EXPERIMENTAL AND POPULATION GENETIC EVIDENCE OF HOST RACE FORMATION IN A SPECIALIZED LYCAENID BUTTERFLY

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Michelle H. Downey, B.S.

San Marcos, Texas
December 2010

EXPERIMENTAL AND POPULATION GENETIC EVIDENCE OF HOST RACE FORMATION IN A SPECIALIZED LYCAENID BUTTERFLY

Committee Members Approved:

_____
Chris C. Nice, Chair

_____
Jim R. Ott

_____
Noland Martin

Approved:

_____
J. Michael Willoughby
Dean of the Graduate College

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

ACKNOWLEDGEMENTS

I would like to thank my advisor, Chris Nice, and my committee members, Noland Martin and Jim Ott, for their guidance and feedback throughout my graduate career at Texas State University-San Marcos. My peers in the Department of Biology, and especially the Population and Conservation program, provided much-appreciated support and encouragement as well. I also want to thank my husband, Lee Downey, for his support, patience, and butterfly-catching skills in the field.

This manuscript was submitted on November 8, 2010.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

EXPERIMENTAL AND POPULATION GENETIC EVIDENCE OF HOST RACE FORMATION IN A SPECIALIZED LYCAENID BUTTERFLY

by

Michelle Hanako Downey, B.S.

Texas State University-San Marcos

December 2010

SUPERVISING PROFESSOR: CHRIS NICE

Host-associated differentiation in phytophagous insects is an important mechanism of speciation. The current study investigates whether adaptation to different hosts drives population genetic divergence in the juniper hairstreak butterfly, *Mitoura gryneus*. *Mitoura* exhibit host plant fidelity, in which males lek and mating occurs on host trees. Female oviposition preference for the natal host, and differential fitness of larvae when reared on natal vs. alternate hosts, was examined to assess specialization. While some evidence of specialization was found, populations varied in their patterns of preference and performance, possibly reflecting differences in the timing and direction of colonization of hosts by *Mitoura*. Molecular genetic data were also examined to test the hypothesis that specialization on three alternate hosts restricts gene flow among different

host-associated populations of *Mitoura.* Combined with the previous experimental results, mitochondrial DNA sequence and AFLP data indicate varying levels of differentiation among host associations, and identify a role for both isolation in allopatry as well as ecological factors in limiting gene exchange. The experimental assessment of specialization and host fidelity, along with population genetics analyses, provides strong support for the hypothesis of ongoing host race formation in these butterflies. The *Mitoura* species complex within North America includes multiple, differentiated lineages at varying stages of divergence, providing an opportunity to examine the multifarious mechanisms that generate biodiversity in phytophagous insects.

CHAPTER I

EXPERIMENTAL EVIDENCE FOR HOST RACE FORMATION

Introduction

The recent advent of ecological speciation theory has placed a renewed emphasis on natural selection in promoting reproductive isolation and population divergence (Schluter 2001, Via 2001, Rundle and Nosil 2005, Funk and Nosil 2008). Phytophagous insects, among the most species-rich groups on the planet, often exhibit specialization and host-associated life history adaptations in traits related to feeding, development, oviposition, and mating (Jaenike 1990, Funk et al. 2002). For specialized phytophagous insects, selection experienced during the switch to a novel host can lead to the initial innovations that drive an adaptive radiation (Schluter 2000). Thus, plant-insect systems are ideal for assessing the role and relative importance of ecological factors in the process of speciation.

The process of speciation is complex and it is unlikely that divergence leading to speciation occurs strictly in allopatric vs. sympatric conditions (Feder et al. 2003, Michel et al. 2007). Ecological speciation restructures the allopatric and sympatric models of speciation in terms of factors such as life history traits and resource use, rather than biogeography (Schluter 2001, Rundle and Nosil 2005, Nosil 2008). Thus ecological speciation can occur in any spatial arrangement of populations, and understanding the

mechanisms of speciation (both those that initiate and also maintain reproductive isolation) are emphasized. Many studies have taken a macroevolutionary approach in examining speciation in phytophagous insects, comparing phylogenies in relation to host plant use (Farrell 1998, Janz and Nylin 1998, Moreau et al. 2006). Taking a population-level approach and shifting the focus to ongoing speciation events at early stages is useful in gaining further insight into the initial mechanisms of the process, as well as how divergence may be maintained or inhibited (Fitzpatrick et al. 2008, 2009, Via 2009).

Plant-insect systems can be used to evaluate the role of ecology in population divergence, as many insect herbivores are specialized on their hosts and this process appears to be ongoing in several well studied examples (Feder et al. 1998, Funk 1998, Via 1999, Nosil et al. 2002). The switch by herbivorous insects to a novel host, and the subsequent evolution of specialization and host fidelity, can lead to reproductive isolation between populations resulting in host race formation. Host plant fidelity describes the close association of phytophagous insects with their host plants, with adults reproducing on the same host species that was used in earlier life history stages (Feder et al. 1994). If both sexes evolve responses to visual and chemical cues from the host, and/or aggregate on the host plant, this can facilitate finding mates and lead to assortative mating on the host. Positive assortative mating based on host plant use is important in initiating and/or maintaining reproductive isolation between different host-associated entities, or “host races.” Host races are considered to be intermediary stages towards speciation, where partial gene flow among diverging populations is possible (Diehl and Bush 1984, Drès and Mallet 2002).

The juniper hairstreak butterflies in the genus *Mitoura* represent a species complex of several nominal species (alternatively considered subspecies to *M. gryneus*) that are distributed throughout North America, using different species of trees in the cypress family (Cupressaceae) as host plants (Johnson 1981, Miller and Brown 1981, Scott 1992). *Mitoura* are closely associated with their particular hosts and exhibit behaviors associated with host fidelity and specialization: males lek and mating occurs on host trees, and females oviposit and larvae develop exclusively on the host (Forister 2004). Therefore, host race formation is a plausible hypothesis to explain the divergence of *Mitoura* into different evolutionary units. Species boundaries within the *Mitoura* complex are not fully resolved (Miller and Brown 1981, Nice and Shapiro 2001), which could indicate that speciation is ongoing or incomplete for these butterflies. While the objective of the current study is not to delineate taxonomic boundaries within this group, the concept of a host race is useful in distinguishing different lineages and in understanding the patterns and processes of divergence.

Mitoura butterflies appear to exhibit a high degree of specialization on alternate host plants (Forister 2004, 2005) and thus are a useful system to examine the evolutionary consequences of host fidelity. Forister (2004, 2005) examined three nominal species of *Mitoura* in northern California associated with four different host plants occurring in both sympatry and parapatry. Evidence of host-associated adaptation in the form of female oviposition preference for the natal host was found. Larval performance, in terms of pupal weight, was higher on the natal host for some, but not all, populations. Variation in preference and performance persist despite close proximity of hosts, and may thus reflect different levels of adaptation to hosts. These studies included butterflies

that differed in morphology and phenology and used host plants across three different genera of Cupressaceae. The current study examines one nominal species of *Mitoura* (*M. gryneus*) that have minimal phenological, and no apparent morphological, differences and are associated with three species of juniper trees (*Juniperus)* that occur both allopatrically and sympatrically in the southern United States.

To assess whether host race formation is taking place within *Mitoura*, specialization on the natal host (i.e., the host plant with which butterflies are associated in nature) must first be evaluated. Many authors have examined specialization to host plants by phytophagous insects in the context of explaining high biodiversity for this group (for reviews see Thompson 1988a, Futuyma and Moreno 1988, Jaenike 1990, Thompson and Pellmyr 1991, Gripenberg et al. 2010). Specialization has frequently been examined in terms of the "preference-performance" hypothesis (also the "naïve adaptationist" hypothesis, Courtney and Kibota 1990). For insects that oviposit directly on the host plant on which larvae will develop, natural selection is predicted to drive female preference for the host on which larval performance is highest (Levins and MacArthur 1969). A concordance between female preference for, and higher larval performance on, the natal host is used to assess the degree of specialization, with two main questions addressed in this study: 1) Do females exhibit significant oviposition preference for their natal host vs. alternate, potential hosts? and 2) Is larval performance, as measured by fitness correlates including percent survival and developmental efficiency, higher when larvae are reared on the natal vs. alternate hosts? For females to exhibit oviposition preference behavior provides evidence for this trait as potentially influencing host-race formation and incipient speciation. Similarly, differential fitness of larvae on natal vs.

alternate hosts reflects a role for natural selection in driving the process of divergence in this system.

Butterflies from populations that are associated with both single and multiple host plants were examined. While the predictions for populations with single natal host associations can be considered straightforward as mentioned above, *a priori* predictions regarding populations with sympatric hosts are more complicated. The presence of two natal hosts could result in several possible evolutionary outcomes, including 1) two host races with distinct preference-performance correlations on alternative hosts; 2) one essentially panmictic population using (and thus preferring) two hosts equally; or 3) some intermediate outcome, which could potentially result from gene flow following secondary contact between two host races. While preference-performance analyses may not be sufficient to distinguish among these alternative outcomes, these experiments are a necessary first step in understanding if the conditions for host race formation are present within this system.

Methods

Butterfly Biology

Butterflies in the genus *Mitoura* (Family Lycaenidae) are found throughout North America, are multivoltine, with species differing in phenology of flights during the year, and have a facultative diapause. The juniper hairstreak, *Mitoura gryneus*, represents a species complex that includes *M. muiri*, *M. nelsoni*, *M. siva*, *M. sweadneri*, *M. thornei* and others, all of which are considered by some taxonomists to be either separate species or subspecies of *M. gryneus* (Johnson 1981, Miller and Brown 1981, Scott 1992). Host plant association is important in many of these taxonomic designations. Throughout

much of the eastern United States, *M. gryneus* uses *Juniperus virginiana* as the sole host plant (with exceptions such as *M. sweadneri* with *J. silicicola* in Florida). In the western and northwestern regions of North America, *Mitoura* are more taxonomically diverse and are associated with a greater number of Cupressaceous hosts in several genera including *Juniperus*, *Cupressus*, and *Calocedrus* (Johnson 1981).

The ecological landscape for *M. gryneus* in Texas is unique because several species of potential host plants (*Juniperus* spp.) are found in both allopatry and sympatry. Three host associations of *M. gryneus* on juniper trees (*J. virginiana, J. ashei,* and *J. pinchotii*) are examined in this study. While the range of eastern red cedar (*J. virginiana*) occurs throughout most of eastern North America, the southwestern-most extent of its range lies in eastern Texas. Butterflies were sampled from areas in which *J. virginiana* is allopatric with respect to the range of the other hosts. Ashe juniper (*J. ashei*) occurs throughout central and northern Texas. Redberry juniper (*J. pinchotii*) occurs primarily in western/northwestern Texas. Butterflies were sampled from areas in which *J. pinchotii* and *J. ashei* occur both allopatrically and sympatrically (Figure 1). For the purposes of this study, a population is considered a discrete area where butterflies were sampled that is approximately ≥ 30 km away from other sampling areas (since these are small butterflies and generally not found far from host plants, this distance was considered sufficiently outside of the normal "cruising range" for this species) (Figure 1).

Female oviposition preference

Butterflies were collected during the spring and summer of 2008 and 2009 from nine different populations, with either a single host association (*J. ashei, J. pinchotii, or J. virginiana*) or with sympatric hosts (*J. ashei-J. pinchotii*) (Figure 1). To test the

prediction that *Mitoura* butterflies are specialized on their associated hosts, oviposition preference trials were conducted. Wild-caught females were placed individually in cages (~30 cm^3) with approximately equal amounts of branch clippings from each of the three species of *Juniperus* (e.g., one from the natal host, plus the two alternate hosts). Butterflies were fed periodically with Gatorade® and misted daily with water. After 72 hours, the number of eggs deposited per host plant per individual female was recorded. In order to minimize any effects from intraspecies variation of host trees, branches were collected haphazardly from trees at all study sites, with no more than one branch taken from an individual tree at a time. Branches were stored in refrigeration at 10°C for up to four weeks (Forister 2005).

The decision to use choice (simultaneous presentation of hosts) instead of no-choice (sequential presentation of hosts, see Singer et al. 1992) in the design of oviposition preference trials was informed by previous work by Forister (2008) who found that (for *Mitoura*) choice tests can provide similar outcomes as no-choice tests. Choice tests were also more efficient in terms of sample size (given the small number of females from some populations).

Larval performance

To test the prediction that larval performance will be greater on natal hosts, larvae were reared in a split brood design on the three different host plants (Forister 2004). Eggs from female oviposition trials were removed from branches and placed in Petri dishes. Once eggs hatched, larvae were placed (up to five per rearing group) on branch clippings of the different host plant treatments, with roughly equal numbers of progeny from each female reared on all three hosts. Larvae were reared in incubation chambers at constant

temperature (27°C) and equal (12 L: 12 D) light-dark cycles. Cups were monitored daily, and plant material was replaced as needed. Pupal weights were taken within 24 h using a Mettler-Toledo scale, and weighed to the nearest milligram. Three fitness correlates were directly measured for each larva: survival to pupation, weight (mg) at pupation, and time (d) to pupation. An index of "developmental efficiency" (DE) was calculated as the ratio of pupal weight to development time (i.e., days to pupation), with the assumption that a faster development translates to greater efficiency of resource use. In addition, less time spent during development translates to less time spent in a more vulnerable larval stage (i.e., "slow growth, high mortality hypothesis" Feeny 1976, Clancy and Price 1987; but see Benrey and Denno 1997, Nylin and Gotthard 1998, Fordyce and Shapiro 2003).

