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Abstract

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Adaptive radiations are thought to be one of the most important processes generating biological diversity on Earth. Although the existence of adaptive radiations is not in doubt, the exact mechanisms via which adaptation to different habitats translates into lineage splitting has been debated for over a century. Since the time of Darwin, biologists have invoked trade-offs during adaptation to environmental gradients as being the key to linking adaptation to species formation. However, identification of the causal gradients, trade-offs and extrinsic selective regimes involved in adaptive or ecological speciation requires detailed fieldwork and experimentation and cannot be inferred using genetic or observational data alone.

This series of studies provides an empirical, experimental basis for the conclusion that adaptation to different habitats is driving divergence in *Encelia* (Asteraceae) a genus of perennial desert shrubs that has radiated extensively throughout the Mojave and Sonoran deserts. *Encelia* is an ideal system for studying the mechanisms of adaptation and speciation because all taxa are completely interfertile, and many are considered habitat specialists and form hybrid zones wherever their distributions abut. Reciprocal transplant field experiments between two taxa, *Encelia palmeri* and *E. ventorum*, showed that extremely strong postzygotic divergent natural selection is primarily responsible for preventing species fusion. A resource manipulation experiment between the same two species further showed that this was due to trade-offs caused by a gradient in water availability between dune and desert habitats. Patterns of seed germination, herbivory, and burial by sand were also important and showed interactions with taxa and habitat indicating that divergent selection at this site is complex and multifaceted.

A second study involving the same two species asked whether a signature of postzygotic selection can be seen in the distribution of phenotypes through time and along a gradient of habitat disturbance. I found that novel phenotypes suggestive of recombination are produced at a high frequency but are not present in the adult population indicating a role for postzygotic natural selection in removing the products of recombination from the population. This conclusion was further strengthened by looking at disturbed versus undisturbed habitats. Disturbed habitats contained novel phenotypes suggestive of recombination that were absent in the undisturbed sites indicating that the hybrid swarms that frequently follow disturbance are likely caused by an alteration of postzygotic selective pressures. The higher resource availability of the disturbed sites suggests that a relaxation of selection is likely responsible.

These results are elaborated on further by conducting a literature review of cases where hybridization, species fusion or hybrid swarm formation are associated with disturbances or changes in environmental forcing factors. I found that there are many cases in the literature that describe taxa maintained primarily if not solely by extrinsic postzygotic selection, although this appeared to be more true for plants than animals. Animals, in contrast, were isolated primarily by prezygotic barriers including allopatry and sexual selection. This discrepancy may help explain the disagreement between zoologists and botanists for the past century about species definitions; zoologists have typically favored prezygotic criteria while many botanists have pointed out that definitions based on reproductive compatibility fail to capture much of the variation observed among plants.

Finally, I conducted a common garden experiment and an analysis of climatic niches with eight species in *Encelia* in order to determine whether there is evidence that selection is driving divergence among other taxa in the genus in addition to *E. palmeri* and *E. ventorum*. All taxa studied showed strong climatic differentiation according to temperature and precipitation, and trait divergence in the group was high with some taxa showing evidence of the evolution of key traits allowing colonization of high altitude and hyper-saline habitats. Despite this, trait variation did not reliably follow the predictions of leaf economic theory either within or among taxa. This may be due to the existence of multiple alternative ecologically equivalent strategies that may introduce noise into low-dimensional analyses of functional traits and climate. There were, however, exceptions to this pattern. Specific leaf area showed coherent variation within taxa but not among taxa, and ecotypes *E. farinosa* also varied in the directions predicted by leaf economic theory for nearly all of the traits examined.

These and previous studies establish *Encelia* as a classic case of adaptive radiation and underscore the importance of empirical, field-based studies for disentangling the complex mechanisms driving adaptation and the formation of new species.

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CHAPTER 1

A history of research on the systematics, ecology and evolution of *Encelia*

The plant genus *Encelia* (Asteraceae: Heliantheae: Enceliinae) is comprised of 22 minimum rank taxa distributed throughout the hot deserts of North America with two disjunct taxa, one inhabiting the Atacama Desert of South America and a second inhabiting the Galapagos Islands (Clark 1998a, Fehlberg and Ranker 2007). Following circumscription of the genus by Adanson (1763), the South American taxon *E. canescens* was described by Lamarck (1786) with subsequent revisions and additions by De Candolle (1836), Vasey and Rose (1890), Blake (1913), Keck (1958) and Wiggins (1965), among others. Modern taxonomic work added the taxa *E. asperifolia* (Clark and Kyhos 1980), *E. densifolia* (Clark et al. 1988, Harrington and Clark 1989), *E. frutescens* ssp. *glandulosa* and *E. resinifera* (Clark 1998b), the systematic implications of which are reviewed in Clark and Sanders (1986), Ehleringer and Clark (1988) and Clark (1998a). Homoploid hybrid speciation is also likely to have occurred in the genus, with several taxa including *E. virginensis* (Allan et al. 1997) and *E. asperifolia* (Clark and Kyhos 1980, Clark 1998a, Clark 1998b) hypothesized as being of hybrid origin.

Biosystematic studies were initiated by Dr. Donald Kyhos of the University of California, Davis who began his career pioneering cytological techniques with the desert annual genus *Chaenactis* (Asteraceae: Heliantheae), addressing questions about speciation and adaptation at a wide variety of taxonomic scales (Raven and Kyhos 1961, Payne et al. 1964, Raven and Kyhos 1965, Kyhos 1965, Ornduff et al. 1967). Beginning in the late 1960's Dr. Kyhos expanded his work in the Heliantheae to include *Encelia*, a genus of perennial desert shrubs known for high morphological, physiological and habitat diversity (Figure 1; Kyhos 1967, Kyhos 1971, Solbrig et al. 1972, Clark and Kyhos 1980, Kyhos et al. 1981, Clark et al. 1988). These studies established that all taxa in the genus have 18 pairs of chromosomes and are fully interfertile. Exhaustive crosses between nearly every member of the genus revealed no evidence of any intrinsic reproductive incompatibilities, hybrid breakdown or heterosis (Kyhos et al. 1981, D.W. Kyhos personal communication) leading to the conclusion that extremely strong postzygotic natural selection resulting from adaptation to different habitats is likely responsible for the maintenance of discrete species despite widespread potential gene flow (Kyhos et al. 1981). These properties make *Encelia* unique among speciation study systems in that at hybrid zones taxa appear to be isolated almost entirely by adaptation to different habitats (Figure 2). This work is the subject of the subsequent chapters in this volume. Later work with his student Dr. Bruce Baldwin examined morphological, cytological and molecular differentiation in the Hawaiian silversword alliance and resulted in a series of now-classic papers (Baldwin et al. 1990, Baldwin et al. 1991) cementing the group as one of the most striking examples of adaptive radiation among both animals and plants (Schluter 2000, Coyne and Orr 2004).

Figure 1: Survey of leaf trait diversity in *Encelia.* All hybrids are putative and diagnosed based on visual examination of morphology and geographic proximity to congeneric taxa. Row 1 (left to right): *E. asperifolia*, *E. palmeri* × *E. ventorum* backcross to *E. palmeri*, *Encelia palmeri* × *E. ventorum* backcross to *E. ventorum*, *E. asperifolia* × *E. farinosa* var. *phenocodonta*. Row 2: *E. actoni*, *E. frutescens*, *E. palmeri* × *E. ventorum*, *E. farinosa* var. *farinosa* Riverside County ecotype. Row 3: *E. ventorum*, *E. farinosa* var. *farinosa* Mojave Desert ecotype, *E. californica*, *E. asperifolia* × *E. ventorum*. All photos by the author.

Several parallel lines of research beginning in the 1970's on the ecophysiology and phytochemistry of *Encelia* contributed much to our understanding of the evolution of the genus. A period of detailed ecophysiological work began with Dr. Hal Mooney, Dr. Jim Ehleringer and Dr. Olle Björkman of Leland Stanford Junior University who studied the adaptive value of a number of morphological and physiological traits such as leaf pubescence and water use efficiency, including making some of the first direct measurements of gas exchange in the field (Mooney et al. 1966, Ehleringer et al. 1976, Ehleringer and Björkman 1977, Ehleringer and Björkman 1978a, Ehleringer and Björkman 1978b, Ehleringer and Mooney 1978, Rundle et al. 1980, Ehleringer and Cook 1980, Ehleringer et al. 1981, Ehleringer and Pearcy 1983). These studies identified leaf pubescence as a key adaptation for reducing leaf temperature using both experimental and comparative approaches.

Continuing this work, Dr. Ehleringer published over 30 additional articles on the physiology of *Encelia* (Ehleringer 1981, Ehleringer 1982, Ehleringer 1983, Werk and Ehleringer 1983, Comstock and Ehleringer 1984, Ehleringer 1984, Ehleringer and Cook 1984, Ehleringer 1985, Comstock and Ehleringer 1986, Ehleringer and Comstock 1987, Ehleringer and Cook 1987, Comstock et al. 1988, Ehleringer 1988a, Ehleringer and Cooper 1988, Ehleringer and Cook 1990, Nilson et al. 1990, Ehleringer 1993, Schuster et al. 1994, Squeo et al. 1994, Sandquist and Ehleringer 1996, Sandquist and Ehleringer 1997, Sandquist and Ehleringer 1998) including pioneering work using stable isotopes in ecology (Ehleringer 1988b, Ehleringer et al. 1992, Schuster et al. 1992, Ehleringer 1993, Sandquist et al. 1993). Recent field studies have additionally shown that ecophysiological differentiation is pronounced both within and among species and identified additional morphological and physiological traits with adaptive functions (Monson et al. 1992, Housman et al. 2002, Knight and Ackerly 2002, Knight and Ackerly 2003a, Knight and Ackerly 2003b, Sandquist and Ehleringer 2003a, Sandquist and Ehleringer 2003b). These studies establish *Encelia* as a model ecophysiology study system for exploring adaptation to drought and temperature.

A second line of research that accelerated in the 1970's sought to document the distinctive and diverse secondary compounds produced by the various members of the genus. The unique secondary chemistry of *Encelia* has been recognized for a long time (Webber 1932, Gray and Bonner 1948) and the common name of the genus in Spanish is "*incienso*" which refers to the aromatic incense-like smell of the amber resins that can often be seen oozing from broken stems. This resin was marketed for a short time in the 1950's as counterfeit frankincense, but this was not commercially viable and phytochemists soon began to analyze the resins for potentially useful compounds. These studies revealed a large number of secondary metabolites including chromenes, benzofurans, lactones and flavonoids with putative anti-herbivore defensive functions, some of which were unique to the genus or specific to certain species such as "encecalin" from *E. farinosa* and "virginin" from *E. virginensis* (Bjeldanes and Geissman 1969, Sims and Berryman 1972, Steelink and Marshall 1979, Proksch and Rodriguez 1982, Bohlmann et al. 1983, Wisdom and Rodriguez 1983, Proksch and Rodriguez 1984,

Figure 2: Major isolating barriers identified for model speciation study systems illustrating the unique property that *Encelia* lacks significant prezygotic or intrinsic isolating mechanisms.

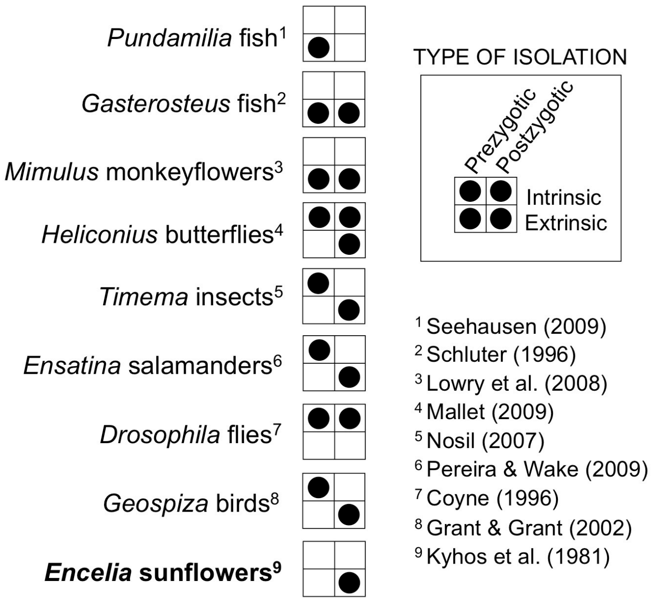
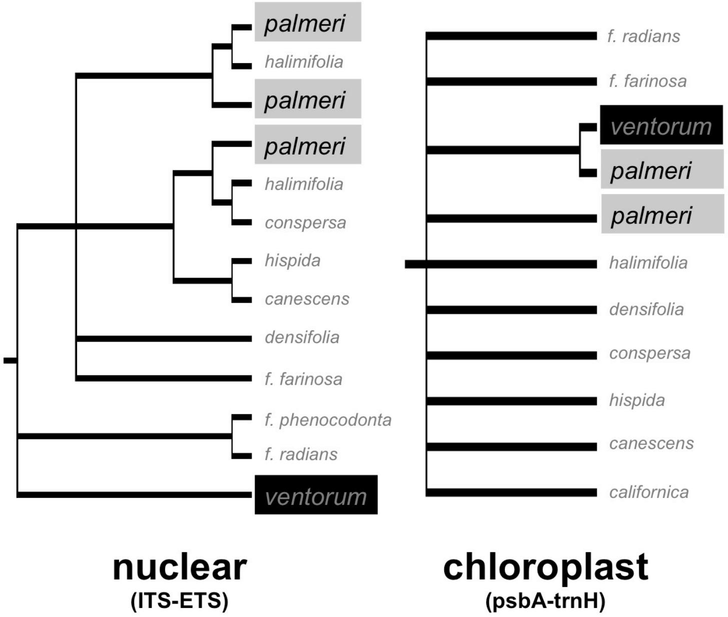


Figure 3: Reanalysis of published molecular genetic data revealed evidence of introgression, with discordance between cladograms estimated from chloroplast and nuclear genes. Data from Fehlberg (2006) and Fehlberg and Ranker (2007), trees estimated using maximum likelihood with arbitrary branch lengths.



