. gigantea McMurray Mdws.
A. o. ovata Acton
0.89
A. o. armiger Grandview
0.60
A. o. armiger Grandview
1.00
A. o. gigantea Lone Pine
A. o. gigantea Lone Pine
1.00
0.95
A. o. longicauda Walker Pass
A. o. longicauda Walker Pass
A. ovata
(large spp.)
A. diminutiva Harris Grade
0.99
A. diminutiva La Panza
A. diminutiva La Panza
1.00
A. d. diminutiva Mt. Diablo
A. diminutiva N4
0.99
A. diminutiva Upper Oso
0.50
A. d. dactyla Nicholas Flat
0.98
A. d. dactyla 23S&Mulholland
1.00
A. d. dactyla Point Mugu
A. d. dactyla Point Mugu
A. diminutiva
Coastal
0.77
0.72
A. diminutiva Sycamore Flat
0.83
A. diminutiva Boulder Oaks
1.00
A. diminutiva Troy Cyn.
0.75
A. diminutiva Pinyon Flat
0.65
A. diminutiva Pinyon Flat
0.94
A. diminutiva Pinyon Flat
0.99
A. diminutiva Whitewater
A. diminutiva
Inland
0.95
A. m. morsei Arroyo Seco
A. m. morsei Cyn.
A. m. morsei Millard Cyn.
A. m. morsei Millard Cyn.
0.51
A. longipennis Fryman Cyn.
A. longipennis Griffith Park
A. longipennis Griffith Park
1.00
A. morsei Kenneth Hahn
A. morsei Kenneth Hahn
A. morsei Malaga Dune
A. morsei Malaga Dune
A. longipennis Big Rock
A. longipennis Topanga Cyn.
A. longipennis Topanga Cyn.
A. d. dactyla Point Mugu
A. longipennis Topanga Cyn.
A. morsei Backbone Tr.
A. m. curtatus Charmlee
A. m. curtatus Charmlee
A. morsei Nicholas Flat
A. morsei Nicholas Flat
1.00
A. m. costalis Point Mugu
A. m. costalis Point Mugu
A. m. costalis Point Mugu
A. m. curtatus 23S&Mulholland
A. m. curtatus 23S&Mulholland
A. m. tectinota Point Dume
A. m. tectinota Point Dume
A. m. tectinota Point Dume
A. morsei-
longipennis