Diapause strategy may be a potential source of bias in regard to evaluating DE, given that there can be intraspecific variation in butterflies that will undergo direct development vs. diapause. Individuals that are "set" to diapause potentially have a greater weight at pupation (Hunter and McNeil 1997, Neve and Singer 2008). *M. gryneus* is multivoltine in the study area and has been observed in the field as late as October (C. Nice, personal observations). Although diapause has not been specifically examined in *Mitoura*, during laboratory rearing, the majority of individuals (>90%) eclosed after approximately 14 days (M. Downey, personal observations). To minimize the chance that individuals collected might undergo a facultative winter diapause, sampling was conducted early in the year (no later than August).

Statistical analyses

Female preference was assessed at the level of natal host association, with populations grouped together within each single natal host association (sympatric host populations were analyzed separately). The number of eggs laid per plant for each female was analyzed in a nonparametric Quade test (analogous to a randomized, blocked ANOVA, Conover 1999). Each preference arena for a female was considered a block, and the number of eggs laid on each host determined the relative ranking of hosts, which was also weighted by the range in number of eggs laid (e.g., for two females that laid the same total number of eggs, the preference ranking of a female that laid most eggs on one plant would be weighted more in the analysis than the female that laid eggs that were more evenly distributed among the three hosts). Preference was also assessed at the population level; if significant differences in preference were detected, then a post-hoc analysis determining relative ranking of host plants by females was conducted. Finally, if post-hoc comparisons revealed two hosts were equally preferred over the third host, then a heterogeneity G-test (Sokal and Rohlf 1995) was conducted to address the question of whether females were laying eggs in roughly equal proportions on both of the more-preferred hosts, or if there were distinct groups of females laying a greater proportion of eggs on one host vs. the other.

Larval performance was evaluated using analysis of variance (ANOVA). Due to space limitations, larvae were reared in small groups; since each individual larva within a cup could not be treated as independent, the “rearing cup” was considered the replicate for statistical analyses, and data were collected for individual pupae and averaged per rearing cup. Populations with a single host association were analyzed separately from the

sympatric-host populations. Percent survival was evaluated as the proportion of larvae within each rearing cup surviving to pupation. The data were not normally distributed, and most cups had a proportion of survival near 0 or 1. Therefore the data were transformed using the empirical logistic transformation (Cox and Snell 1989). The response variable was *z* (transformed average per-cup survival) weighted by *w* (that takes into account the number of larvae initially in each cup). Other performance response variables examined included weight at pupation, time to pupation, and the ratio of weight: time to pupation (DE). These measurements were recorded for each pupa, and then an average rearing cup value was calculated. Percent survival, weight at pupation, time to pupation, and DE were examined in separate ANOVAs, with natal host association, population (nested within natal host association), and treatment as fixed factors. Interactions examined included population crossed with treatment, and natal host association crossed with treatment. ANOVAs were conducted using JMP-IN software, version 8.0 (SAS Institute 2008).

Results

Female oviposition preference

A total of 138 preference trials were conducted for female *Mitoura gryneus* from seven populations associated with a single host, and two populations associated with both *Juniperus ashei* and *J. pinchotii*. Female preference varied among the different host associations. Females from *J. ashei*-associated populations showed a clear host plant preference hierarchy, and significantly preferred their natal host with *J. pinchotii* ranked second and *J. virginiana* last (Figure 2, Table 1). Females from *J. pinchotii*-associated populations showed equal preference for their natal host and *J. ashei,* with both of these

plants preferred over *J. virginiana*. In contrast, butterflies sampled from populations associated with *J. virginiana* did not exhibit significant oviposition preference for any hosts (Figure 2, Table 1).

Female oviposition preference differed between the two populations in which *J. ashei* and *J. pinchotii* host plants are sympatric. Female preference in the Junction population was similar to that found with *J. ashei*-only associated populations, with a preference hierarchy of *J. ashei*, followed by *J. pinchotii*, and finally *J. virginiana*. Females at Independence Creek, however, preferred both *J. ashei* and *J. pinchotii* equally over *J. virginiana*, similar to the *J. pinchotii*-only associated populations (Figure 2, Table 1).

The overall pattern of preference observed for butterflies from Independence Creek was further examined in a heterogeneity G-test to assess whether individual females laid eggs in roughly equal proportions among the two preferred hosts, or whether individual females laid more eggs on one host vs. the other. Significant heterogeneity was found among females for the proportion of eggs laid on *J. ashei* vs. *J. pinchotii* (heterogeneity G = 1496.92, df = 27, $P < 0.001$), with a post-hoc test revealing some females laying a greater proportion on *J. ashei*, and others laying a greater proportion on *J. pinchotii*. There were also females from Independence Creek that were intermediate in their preference, including a few individuals that laid equal proportions on both hosts (which is the pattern predicted if individual females equally preferred two hosts). Significant heterogeneity of preference among individual females was also found within the *J. ashei*-associated Freeman Ranch and the *J. pinchotii*-associated San Angelo populations, suggesting that not all individual females were expressing preference for *J.*

ashei and *J. pinchotii* in the same way, and that the finding of "equal preference" represents the composite preference of individual females.

Larval performance: Percent survival

A total of 3,640 larvae housed in 1,238 rearing cups were established across all host plant treatments. No larvae survived to pupation in approximately 25% of rearing cups; in general, larvae that did not survive to pupation died at an early instar, without establishing a feeding site on the plant. For *J. ashei*- and *J. pinchotii*-associated populations, mean percent survival did not differ between the *J. ashei* and *J. pinchotii* treatments, although each of these treatments resulted in greater survival when compared with *J. virginiana*. For *J. virginiana*-associated populations mean percent survival did not differ when larvae were reared on natal vs. non-natal hosts (Figure 3, Table 3).

For host-sympatric populations, the patterns observed in larval survival to pupation mirrored female preference: larvae at Junction had highest survivorship on *J. ashei*, followed by *J. pinchotii* and *J. virginiana*, while at Independence Creek larvae survived equally well on *J. ashei* or *J. pinchotii*, and survivorship on both hosts was greater than on *J. virginiana* (Figure 4, Table 3).

Larval performance: Weight at pupation

Rearing cups for which ≥1 larva survived to pupation were used in subsequent analyses of larval performance, including weight at pupation, time to pupation, and DE (the ratio of weight : time to pupation). For insects, weight at pupation can be used as a fitness correlate related to fecundity, since body size at adulthood has been found to be strongly positively correlated with egg load (reviewed in Honek 1993). *J. ashei*-associated larvae had higher pupal weights when reared on their natal host and *J.*

pinchotii; weights were significantly lower when reared on *J. virginiana* (Table 2). Progeny of *J. virginiana*-associated butterflies had significantly higher pupal weights when reared on the natal host in comparison to both *J. ashei* and *J. pinchotii*. Larvae from *J. pinchotii*-associated populations attained the highest weights at pupation on *J. ashei*, and pupal weights did not significantly differ when reared on their natal host and *J. virginiana* (Table 2).

The patterns of weight at pupation differed between the Junction and Independence Creek populations, where *J. ashei* and *J. pinchotii* are sympatric. Butterflies from Junction produced progeny that weighed significantly more at pupation when reared on *J. ashei* than when reared on *J. pinchotii* or *J. virginiana*. Independence Creek larvae had highest weights at pupation when reared on either *J. ashei* or *J. pinchotii*, with larvae reared on *J. virginiana* having lower weights (Table 2).

Larval performance: Time to pupation

Time to pupation gauges how efficiently nutrients are acquired and metabolized in insects that undergo complete metamorphosis. *J. ashei*-associated larvae reached pupation fastest when reared on their natal host (Table 4). *Juniperus pinchotii*-associated larvae pupated on *J. ashei* and *J. pinchotii* after a similar length of time, and this was faster than when reared *J. virginiana*. Butterflies from populations associated with *J. virginiana* had the shortest development time when reared on their natal host, although this did not differ significantly from those reared on *J. ashei* (Table 4).

Butterflies from areas of host sympatry again differed in their patterns of time to pupation according to the source population. Larvae from the Junction population pupated fastest on *J. ashei*; whereas for larvae from Independence Creek, time to

pupation did not differ significantly between larvae reared on *J. ashei* or *J. pinchotii*, but was faster on either over *J. virginiana* (Table 4).

Larval performance: Developmental efficiency

While both weight at, and time to, pupation can be considered individually as fitness correlates, the composite metric (pupal weight : development time) provides an estimate of relative DE for larvae. For both *J. ashei*- and *J. virginiana*- associated populations, DE was significantly higher on the natal host. For *J. pinchotii*-associated populations, DE did not differ between the natal host and *J. ashei*, but these DE values were significantly higher than when larvae were reared on *J. virginiana* (Figure 3, Table 5).

For those populations that are associated with both *J. ashei* and *J. pinchotii* host plants, DE differed between the two populations, but mirrored the patterns of female preference. Junction larvae had the highest DE on *J. ashei*, followed by *J. pinchotii*, with the lowest DE on *J. virginiana*. For Independence Creek butterflies, DE on *J. ashei* and *J. pinchotii* did not significantly differ, and was higher than when larvae are reared on *J. virginiana* (Figure 5, Table 5).

Discussion

Host plant specialization

Phytophagous insects that both mate and oviposit on their host plant have in place the conditions that can lead to specialization and host race formation, considered an intermediate stage in the evolution of new species (Drès and Mallet 2002). This study tested for evidence of specialization by examining patterns of preference and performance for multiple populations of a single nominal species of hairstreak butterfly,

Mitoura gryneus, distributed across multiple hosts of *Juniperus* that occur both allopatrically and sympatrically. Butterflies varied in host preference, with *J. ashei*-associated females preferring their natal host, *J. pinchotii*-associated females equally preferring both the natal host and *J. ashei*, and *J. virginiana*-associated females not expressing significant preference for their natal host over the alternate hosts. Larvae exhibited differential fitness, as measured by survival and developmental efficiency (DE), according to host plant treatment. *Juniperus ashei*-associated populations had highest larval survival and DE on the natal host. *Juniperus pinchotii*-associated populations had higher survival and shorter time to pupation on the natal host as well as on *J. ashei*. For *J. virginiana* populations, larval survivorship did not differ among the different host treatments, although time to pupation was shortest on the natal host, and mean pupal weight was reduced by a third when larvae were reared on alternate hosts. Observations of both female oviposition preference and increased larval performance on natal vs. alternate hosts provide evidence for specialization and indicates that the conditions for host race formation are present.

The results presented herein suggest that these putative host races are at different stages of adaptation to their natal host, possibly a reflection of the amount of time accumulated in association with a particular host (Thompson 1988b, Keeler and Chew 2008), differences among populations in the strength of selection leading to local adaptation, and/or varying levels of gene flow between different host-adapted populations. All of these processes in turn may be the result of past biogeographical patterns, both of host plant range and the direction and timing of colonization of these hosts by *Mitoura*. In addition, past host plant use in “deep” evolutionary time may help to

explain the plasticity in these butterflies' ability to use different hosts (Nylin and Wahlberg 2008, Nylin and Janz 2009; see also concepts of "ecological fitting," Janzen 1985, Agosta 2007). Much work has been done to elucidate the role of oscillations in host plant range and diversification of butterfly lineages on a macroevolutionary scale (e.g., "oscillation hypothesis" Janz et al. 2006, Janz and Nylin 2008). However the population-level processes described here represent an important transitional stage between host range expansion and a potential host shift, which in turn will shape evolutionary trajectories and the direction of large-scale processes such as lineage splitting.

When considered in combination with previous experimental and population genetic work with *Mitoura* (Nice and Shapiro 2001, Forister 2004, 2005), the results of this study contribute to an emerging body of evidence that suggests that specialization and host race formation are occurring within *Mitoura* on Cupressaceous hosts in North America, with conditions in place for parallel ecological speciation events occurring at different stages in different areas of host association. Forister (2004) examined three nominal species of *Mitoura* (*M. muiri, M. nelsoni*, and *M. siva*) associated with four different hosts in northern California (two species of *Cupressus*, one *Juniperus*, and one *Calocedrus*). Female oviposition preference was correlated with larval performance for some but not all host associations; as in this study, the relationship between preference and performance measures in each population was not straightforward, possibly a reflection of different levels of adaptation to hosts, or asymmetrical gene flow between different host races. Population genetic analyses by Nice and Shapiro (2001) using allozymes and mtDNA sequence data of the same taxa examined by Forister (2004, 2005) revealed little genetic differentiation among the nominal taxa, indicating recent

divergence and/or ongoing gene flow for these butterflies. However, coastal *M. muiri* was found to be significantly genetically differentiated from other taxa, despite close geographic proximity. Limited gene flow due to host plant fidelity and phenological differences (Nice and Shapiro 2001) could be an explanation, as non-ecological barriers to gene flow were low.