Isman and Proksch 1985, Mitsakos and Proksch 1985, Proksch et al. 1985, Proksch et al. 1986, Proksch et al. 1988, Isman et al. 1990). Some compounds were even found only in hybrids (Bjeldanes and Geissman 1970, Proksch et al. 1983) suggesting that transgressive segregation for secondary chemistry may be occurring. The unique benzofuran chemistry of some of the taxa was also used as a character to delineate the northern Mojave Desert clade from a more southern Baja California clade (Proksch and Clark 1987).

These phytochemical studies were accompanied by entomological research into the toxicity of some of the secondary compounds to herbivores and the allelopathic effects on other plants (Gray and Bonner 1948, Proksch et al. 1983, Isman and Yan 1986, Kunze et al. 1995). One beetle in particular, the Chrysomelid *Trirhabda geminata*, is a specialist on *Encelia farinosa* and is known for outbreaks that can defoliate entire populations (Redak et al. 1995, Redak et al. 1997, Sorensen et al. 2010). Feeding trials were performed with multiple species of *Encelia* that documented the anti-herbivore properties of the secondary compounds produced but also demonstrated adaptation of several insect metabolisms to specific species of *Encelia* (Paine et al. 1993, Redak et al. 1995, Kunze et al. 1996, Redak et al. 1997, Sorensen et al. 2010). Furthermore, the *T. geminata* lineage appears to have made an evolutionary host shift to *Encelia* from *Solidago* (Asteraceae: Astereae), which is the preferred host for the rest of the species of *Trirhabda* (Swigonova et al. 2004). A beetle visually indistinguishable from *T. geminata* was observed infesting populations of *E. palmeri* in the Vizcaíno Desert of Baja California Sur, México (C.T. DiVittorio personal observation) however *T. geminata* is not known from this region nor is it known to infest *E. palmeri* thus this occurrence may represent either an introduction or another undescribed species of beetle. No further investigations of the coevolution of secondary chemistry and herbivory have been performed, and this is a promising area for future research.

Finally, recent molecular genetic work conducted by Dr. Shannon Fehlberg verified the monophyly of the *Encelia* alliance which includes *Encelia* and closely related genera *Enceliopsis* and *Geraea* (Fehlberg 2006, Fehlberg and Ranker 2007). Phylogenies were constructed from several nuclear and chloroplast genes, the results of which also clearly showed accelerated rates of speciation relative to *Enceliopsis* and *Geraea*. The lack of resolution among taxa within *Encelia* is noteworthy with populations sometimes grouping according to geography instead of taxon. This, as well as the presence of topological discordance between estimated nuclear and chloroplast cladograms (Figure 3), points to periods of historic and potentially current gene flow among taxa. Population genetic analysis of inter-simple sequence repeat markers also provided evidence of a complex biogeographic history for *E. farinosa*, the most widespread and variable taxon in the genus, that was inferred to have expanded from multiple isolated Pleistocene refugia and subsequently exchanged haplotypes among regions (Fehlberg and Ranker 2009).

Despite this large body of research, there have been few focused investigations into the mechanisms of speciation in this group. Two notable exceptions to this generalization exist. First, Kyhos et al. (1981) performed detailed field investigations of hybrid zones between *Encelia palmeri* and *E. ventorum*. They explicitly cited postzygotic divergent natural selection in maintaining high levels of phenotypic divergence between two parapatric yet fully interfertile taxa. Later, Ehleringer and Clark (1988) and Clark (1998) approached the problem from a comparative perspective and synthesized the diverse body of ecophysiological and systematic studies to identify patterns of divergence across the entire genus. These studies established *Encelia* as a textbook example of adaptive radiation (Schluter 2000), and laid the foundation for a new wave of synthetic studies combining genomic analyses with field experiments and detailed natural history observations.

The following four chapters investigate the patterns and mechanisms of adaptation speciation in *Encelia* using observational and experimental approaches. Chapter 2 presents the results of a reciprocal transplant experiment between *Encelia palmeri*, *E. ventorum* and their hybrids. This experiment was designed to measure the magnitude and strength of natural selection across a dune to desert habitat transition, and additionally to test the importance of a gradient in water availability for local adaptation. Chapter 3 examines hybrid zones and hybrid swarms between the same two species, this time taking an observational approach. The goal of this study was to examine patterns of trait variation and covariation at different life stages and in undisturbed versus disturbed hybrid zones in order to look for signatures of postzygotic natural selection. Chapter 4 expands these results into a theoretical framework for classifying hybrid swarms and species fusion according to the mechanisms via which they formed, and includes a literature review to weigh the evidence for the prevalence of different mechanisms of hybrid zone structure. Finally, Chapter 5 broadens the taxonomic scope to look for patterns consistent with adaptive radiation among nine different species in *Encelia* based on the results of a common garden experiment and analysis of each species' climatic distributions. Together, these results provide independent, empirical tests of the role of natural selection in driving the adaptive radiation of *Encelia*.

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CHAPTER 2

Mechanisms of natural selection at a brittlebrush hybrid zone

Abstract

Ecologically based divergent natural selection is presumed to be involved in most speciation events. However, the magnitude of natural selection required in many models of parapatric or sympatric speciation may be unrealistically high, and the specific ecological mechanisms are rarely tested. Additionally, most current model systems exhibit multiple intrinsic and extrinsic barriers to gene flow making it difficult to disentangle the direct effects of adaptation from other isolating barriers. Here I show using reciprocal transplant and resource addition field experiments between highly divergent yet fully interfertile dune and desert shrubs from the genus *Encelia* (Asteraceae) that selection coefficients of 0.10-0.70 against hybrids and 0.76-0.98 against parental migrants can occur over very short geographic distances despite an interspecific hybridization rate of 17.9%. Divergent selection is additionally shown to be dependent on a gradient in water availability such that adding water caused one species to perform best in all habitats. Seed germination, herbivory and burial by sand also exhibited habitat-by-genotype interactions illustrating the complexity of ecological mechanisms at this site. Physiological measurements further indicate that local adaptation is at least partly caused by a trade-off between growth and drought tolerance. Rapid apical growth likely functions as a key trait allowing the dune species to avoid burial, however the low-resistance hydraulic architecture required for rapid growth may be deleterious in the low-water desert habitat. The lack of species fusion at this site thus appears to represent an example of extreme phenotypic divergence maintained almost entirely by adaptation to different habitats.

Key Words: hybrid zone, reciprocal transplant, ecological speciation, divergent selection, parapatry

Introduction

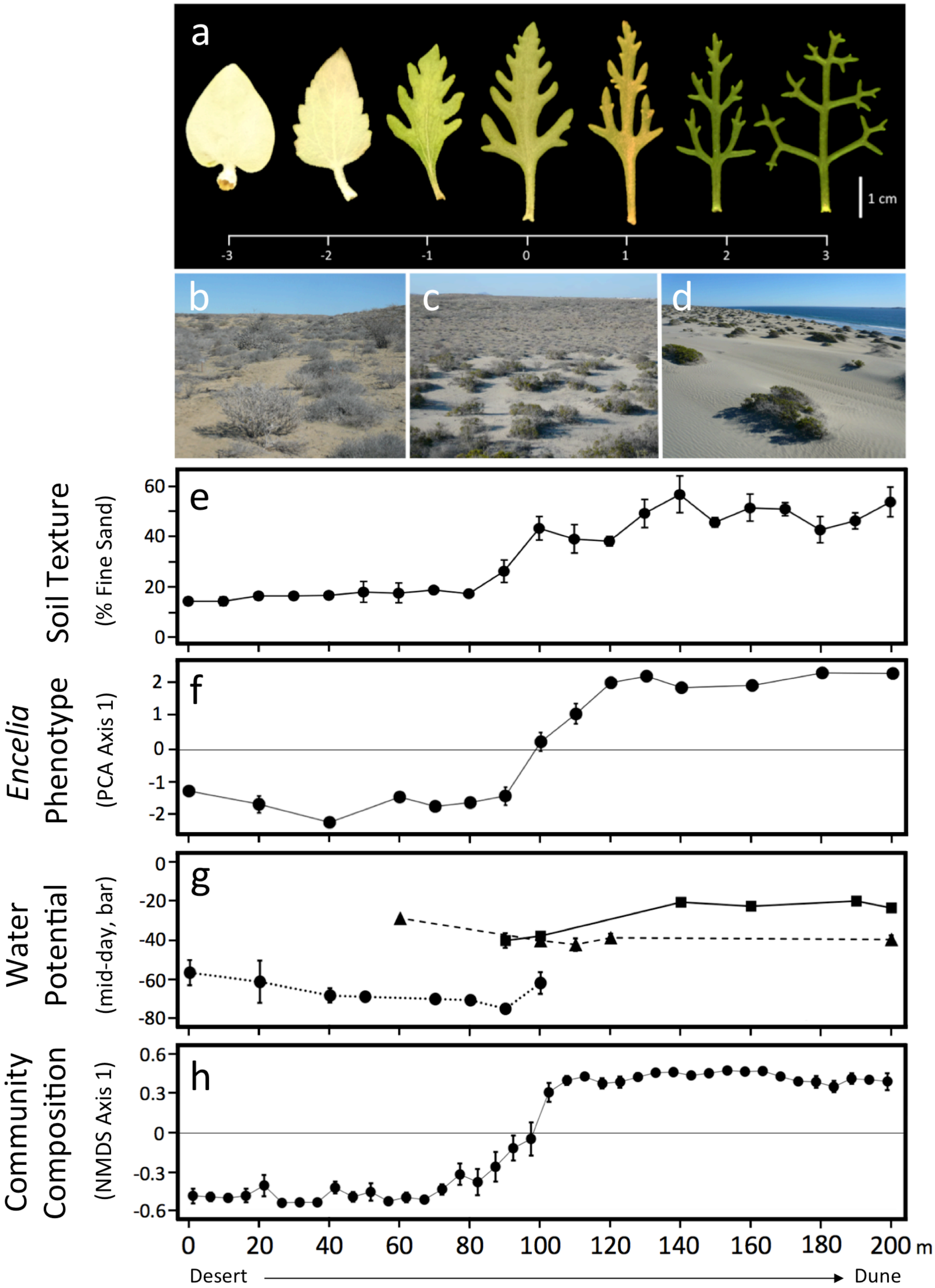
One of the longest standing debates in evolutionary biology is whether natural selection alone is strong enough to cause speciation despite high amounts of gene flow (Dobzhansky 1937, Clausen 1951, Schluter 2000, Coyne and Orr 2004, Bolnick and Fitzpatrick 2007, Pinho and Hey 2010). Theory predicts that strong selection can generate and maintain locally adapted gene complexes despite high rates of recombination (Slatkin 1973, Endler 1977, Dieckmann and Doebeli 1999, Gavrilets 2004) however it is not known whether the magnitude of natural selection required is biologically realistic (Endler 1986, Kirkpatrick and Ravigné 2002, Coyne and Orr 2004, Pinho and Hey 2010). Empirical measurements of natural selection and gene flow provide direct tests of speciation theory (e.g. McNeilly and Antonovics 1968, Harrison 1986, Nagy and Rice 1997, Hatfield and Schluter 1999, Martin and Willis 2007, Nosil 2007, Mallet 2009, Seehausen 2006, Ramsey et al. 2003), however most model systems exhibit multiple intrinsic and extrinsic barriers to gene flow making it difficult to disentangle the direct effects of adaptation from other reproductive isolating barriers (Coyne and Orr 2004, Ramsey et al. 2003, Sobel et al. 2009). A missing link in speciation research stems from the lack of examples of locally adapted lineages that are not predominantly isolated by intrinsic incompatibilities, nonadaptive prezygotic barriers or geographic distance (Chapter 1: Figure 2).

The radiation of *Encelia* (Asteraceae) presents a rare opportunity to experimentally investigate the role of extrinsic natural selection in speciation (Blake 1913, Kyhos et al. 1981, Ehleringer and Clark 1988, Clark 1998, Fehlberg and Ranker 2007). *Encelia* comprises approximately 22 described perennial desert shrubs that are known for their habitat specialization and high phenotypic diversity despite all taxa maintaining full interfertility and readily forming hybrids at ecotones between habitats (Kyhos 1967, Kyhos et al. 1981, Clark 1998). Detailed biosystematic studies by Dr. Donald Kyhos and colleagues found no evidence of intrinsic reproductive isolation between any taxa with crosses exhibiting normal pollen-pistil behavior, chromosome pairing, hybrid fertility and hybrid vigor (Kyhos 1971, Kyhos et al. 1981). Additionally, hybrids are almost exclusively restricted to ecotones and disturbed areas, a pattern indicative of strong extrinsic selection (Anderson 1948, Barton and Hewitt 1985, Chapters 3 and 4). The maintenance of phenotype-environment correlations despite widespread hybridization suggests that adaptation to different habitats is driving divergence in *Encelia*.