Figure 3

b

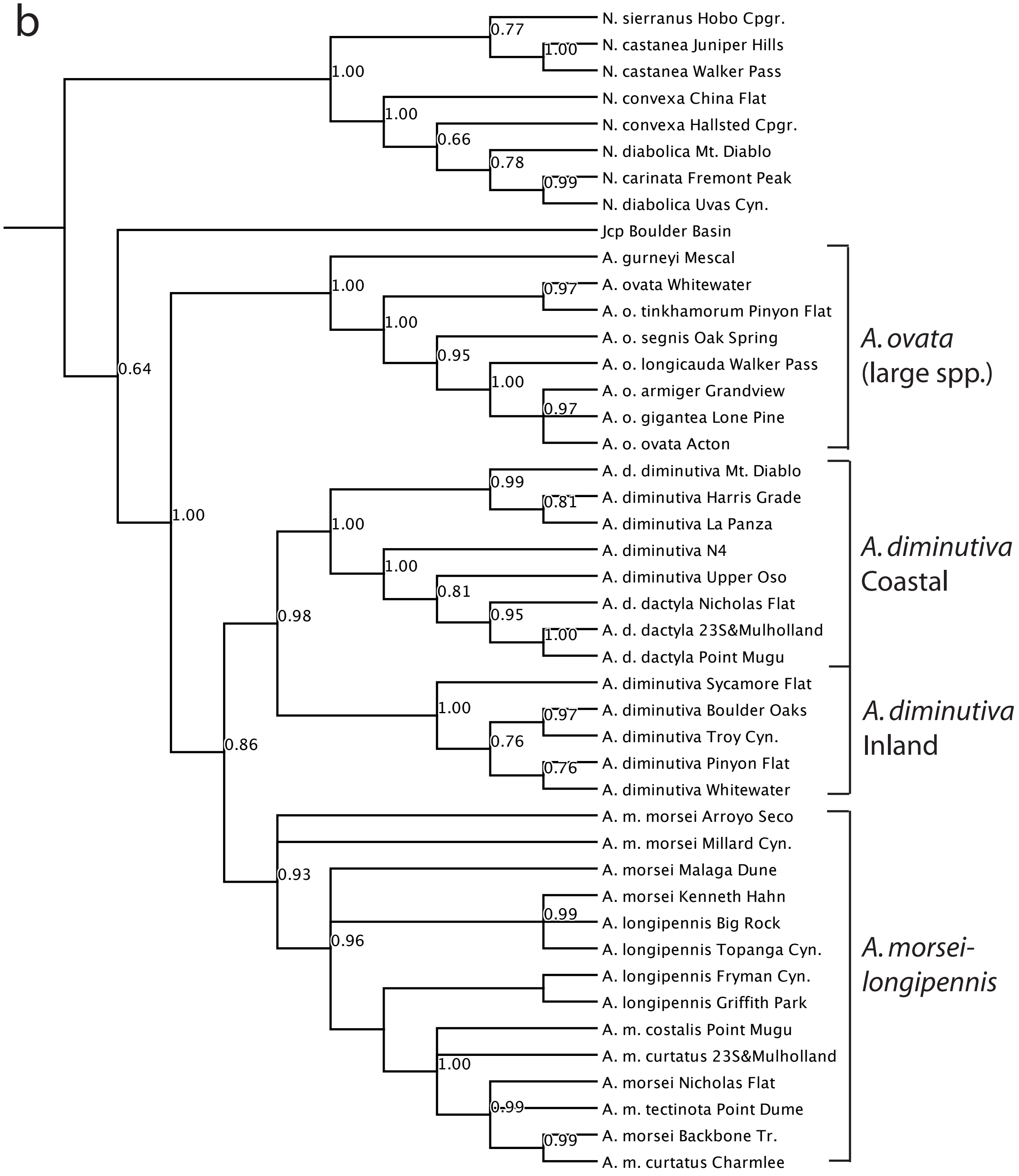

N. sierranus Hobo Cpgr.
N. castanea Juniper Hills
N. castanea Walker Pass
N. convexa China Flat
N. convexa Hallsted Cpgr.
N. diabolica Mt. Diablo
N. carinata Fremont Peak
N. diabolica Uvas Cyn.
Jcp Boulder Basin
A. gurneyi Mescal
A. ovata Whitewater
A. o. tinkhamorum Pinyon Flat
A. o. segnis Oak Spring
A. o. longicauda Walker Pass
A. o. armiger Grandview
A. o. gigantea Lone Pine
A. o. ovata Acton
A. d. diminutiva Mt. Diablo
A. diminutiva Harris Grade
A. diminutiva La Panza
A. diminutiva N4
A. diminutiva Upper Oso
A. d. dactyla Nicholas Flat
A. d. dactyla 23S&Mulholland
A. d. dactyla Point Mugu
A. diminutiva Sycamore Flat
A. diminutiva Boulder Oaks
A. diminutiva Troy Cyn.
A. diminutiva Pinyon Flat
A. diminutiva Whitewater
A. m. morsei Arroyo Seco
A. m. morsei Millard Cyn.
A. morsei Malaga Dune
A. morsei Kenneth Hahn
A. longipennis Big Rock
A. longipennis Topanga Cyn.
A. longipennis Fryman Cyn.
A. longipennis Griffith Park
A. m. costalis Point Mugu
A. m. curtatus 23S&Mulholland
A. morsei Nicholas Flat
A. m. tectinota Point Dume
A. morsei Backbone Tr.
A. m. curtatus Charmlee
0.77
1.00
1.00
1.00
0.66
0.78
0.99
0.64
1.00
0.97
1.00
0.95
1.00
0.97
0.99
0.81
1.00
1.00
1.00
0.81
0.98
0.95
1.00
1.00
0.97
0.86
0.76
0.76
0.93
0.99
0.96
1.00
0.99
0.99
A. ovata (large spp.)
A. diminutiva Coastal
A. diminutiva Inland
A. morsei-longipennis