Single host-associated populations

For butterflies from *J. ashei*-associated populations, both female oviposition preference and increased larval performance on the natal host are consistent with natural selection for increased fitness on the natal host. For populations associated with the host *J. pinchotii*, however, both the natal host and *J. ashei* were preferred as host plants for oviposition, and larvae had similar levels of performance on these two hosts relative to *J. virginiana*. In fact, after their natal host, *J. ashei*-associated butterflies both preferred and performed better on *J. pinchotii*—despite the relative geographic proximity of *J. virginiana*-associated populations. Several factors might explain the differences in adaptation to the natal host for these populations. Butterflies may have been associated with *J. ashei* longer, allowing more time for selection on preference and performance. *Mitoura* from *J. ashei*-adapted populations may have colonized areas with *J. pinchotii* more recently, and adaptation to the new host is ongoing, or populations of *Mitoura* associated with *J. pinchotii* may have had recent or ongoing gene flow with *J. ashei*-adapted butterflies. Alternatively, *J. ashei* and *J. pinchotii* may be similar in terms of their suitability as host plants for *Mitoura*. A population genetics approach would be useful in testing these alternative (although not mutually exclusive) hypotheses, and would provide the information needed to understand geographic patterns of genetic

differentiation among populations and test whether gene flow is restricted based on host plant use.

J. virginiana-associated populations did not exhibit a concordance between oviposition preference and larval performance. Females did not have a clear signal of preference for the natal host, and larval survival did not significantly differ among host treatments; however, larval DE was significantly higher on the natal host. One possible explanation for the lack of female preference in these populations is related to past biogeographic patterns. If these butterflies have colonized the region from the east, and have only had experience with one host plant (*J. virginiana*) in the recent evolutionary past, then these populations have not experienced the selective pressures associated with the presence of alternate hosts, and therefore have not had a need to "fine-tune" preference for their host plant (Thompson and Pellmyr 1991, Keeler and Chew 2008).

Populations in areas of host sympatry

Examining preference and performance in *Mitoura* from areas of host sympatry can provide further clues to the evolution of adaption to each individual host. Butterflies sampled from two different locations (Junction and Independence Creek) where the host plants *J. ashei* and *J. pinchotii* are sympatric differed in their respective patterns of female oviposition preference and larval performance (Figures 3 – 5). At Junction, patterns of preference and performance were similar to those found with *J. ashei*-associated populations, with a strong association of female preference and increased larval performance (for all variables measured) on *J. ashei* (followed by *J. pinchotii* and then *J. virginiana*). Butterflies at Independence Creek, however, expressed similar patterns of preference and performance as those found in *J. pinchotii*-only populations.

Female preference, and larval survival and DE, did not statistically differ between the two potential hosts, *J. ashei* and *J. pinchotii*.

Evidence of specialization for *Mitoura* in allopatry allows for the possibility that distinct host races exist in areas of sympatry, where gene flow is possible, but selection favoring specialization, along with assortative mating, can drive divergence. If gene flow is occurring freely in areas of host sympatry, then these areas are predicted to resemble the population at Independence Creek, where both *J. ashei* and *J. pinchotii* were equally preferred hosts. However, if this were the case, then the prediction could further be made that females would lay roughly equal proportions of eggs on both *J. ashei* and *J. pinchotii* in preference trials. A heterogeneity G-test revealed that distinct groups were laying more eggs on one potential host than the other, and vice versa. If preference in *Mitoura* has been found to be an independently inherited (potentially dominant) trait (Forister 2005), then the observed pattern could be a result of two host races coming into secondary contact, with some initial gene flow but also the presence of individuals still expressing preference for one host over the other. Again, population genetics data would be helpful in testing the likelihood of this scenario.

If there are different host races present in areas of host plant sympatry, then the relative frequency of the hosts could influence the composition of *Mitoura* host races, with selection favoring butterflies adapted to the more frequently occurring host. The Junction site is at the easternmost edge of the range of *J. pinchotii*, where it begins to come into contact with *J. ashei*, which appears to be the more abundant host (M. Downey, personal observations). Conversely, Independence Creek is located closer to the center of the range for *J. pinchotii*, and the two trees appear to be in relatively equal

abundance (M. Downey, personal observations); therefore, two distinct host races may be able to be maintained in this area. The results for the Junction population, in which there is a strong signal of adaptation to one host (*J. ashei*) despite two potential hosts being present, could be due to relative host frequency. Alternatively, the Junction population could have been more recently colonized by *J. ashei*- adapted *Mitoura*, and incorporation of *J. pinchotii* as a suitable host is incomplete or hindered by host fidelity.

The concept of a host race is useful in distinguishing different incipient lineages and in understanding the process of divergence at a microevolutionary scale. Preference-performance relationships among the different host-associated populations examined in this study varied from a concordance between female preference and increased larval fitness on the natal host (*J. ashei*-associated populations), to roughly equivalent preference and performance on both the natal and an alternate host (*J. pinchotii*-associated populations), to a lack of oviposition preference but highest larval performance on the natal host (*J. virginiana*-associated populations). *Mitoura* are capable of using all three host plants considered in this study, have no apparent morphological differences, and in a laboratory setting, individuals from different host-associated populations are able to successfully interbreed (authors, personal observations). In the absence of physical boundaries to migration, the patterns observed in this study may be partially explained by host fidelity and specialization driving reproductive isolation between host associated groups—host race formation—although what is taking place here appears to be in the earliest stages of divergence. *Mitoura gryneus* species are New World taxa, and their Cupressaceous hosts are more diverse in western areas of North America. Future research that examines patterns of geographic genetic differentiation of

Mitoura, and whether these are in alignment with host plant associations, may reveal if gene flow is limited based on host plant use. Examining these patterns on a broader scale—both geographically as well as phylogenetically—will be valuable in understanding the importance of ecological interactions in driving the diversification of phytophagous insect species on their hosts.

Table 1. Oviposition preference results. Quade test, *n* = number of females. Populations pooled within host plant association at top of table, followed by individual population results. If significant differences among treatments were found, a post-hoc analysis was conducted (indicated by superscript letters).

					Mean % of eggs laid on:		
	n	$df_{(N,D)}$	T_3	*P* value	*J. ashei*	*J. pinchotii*	*J. virginiana*
J. ashei	61	2, 120	22.55	< 0.001	57.18[a]	33.10[b]	9.72[c]
J. pinchotii	17	2, 32	10.18	< 0.001	43.78[a]	41.23[a]	14.99[b]
J. virginiana	20	2, 38	1.31	0.28	30.32	20.63	49.06
J. ashei host association							
Guadalupe	20	2,38	13.70	< 0.001	65.54[a]	28.88[b]	5.59[c]
Pedernales Falls	12	2,22	6.79	0.005	61.12[a]	22.17[b]	16.71[b]
Freeman Ranch	29	2,56	9.03	< 0.001	49.79[a]	40.53[a]	9.69[b]
J. pinchotii host association							
Big Spring	6	2,10	3.58	0.07	55.39	27.51	17.1
San Angelo	11	2, 20	5.72	0.010	37.45[a]	48.71[a]	13.84[b]
J. ashei-J. pinchotii host association							
Independence Creek	28	2,54	10.79	< 0.001	53.60[a]	43.29[a]	3.11[b]
Junction	25	2,48	19.83	< 0.001	72.04[a]	22.70[b]	5.26[c]
J. virginiana host association							
Welsh	14	2,26	3.18	0.058	27.00	17.03	55.97
Oak Thicket	6	2,10	0.30	0.74	38.05	29.02	32.93

Table 2. Mean weight at pupation (mg) of larvae reared on different hosts. Populations pooled within host plant association at top of table, followed by individual population results. Superscript letters indicate results of post-hoc test if significant differences were found.

					Mean weight (mg) ± SE when reared on:		
	n	$df_{(N,D)}$	F	*P* value	*J. ashei*	*J. pinchotii*	*J. virginiana*
J. ashei	338	8,335	9.57	<0.001	95.56 (1.39)[a]	89.79 (1.51)[b]	79.33 (1.98)[c]
J. pinchotii	160	5,157	6.10	<0.001	97.96 (1.91)[a]	88.31 (1.89)[b]	87.89 (2.14)[b]
J. virginiana	91	5,88	18.82	<0.001	69.45 (2.21)[a]	67.92 (2.08)[a]	92.00 (2.31)[b]
J. ashei host association							
Guadalupe	156	2,153	39.04	<0.001	101.78 (1.98)[a]	93.44 (1.80)[b]	80.32 (2.33)[c]
Pedernales Falls	64	2,61	4.54	0.0145	99.46 (3.65)	91.04 (3.79)	86.79 (4.81)
Freeman Ranch	118	2,115	6.68	0.0018	89.43 (2.18)[a]	83.78 (2.55)[ab]	75.85 (2.94)[b]
J. pinchotii host association							
Big Spring	54	2,51	17.13	<0.001	94.59 (3.72)[a]	81.45 (3.71)[ab]	85.17 (2.90)[b]
San Angelo	106	2,103	7.08	0.0013	103.83 (2.25)[a]	90.45 (2.53)[b]	89.01 (2.20)[b]
J. ashei-*J. pinchotii* host association							
Independence Creek	197	2,194	26.16	<0.001	89.55 (2.02)[a]	89.67 (2.14)[a]	78.63 (2.26)[b]
Junction	64	2,61	46.85	<0.001	107.98 (2.06)[a]	96.77 (1.84)[b]	94.49 (2.06)[b]
J. virginiana host association							
Welsh	68	2,65	33.09	<0.001	70.07 (2.41)[b]	66.55 (2.33)[b]	88.66 (2.96)[a]
Oak Thicket	23	2,20	22.30	<0.001	64.92 (3.54)[b]	68.50 (2.85)[b]	95.26 (3.15)[a]

Table 3. Mean percent survival of larvae reared on different hosts. ANOVA results, *n* = number of rearing groups. Populations pooled within host plant association at top of table, followed by individual population results. Superscript letters indicate results of post-hoc test if significant differences were found.

					Mean % survival of larvae (±SE) reared on:		
	n	$df_{(N,D)}$	F	*P* value	*J. ashei*	*J. pinchotii*	*J. virginiana*
J. ashei	466	2, 463	27	<0.001	54.07 (2.26)[a]	47.74 (2.40)[a]	29.62 (2.51)[b]
J. pinchotii	180	2, 177	16.1	<0.001	66.08 (3.58)[a]	73.33 (3.61)[a]	44.77 (3.57)[b]
J. virginiana	167	2, 164	1.1	0.35	44.14 (4.15)	34.56 (4.40)	39.42 (4.55)
J. ashei host association							
Guadalupe	198	2,195	11.5	<0.001	61.83 (3.59)[a]	60.96 (3.72)[a]	39.32 (3.86)[b]
Pedernales Falls	94	2,91	10.5	<0.001	55.78 (4.77)[a]	46.46 (4.80)[a]	23.45 (5.27)[b]
Freeman Ranch	173	2,170	11.4	<0.001	45.82 (3.21)[a]	33.58 (3.60)[b]	21.96 (3.70)[b]
J. pinchotii host association							
Big Spring	65	2, 62	8.7	<0.001	63.15 (5.95)[a]	70.15 (6.13)[a]	33.70 (5.95)[b]
San Angelo	115	2, 112	7.7	<0.001	67.82 (4.42)[a]	73.53 (4.41)[a]	51.30 (4.40)[b]
J. ashei-J. pinchotii host association							
Independence Creek	250	2, 247	11.3	<0.001	63.42 (3.38)[a]	56.11 (3.37)[a]	41.18 (3.49)[b]
Junction	198	2, 195	16.9	<0.001	76.53 (3.58)[a]	61.58 (3.56)[b]	47.39 (3.38)[c]
J. virginiana host association							
Welsh	101	2, 99	1.4	0.25	42.72 (4.90)	29.49 (5.38)	39.39 (5.55)
Oak Thicket	33	2, 30	0.63	0.54	49.52 (10.04)	55.73 (10.38)	40.17 (10.43)

Table 4. Mean time to pupation (d) of larvae reared on different hosts. ANOVA results, *n* = number of rearing groups). Populations pooled within host plant association at top of table, followed by individual population results. Superscript letters indicate results of post-hoc test if significant differences were found.