Hybrid zones between *Encelia palmeri* and *E. ventorum* illustrate these patterns particularly well (Figure 1). Both taxa are self-incompatible diploid (*n*=18; Solbrig et al. 1972) perennial shrubs that are parapatric throughout their ranges and exhibit radically divergent phenotypes yet no evidence of phenotypic fusion despite the abundant production of phenotypically hybrid offspring at hybrid zones (Figures 2 and 3). Both species are dominant members of the community where they occur (Peinado et al. 2005) with *Encelia ventorum* inhabiting relatively mesic active coastal sand dunes and *E. palmeri* inhabiting xeric interior desert habitats (Figure 4; Shreve and Wiggins 1964, Johnson 1977). Hybrids are phenotypically intermediate and narrowly restricted to the linear ecotone between habitats. The steep gradient in soil type and water availability corresponds with differences in temperature, relative humidity and wind speed (Figure 5) and is concordant with clines in *Encelia* morphology and physiological functioning and with a complete turnover of the rest of the perennial plant community (Figure 2 and Table 1). The co-incidence of morphology, soil type and the abundance of the rest of the community would be highly unlikely if environmental gradients were not primarily important in structuring this hybrid zone.

Figure 1: *Encelia palmeri* (left), F_1 hybrid (middle) and *E. ventorum* (right). All individuals were growing within three meters of each other and were flowering simultaneously. No backcrosses were observed. Photo by the author, Bahía Asunción, México, June 2009.

Figure 2 (following page): Phenotypes, habitats and clines at the hybrid zone. Leaf phenotypic spectrum (a) illustrating *E. palmeri* (far left), *E. ventorum* (far right) and hybrid phenotypes (middle) with the first PCA axis of four leaf functional traits shown at bottom. Photographs illustrate the desert habitat of *E. palmeri* (b), linear ecotone (c) and dune habitat of *E. ventorum* (d). A steep gradient in soil type (e) is concordant with clines in *Encelia* phenotype (f) and physiological functioning (g) of *E. palmeri* (circles), *E. ventorum* (squares) and hybrids (triangles). NMDS ordination of all other perennial vascular plant species (h) shows complete turnover in species composition across the narrow ecotone. All error bars +/- 1 standard error, from 4-7 replicate 200 meter transects.


a
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Soil Texture
(% Fine Sand)
60
40
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f
Encelia
Phenotype
(PCA Axis 1)
2
1
0
-1
-2
g
Water
Potential
(mid-day, bar)
0
-20
-40
-60
-80
h
Community
Composition
(NMDS Axis 1)
0.6
0.3
0
-0.3
-0.6
0
20
40
60
80
100
120
140
160
180
200 m
Desert
Dune

Hybridization rates of 17.9% in this study and 4% at 200 meters distant from the hybrid zone in (Kyhos et al. 1981) further suggest that extrinsic prezygotic isolation is insufficient to explain the lack of phenotypic fusion since migration rates in this range arepredicted in theoretical models to rapidly cause deterioration of differences between populations for all but the highest levels of selection (Slatkin 1973, Endler 1977, Pinho and Hey 2010). Pollinators frequently cross between habitats and flowering times are largely overlapping, although the higher water availability in the dune habitat corresponds with maintenance of some flowers by *E. ventorum* throughout the year whereas *E. palmeri* flowers only in response to rain (Kyhos et al. 1981). Hundreds of experimental crosses performed by Donald Kyhos and colleagues between nearly every species pair in *Encelia* further found no evidence of any intrinsic isolation. These observations led to the hypothesis that only extremely strong extrinsic postzygotic selection can explain the maintenance of discrete phenotypic entities at these sites (Kyhos et al. 1981). If this hypothesis is correct, *E. palmeri* and *E. ventorum* would represent a case of extreme phenotypic divergence maintained almost entirely by extrinsic selection.

To test this hypothesis, reciprocal transplant and resource manipulation field experiments were conducted at a natural hybrid zone between *E. ventorum* and *E. palmeri*. Fertile fruits were collected from naturally occurring parental and hybrid plants, germinated and reciprocally planted as seedlings into dune, desert and ecotone habitats (Figure 3). This design allowed measurement of the magnitude and direction of natural selection acting on a spectrum of phenotypes at three points along a steep environmental gradient. In order to further test the importance of the gradient in water availability in generating divergent selection I added water to a subset of the experimental plants in dune and desert habitats. The specific predictions were: (1) genotype × environment interactions are sufficient to maintain divergence despite frequent hybridization, (2) hybrids have intermediate fitness in parental habitats and the ecotone and (3) divergent selection is dependent on a gradient in water availability.

Methods

Site Characteristics

This study took place on the Pacific coast of the Vizcaíno Desert, Baja California Sur, México. The Baja California peninsula was formed between 5 and 12 million years ago due to transfer of the peninsular batholith to the Pacific plate (Oskin et al. 2001, Oskin and Stock 2003). The resulting geographic isolation and increase in ecological opportunity due to the onset of the desert climate caused the peninsula to become a hotspot of evolutionary diversification (Murphy and Aguirre-Leon 2002, Jacobs et al. 2004, Peinado et al. 2005). Coastal sand dunes at the study site are formed by onshore winds that deposit sand inland (Maun 1998). Paleogeomorphic evidence indicates that sand dunes on the Pacific Coast tracked variation in sea level and wind direction has been constant since at least the middle Pleistocene (Murillo De Nava et al. 1999). Strong onshore winds blow year-round from between approximately 270° to 330° creating a stable ecotone between dune and desert habitats defined by the tangent of the direction of the wind to the curvature of the beach (Figure 3; Koracin et al. 2004, Peinado et al. 2005). Paleogeographic evidence further suggests that this wind pattern has been stable throughout the Pleistocene and likely throughout the late Miocene as well (Moreno-Ruiz and Carreno 1994, Murillo de Nava et al. 1999, Ledesma-Vazquez et al. 2006).

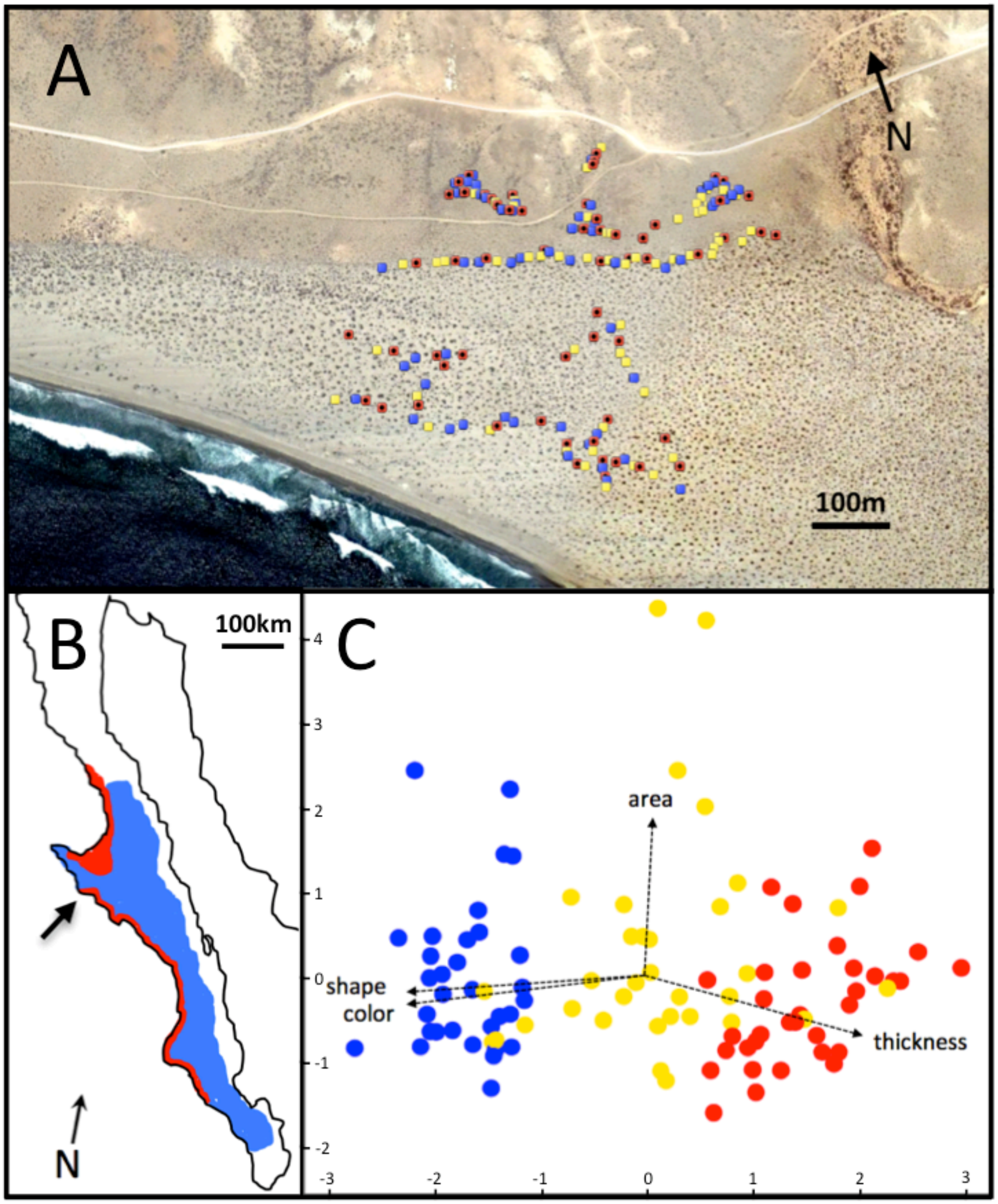
The study site is composed of aeolian sand dunes overlaying mid-Miocene sedimentary marine formations with little topographic relief, punctuated by terrestrial volcanic deposits of later Pliocene and Pleistocene age (Moreno-Ruiz and Carreno 1994, Peinado et al. 2005). It exhibits a coastal desert climate with the majority of precipitation falling in the winter as the remnants of frontal systems originating in the north Pacific Ocean, although infrequent convective storms can contribute significant amounts of summer rain (Peinado et al. 2005, Johnson 1977). Summer fog is likely a substantial contributor to local water balance although no studies have quantified the amount of fog utilization by vegetation at this site (Peinado et al. 2005, Stone 1963). Precipitation is highly interannually variable and averages less than 80 mm per year (30-second precipitation maps, www.worldclim.org).

Relative humidity, temperature and wind speed were measured in dune and desert habitats by installing paired weather stations 250 m apart (Onset HOBO data loggers and sensors; Figures 4 and 5). Both habitats also likely exhibit differences in soil salinity and moisture due to fog and dew interception (Johnson 1977, Wells and Shunk 1938, Stone 1963) although these factors were not measured. Burial by wind blown sand was also hypothesized to be a significant factor (Maun 1998) although rates of sediment aggradation were not measured directly. Soil water content was measured in dune and desert habitats by deploying soil moisture sensors at 0.2, 1.0 and 2.0 meters (Onset Corporation HOBO loggers with ECH2O soil moisture probes). In arid regions soil moisture is consistently higher in sand dune habitats relative to adjacent non-dune soils due to higher rates of infiltration, and this effect is independent of proximity to the ocean (Noy-Meir 1973).

Trait Measurements

Leaf area, shape and brightness were quantified by photographing leaves under constant illumination. Shape was quantified by calculating the percent of the smallest convex hull enclosing the entire leaf that is covered by the leaf lamina using the solidity metric in the computer program Image-J (http://imagej.nih.gov/ij/), with low values indicating highly dissected leaves and high values indicating entire leaves. Leaf area was calculated with Image-J using the measure particles function. Brightness is an indication of the amount of reflective pubescence covering the leaf and has been shown to be adaptive in other species in the genus (Ehleringer and Björkman 1978, Ehleringer 1982). Brightness was quantified by converting the original leaf photographs to 8-bit greyscale images in Image-J, standardizing the exposure using a color reference included in the photograph and

Figure 3: Map of the transplant site (a) showing desert, ecotone and coastal dune habitats and the locations of individual experimental plants of *Encelia ventorum* (red), hybrids (yellow) and *E. palmeri* (blue). Location of the study site in Baja California Sur, México (b) is indicated by a bold arrow, with the geographic distribution of *E. ventorum* shown in red and the distribution of *E. palmeri* in blue. Axis 1 (horizontal) and axis 2 (vertical) of a principal components analysis of four leaf functional traits (c) on all plants measured at the end of the experiment and colored by maternal phenotype shows that the phenotypes of *E. palmeri* (blue) and *E. ventorum* (red) are non-overlapping, however hybrids (yellow) exhibit phenotypes that span the full range of variability between parental taxa. PCA axis 1 is used as a phenotypic hybrid index in Figure 9.



reading the pixel intensity off the lamina in the center of the leaf using Photoshop (Adobe Systems Inc.). Leaf thickness was measured with a digital caliper as the average of two measurements (middle lamina and tip) avoiding all major veins. Mid-day shoot water potentials were measured on healthy 10 cm long shoots with a Scholander-type pressure bomb (PMS Model 1000) between 13:00 and 15:00 using standard protocols. Soil samples were sifted to obtain the proportion of fine sand (particle diameter 500-250 μm; U.S. Standard sieves #60 and #35). Community composition was quantified by using the first axis of a non-metric multidimensional scaling (NMDS) ordination of abundance of all 11 perennial vascular plant species encountered during sampling of both dune and desert habitats excluding *Encelia* taxa (Table 1). Hybridization rate was measured by progeny testing fertile achenes collected from mature, phenotypically parental plants growing at the hybrid zone. Out of 134 successful progeny from 11 maternal plants (six *E. palmeri* and five *E. ventorum*) I scored whether the progeny possessed diagnostic traits from both species. Plants were scored as hybrids if they exhibited combinations of leaf dentition and pubescence, traits diagnostic for *E. ventorum* and *E. palmeri* respectively. Phenotypes suggestive of backcrossing were not observed during progeny testing thus it is presumed that all hybrids were F_1 generation.