Figure 4

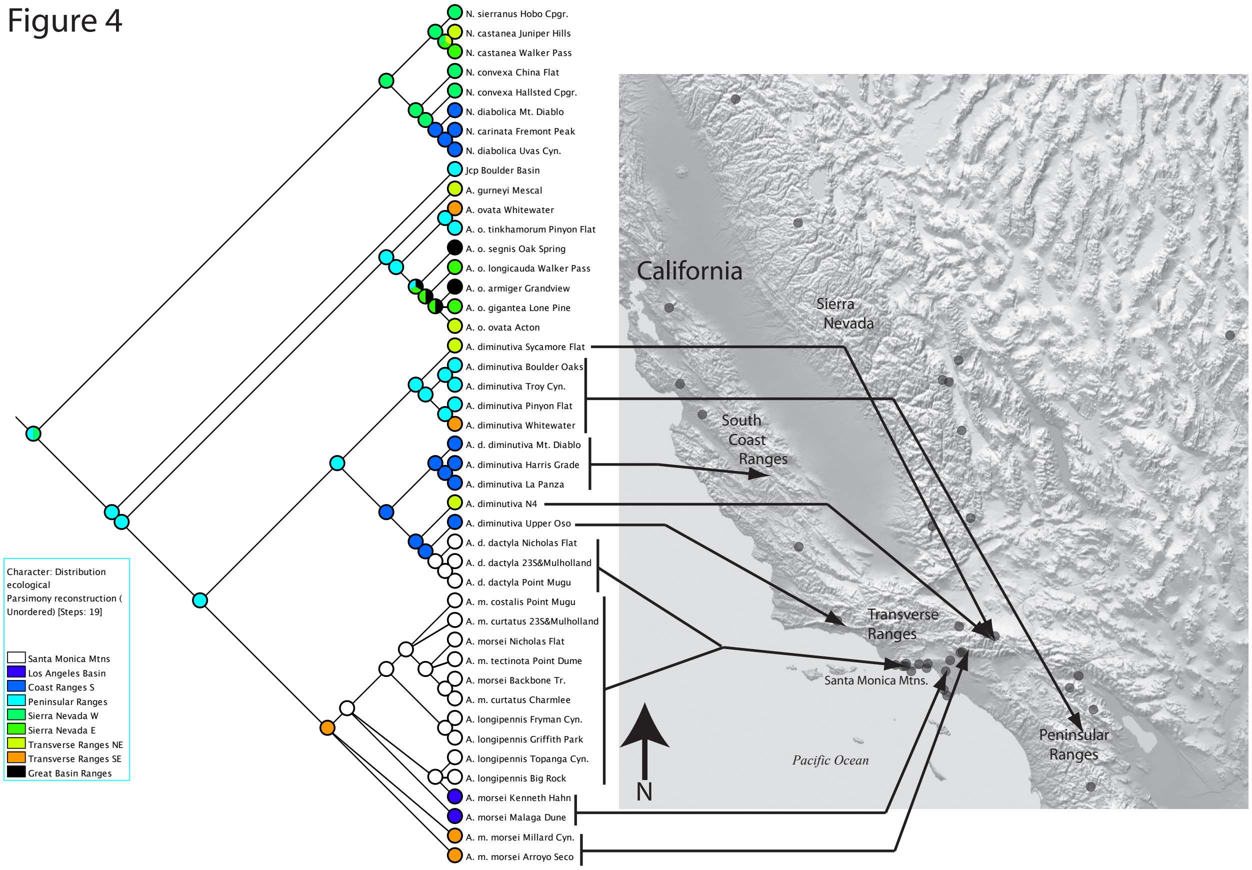

N. sierranus Hobo Cpgr.
N. castanea Juniper Hills
N. castanea Walker Pass
N. convexa China Flat
N. convexa Hallsted Cpgr.
N. diabolica Mt. Diablo
N. carinata Fremont Peak
N. diabolica Uvas Cyn.
Jcp Boulder Basin
A. gurneyi Mescal
A. ovata Whitewater
A. o. tinkhamorum Pinyon Flat
A. o. segnis Oak Spring
A. o. longicauda Walker Pass
A. o. armiger Grandview
A. o. gigantea Lone Pine
A. o. ovata Acton
A. diminutiva Sycamore Flat
A. diminutiva Boulder Oaks
A. diminutiva Troy Cyn.
A. diminutiva Pinyon Flat
A. diminutiva Whitewater
A. d. diminutiva Mt. Diablo
A. diminutiva Harris Grade
A. diminutiva La Panza
A. diminutiva N4
A. diminutiva Upper Oso
A. d. dactyla Nicholas Flat
A. d. dactyla 23S&Mulholland
A. d. dactyla Point Mugu
A. m. costalis Point Mugu
A. m. curtatus 23S&Mulholland
A. morsei Nicholas Flat
A. m. tectinota Point Dume
A. morsei Backbone Tr.
A. m. curtatus Charmlee
A. longipennis Fryman Cyn.
A. longipennis Griffith Park
A. longipennis Topanga Cyn.
A. longipennis Big Rock
A. morsei Kenneth Hahn
A. morsei Malaga Dune
A. m. morsei Millard Cyn.
A. m. morsei Arroyo Seco
Character: Distribution
ecological
Parsimony reconstruction (
Unordered) [Steps: 19]
Santa Monica Mtns
Los Angeles Basin
Coast Ranges S
Peninsular Ranges
Sierra Nevada W
Sierra Nevada E
Transverse Ranges NE
Transverse Ranges SE
Great Basin Ranges
California
Sierra Nevada
South Coast Ranges
Transverse Ranges
Santa Monica Mtns.
Peninsular Ranges
Pacific Ocean
N