					Mean time (d) ± SE when reared on:		
	n	$df_{(N,D)}$	F	*P* value	*J. ashei*	*J. pinchotii*	*J. virginiana*
J. ashei	338	2,335	4.09	<0.001	31.40 (0.44)[a]	33.97 (0.47)[b]	33.76 (0.62)[b]
J. pinchotii	160	2,157	8.20	<0.001	30.24 (0.52)[a]	29.58 (0.52)[a]	33.11 (0.59)[b]
J. virginiana	91	2,88	5.11	<0.001	34.07 (1.23)[ab]	38.11 (1.16)[a]	31.04 (1.28)[b]
J. ashei host association							
Guadalupe	156	2,153	18.21	<0.001	29.96 (0.51)[a]	33.29 (0.46)[b]	34.22 (0.59)[b]
Pedernales Falls	32	2,61	0.38	0.680	33.01 (1.03)	33.82 (1.07)	34.46 (1.35)
Freeman Ranch	118	2,115	2.87	0.060	31.96 (0.97)	35.15 (1.00)	32.60 (1.06)
J. pinchotii host association							
Big Spring	54	2,51	10.20	<0.001	27.75 (1.16)[a]	30.80 (1.16)[ab]	34.30 (0.90)[b]
San Angelo	106	2,103	4.90	0.009	32.40 (0.64)[a]	29.60 (0.72)[b]	32.05 (0.62)[a]
J. ashei-*J. pinchotii* host association							
Independence Creek	197	2,194	15.77	<0.001	30.71 (0.54)[a]	32.31 (0.57)[a]	35.25 (0.61)[b]
Junction	64	2,61	58.84	<0.001	27.49 (0.68)[a]	34.26 (0.61)[b]	37.64 (0.68)[c]
J. virginiana host association							
J. virginiana Welsh	68	2,65	8.32	<0.001	33.16 (1.37)[a]	39.68 (1.33)[b]	32.22 (1.68)[a]
J. virginiana Oak Thicket	23	2,20	11.32	<0.001	35.57 (1.56)[a]	35.06 (1.25)[a]	27.20 (1.39)[b]

Table 5. Mean developmental efficiency (DE) of larvae reared on different hosts. ANOVA results, *n* = number of rearing groups. Populations pooled within host plant association at top of table, followed by individual population results. Superscript letters indicate results of post-hoc test if significant differences were found.

					Mean DE (±)SE when reared on:		
	n	$df_{(N,D)}$	F	*P* value	*J. ashei*	*J. pinchotii*	*J. virginiana*
J. ashei	338	2,335	35.14	<0.001	3.06 (0.05)[a]	2.72 (0.05)[b]	2.37 (0.07)[c]
J. pinchotii	160	2,157	15.96	<0.001	3.24 (0.06)[a]	3.05 (0.06)[a]	2.68 (0.08)[b]
J. virginiana	91	2,88	50.62	<0.001	2.12 (0.07)[a]	1.87 (0.09)[a]	3.02 (0.08)[b]
J. ashei host association							
Guadalupe	156	2,153	39.04	<0.001	3.26 (0.06)[a]	2.87 (0.06)[b]	2.40 (0.08)[c]
Pedernales Falls	32	2,61	4.54	0.015	3.04 (0.12)[a]	2.66 (0.14)[b]	2.40 (0.21)[b]
Freeman Ranch	118	2,115	6.68	0.002	2.79 (0.08)[a]	2.44 (0.11)[b]	2.26 (0.14)[b]
J. pinchotii host association							
Big Spring	54	2,51	17.13	<0.001	3.46 (0.10)[a]	2.85 (0.10)[b]	2.45 (0.15)[b]
San Angelo	106	2,103	7.08	0.001	3.13 (0.07)[a]	3.16 (0.07)[a]	2.77 (0.09)[b]
J. ashei-*J. pinchotii* host association							
Independence Creek	197	2,194	26.16	<0.001	3.05 (0.07)[a]	2.85 (0.07)[a]	2.27 (0.09)[b]
Junction	64	2,61	46.85	<0.001	4.03 (0.10)[a]	2.99 (0.13)[b]	2.41 (0.15)[c]
J. virginiana host association							
Welsh	68	2,65	33.09	<0.001	2.10 (0.08)[b]	1.86 (0.11)[b]	2.94 (0.09)[a]
Oak Thicket	23	2,20	22.30	<0.001	2.16 (0.15)[b]	1.89 (0.15)[b]	3.41 (0.18)[a]

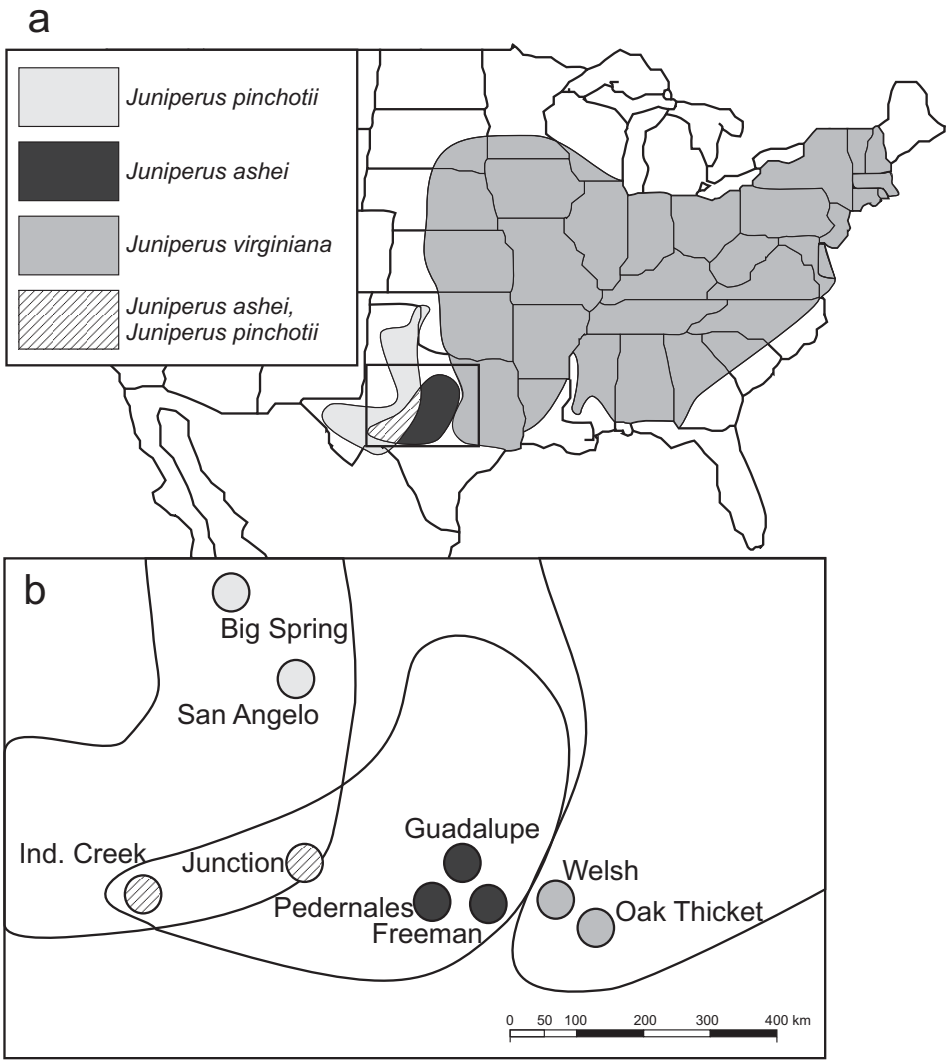

Figure 1. Map of study area. **a**. Range of *Juniperus* hosts examined in this study and **b**. locations of study populations.

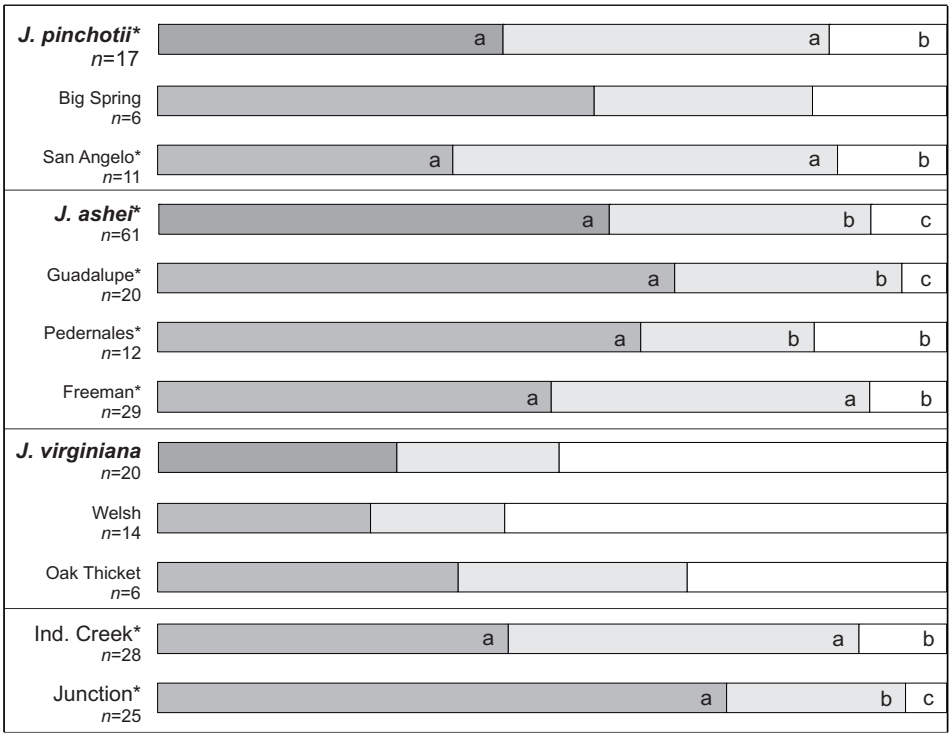

Figure 2. Female oviposition preference for individual populations and by natal host association (in bold). Bars indicate the proportion of total eggs laid on each plant; dark grey is *Juniperus ashei*, light grey is *J. pinchotii*, and white is *J. virginiana*. Asterisks next to population names indicate significant preference, and lowercase letters on graph indicate results of post-hoc analysis.

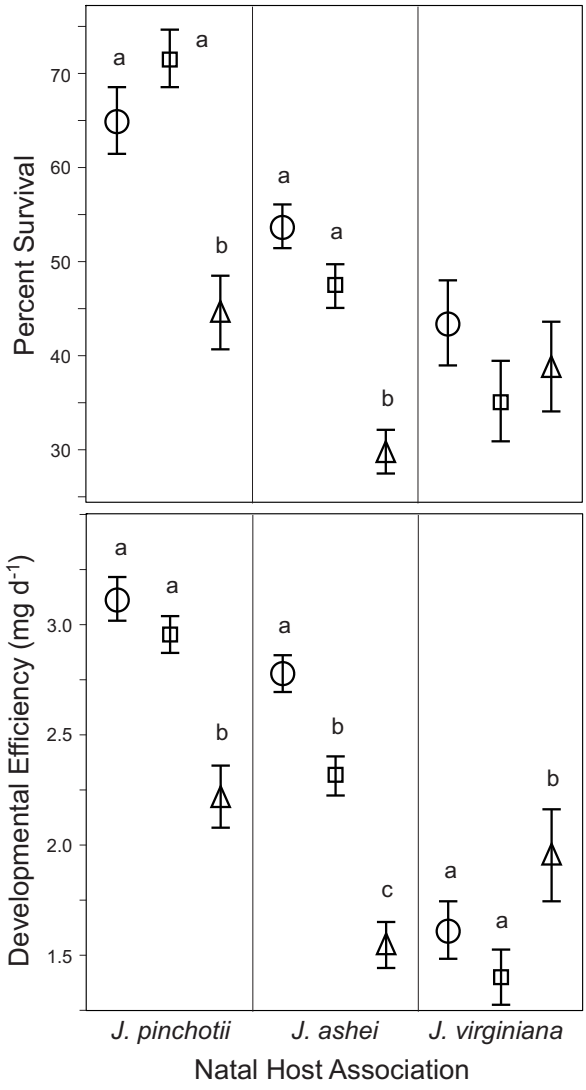

Figure 3. Larval performance results by natal host association. Lowercase letters represent results of post-hoc analysis if significant differences were found. Symbols are experimental plant treatments: circles = *Juniperus ashei*; squares = *J. pinchotii*; triangles = *J. virginiana*.

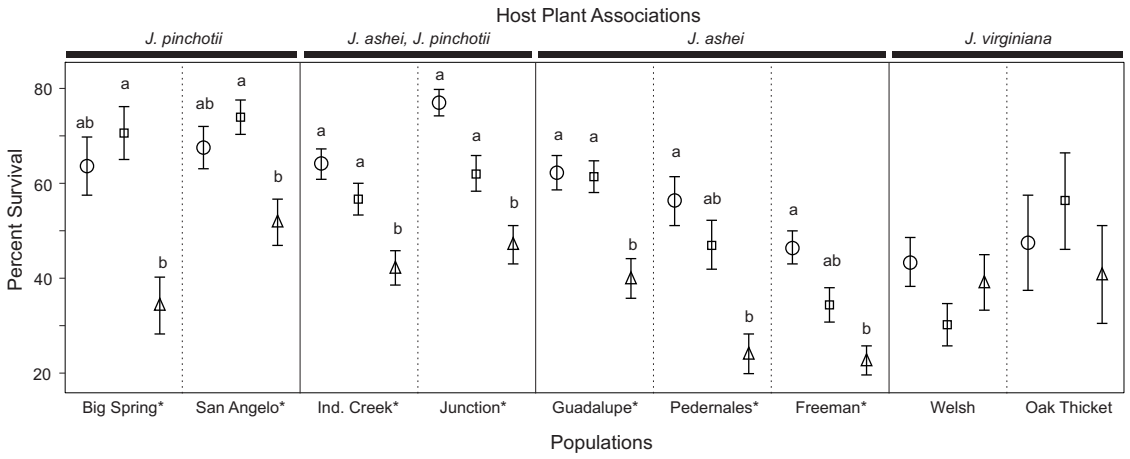

Figure 4. Larval survival for all populations. Asterisks next to population names indicate significant preference, and lowercase letters represent results of post-hoc analysis. Symbols are experimental plant treatments: circles = *Juniperus ashei*; squares = *J. pinchotii*; triangles = *J. virginiana*.