Field Experiments

Measurements of population parameters at hybrid zones are one of the most powerful ways to infer the role of natural selection in population divergence (e.g. Briggs 1962, Byatt 1975, Fritsche and Kaltz 2000, Bleeker and Hurka 2001, Kay et al. 2006, Campbell and Waser 2007, Abadie et al. 2011, Kamiya et al. 2011). A reciprocal transplant experiment was conducted to measure the magnitude of selection on parental taxa and hybrids across a range of environmental conditions (Turreson 1922, Clausen et al. 1940, Clarkson 1959, Geber and Eckhart 2005, Angert and Schemske 2005, Emms and Arnold 1997, Wang et al. 1997). Fertile achenes were collected from phenotypically pure parental taxa and phenotypically intermediate hybrid plants and germinated in greenhouse flats until all plants were five centimeters tall or exhibited five true leaves. Parental taxa were easily distinguishable in the field due to discrete, non-overlapping phenotypes (Figure 3) and hybrids were identified visually by possessing combinations of pubescence and leaf dentation. Propagules were not collected from plants that exhibited phenotypes indicative of backcrossing or segregation toward one parental species. A total of 276 plants were reciprocally planted into dune, desert and ecotone habitats beginning in September 2010. Individuals that were planted later due to later emergence from the germination flats were watered correspondingly longer to standardize the total time spent under irrigation. Planting order was randomized by habitat and taxon, and linear regression of total ln-transformed biomass versus planting date was not significant (n=72, R^2=0.013, p=0.355). Watering was terminated in December and all plants allowed to grow under ambient conditions for another four months. Cages were used on all plants in all treatments to prevent disturbance from coyotes that were attracted to the watering treatment (Figure 6). Cages did not substantially affect solar insolation or wind speed and leporids are the only herbivores at the site that may have been prevented access to the

Figure 4: Soil water content at -0.2 meters below the soil surface in the desert, -1.0 meters in the desert and -1.0 meters in the dune as measured by time delay refractometry probes. Higher infiltration rates into dune sand results in deeper percolation in the dunes after small rain events (November and January) resulting in higher water availability than in adjacent desert soils despite the dune sand's lower overall water holding capacity. However, large infrequent rain events may percolate deeper into desert soils (March) and the effects may be detectable for several months.

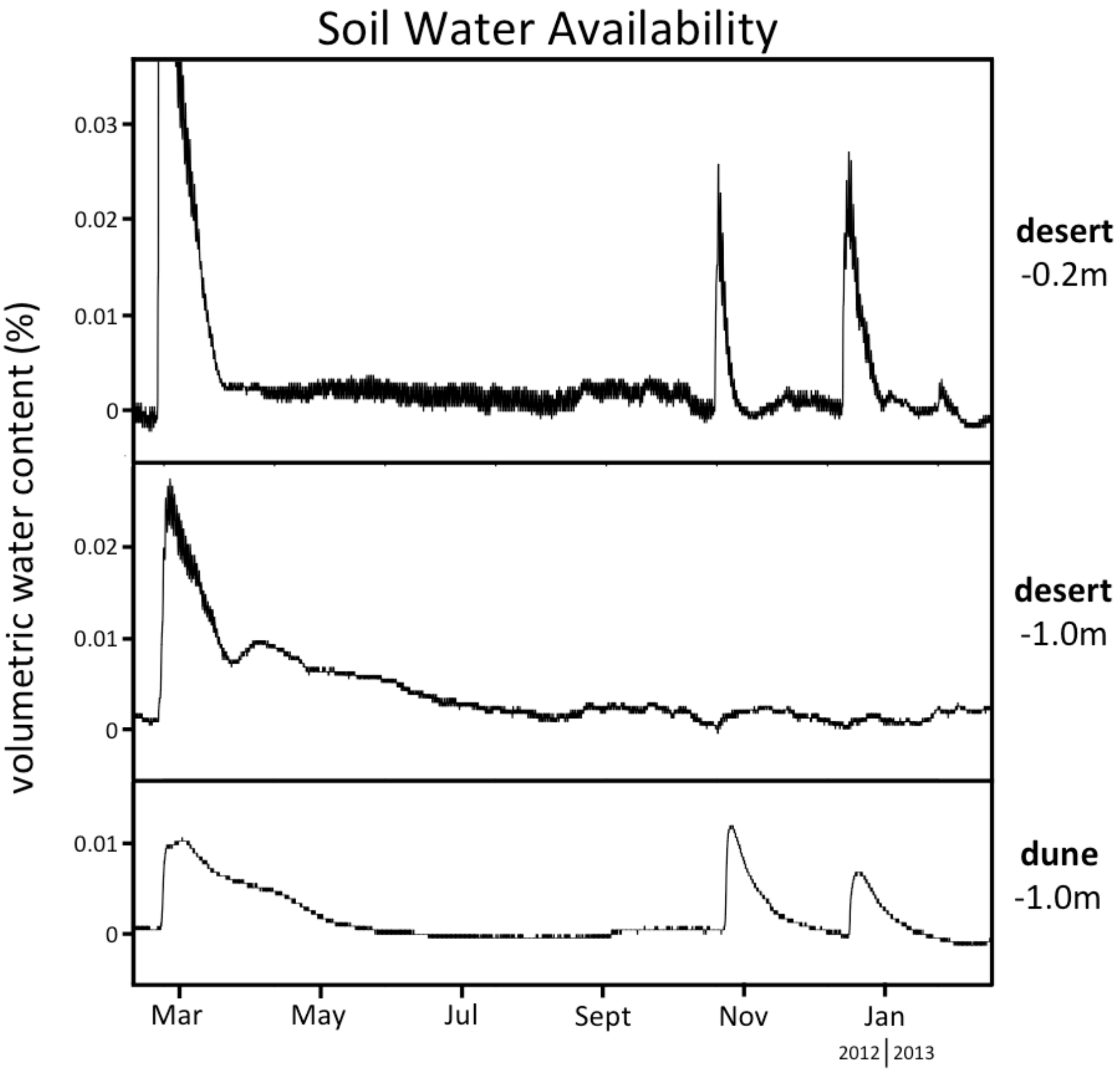
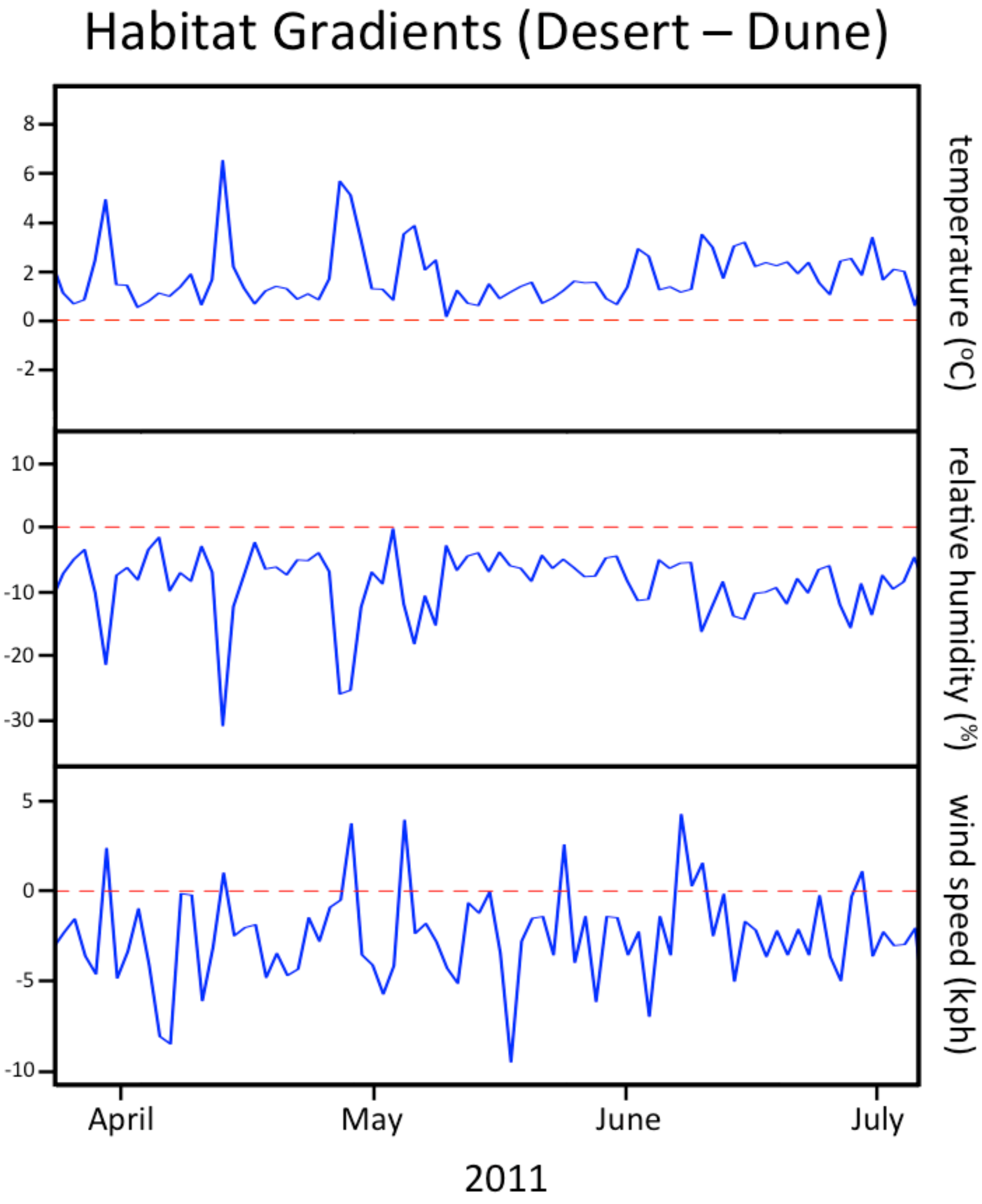


Figure 5: Temperature (a), relative humidity (b) and wind speed (c) differentials (desert minus dune) measured using paired weather stations separated by a distance of 250 meters. The desert habitat is on average 3.20 degC hotter, 9.24% less humid and experiences 2.54 kph slower afternoon wind speeds than the dune habitat. Data are subsetted to daytime measurements between 15:00 and 16:00 during the peak growing season.



experimental plants. All other known herbivores are smaller than the mesh size or could enter through the open top. The effect of water availability was tested by adding water to a subset of the experimental plants in dune and desert habitats for the duration of the experiment. Added water quickly dissipated and no evidence of watering was apparent 48 hours after application, thus the amount of water added created soil moisture levels that were above what other experimental plants received but were still below what would be expected during a wet recruitment year. Instances of mortality due to herbivory and burial by sand was also recorded. Herbivory was evidenced by the complete destruction of the plant with obvious signs of herbivore activity nearby. This is expected to be a conservative estimate of herbivory because a plant had to be completely killed and to have obvious signs of herbivore damage to be classified as affected by herbivory. Burial was evidenced by the entire plant being completely buried by sand and lacking turgor pressure when excavated. Patterns of seed germination were quantified by reciprocally sowing 500 seeds of each parental species into eight plots per species in each in of dune and desert habitats for a total of 16,000 sown seeds. Seedling plots were watered by hand twice per week for four months and the number of germinated seedlings counted and removed as they emerged. At the end of the experiment aboveground and belowground biomass were harvested by excavating the plants to a depth of one meter and removing roots with a sieve and tweezers. Several physiological and morphological measurements were made at the end of the experiment on all plants with assayable tissues. Mid-day shoot water potentials were measured on healthy ten centimeter long shoots with a Scholander-type pressure bomb using standard protocols. Leaf brightness, thickness, area and shape were measured as described above. For all plant-level traits, values represent the average of approximately four mature leaves per plant (range 1-11 leaves) except water potential measurements that were measured on one shoot per plant.

Statistical Analyses

Growth was calculated as combined aboveground and belowground biomass and was ln+1 transformed. Differences in growth were analyzed by using two-way analysis of variance (ANOVA) with habitat and maternal taxon as predictor variables and ln-transformed growth as the dependent variable. Differences in survival were analyzed using logistic regression with habitat and maternal taxon as predictor variables and survival as the dependent variable. Relative fitness was calculated by adding dead individuals to the transformed growth data as zeroes, calculating the arithmetic mean within taxa and setting the fitness of the most fit phenotype in each habitat equal to one. Selection coefficients were calculated as one minus relative fitness. Analysis of fitness using ASTER models (Geyer et al. 2007) would have been preferable but was not possible due to lack of sufficiently detailed life history data.