Figure 5

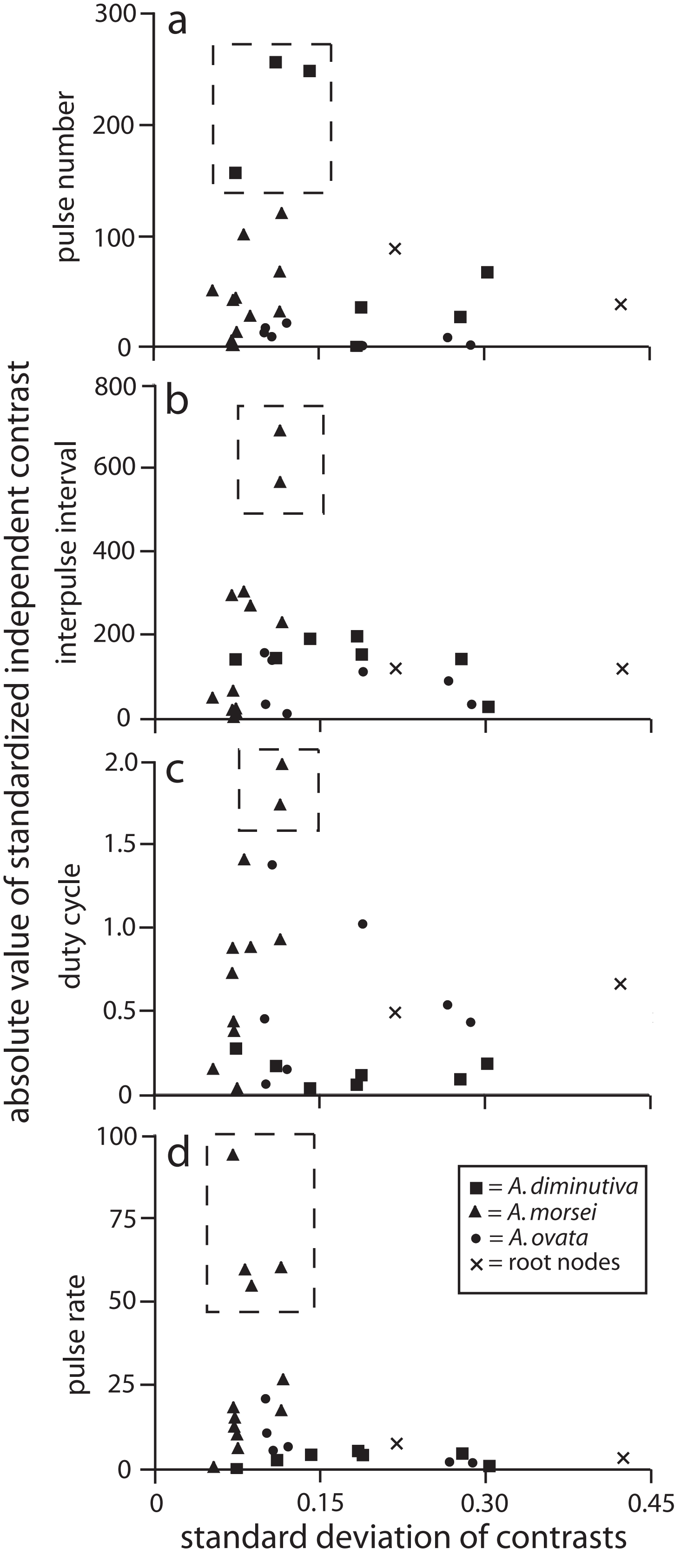
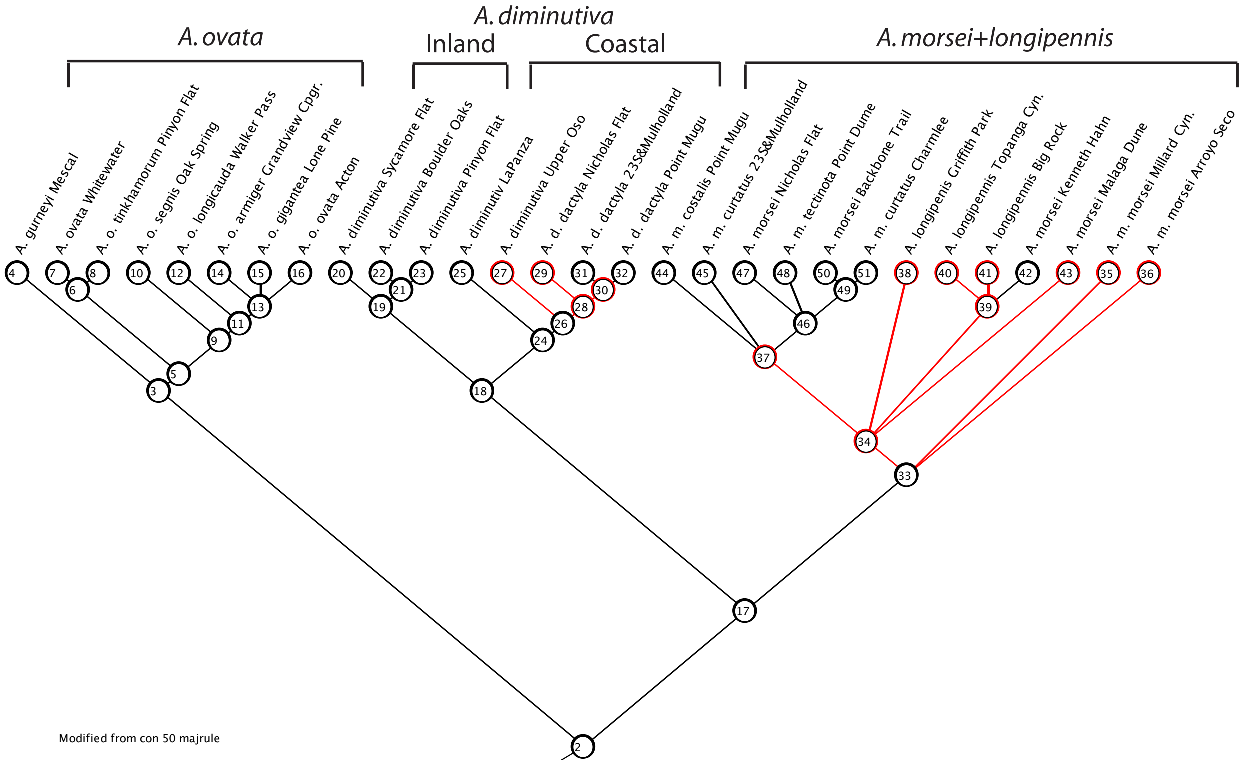

a
b
c
d
pulse number
interpulse interval
duty cycle
pulse rate
absolute value of standardized independent contrast
standard deviation of contrasts
300
200
100
0
800
600
400
200
0
2.0
1.5
1.0
0.5
0
100
75
50
25
0
0
0.15
0.30
0.45
■ = A. diminutiva
▲ = A. morsei
● = A. ovata
× = root nodes