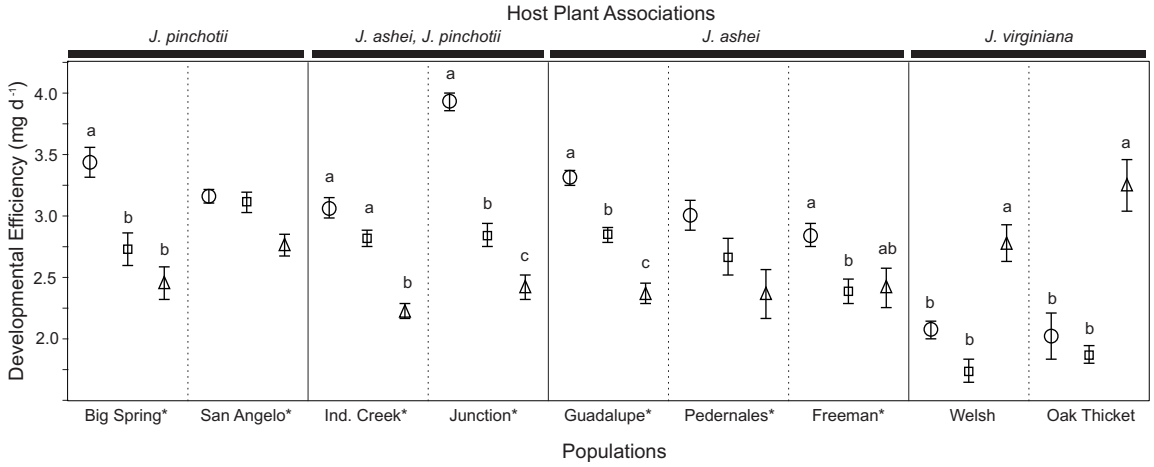

Figure 5. Developmental efficiency (DE) for all populations. Asterisks next to population names indicate significant preference, and lowercase letters represent results of post-hoc analysis. Experimental plant treatments: circles = *Juniperus ashei*; squares = *J. pinchotii*; triangles = *J. virginiana* treatments.

CHAPTER II

POPULATION GENETIC EVIDENCE FOR HOST RACE FORMATION

Introduction

Recent studies of speciation have examined more closely the influence ecological factors have on the process of population genetic divergence under varying degrees of spatial isolation and gene flow (Nosil et al. 2006, Thorpe et al. 2008, Berner et al. 2009). Ecological speciation theory proposes divergent natural selection as the mechanism that initiates and drives reproductive isolation between groups, either through acting directly on traits associated with reproduction or indirectly on genetically correlated traits (Schluter 2001, Rundle and Nosil 2005, Funk and Nosil 2008). These studies have highlighted fundamental questions of evolutionary biology: what is the relative influence of natural selection or genetic drift on the process of divergence among populations (Funk 1998, Gavrilets et al. 1998, Coyne and Orr 2004)? How strong must selection be to overcome gene flow (Felsenstein 1980, Slatkin 1987, Lenormand 2002)? The challenge of speciation studies is to characterize the balance between local adaptation and gene flow, and link proximate ecological processes to the larger phylogeographic history of the natural system of interest (Feder et al. 2005, Butlin et al. 2008, Raesaenen and Hendry 2008).

In order to untangle the complexity of speciation, then, it would be helpful to examine a system of closely related taxa for which different stages of speciation are taking place and for which different mechanisms are responsible for divergence. This would allow comparisons to be made to attempt to tease apart the relative contributions of different reproductive isolating barriers under different conditions of spatial and ecological context (Tregenza 2002, Nosil et al. 2009, Via 2009). Plant-insect systems have frequently been examined to address speciation questions, as phytophagous insects are often closely associated with their host plants, and host-associated divergence [is central to] sympatric or ecologically-based speciation processes (Funk et al. 2002, Drès and Mallet 2002).

Butterflies belonging to the *Mitoura gryneus* species complex represent an informative group to study the mechanisms of divergence because they are found only in North America, and taxonomic designations are not fully agreed upon in the literature—possibly indicating recent or ongoing speciation (Johnson 1980, Miller and Brown 1981, Scott 1992). *Mitoura* are closely associated with their respective hosts (trees in the family Cupressaceae) and exhibit behaviors such as female oviposition preference and male lekking, which may influence assortative mating based on host plant use (Scott 1992, Forister 2004). The geographic distribution of *Mitoura* hosts occurs in varying degrees, from complete allopatry, to parapatry, to sympatry; therefore, these butterflies provide the opportunity to study the potential interaction between geographic and ecological reproductive isolating barriers.

Population genetic (Nice and Shapiro 2001) and experimental (Forister 2004, 2005) work in northern California examined three nominal species of *Mitoura* that differ

in host plant association, morphology, and phenology of flight time during the year. Nice and Shapiro (2001) found population genetic evidence for host race formation associated with some of these ecological factors, while taxonomic designations were not indicative of levels of population genetic divergence. Forister (2004, 2005) tested the hypothesis of host-associated divergence among these same populations using both female preference and larval performance as evidence of specialization on hosts. Preference and performance varied, with one host association exhibiting a concordance of preference and performance for the natal host, while for other host associations the relationship was more complex.

The current study complements previous work on *Mitoura*; however, in contrast to the butterflies in the northwest, *Mitoura* in the south-central portion of North America are morphologically similar and are considered one nominal taxon (*M. gryneus*). In addition, they are associated with different species of trees within a single genus, *Juniperus*, that occur both allopatrically and sympatrically. Previous experimental work in Texas (Downey and Nice 2010) found variation in female oviposition preference and larval performance among different host-associated populations, indicating incipient (or incomplete) host race formation. This study tests the hypothesis that populations with different host associations experience restricted gene flow, and exhibit significant population genetic divergence. Furthermore we ask, are the patterns of ecological divergence (i.e., patterns of specialization in terms of preference and performance) coincident with genetic differentiation?

Methods

Biology of Mitoura gryneus *and Host Plants*

Three host associations of *Mitoura* on *Juniperus* in Texas were examined in this study. Ashe juniper (*J. ashei)* occurs primarily in the Edwards Plateau region of central Texas, and red-berried juniper (*J. pinchotii*) occurs primarily in western-northwestern Texas. Eastern red cedar (*J. virginiana*) occurs throughout eastern-northeastern regions of North America; its range in Texas is primarily east of the Balcones fault line. For the populations examined in this study, *J. ashei* and *J. virginiana* are allopatric, and *J. pinchotii* and *J. ashei* are both allopatric and sympatric at various points across their range (Downey and Nice 2010, Fig. 1). Since differentiation according to host plant association is the characteristic of interest for this study, *M. gryneus* is referred to by host plant association (*J. ashei*, *J. pinchotii*, sympatric *J. ashei*-*J. pinchotii*, or *J. virginiana*).

Previous studies with these populations examined female oviposition preference and larval performance to test the hypothesis that specialization on hosts, and host race formation, was taking place among populations of *Mitoura* in Texas (Downey and Nice 2010). Different host associations exhibited different degrees of specialization. Females of *J. virginiana*-associated populations exhibited no preference for, but larvae were observed to have increased developmental efficiency on, their natal host. *J. ashei*-associated populations (and one sympatric population) exhibited a concordance between preference and performance for their natal host, while *J. pinchotii*-associated populations (and one sympatric population) preferred and had equally high performance on *J. ashei* and *J. pinchotii* (Downey and Nice 2010).

Adult specimens of *M. gryneus* were collected from ten locations (hereafter "populations") during 2008-2009. Populations were the same as those used in earlier experimental work by Downey and Nice (2010), with the addition of one *J. ashei*-associated population (Bandera); locations and approximate range of host plants are indicated in Figure 6 and Table 6. Thoracic tissue from adult butterflies was used for genomic DNA extraction (head, abdomen, and wings stored as voucher specimens at -80° C). Regions of the mitochondrial DNA (cytochrome oxidase subunits I and II) were amplified and sequenced for a subset of 8-14 individuals per sampling population using the primer pairs RON and NANCY (COI) and PATRICK and EVA (COII) (Catarino and Sperling 1999). PCR products were purified using Promega Wizard SV Genomic DNA Purification System, followed by cycle sequencing using either a CEQ8800 Genetic Analysis System and protocols (Beckman Coulter) or the BigDye Terminator v3.1 Reaction Kit and ABI3730 DNA Analyzer (for the latter, service provided by Nevada Genomics Center, www.ag.unr.edu/genomics). Sequence data were edited by eye and aligned using Geneious v5.0 (Drummond et al. 2010).

To obtain genome-wide nuclear markers, AFLP data were also collected for 18-27 individuals per sampling population (184 individuals total) following a modification of the protocol described by Vos et al. (1995, see also Gompert et al. 2006). Two selective primer pairs were used, a FAM-labeled EcoRI-ACA primer paired with MseI-CAGA and MseI-CAGC, respectively. Reaction products were mixed with a 500 MW LIZ size standard and analyzed on an ABI Prism 3730 DNA Analyzer (service provided by Nevada Genomics Center). Eight samples were run twice (on each 96-well plate) to check that between-plate variability was minimized during selective reactions. Raw

electropherograms were analyzed using ABI PeakScanner v.1.0 to detect presence/ absence and size of bands for each individual (with "light peak smoothing"; all other settings were default). A binary matrix of presence (1)/absence (0) for AFLP bands was constructed using the automated scoring RawGeno package in R (R CRAN; Arrigo et al. 2009) with the following parameters: scoring range, 100 – 500 bp; maximum bin width, 2 bp; low intensity threshold, 50 rfu's; with 5% low frequency bins eliminated. Each selective primer pair data set was scored separately, with binary matrices of data combined for subsequent analyses.

mtDNA Data Analysis

An analysis of molecular variance (AMOVA) was used to analyze population structure according to host plant association by examining mtDNA sequence data within the software ARLEQUIN v.3.5 (Excoffier et al. 2005). Three levels or hierarchies of genetic variance were examined: 1) within-population variance; 2) variance among populations, within groupings by host associations ("within-hosts"); and 3) variance among groupings of host associations ("among-hosts"). Significance of ϕ-statistics was assessed with 10,000 permutations of each level of the hierarchy. If populations using alternative hosts are differentiated (i.e., "among-hosts" variation is significant), then host plant fidelity is implicated in maintaining reproductive isolation among populations. The results from previous experimental work were used to determine how to include the populations in which *J. ashei* and *J. pinchotii* hosts are sympatric (Junction and Independence Creek), since the AMOVA examined three single host-association groupings. Two analyses were conducted: one AMOVA included Junction with the *J. ashei*-associated populations, and Independence Creek with the *J. pinchotii*-associated

populations; a subsequent AMOVA was conducted excluding both of these populations from the analysis.

While an AMOVA describes variance partitioning for genetic differentiation in terms of nested hierarchies, additional analyses were employed to examine other spatial relationships (if any) within the data. A spatial analysis of molecular variance (SAMOVA) in the molecular data (Dupanloup 2002) was used to assess and define the structure of populations in both spatial and genetic terms; the number of host population groups hypothesized ($K = 3$) was tested along with other possible numbers of groupings (from 1 through 9). The SAMOVA assigns populations to groups using a simulated annealing procedure that detects areas of local maxima of genetic variance; groups are geographically adjacent and genetically homogeneous (Dupanloup 2002). If host-associated differentiation is occurring, then the value of *K* that maximizes among-group differentiation should correspond to the AMOVA ϕ_{ST} designation of host-associated population groupings.

An alternative pattern of geographical genetic variation that may be detected is isolation by distance (IBD), which is in contrast to the hypothesis of restricted gene flow between different host-associated populations. A Mantel test (Sokal and Rohlf 1995) was used to test for IBD by comparing a genetic distance matrix of pairwise ϕ_{ST}'s calculated from mtDNA data with a geographic distance matrix. A lack of a strong signal of IBD indicates that other factors could be influencing the distribution of genetic variation (i.e., host plant association).