Additionally, I did not use a multiplicative framework for comparing the contributions of different prezygotic and postzygotic isolating barriers to total reproductive isolation. This approach is analogous to multiplying life history stage transition probabilities to calculate population growth rate and to multiplying the components of fitness to calculate selection. However, unlike these examples current frameworks for calculating reproductive isolation include overlapping and/or nonessential components that preclude the use of a multiplicative framework (Ramsey et al. 2003, Nosil et al. 2005, Lowry et al. 2008). For example, inclusion of selection against parental migrants in calculation of total reproductive isolation is invalid for many plant populations because pollen dispersal obviates the need for individuals to migrate across habitat boundaries (McNeilly 1968, Hall and Willis 2006, Sobel et al. 2009, Harrison 2012). While selection against hybrids is an essential component of gene flow since all alleles must pass through hybrids in order to pass between populations, selection against parental migrants (i.e. "immigrant inviability") can be absolute yet introgression may still occur. Another option would be to try to disentangle adaptive from nonadaptive causes of reduced hybridization as a function of geographic and "ecological" distance by computing isolation by distance versus isolation by adaptation/ecology/environment (Shafer and Wolf 2013, Andrew et al. 2012), although this approach may be impractical to apply in the field. See Chapter 4 for a discussion on comparing reproductive isolating barriers. Differences in fitness due to the watering treatment were analyzed separately for each habitat by adding dead individuals as zeroes to the ln-transformed growth data and performing two-way ANOVA using maternal taxon and watering treatment as predictor variables. Differences in burial, herbivory and seedling germination were analyzed by performing backward stepwise log-linear analysis on three-way contingency tables and comparing AIC scores and performing analysis of deviance tests for models with and without burial, herbivory and interaction terms (Christensen 1997). Water potential measurements performed on experimental plants at the end of the experiment in the desert habitat were analyzed with a one-way ANOVA with maternal taxon as a factor (Figure 7). All analyses were conducted in R (http://r-project.org/).

Results

The reciprocal transplant revealed extremely strong divergent natural selection with selection coefficients against hybrids ranging from 0.10 in the desert to 0.70 in the dunes and selection coefficients against parental migrants ranging from 0.76 in the desert to 0.98 in the dunes (Figure 8). These results are qualitatively the same whether performance is measured as growth or survival, and whether analyzing growth as a function of maternal phenotype (Figure 8A) or the phenotype of the individual experimental plant (Figure 9). These results show that the direct effect of natural selection on reproductive isolation is sufficient to maintain dramatic phenotypic divergence despite very high rates of hybridization (Endler 1977, Dieckmann and Doebeli 1999, Gavrilets 2004). As predicted by models of ecological speciation, growth. and survival of hybrids was intermediate to that of parental taxa in parental habitats and not significantly different from parental taxa in the ecotone indicating a primary role for environmental gradients in structuring the spatial distribution of phenotypes (Barton and Hewitt 1985, Schluter 2000).

Figure 6: Photographs of *Encelia palmeri* (a) and *E. ventorum* (b) from the desert water addition treatment immediately prior to harvest. *Encelia ventorum* grew larger than *E. palmeri* in the desert with water but suffered high mortality without water.

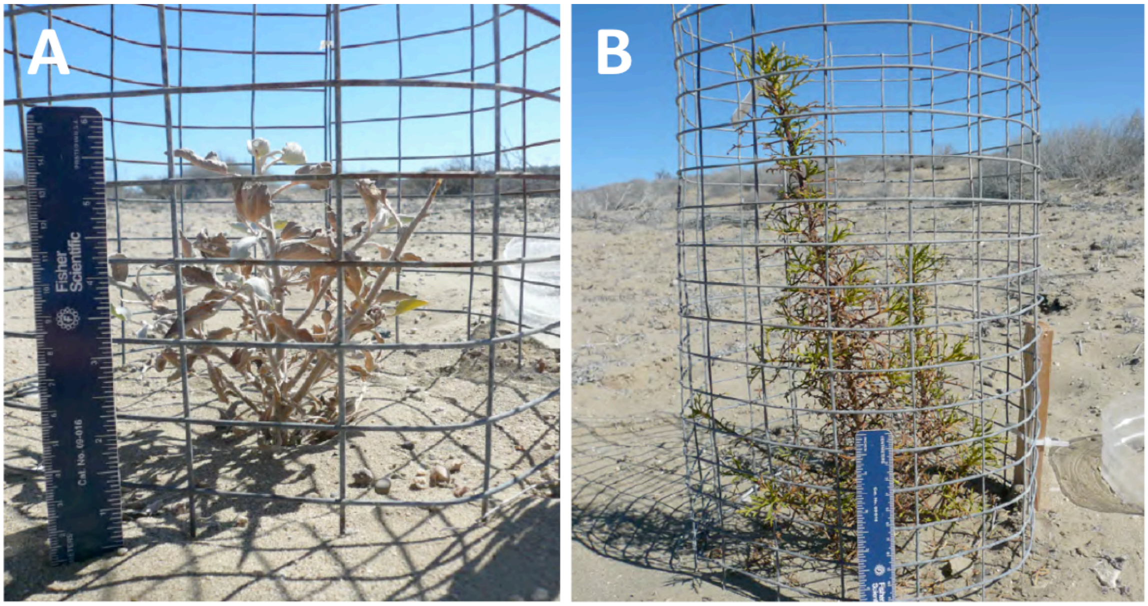


Figure 7: Mid-day stem water potential of experimental plants alive in the desert habitat at the end of the experiment illustrating significantly more negative water potentials of plants grown from *Encelia palmeri* mothers compared to *E. ventorum* or hybrid mothers. One-way ANOVA detected a significant effect of maternal taxon (n=35, F=14.26, p<0.001) with Tukey post hoc tests indicated by letters.

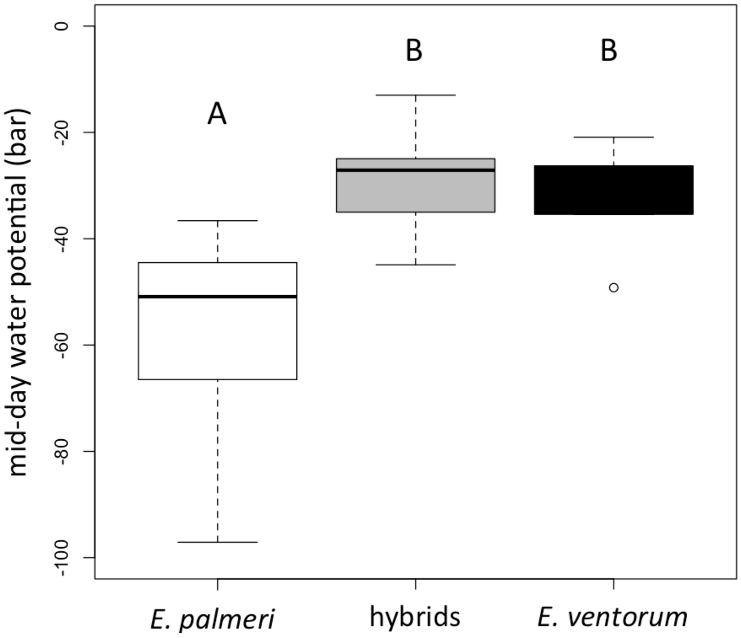
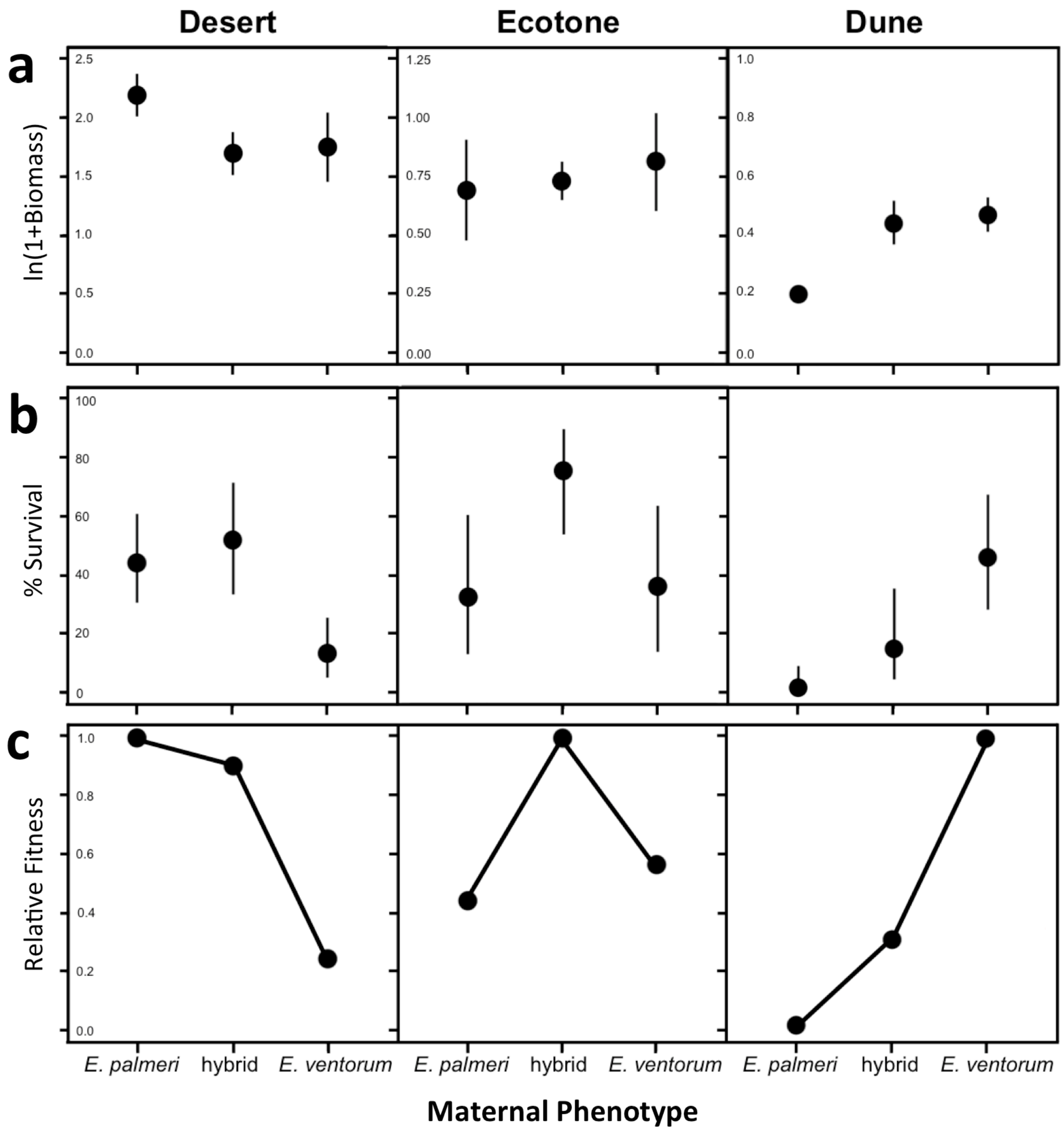


Figure 8: Growth (a), survival (b) and relative juvenile fitness (c) of plants grown in desert, ecotone and dune habitats and categorized by maternal phenotype. Significant habitat × taxon interactions were detected for growth (two-way ANOVA: F=5.812, n=72, p=0.0186) and survival (logistic regression: n=243, z=-5.240, p<0.001). Growth analyzed by the phenotype of the experimental plant yielded identical results (Figure 9). Neither growth or survival of hybrids was different from that of parental taxa when the ecotone was analyzed separately. Error bars for growth are +/- one standard error and error bars for survival are binomial 95% confidence intervals.



Adding water eliminated divergent selection such that *E. ventorum* performed as well as or better than *E. palmeri* in all habitats (Figure 10), indicating that physiological trade-offs related to water use are likely important components of local adaptation. However, other factors also contributed to patterns of divergent selection. In particular, herbivory, burial and seed germination were examined by reciprocally sowing seed of each taxon and by recording cases of herbivory or burial in the transplant experiment. Significant habitat × taxon interactions were associated with herbivory and seed germination while habitat and taxon were significantly associated with burial (Figure 12). Interestingly, selective mechanisms were asymmetrical in that each factor was important in one habitat but not important in the other. For example, adding water eliminated fitness differences in the desert habitat but had no effect on growth or survival in the dune habitat. Similarly, burial affected *E. palmeri* and hybrids disproportionately in the dune and ecotone habitat These results combined with additional physiological measurements strongly suggest that local adaptation at this site is at least partly explained by a trade-off between drought tolerance and growth rate. *Encelia palmeri* exhibited more negative mid-day shoot water potentials at the end of the experiment (Figure 7) and outperformed *E. ventorum* in the desert habitat, however *E. ventorum* grew larger and taller than *E. palmeri* in the desert when given supplemental water (Figure 10). In this system the rapid apical growth of *E. ventorum* likely functions as a key adaptation preventing burial in the active dune habitat, a pattern observed in other close relatives adapting to sand dune habitats (Andrew et al. 2013, Roda et al. 2013b), however the low-resistance hydraulic architecture required for rapid growth may be deleterious in the arid desert habitat due to increased risk of xylem cavitation and embolism. Similar trade-offs have been reported between other species in the genus (Ehleringer 1988, Ehleringer and Cook 1990) suggesting that this may be a general axis of ecological strategy variation in this group.

Phenotypic plasticity may also play a role in facilitating invasion of different habitats (Schlichting 1986). Plasticity for the three most diagnostic leaf morphological traits distinguishing parental taxa showed that the variability within taxa was always lower than the variability between taxa, and the ranges of variation between taxa were almost completely non-overlapping (Figure 11). For leaf thickness and leaf shape, *Encelia palmeri* and *E. ventorum* exhibited counter-gradient variation such that when planted in a common environment they became less similar morphologically, indicating that plasticity for these traits is not adaptive. For leaf color, plasticity was co-gradient indicating that when planted in a common environment both taxa became more similar. In this study as in some previous studies, the direction of plasticity is as often counter-gradient as it is co-gradient indicating that plasticity is frequently not in the directions that would be adaptive (e.g. Eckhart et al. 2004), however this pattern is not universal (e.g. Lusk et al. 2008). For leaf color, thickness and shape, the co- or counter-gradient plasticity was caused by only one of the taxa. For leaf thickness and leaf color, *E. ventorum* did not exhibit significantly different leaf morphologies when planted into different habitats, whereas *E. palmeri* exhibited substantial plasticity. Similarly, *E. palmeri* showed no plasticity for leaf shape when planted into the dune habitat although *E. ventorum* exhibited plasticity when planted in the desert environment.