Figure 6

	contrast name				unstandardized independent contrasts				absolute value standardized independent contrasts			
clade	tip node-node	base node	rel. node height	standard deviation	pulse number	interpulse interval	duty cycle	pulse rate	pulse number	interpulse interval (ms)	duty cycle	pulse rate (s^{-1})
root - all taxa	3-17	2	7	0.4249	-16.1666	-50.3066	0.2803	1.3843	38.0491	118.3998	0.6598	3.2581
A. ovata	4-5	3	5	0.2877	-0.5336	-9.8268	0.1239	-0.5615	1.8545	34.1516	0.4306	1.9515
A. ovata	6-9	5	4	0.1892	-0.2334	-21.2022	0.1936	-0.7581	1.2335	112.0550	1.0232	4.0068
A. ovata	7-8	6	1	0.0997	-1.3000	15.7000	0.0450	-2.1000	13.0451	157.5445	0.4516	21.0728
A. ovata	10-11	9	3	0.2667	-2.2960	24.0511	-0.1428	0.5871	8.6077	90.1693	0.5354	2.2013
A. ovata	12-13	11	2	0.1201	2.6012	1.4278	-0.0177	-0.8105	21.6560	11.8870	0.1470	6.7478
A. ovata	p3-16	13	1	0.1007	-1.7474	3.4541	-0.0058	1.0884	17.3446	34.2859	0.0579	10.8038
A. ovata	14-15	p3	1	0.1065	-1.0000	-14.9000	0.1470	0.6000	9.3856	139.8460	1.3797	5.6314
root - small taxa	18-33	17	6	0.2192	19.2905	26.1002	-0.1068	-1.6570	88.0115	119.0800	0.4871	7.5599
A. diminutiva	19-24	18	5	0.3030	-20.3317	-8.5052	0.0548	0.2869	67.1069	28.0723	0.1808	0.9469
A. diminutiva	20-21	19	2	0.1838	-0.0197	-36.1693	0.0099	0.9991	0.1069	196.8034	0.0539	5.4365
A. diminutiva	22-23	21	1	0.1880	-6.7000	28.8000	0.0210	-0.8000	35.6312	153.1612	0.1117	4.2545
A. diminutiva	25-26	24	4	0.2784	-7.5183	-39.6367	0.0241	1.3089	27.0032	142.3621	0.0866	4.7010
A. diminutiva	27-28	26	3	0.1411	-34.9830	26.9388	-0.0045	-0.6103	**247.8965**	190.8937	0.0320	4.3250
A. diminutiva	29-30	28	2	0.1101	28.1652	-15.9321	0.0183	0.3000	**255.7297**	144.6569	0.1664	2.7239
A. diminutiva	31-32	30	1	0.0735	11.5000	-10.4000	0.0200	0.0000	156.4372	141.4737	0.2721	0.0000
A. morsei-longipennis	p4-36	33	5	0.1141	3.6028	78.4831	-0.1985	-6.8664	31.5781	**687.8891**	**1.7401**	**60.1828**
A. morsei-longipennis	34-35	p4	5	0.1142	-7.7000	64.5000	-0.1060	-2.0000	67.4195	**564.7475**	0.9281	17.5116
A. morsei-longipennis	p18-43	36	4	0.0872	-2.4141	23.4931	-0.0769	-4.7717	27.6694	269.2661	0.8815	**54.6905**
A. morsei-longipennis	39-p5	p18	4	0.0813	-8.1874	-24.6027	0.1145	4.8399	100.7441	302.7311	**1.4095**	**59.5542**
A. morsei-longipennis	p19-42	39	1	0.0528	2.6684	-2.5920	-0.0078	0.0293	50.4959	49.0493	0.1471	0.5551
A. morsei-longipennis	40-41	p19	1	0.0704	0.4000	-20.7000	0.0510	6.6000	5.6854	294.2170	0.7249	**93.8083**
A. morsei-longipennis	37-38	p5	4	0.1159	-13.9000	-26.6000	0.2300	3.1000	119.8944	229.4383	**1.9839**	26.7390
A. morsei-longipennis	p20-46	43	3	0.0741	3.2475	-1.7115	-0.0012	0.7600	43.8078	23.0881	0.0161	10.2518
A. morsei-longipennis	44-45	p20	3	0.0720	-0.1000	0.2000	-0.0270	1.1000	1.3886	2.7772	0.3749	15.2748
A. morsei-longipennis	p70-49	46	2	0.0749	0.9850	0.8025	0.0023	-0.4635	13.1595	10.7207	0.0307	6.1919
A. morsei-longipennis	47-48	p70	2	0.0716	-3.0000	4.7000	-0.0310	-0.9000	41.9222	65.6781	0.4332	12.5767
A. morsei-longipennis	50-51	49	1	0.0708	-0.1000	1.4000	-0.0620	1.3000	1.4129	19.7812	0.8760	18.3683



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