AFLP Data Analysis

AFLP data were analyzed using STRUCTURE (Pritchard et al. 2000), a Bayesian clustering method that assigns individuals to groups (or clusters, K) that does not require *a priori* information. Ten replicates for each value of K (from K = 1 through K = 10) were conducted, with the mean lnP(data|K) and variance calculated (for each replicate, a burnin of 50,000 with 500,000 MCMC was used). An initial model was run with admixture and correlated allele frequencies, allowing for recessive alleles (Falush et al. 2003). An additional model incorporating population sampling location information was used. Although not explicitly spatial, the LOCPRIOR model in STRUCTURE optionally incorporates sampling information if there is a correlation between cluster assignment and population information (Hubisz et al. 2009). The number of K selected as best describing the data was chosen by examining the lnP (data|K) (Pritchard et al. 2000) as well as the calculation of delta K as described by Evanno et al. (2005); the value of K with the highest log likelihood and delta K statistic was selected.

Results

mtDNA Haplotypes and Analyses

A total of 396 bp of COI and 436 bp of COII sequence data were obtained; these data were combined and analyzed as a single unit after a partition homogeneity test found no significant difference in phylogenetic signal between the two markers (P = 0.23) (PAUP v.4.0beta10, Swofford 2003). Fourteen unique haplotypes were identified for the combined COI-COII data and a statistical parsimony haplotype network was constructed using the program TCS v.1.21 (Clement 2000) (Figure 6).

In an AMOVA, 56.6 % of the total variation was explained when all populations were grouped according to host plant association (df = 2, P = 0.002; Table 7). This proportion was relatively unchanged even after the host-sympatric populations were excluded (explaining 62.4% of the total variation, df = 2, P = 0.005; Table 7). An examination of the haplotype network reveals substantial divergence (8 nucleotide substitutions) between haplotypes from *J. virginiana* populations and haplotypes from all other populations, and a greater number of private alleles in the western populations. Therefore an additional AMOVA was conducted excluding the *J. virginiana* populations, to determine if grouping by host association might still account for some amount of the observed variation. When considering only the *J. ashei* and *J. pinchotii*-associated populations, 18.6 % of the variation was explained by host association (df = 1, P = 0.02; Table 7). Isolation by distance was not detected, either when all populations were included (grouping Junction with *J. ashei* and Independence Creek with *J. pinchotii* populations), $r^2 = 0.06$, P = 0.23; or when excluding *J. virginiana*-associated populations from the analysis, $r^2 = 0.15$, P = 0.22, suggesting that host plant association influences population genetic structure.

When including spatial data in the analysis using an SAMOVA, the number of groups that explained the highest proportion of among-group variation was K = 2, in which *J. virginiana* populations grouped together and were separate from all other populations (Figures 6 and 7). To examine whether there was any substructure in the data for *J. ashei* and *J. pinchotii* populations, *J. virginiana* populations were excluded from a subsequent SAMOVA. The amount of variation explained by different values of K was similar, with K = 4 accounting for the highest percent of variation (Figure 7). *Juniperus*

ashei-associated populations were separate from *J. pinchotii*-associated populations, although the Guadalupe population was considered a separate group. With the sympatric *J. ashei*-*J. pinchotii*-associated populations, Junction grouped with *J. ashei* populations and Independence Creek was considered a separate group (Figure 6).

AFLP Analyses

The EcoRI-Mse-CAGA and Mse-CAGC selective primer pairs yielded 223 and 234 variable loci, respectively, for a total of 457. Interpretation of results for both the log likelihood values, and for calculating delta K, identified the number of clusters at K = 2 (Figures 8 and 9). Additionally, this was the result for both STRUCTURE models—those that included population sampling information (LOCPRIOR) and did not include this information.

Discussion

Patterns of Population Genetic Divergence

For phytophagous insects that both mate and oviposit on their host plant, positive assortative mating and selection for increased fitness on natal hosts can generate reproductive isolation between different host-associated groups, facilitating host race formation (Funk 1998, Via 1999, Drès and Mallet 2002). Host-associated differentiation should also be considered within the larger framework of historical biogeography, incorporating both the spatial and temporal context for population dynamics over time. This study examined patterns of genetic diversity at the scale of a group of potentially interbreeding populations of one nominal taxon, *M. gryneus*, testing for evidence of host-associated differentiation in the form of restricted gene flow among populations corresponding with the three different host plant associations.

Patterns of genetic divergence were found to be consistent with the hypothesis of host-associated differentiation. The results of the AMOVA indicate that gene flow is restricted among populations based on host plant association, and no pattern of IBD was detected. The AFLP analysis using STRUCTURE also supports restricted gene flow between *J. virginiana*-associated populations and all others. Introgression from *J. ashei* populations to *J. virginiana* populations may have occurred at some point in the past, given the presence of one shared haplotype (A3); however, this may also be an indication of ancestral polymorphism. In comparison, patterns of divergence between *J. ashei*- and *J. pinchotii*-associated populations appear more recent in evolutionary time, with shared haplotypes and lack of structure in the AFLP analysis suggesting recent or ongoing gene flow. However, *J. pinchotii* and sympatric *J. ashei*-*J. pinchotii*-associated populations (especially San Angelo and Independence Creek) do have more haplotype diversity in terms of both the number of haplotypes and the presence of private alleles than *J. virginiana* or *J. ashei*-associated populations, indicating some degree of differentiation from other host-associated populations.

In order to examine whether any structure between *J. ashei*- and *J. pinchotii*-associated populations was obscured by the degree of divergence between *J. virginiana* and these other host associations, we removed *J. virginiana* –associated populations from analyses. When excluding *J. virginiana*-associated populations in an AMOVA, grouping populations by host association explained 18.6 % of the variation and IBD was again not observed, suggesting that host plant association is still important in structuring genetic variation between *J. ashei* and *J. pinchotii*-associated populations. Analysis of AFLP data did not reflect the finer population structure evident in the mtDNA data in that there was

little support for clusters at K greater than two in the AFLP data. It is not unusual to find discrepancies between mitochondrial and nuclear markers (Chan and Levin 2005, Gompert et al. 2006). This may be due to a lack of resolution in the AFLP data in cases where divergence is recent and there is limited differentiation among populations (Waples and Gaggiotti 2006). This situation might also be exacerbated when the sexes exhibit differential dispersal. Female philopatry with greater male dispersal can produce differentiation in maternally-inherited markers that would be obscured in data from biparentally-inherited markers (Ohshima and Yoshizawa 2010).

Comparing these patterns of population genetic differentiation with the experimental results of oviposition preference and larval performance (Downey and Nice 2010) can provide a more complete picture of the evolutionary history of these butterflies and identify possible mechanisms of divergence. While host-associated differentiation is a plausible hypothesis to explain some of the patterns observed within this system, other factors could have influenced divergence in *Mitoura* and their host plants. From a historical biogeographical perspective, different spatial arrangements of hosts affect the types of reproductive isolating barriers that occur between populations. Molecular data can be used to make inferences about the relative ages of different populations (based on the accumulation of neutral mutations) or degree of isolation over time (given the presence of private alleles).

For *J. virginiana*-associated populations, females did not exhibit significant preference for the natal host, although larvae had highest fitness when reared on *J. virginiana* (Downey and Nice 2010). Given eight nucleotide substitution differences between the *J. virginiana* mtDNA haplotypes and all others, it is likely that these

populations experienced a relatively longer time in allopatry associated only with their *J. virginiana* host plant. In terms of larval performance, the strongest division occurs between *J. virginiana* vs. *J. pinchotii*- and *J. ashei*-associated populations, and this is congruent with observed genetic differentiation. Local adaptation as measured by increased larval fitness on the natal host has evolved in allopatry. However, without alternative hosts, *J. virginiana*-associated females might not have experienced the selective pressure via decreased larval fitness when ovipositing on the "wrong" host necessary for preference evolution. The distinction between the *J. ashei* and *J. pinchotii* populations in terms of preference and performance varies, with *J. ashei*-associated butterflies exhibiting specialization to the natal host for both preference and performance, while *J. pinchotii*-associated populations did not distinguish between *J. ashei* and *J. pinchotii* in either preference or larval performance (although they clearly preferred these hosts over *J. virginiana*). This overlap in preference and performance behaviors is paralleled in the AFLP data, which failed to distinguish populations using *J. pinchotii* from populations using *J. ashei*.

Other factors might explain the maintenance of a strong signal of specialization for *J. ashei* populations when compared to the other host associated populations. Because they contain only a limited subset of haplotypes found also in western populations, and no private alleles, *J. ashei*-associated populations may represent a more recent colonization from west to east. This would mean that adaptation to *J. ashei* is the derived condition, and *J. pinchotii* is the "ancestral" host (at least when considered within the range of this study). For *J. ashei* populations, the partially sympatric condition with *J. pinchotii* and proximity to *J. virginiana* hosts may translate into an increased likelihood

for disruptive selection or post-zygotic isolating barriers to have a role in driving specialization to *J. ashei*.

However, for sympatric *J. ashei*-*J. pinchotii*-associated populations, in which some degree of adaptation and preference for both hosts exists, gene flow from specialized *J. ashei*-populations to the west would represent secondary contact. If the sympatric condition of host plants facilitates gene flow among populations of *Mitoura,* this could inhibit specialization and host race formation. Butterflies from the sympatric *J. ashei*-*J. pinchotii*-associated population Independence Creek did not distinguish between these two hosts in preference trials, and larvae did not exhibit a clear fitness difference when reared on either host. As mentioned, AFLP analyses indicate that gene flow may be taking place between *J. pinchotii* populations and the Independence Creek population, which would break down any co-adapted gene complexes that increase fitness on one host over another. Another factor that might inhibit divergence between these different host-associated populations is a failure of *Mitoura* to discriminate between *J. ashei* and *J. pinchotii* as suitable hosts.

The underlying genetic architecture for the traits of preference and performance may also influence how these traits respond to selection, and what might happen under different conditions of host plant proximity. In a series of ecological genetics experiments, Forister (2005) conducted crosses between two nominal species that use alternate hosts, *Mitoura nelsoni* and *Mitoura muiri*, and found evidence of independent inheritance of adult preference and larval performance. Preference for incense cedar, the host of *M. nelsoni*, was dominant to preference for the alternate host (a cypress). Parental *M. nelsoni* had higher survivorship on their natal host compared with hybrids reared on

incense cedar, suggesting that performance on the alternate host (cypress) is dominant to performance on cedar. These findings have implications for the results of *Mitoura* studies in Texas populations. If genes underlying preference and performance are unlinked and can evolve independently, then local adaptation in allopatry (via enhanced larval performance) can take place without the evolution of preference, as observed with butterflies from *J. virginiana* populations. Evidence for dominance in preference for one host over another may also help to explain the lack of two distinct host races between *J. ashei* - and *J. pinchotii*-associated populations. If secondary contact is taking place between *J. pinchotii*- and *J. ashei*-adapted butterflies in areas of host sympatry, and if preference for *J. ashei* is dominant (or codominant) to preference for *J. pinchotii*, then this will influence the genetic makeup and behavior of these populations. Interestingly, patterns of preference for butterflies from Independence Creek were "heterogeneous" in the sense that there appeared to be distinct groups of females, some preferring *J. ashei* and others preferring *J. pinchotii* (Downey and Nice 2010). Conducting ecological genetics experiments with the populations examined in this study, such as crossing individuals from different host-adapted backgrounds and examining preference and performance of hybrid progeny, could help distinguish between hypotheses of secondary contact, or dominance of *J. ashei* preference, in influencing behavior and genetic differentiation among western populations.

Population genetic divergence can result from both local adaptation taking place in allopatry as well as from contemporary ecological interactions (Nosil et al. 2009). These can both work in concert over time to influence reproductive isolation among populations and potentially lead to speciation. While speciation occurs along a

continuum, at early stages there are many factors that can work to inhibit, as well as drive, divergence. Areas of host sympatry present an opportunity for gene flow to take place among populations for an insect that otherwise has a tendency to specialize on its natal host. However, independent inheritance of preference and performance traits means that preference might not always evolve in concert with local adaptation to a host. Exploring these issues might also help to address the apparent "paradox" between the capacity of phytophagous to specialize on the one hand, but on the other, have the potential to undergo a host switch. The *Mitoura* species complex offers many opportunities to compare different mechanisms of speciation—drift in allopatry, vs. selection based on ecological interactions—with divergence taking place at different stages in different spatial scales.

Table 6. Collection information for *M. gryneus* specimens. Number of individuals in parentheses following mtDNA haplotype information.