Figure 9: Scatterplots of ln-transformed growth versus phenotypic hybrid index for all plants alive at the end of the transplant experiment with 5, 10, 25, 50, 75, 90 and 95th quantile regression lines shown for dune (a), ecotone (b) and desert (c). Hybrid index is PCA axis 1 in Figure 8A. Regression revealed a marginally significant negative slope in the desert habitat (n=35, R^2=0.055, p=0.094), a flat slope in the ecotone (n=20, R^2=0.003, p=0.805) and a significantly positive slope in the dune (n=17, R^2=0.330, p<0.01).

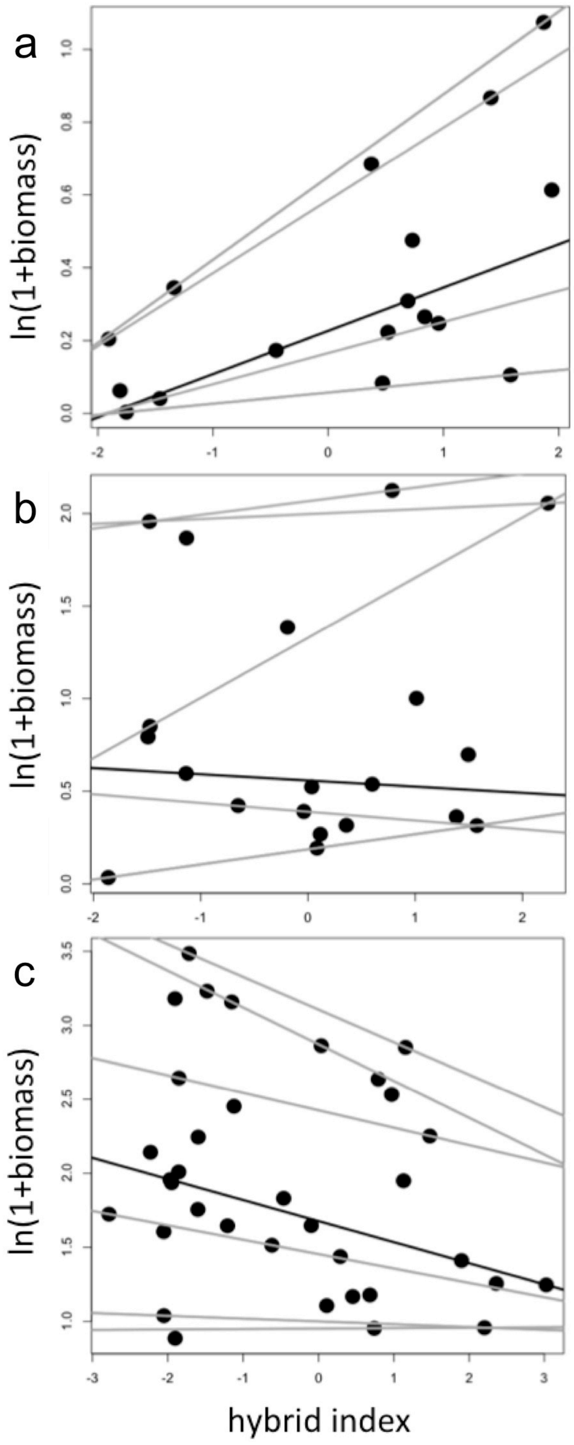


Figure 10: Relative fitness of *Encelia palmeri* (circles) and *E. ventorum* (squares) without supplemental water (a) and with supplemental water (b). Separate two-way ANOVA of taxa and watering treatment on unrelativized combined growth and survival indicated a significant effect of water in the desert (F=92.26, n=120, p<0.001) but not in the dunes (F=1.16, n=108, p=0.284).

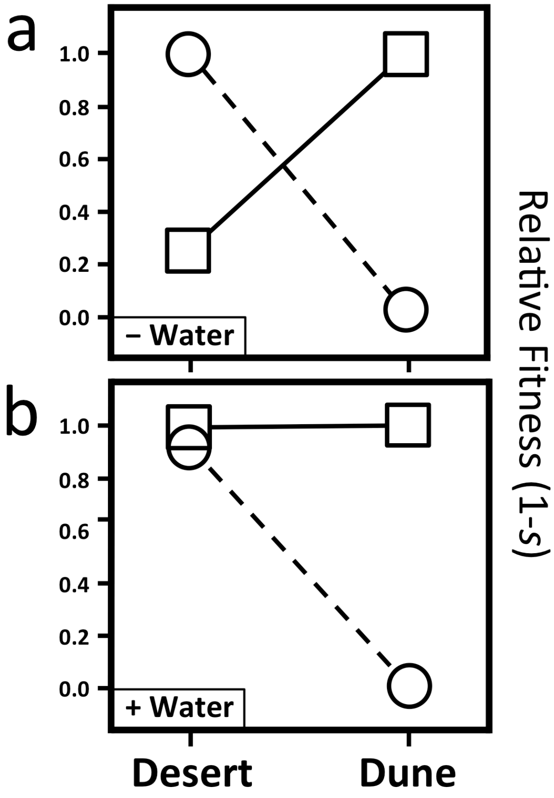


Table 1: List of all perennial vascular plant species encountered during community sampling and their habitat associations. Data illustrated in Fig. 2H.

species	family	habitat association
Jatropha cinerea	Euphorbiaceae	desert
Fouquieria diguetii	Fouquieriaceae	desert
Frankenia palmeri	Frankeniaceae	desert
Lycium andersonii	Solanaceae	desert
Funastrum arenarium	Apocynaceae	dune
Helianthus niveus	Asteraceae	dune
Atriplex julacea	Chenopodiaceae	dune
Euphorbia misera	Euphorbiaceae	dune
Stillingia linearifolia	Euphorbiaceae	dune
Astragalus magdalenae	Fabaceae	dune
Errazurizia benthamii	Fabaceae	dune

Discussion

These results establish *Encelia palmeri* and *Encelia ventorum* as a classic example of ecological speciation by measuring strong extrinsic divergent natural selection, identifying underlying causal environmental gradients and suggesting the ecophysiological trade-offs responsible (Schluter 2000, Coyne and Orr 2004, Nosil 2012). This experiment revealed some of the strongest divergent natural selection measured in the field between actually hybridizing populations (Nosil et al. 2005, Rundle and Nosil 2005, Lowry et al. 2008, Schluter 2009). Together these results support the plausibility of models of divergence with gene flow by demonstrating that extremely strong divergent natural selection can occur over very short geographic distances and that direct effect of adaptation to different habitats alone is capable of maintaining dramatic phenotypic differentiation despite high rates of hybridization. The magnitude of selection and the spatial scale over which it is measured is comparable to that found in classic studies of adaptation of plants to the extreme environment of mine tailings (McNeilly 1968, McNeilly and Antonovics 1968) however this study shows that selection of this magnitude can occur naturally via multiple adaptive mechanisms. This study is also similar to others that found an extrinsic basis for hybrid zone structure (Ownbey and Weber 1943, Rieseberg et al. 1999, Miglia et al. 2005, Zha et al. 2010, Yost et al. 2012) and adds to the evidence that natural selection may be a ubiquitous driver of speciation.

The diversity of mechanisms generating selection additionally suggests that the functional traits involved in adaptation are likely numerous and may have a correspondingly complex genetic basis (Roda et al. 2013a). For example, drought tolerance is a quantitative trait that responds to variation in water deficit, however drought tolerance is also a complex epistatic phenomenon that involves the interactive effects of dozens of sub-traits including xylem architecture, stomatal conductance and deciduousness, many of which are likely to be under complex genetic control themselves (Rockman 2011).

These data also suggest that the majority of reduction in realized gene flow is postzygotic. While selection against parental migrants was stronger than selection against hybrids, in this system the importance of selection against hybrids in blocking gene flow is likely to be much greater than that of selection against parental migrants. Alleles must transit through a hybrid in order to introgress from one population into another, whereas in plants the parental migration phase is typically bypassed via pollen dispersal, precluding direct comparison of selection coefficients and questioning the utility of multiplicative frameworks for calculating total reproductive isolation as a proxy for progress toward speciation. Despite this, selection against parental migrants should still reduce the frequency of hybridization and knowledge of the magnitude of selection is useful for establishing the ends of the phenotypic selection gradients in each habitat, since both ecological speciation and bounded hybrid superiority models predict linear and crossing reaction norms for reciprocally transplanted taxa.

Interestingly, both species maintain complete interfertility despite strong selection against hybrid phenotypes which theory predicts should eventually lead to reinforcement (Kirkpatrick and Ravigné 2002, Servedio and Noor 2003, Coyne and Orr 2004). One potential explanation is that high hybrid fitness in the ecotone, low rates of seedling survival and low maternal investment reduces the cost of gametic wastage weakening the feedbacks required for reinforcement occur. It is thought to be difficult for intrinsic prezygotic isolating barriers to evolve due to postzygotic natural selection alone (Kirkpatrick and Ravigné 2002) suggesting that both taxa may continue to coexist without evolving the intrinsic isolation characteristic of traditionally defined biological species (Epling 1947). In fact, if hybrids are favored in some habitat or if phenotypic plasticity is advantageous there may actually be selection against the evolution of intrinsic isolating barriers, akin to reinforcement in reverse. While we do not know the precise sister species relationships of any of the taxa in the genus, this distinction may be irrelevant considering that all species continue to hybridize and segregate along environmental gradients, similar to what have been described as "syngameons" (Grant 1971) or "ecological species" (Van Valen 1976). Many plant clades exhibit a pronounced lack of prezygotic or intrinsic isolating barriers such as in *Quercus* and *Rhododendron* where taxa maintain interfertility despite fossil evidence placing minimum clade ages in the Cretaceous and Paleocene, respectively (Collinson and Crane 1978, Van Boskirk 1998). In these groups strong selective filters appear to maintain favorable gene combinations associated with environmental gradients despite free introgression of neutral gene regions (Benson et al. 1967, Whittemore and Schaal 1991, Milne et al. 2003, Mitsui et al. 2010, Levin 2012). Thus, the situation illustrated by *Encelia* may be general to other lineages that diverge along strong ecological gradients despite the potential for high rates of gene flow.

Figure 11: Phenotypic plasticity of six functional traits for *Encelia palmeri* (left panels) and *E. ventorum* (right panels) in desert, dune and ecotone habitats. Note the change in scale of the y-axes.