County	Population Locality	Coordinates	Host Plant Association	Haplotype
Howard	Big Spring	32° 14'59.74" N 101° 29'02.75" W	*Juniperus pinchotii*	A1 (6), B2 (2), E1 (1)
Tom Green	San Angelo	31° 29'02.75" N 100° 29'48.92" W	*Juniperus pinchotii*	A1 (8), A6 (1), A8 (1), B2 (1), B7 (1), E5 (1), F2 (1)
Terrell	Independence Creek	30° 29'24.57" N 101° 47'54.33" W	*Juniperus ashei*, *Juniperus pinchotii*	A1 (3), A2 (1), A3 (1), B2 (4), D5 (1)
Kimble	Junction	30° 29'53.19" N 99° 44'03.20" W	*Juniperus ashei*, *Juniperus pinchotii*	A1 (6), A3 (1), D1 (1)
Bexar	Bandera Rd	29° 35'48.26" N 98° 38'4.17" W	*Juniperus ashei*	A1 (8)
Kendall	Guadalupe	29° 53'9.87" N 98° 32'4.49" W	*Juniperus ashei*	A1 (3), A3 (4)
Blanco	Pedernales	30° 16'39.18" N 98° 15'23.73" W	*Juniperus ashei*	A1 (7), A3 (1)
Hays	Freeman	29° 55'23.48" N 98° 1'13.27" W	*Juniperus ashei*	A1 (11), A3 (1)
Bastrop	Welsh	30° 13'58.19" N 97° 15'41.53" W	*Juniperus virginiana*	A3 (4), C4 (5), G4 (1)
Fayette	Oak Thicket	29° 56'55.08" N 96° 43'49.44" W	*Juniperus virginiana*	A3 (8), C4 (4)

Table 7. AMOVA results. Significant results are in bold.

Source of Variation	df	Sum of Squares	Percentage of Variation	P value
All populations				
Among groups	2	81.54	**56.59**	0.002
Among populations within groups	9	7.79	1.32	0.43
Within populations	89	67.72	**42.09**	< 0.001
Excluding sympatric *J. ashei*-*J. pinchotii* populations				
Among groups	2	60.28	**60.02**	0.038
Among populations within groups	5	2.12	1.99	0.45
Within populations	71	57.79	**40.38**	< 0.001
Excluding *J. virginiana* populations				
Among groups	1	4.17	**18.63**	0.018
Among populations within groups	6	3.20	2.75	0.116
Within populations	69	27.72	**78.62**	0.001

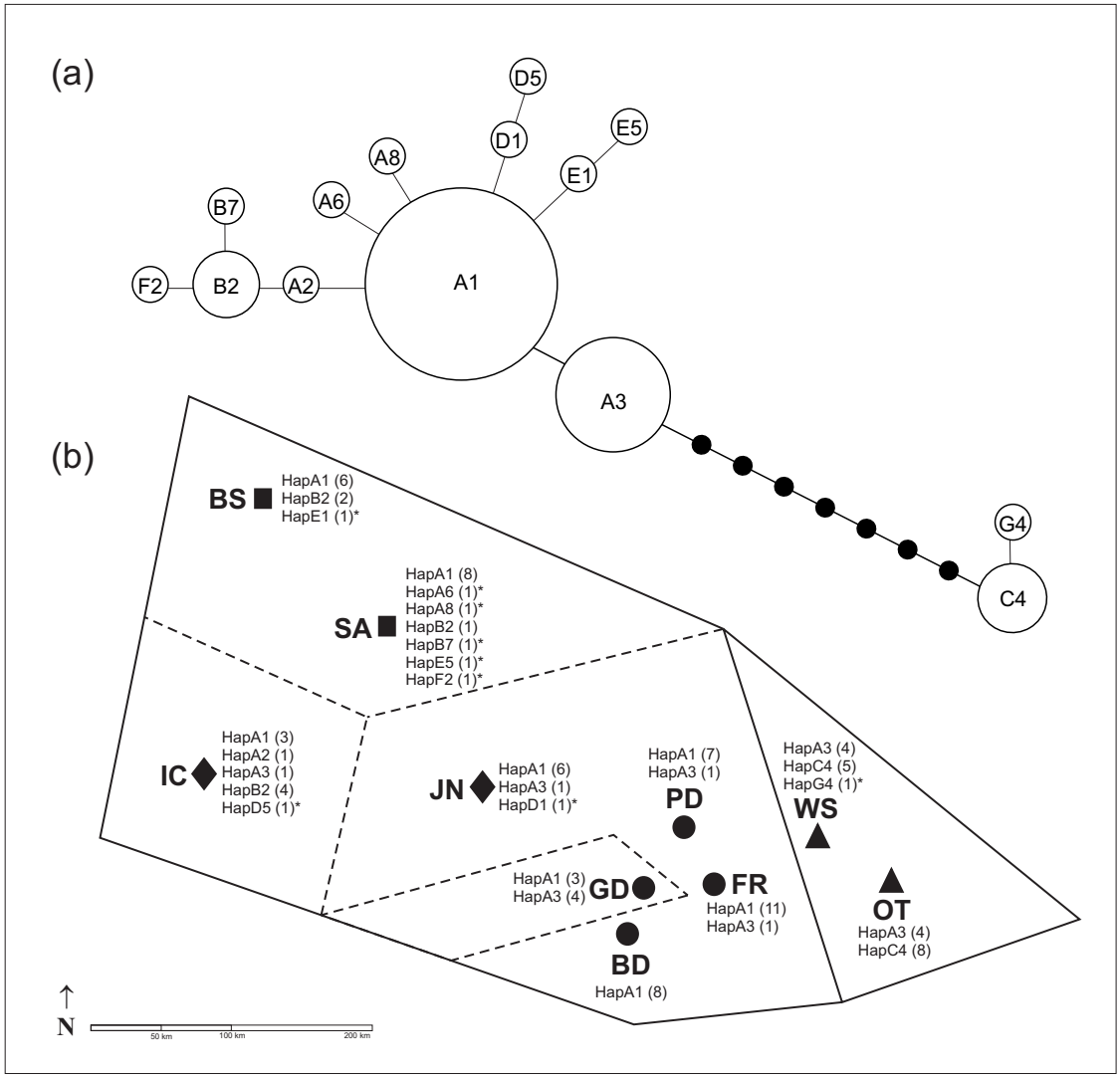

Figure 6. Results of mtDNA analyses. (a) Haplotype network of combined COI-COII sequence. Connections are 1 nucleotide substitution difference. Filled circles are unsampled intermediate haplotypes. (b) Map of sampling locations indicating relative position of populations. Number of individuals with each haplotype in parentheses. Private alleles are indicated by asterisks. Square symbols = *J. pinchotii* host association; diamonds = *J. pinchotii* and *J. ashei*; circles = *J. ashei*; and triangles = *J. virginiana*. Population labels are the same as in Table 6. Solid-line polygons around populations are SAMOVA results when K = 2; dashed line indicates SAMOVA results (excluding *J. virginiana* populations) when K = 4.

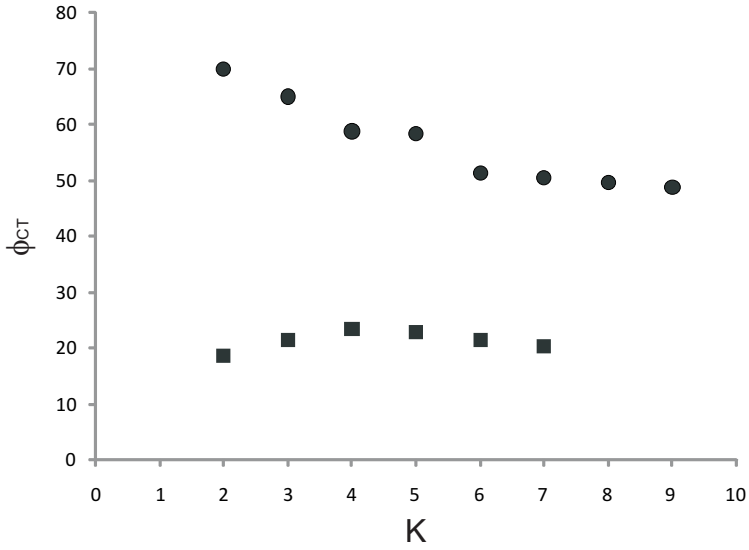

Figure 7. SAMOVA results. Percentage of among-hosts variation (ϕ_{CT}) on the *x*-axis for each increasing value of number of clusters, K, on the *y*-axis. Circles are results including all populations; squares are results of SAMOVA excluding *J. virginiana*-associated populations.

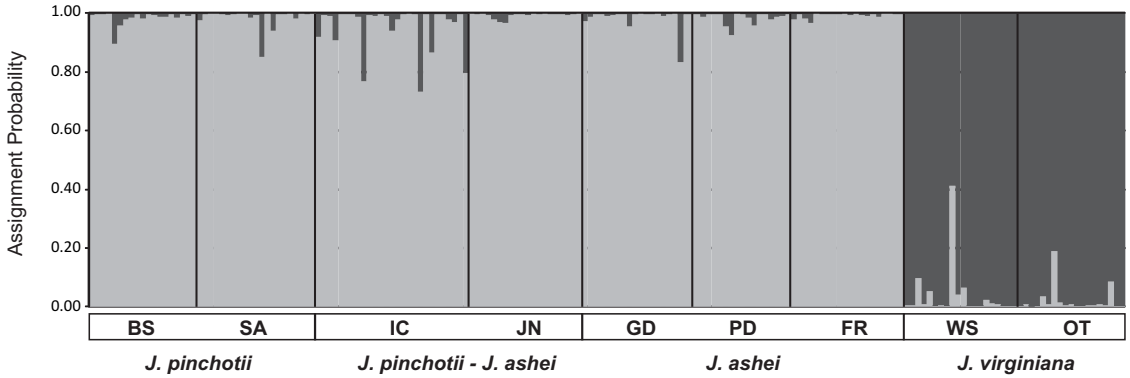

Figure 8. STRUCTURE bar plot when K = 2. Each bar represents an individual, with probability of assignment to each cluster in light grey (Cluster 1) and dark grey (Cluster 2). Population labels correspond with Table 6 and Figure 6.

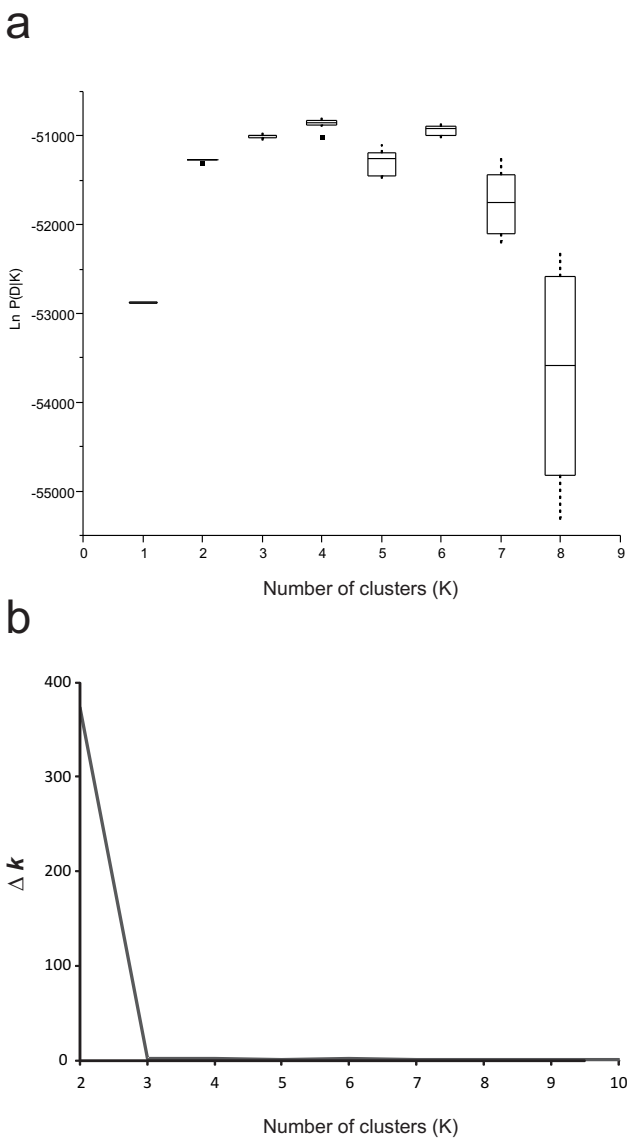

Figure 9. Evaluation of number of clusters (K) from STRUCUTRE results. (a) Plot of log likelihood vs. number of K. (b) Plot of statistic delta K vs. number of K.

LITERATURE CITED

Agosta, S. J. 2006. On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. - Oikos 114: 556-565.

Arrigo, N., Tuszynski, J. W., Ehrich, D., Gerdes, T. and Alvarez, N. 2009. Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. - BMC Bioinformatics 10: 33.

Benrey, B. and Denno, R. F. 1997. The slow-growth-high-mortality hypothesis: A test using the cabbage butterfly. - Ecology 78: 987-999.

Bernays, E. and Graham, M. 1988. On the Evolution of Host Specificity in Phytophagous Arthropods. - Ecology 69: 886-892.

Berner, D., Grandchamp, A., and Hendry, A. P. 2009. Variable Progress Toward Ecological Speciation in Parapatry: Stickleback Across Eight Lake-Stream Transitions. - Evolution 63: 1740-1753.

Bolnick, D. I. and Fitzpatrick, B. M. 2007. Sympatric speciation: Models and empirical evidence. - Annual Review of Ecology Evolution and Systematics 38: 459-487.

Bush, G. L. 1975. Modes of Animal Speciation. - Annu Rev Ecol Syst 6: 339-364.

Bush, G. L. 1994. Sympatric Speciation in Animals - New Wine in Old Bottles. - Trends in Ecology and Evolution 9: 285-288.

Butlin, R. K., Galindo, J. and Grahame, J. W. 2008. Sympatric, parapatric or allopatric: the most important way to classify speciation? - Philosophical Transactions of the Royal Society B-Biological Sciences 363: 2997-3007.

Caterino, M. S. and Sperling, F. A. H. 1999. Papilio phylogeny based on mitochondrial cytochrome oxidase I and II genes. - Mol Phylogenet Evol 11: 122-137.

Chan, K. M. A. and Levin, S. A. 2005. Leaky prezygotic isolation and porous genomes: Rapid introgression of maternally inherited DNA. - Evolution 59: 720-729

Clancy, K. M. and Price, P. W. 1987. Rapid Herbivore Growth Enhances Enemy Attack - Sublethal Plant Defenses Remain a Paradox. - Ecology 68: 733-737.

Clement, M., Posada, D., Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. - Mol Ecol 9: 1657-1659.

Conover, W. J. 1999. Practical nonparametric statistics. - Wiley.

Courtney, S. P. and Kibota, T. T. 1990. Mother doesn't know best: host selection by ovipositing insects. - In: Bernays, E. A. (ed.), Insect-plant interactions. CRC Press, pp. 161-188.

Cox, D. R. and Snell, E. J. 1989. Analysis of binary data. - Chapman and Hall.

Coyne, J. and Orr, H. 2004. Speciation. - Sinauer Associates.

Diehl, S. R. and Bush, G. L. 1984. An Evolutionary and Applied Perspective of Insect Biotypes. - Annu Rev Entomol 29: 471-504.

Drès, M. and Mallet, J. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. - Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 357: 471-492.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., and Wilson, A. 2010. Geneious v5.1, available from http://www.geneious.com

Dupanloup, I., Schneider, S. and Excoffier, L. 2002. A simulated annealing approach to define the genetic structure of populations. - Mol Ecol 11: 2571-2581.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. - Mol Ecol 14: 2611-2620.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. - Evolutionary Bioinformatics 1: 47-50.

Farrell, B. D. 1998. "Inordinate fondness" explained: Why are there so many beetles? - Science 281: 555-559.

Feder, J. L., Opp, S. B., Wlazlo, B., Reynolds, K., Go, W., Spisak, S. 1994. Host Fidelity is an Effective Premating Barrier between Sympatric Races of the Apple Maggot Fly. - Proc Natl Acad Sci U S A 91: 7990-7994.

Feder, J. L. 1998. The apple maggot fly Rhagoletis pomonella: flies in the face of conventional wisdom about speciation? - In: Howard, D. J. and Berlocher, S. H. (eds.), Endless forms: species and speciation. University Oxford Press, pp. 130-144.

Feder, J. L.. Berlocher, S. H., Roethele, J. B., Dambroski, H., Smith, J., Perry, W., Gavrilovic, V., Filchak, K., Rull, J., and Aluja, M. 2003. Allopatric genetic origins for sympatric host-plant shifts and race formation in Rhagoletis. - Proc Natl Acad Sci U S A 100: 10314-10319.

Feder, J. L., Xie, X. F., Rull, J., Velez, S., Forbes, A., Leung, B., Dambroski, H., Filchak, K., and Aluja, M. 2005. Mayr, Dobzhansky, and Bush and the complexities of sympatric speciation in Rhagoletis. - Proc Natl Acad Sci U S A 102: 6573-6580.

Feeny, P. 1976. Plant apparency and chemical defense. - In: Nansel, R. L. (ed.), Advances in phytochemistry. Plenum Press, pp. 1-40.

Felsenstein, J. 1981. Skepticism Towards Santa Rosalia, Or Why are there so Few Kinds of Animals. - Evolution 35: 124-138.

Fitzpatrick, B. M., Fordyce, J., and Gavrilets, S. 2008. What, if anything, is sympatric speciation? - J Evol Biol 21: 1452-1459.

Fitzpatrick, B. M., Fordyce, J., and Gavrilets, S. 2009. Pattern, process and geographic modes of speciation. - J Evol Biol 22: 2342-2347.

Fordyce, J. A. and Shapiro, A. M. 2003. Another perspective on the slow-growth/high-mortality hypothesis: Chilling effects on swallowtail larvae. - Ecology 84: 263-268.

Forister, M. L. 2004. Oviposition preference and larval performance within a diverging lineage of lycaenid butterflies. - Ecol Entomol 29: 264-272.

Forister, M. L. 2005. Independent inheritance of preference and performance in hybrids between host races of Mitoura butterflies (Lepidoptera : Lycaenidae). - Evolution 59: 1149-1155.

Forister, M. L. 2008. Experimental design and the outcome of preference-performance assays, with examples from *Mitoura* butterflies (Lycaenidae). - J Lepid Soc 62: 99-105.

Forister, M. L., Nice, C., Fordyce, J., and Gompert, Z. 2009. Host range evolution is not driven by the optimization of larval performance: the case of *Lycaeides melissa* (Lepidoptera: Lycaenidae) and the colonization of alfalfa. - Oecologia 160: 551-561.

Funk, D. J. 1998. Isolating a role for natural selection in speciation: Host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. - Evolution 52: 1744-1759.

Funk, D. J., Filchak, K. E., and Feder, J. L. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. - Genetica 116: 251-267.

Funk, D. J. and Nosil, P. 2008. Comparative analyses of ecological speciation. - In: Tilmon, K. J. (ed.), Specialization, speciation and radiation: the evolutionary biology of herbivorous insects. The University of California Press, pp. 117-135.

Futuyma, D. J. and Moreno, G. 1988. The Evolution of Ecological Specialization. - Annu Rev Ecol Syst 19: 207-233.

Gavrilets, S., Li, H. and Vose, M. D. 1998. Rapid parapatric speciation on holey adaptive landscapes. - Proceedings of the Royal Society of London Series B-Biological Sciences 265: 1483-1489.

Gompert, Z., Nice, C. C., Fordyce, J. A., Forister, M. L. and Shapiro, A. M. 2006. Identifying units for conservation using molecular systematics: the cautionary tale of the Karner blue butterfly. - Mol Ecol 15: 1759-1768.

Gripenberg, S., Mayhew, P., Parnell, M., and Roslin, T. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. - Ecol Lett 13: 383-393.

Honek, A. 1993. Intraspecific Variation in Body Size and Fecundity in Insects - a General Relationship. - Oikos 66: 483-492.

Howard, M. D. and Berlocher, S. 1998. Endless forms: species and speciation. - Oxford University Press.

Hubisz, M. J., Falush, D., Stephens, M. and Pritchard, J. K. 2009. Inferring weak population structure with the assistance of sample group information. - Molecular Ecology Resources 9: 1322-1332.

Hunter, M. D. and McNeil, J. N. 1997. Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. - Ecology 78: 977-986.

Jaenike, J. 1990. Host Specialization in Phytophagous Insects. - Annu Rev Ecol Syst 21: 243-273.

Jansen, D. H. 1985. On Ecological Fitting. - Oikos 45: 308-310.

Janz, N. and Nylin, S. 1998. Butterflies and plants: A phylogenetic study. - Evolution 52: 486-502.

Janz, N., Nylin, S., and Wahlberg, N. 2006. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. - Bmc Evolutionary Biology 6: 4.

Janz, N. and Nylin, S. 2008. The oscillation hypothesis of host-plant range and speciation. - In: Tilmon, K. J. (ed.), Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. The University of California Press, pp. 203-215.

Jiggins, C. D., Emelianov, I., and Mallet, J. 2006. Assortative mating and speciation as pleiotropic effects of ecological adaptation: examples in moths and butterflies. - In: Fellowes, M.D.E., Holloway, G.J., and Rolff J. (eds.), Insect evolutionary ecology: proceedings of the Royal Entomological Society's 22nd Symposium. CABI Publishing.

Johnson, K. D. 1981. Revision of the Callophryina of the world with phylogenetic and biogeographic analyses (Lepidoptera: Lycaenidae)

Keeler, M. S. and Chew, F. S. 2008. Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. - Oecologia 156: 559-568.

Levins, R. and MacArthur, R. 1969. An hypothesis to explain the incidence of monophagy. - Ecology 50: 910-911.

Mayr, E. 1942. Systematics and the origin of species. - Columbia University Press.

Michel, A. P., Rull, J., Aluja, M., and Feder, J. 2007. The genetic structure of hawthorn-infesting *Rhagoletis pomonella* populations in Mexico: implications for sympatric host race formation. - Mol Ecol 16: 2867-2878.

Miller, L. D. and Brown, F. M. 1981. Memoir No. 2, The Lepidopterist's Society.

Moreau, C. S., Benrey, B., and Thiery, D. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. - Science 312: 101-104.

Neve, G. and Singer, M. C. 2008. Protandry and postandry in two related butterflies: conflicting evidence about sex-specific trade-offs between adult size and emergence time. - Evol Ecol 22: 701-709.

Nice, C. C. and Shapiro, A. M. 2001. Population genetic evidence of restricted gene flow between host races in the butterfly genus Mitoura (Lepidoptera : Lycaenidae). - Ann Entomol Soc Am 94: 257-267.

Nosil, P., Crespi, B., and Sandoval, C. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. - Nature 417: 440-443.

Nosil, P., Sandoval, C. P. and Crespi, B. J. 2006. The evolution of host preference in allopatric vs. parapatric populations of Timema cristinae walking-sticks. - J Evol Biol 19: 929-942.

Nosil, P. 2008. Ernst Mayr and the integration of geographic and ecological factors in speciation. - Biol J Linn Soc 95: 26-46.

Nosil, P., Harmon, L. J., and Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. - Trends in Ecology and Evolution 24: 145-156.

Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. - Annu Rev Entomol 43: 63-83.

Ohshima, I. and Yoshizawa, K. 2010. Differential introgression causes genealogical discordance in host races of Acrocercops transecta (Insecta: Lepidoptera). - Mol Ecol 19: 2106-2119.

Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. - Genetics 155: 945-959.

Raesaenen, K. and Hendry, A. P. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. - Ecol Lett 11: 624-636.

Rundle, H. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. - Evolution 56: 322-329.

Rundle, H. D. and Nosil, P. 2005. Ecological speciation. - Ecol Lett 8: 336-352.

SAS Institute. 2008. JMP v8.0.

Schluter, D. 2000. The ecology of adaptive radiation. - Oxford University Press.

Schluter, D. 2001. Ecology and the origin of species. - Trends in Ecology and Evolution 16: 372-380.

Scott, J. 1992. The butterflies of North America: a natural history and field guide. - Stanford University Press.

Singer, M. C., Vasco, D., Parmesan, C., Thomas, C., and Ng, D. 1992. Distinguishing between Preference and Motivation in Food Choice - an Example from Insect Oviposition. - Anim Behav 44: 463-471.

Slatkin, M. 1987. Gene Flow and the Geographic Structure of Natural-Populations. - Science 236: 787-792.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry: the principles and practice of statistics in biological research. - W.H. Freeman and Company.

Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thompson, J. N. 1988a. Evolutionary Ecology of the Relationship between Oviposition Preference and Performance of Offspring in Phytophagous Insects. - Entomol Exp Appl 47: 3-14.

Thompson, J. N. 1988b. Evolutionary Genetics of Oviposition Preference in Swallowtail Butterflies. - Evolution 42: 1223-1234.

Thompson, J. N. and Pellmyr, O. 1991. Evolution of Oviposition Behavior and Host Preference in Lepidoptera. - Annu Rev Entomol 36: 65-89.

Thorpe, R. S., Surget-Groba, Y. and Johansson, H. 2008. The relative importance of ecology and geographic isolation for speciation in anoles. - Philosophical Transactions of the Royal Society B-Biological Sciences 363: 3071-3081.

Tregenza, T. 2002. Divergence and reproductive isolation in the early stages of speciation. - Genetica 116: 291-300.

Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. - Evolution 53: 1446-1457.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. - Trends in Ecology and Evolution 16: 381-390.

Via, S. 2009. Natural selection in action during speciation. - Proc Natl Acad Sci U S A 106: 9939-9946.

Waples, R. S. and Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. - Mol Ecol 15: 1419-1439.

VITA

Michelle Hanako Downey graduated from Southwestern University in Georgetown, Texas in 1998 with a Bachelor of Arts (with a double major in Biology and Studio Art). While enrolled in the Population and Conservation Biology Master's program at Texas State, Michelle has been an instructional assistant for Functional Biology and Genetics labs, and has been active in the Biology Graduate Student Organization and Tri Beta Biological Honor Society. She has presented her research at regional and national conferences, including the 2009 and 2010 annual meetings of the Society for the Study of Evolution. Michelle has received funding for her research from the Southwestern Association of Naturalists, the American Museum of Natural History, and the Texas Academy of Science. She has also received several scholarships while at Texas State, including the W.E. Norris, Jr. Scholarship, the Durrenberger Scholarship for Women in Science, and the George Strait-Freeman Fellows Graduate Scholarship. She plans to continue her studies and pursue a PhD in ecology and evolutionary biology.

Permanent Address (email): mhdowney@gmail.com

This thesis was typed by Michelle H. Downey