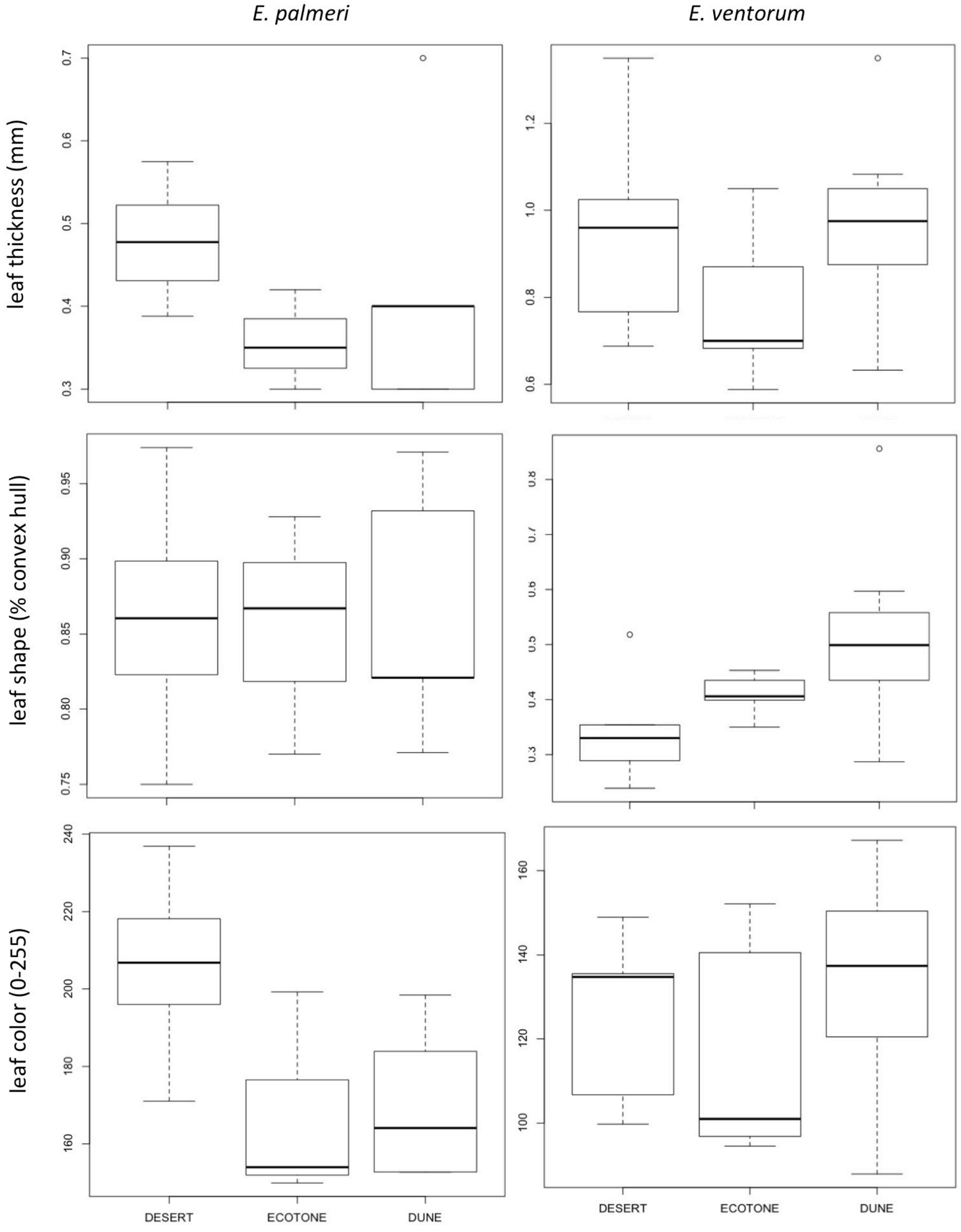
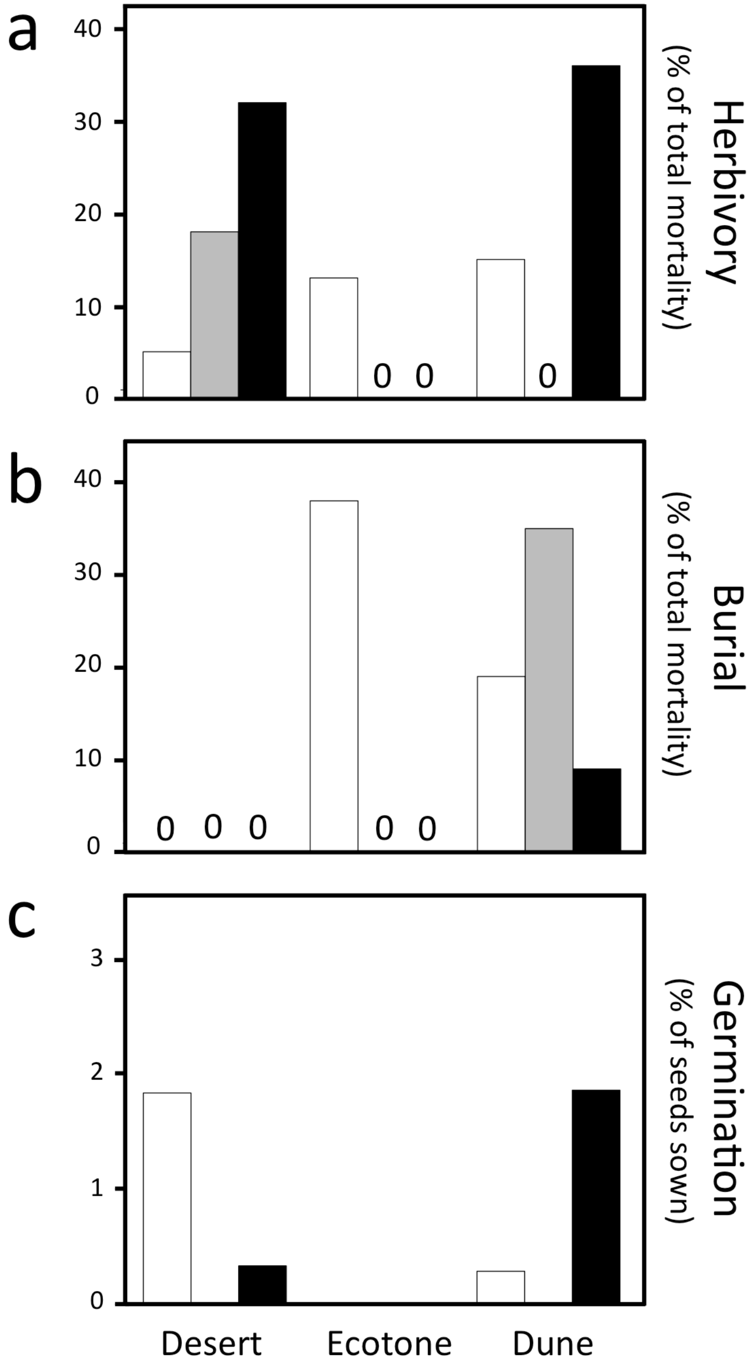


Figure 12: Herbivory (a), burial (b) and seed germination (c) for *Encelia palmeri* (white), hybrids (grey) and *E. ventorum* (black) in desert, ecotone and dune habitats. Backward stepwise log-linear regression and comparison of AIC values indicated taxon × habitat interactions were associated with patterns of herbivory (n=171, AIC=36.00) and seed germination (n=16,000, AIC=16.00) while burial was associated with habitat (n=171, AIC=33.45). Hybrids and the ecotone were not included in the germination experiment.



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CHAPTER 3

Patterns of phenotypic variation and covariation at hybrid swarms in *Encelia*

Abstract

Hybrid swarms frequently result from habitat disturbance, although the underlying mechanisms are frequently not known. At hybrid zones between the desert shrub *Encelia palmeri* and the sand dune specialist *E. ventorum*, individuals with intermediate phenotypes are absent at undisturbed sites while they are common at disturbed sites. Moreover, at undisturbed sites, young seedlings appear to exhibit recombinant phenotypes not present in the adult population. Thus, we hypothesized that extrinsic postzygotic natural selection is removing recombinant phenotypes from the population in the undisturbed habitat but that in the disturbed habitats relaxed selection allows these recombinant phenotypes to persist resulting in a hybrid swarm. To test this we studied the phenotypic variance-covariance structure of ten leaf morphological traits measured on individual plants growing in a range of habitats and different life history stages. The results indicate that recombinant progeny are produced at high frequency but that at undisturbed sites they are absent among adults, and thus apparently removed by postdispersal natural selection. In disturbed sites, higher overall water availability likely results in relaxed selection, allowing the recombinant phenotypes to persist. These results support a neutral hybrid swarm as envisioned by Grant (1971) and suggest that postzygotic natural selection may be a powerful force maintaining phenotypic coherence between locally adapted lineages.

Key words: hybrid swarm, hybrid zone, postzygotic isolation, extrinsic selection, recombination

Introduction

Hybrid swarms and species fusion have long been observed to accompany habitat disturbance (Kölreuter 1761, Focke 1881, Kerner von Marilaun 1888, Tedin 1925, Roberts 1929, Wiegand 1935, Anderson 1948, Grant 1971, Arnold 1997, Rieseberg and Carney 1998) but the causal mechanisms are not well understood. Although disturbances and other environmental perturbations can promote hybridization through a variety of different mechanisms, the reproductive isolating barriers broken are presumed to be ecologically or extrinsically based, since extrinsic isolation is by definition environment-dependent (Grant 1971, Coyne and Orr 2004). The presence of advanced generation hybrids also indicates that there are few or no intrinsic barriers to gene flow, as this would prevent a hybrid population from persisting through time as in tension zone models of hybrid zone structure (Stebbins 1950, Barton and Hewitt 1985).

Extrinsic isolation can be further divided into prezygotic and postzygotic barriers, and of these postzygotic isolation is thought to be especially important for cases of adaptive or ecological speciation. Postzygotic extrinsic selection against hybrids is seen as a hallmark of ecological speciation because of trade-offs in resource use and allocation that arise during adaptation to different habitats (Schluter 2000). Taxa that are evolving via ecological speciation are predicted to exhibit selection against hybrids since postzygotic selection against hybrids is the only direct way that local adaptation can reduce gene flow (Hatfield and Schluter 1999, Schluter 2000, Nosil 2012). Even if recombination were to completely reshuffle the genome each generation, near-absolute postzygotic selective filters could reassemble locally adapted phenotypes from standing genetic variation over the course of a single generation. While selection of this strength is widely considered to be unrealistic (Kirkpatrick and Ravigné 2002, Coyne and Orr 2004) the potential role of postzygotic natural selection in structuring hybrid zones and preventing species fusion is not known (Bolnick and Fitzpatrick 2007, Schluter 2009, Pinho and Hey 2010).

The most compelling evidence for the importance of postzygotic selection in maintaining adaptive divergence is the case where species fusion or collapse ensues after an alteration or relaxation of extrinsic selective pressures. For example, natural anthropogenic disturbances can create a conduit for gene flow across otherwise impermeable species boundaries (Kruckeberg 1977, Nason et al. 1992, Bleeker and Hurka 2001, Mitsui et al. 2010) and can lead to a reduction in phenotypic diversity due to species fusion, or an increase in diversity through the production of recombinant hybrid lineages (Stebbins 1959, Arnold 1997, Rieseberg and Carney 1998). Anderson (1948, 1949) documented many cases where disturbance led to an increased prevalence of advanced generation hybrids and backcrosses expressing a wide variety of phenotypes, a special type of hybrid zone known as a hybrid swarm. Hybrid swarms are typically characterized by an increase in phenotypic diversity, distinguishing them from the process of species fusion which implies a decrease in phenotypic diversity (Stebbins 1950, Coyne and Orr 2004).

Beyond simply noting the pattern, Anderson (1948) provided a clear hypothesis for the formation of hybrid swarms that invoked strong selection and the creation of novel habitats, a process he termed "hybridization of the habitat." Anderson envisioned disturbances as creating novel combinations of environmental parameters that would provide suitable niches for recombinant hybrids that possess new combinations of traits that matched the novel environments. Anderson's hypothesis does not necessarily predict that trait variances will increase, but it does predict that trait covariance should decrease due to the decoupling of parental trait correlations by recombination. Habitat diversity should increase and correlations should be present between hybrid phenotypes and habitat characteristics indicating sorting along environmental gradients. This strong selection hypothesis requires that novel habitats be created and is supported by the observation that hybrid species typically inhabit habitats different than those of the parental lineages (Rieseberg and Carney 1998).

An alternative to Anderson's hypothesis was proposed by Grant (1971), who noted that relaxed selection due to increased resource availability or decreased competition could explain the proliferation of recombinant hybrid phenotypes at disturbed sites. An extreme case would be a competitively neutral hybrid zone where all phenotypes performed equally well provided they are able to disperse to the high-resource habitat. This type of hybrid zone should exhibit decreased trait covariance as in an Andersonian hybrid swarm, but there should be no correlations between hybrid phenotypes and microhabitat characteristics. There should also be evidence of an increase in resource availability due to direct inputs of limiting resources or indirect effects of a reduction in competition.

The hybrid zones between *Encelia palmeri* and *E. ventorum* (Asteraceae) are ideal systems in which to test the importance of postzygotic selection in maintaining divergence. All species in the genus *Encelia* maintain complete interfertility and readily hybridize (Clark 1998). However, phenotypic fusion is not observed and hybrids are associated only with disturbances and ecotones between habitats. Detailed observations by Kyhos et al. (1981) indicated that while hybrids with intermediate F_1-like phenotypes were common at the ecotone between habitats, there was a conspicuous absence of backcross phenotypes and hybrids did not exist outside of the narrow ecotone. Greenhouse crossing studies further corroborated the ability to produce viable F_2, backcross and advanced generation hybrids with no decrease or increase of fertility or vigor (Kyhos et al. 1981). However, they also noted that hybrid swarms containing phenotypes indicative of backcrossing and advanced generation hybridization could be found in numerous locations always associated with natural or anthropogenic disturbances. Thus, the authors hypothesized that the maintenance of postzygotic barriers across the ecotone was almost exclusively controlled by strong postzygotic natural selection due to adaptation to highly divergent habitats.

This hypothesis was supported in recent experimental work, showing that extremely strong divergent selection exists between the desert and dune habitats inhabited by *E. palmeri* and *E. ventorum*, and that selection was the result of at least four independent ecological mechanisms (Chapter 2). Thus, we hypothesized that despite the continuous production of recombinant offspring, postzygotic natural selection is effective in removing these individuals from the population, thereby maintaining the phenotypic discreteness of parental taxa. In contrast, alteration of selective pressures such as through a relaxation of selection or the creation of novel habitats could result in the increased prevalence of recombinant forms.

I sought to infer the processes maintaining species boundaries between *E. palmeri* and *E. ventorum* by examining the patterns of phenotypic variation at hybrid swarms and undisturbed hybrid zones. We additionally examined trait variation at juvenile and adult life stages. If divergence between these species is being maintained by postzygotic natural selection then I predict abundant progeny of recombinant phenotypes to be produced but for these phenotypes to be absent among mature plants. At disturbed hybrid zones, we hypothesize that the increase in water availability, a limiting resource, relaxes selection such that the recombinant progeny being produced are able to persist relative to the strong selection present in undisturbed sites. Alternatively, the distribution of phenotypes present in disturbed and undisturbed habitats may be the same, or there may be no evidence of increased resource availability which would not support a relaxed selection hybrid zone as envisioned by Grant (1971).

In order to address this hypothesis we performed a quantitative analysis of phenotypic variation at different life history stages and across a range of disturbance histories in order to answer the following questions: 1) Are recombinant phenotypes present in seedlings that do not occur in the adult population? 2) Does disturbance result in an increased prevalence of putatively recombinant phenotypes? Comparison of phenotypic variance and covariance in areas with and without altered selection pressures provides a natural experiment with which to test the importance of extrinsic isolation in maintaining divergence. We predict that decreased phenotypic covariance will be seen for traits under selection but not for performance or putatively neutral traits. We also predict that traits that can function as fitness proxies should exhibit an increase in variance or a shift toward higher values in the disturbed habitats without necessarily showing a decrease in trait covariance, which would indicate an increase in resource availability. Finally, we predict that there should be no conclusive pattern among traits that are selectively neutral and that do not reflect overall plant health.

Previous uses of phenotypic correlation structures and variance-covariance matrices include studying phenotypic or morphological integration (e.g. Pigliucci 2003) and inference of the evolutionary trajectories of populations through time (e.g. the "P-matrix" in quantitative evolutionary genetics; Cheverud 1988, Eroukhmanoff and Svensson 2008). However, for this study we are only using patterns of trait variance and covariance to test specific hypotheses about how the multivariate trait space occupied by a population should change under different selective regimes if postzygotic selection is a strong factor maintaining species boundaries. Specifically, we predict that the average strength of trait correlations will be lower in juvenile progeny than adult plants, and lower in disturbed than in undisturbed hybrid zones. Furthermore, phenotypes indicative of recombination should be present in disturbed sites and in juvenile progeny but not mature plants in undisturbed hybrid zones.

Methods

Site descriptions

Five hybrid zones were selected for detailed quantification of phenotypic variation (Figure 1). Two of these sites were undisturbed and three sites were subject to either natural or anthropogenic disturbance. Disturbance in this case is used sensu Sousa (1984) to refer to discrete events that remove individuals opening up sites available for colonization. However, as recognized by Sousa (1984), disturbance lies on a continuum of environmental perturbations that includes at one end punctuated events that do not

Figure 1: Site map of the Vizcaíno Peninsula in Baja California Sur, México showing the locations of the five hybrid zones studied. Yellow indicates undisturbed hybrid zones and red indicates undisturbed hybrid zones.

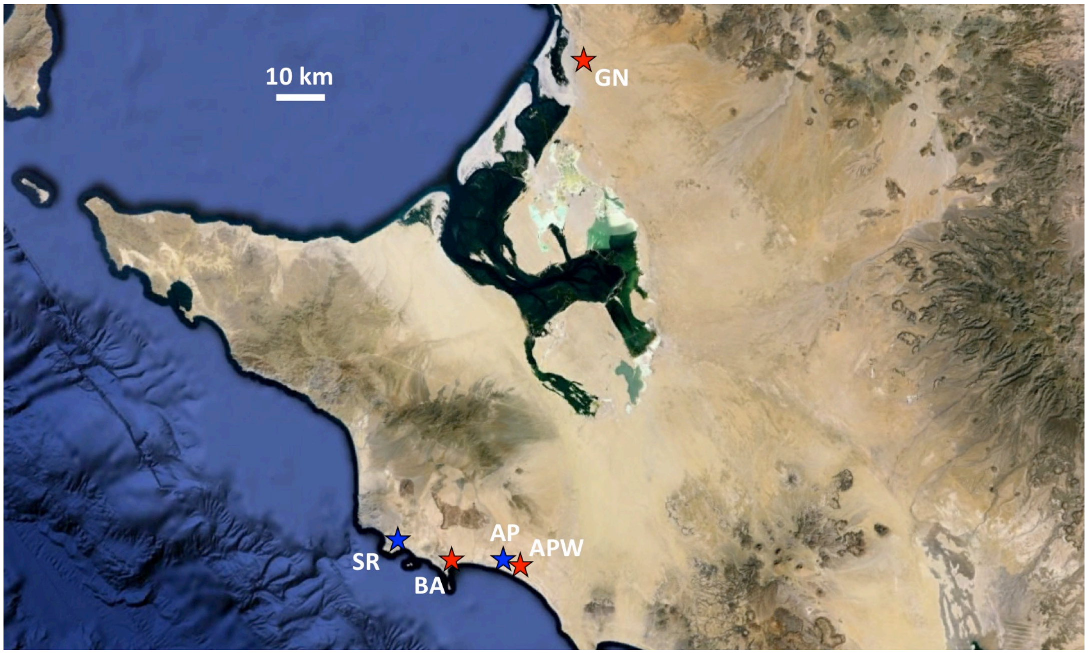
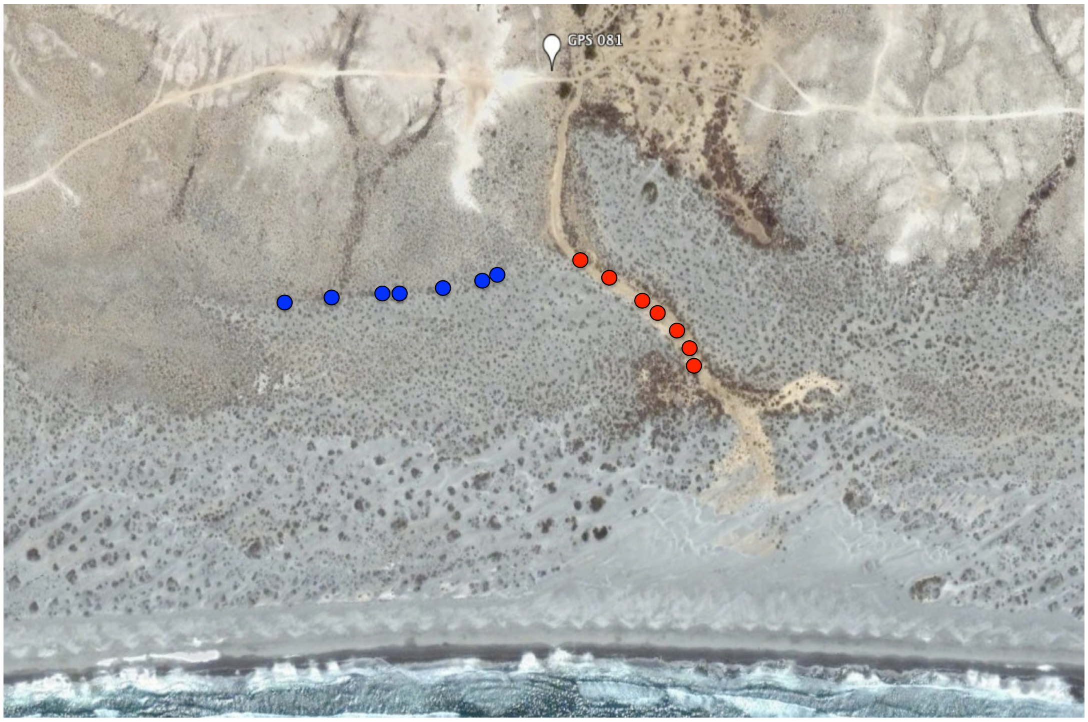


Figure 2: Map of the Arroyo Polvoso site (AP/APW) with the locations of the plots in the disturbed wash habitat in red and the adjacent undisturbed site in blue.



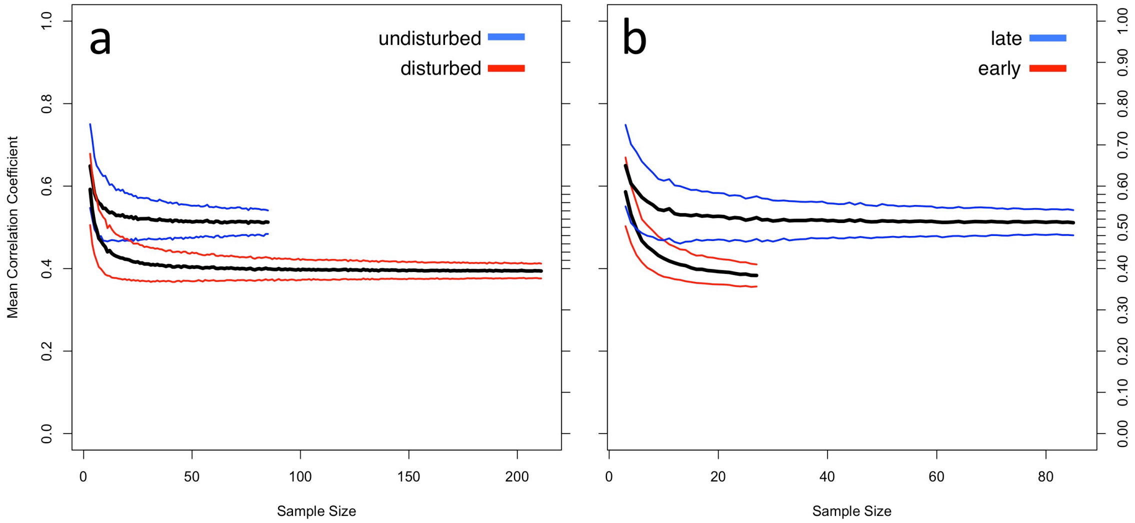
permanently change the long-term underlying environmental conditions, and at the other end includes gradual changes to abiotic or biotic forcing conditions. All of the disturbed sites in this study fit the narrow-sense definition of disturbance, but both punctuated and gradual changes have the potential to change the outcome of ecological or evolutionary processes thus the results of this study may apply more broadly to environmental perturbations in general.

The two undisturbed sites were located ten kilometers apart and consist of active sand dunes that end abruptly where the back-dune desert habitat begins, with a linear ecotone separating the two habitats where the majority of hybrids are found. *Encelia palmeri* is limited to the back-dune desert habitats and *E. ventorum* is limited to the dune habitats. The first undisturbed site, San Roque (SR), is the location of the reciprocal transplant experiment described in Chapter 1 and at this location both mature plants and juvenile seedlings were phenotyped. The second undisturbed site, Arroyo Polvoso (AP) is located ten kilometers south of SR. Only mature plants were phenotyped at AP and the rest of the hybrid zones. The undisturbed AP site was paired with a disturbed site, Arroyo Polvoso Wash (APW), that consists of an intermittent stream bed bisecting the dune and desert habitats that is immediately adjacent to undisturbed habitat (Figure 2 and 3). The natural disturbances at this site are flash floods that occur infrequently but remove all vegetation in the wash bottoms. While no flooding occurred during the course of the study, evidence of recent flooding included flotsam stuck in the branches of shrubs up to one meter above the wash bottom. The other disturbed sites were Bahía Asunción (BA) and Guerrero Negro (GN). The BA site is located between AP/APW and SR on the south end of the town of Bahía Asunción. This site exists on a patch of land that had been graded for development but subsequently abandoned. The GN site is located approximately 100 kilometers east of the BA site, on the side of Highway 1 approximately fifteen kilometers south of the town of Guerrero Negro. This population occurred in mixed soil types in an area that exhibited evidence of grading from the construction of the highway as well as more recent evidence of off-road vehicle traffic and dumping of garbage. Undisturbed dune-desert habitat was observed approximately 200 meters to the west of the population under study but no plots were established there due to time constraints.

The phenotypes of the parental taxa are well differentiated and represented in the upper left and lower right corners of Figure 4. *Encelia palmeri* has densely pubescent entire leaves while *E. ventorum* has highly dissected, glabrous succulent leaves. At disturbed sites, populations of *Encelia* exhibited a wide variety of phenotypes that spanned the range of phenotypes exhibited by *E. ventorum* and *E. palmeri*, and each of these sites was within close proximity to undisturbed, phenotypically "pure" populations of both putative parental taxa. Disturbed sites were chosen based on proximity to undisturbed sites and the existence of both parental taxa nearby. In no case did we observe a disturbance with both parental taxa present that did not include intermediate phenotypes. Despite this, no evidence of phenotypic blending is apparent in parental taxa growing in undisturbed habitats regardless of their proximity, although intermediate phenotypes do exist where habitats abut (Kyhos et al. 1981).

Figure 3: Photograph of the disturbed Arroyo Polvoso wash site (APW). The four large plants in the center of the photograph are what phenotypically resemble *Encelia palmeri* (flowering in the foreground), *E. ventorum* (light green plant in the background) and two hybrid plants in between, illustrating the range of phenotypes present.

Figure 5: Bootstrapped mean pairwise correlation coefficients and standard errors for plants in disturbed (red) versus undisturbed (blue) hybrid zones (a) and at juvenile (red) versus mature (blue) life history stages (b). Means are the average of 10 pairwise trait correlations, and estimates are the average of 1000 permutations for each sample size.



Field sampling and trait measurement

At each of the two undisturbed and three disturbed sites, mature naturally-occurring plants were assayed for a suite of morphological traits. At one of the undisturbed sites juvenile progeny were also assayed for the same suite of traits. The purpose of this comparison is to determine whether there are differences between the phenotypes of mature plants and the pool of phenotypes that are being produced under natural conditions. Phenotypes of juvenile plants were obtained by collecting seed from naturally-occurring hybrid plants growing at the San Roque hybrid zone and germinating them in greenhouse flats. These plants were used in a different experiment and were planted into the field and allowed to grow under natural conditions for one growing season (see Chapter 1 for details). Traits were assayed on the one-year-old plants at the end of the growing season, thus the distribution of phenotypes remaining may be affected by selection acting during this time interval. However, selection should result in a conservative estimate of the true range of recombinant phenotypes being produced. I hypothesize that phenotypic variance should be higher in juvenile plants than in mature plants because selection will remove recombinant phenotypes from the population whereas selection acting on juvenile plants before trait measurement would tend to reduce differences in the variance of juvenile versus mature plants. While this source of juvenile plants is biased in favor of hybrid phenotypes and thus cannot be used to determine the relative abundance of phenotypes being produced across the entire hybrid zone, the goal of this comparison is to determine if progeny are being produced with phenotypes that exceed the range of what is present in the adult population, suggesting that postzygotic selection is enforcing trait correlations among parental taxa.

Diagnosis of hybrid plants based on phenotype was made visually in the field based on the intermediacy of phenotypes. Samples of all plants measured were preserved on silica gel for future genotyping, however no genetic information is presently available to determine the exact composition of taxa that appear phenotypically "hybrid" or "parental". In this system, the distinction between "parental" and "hybrid" phenotypes are often continuous and it is not known to what degree a plant's phenotype is indicative of its genotype. However, because phenotypic plasticity is expected to play a minor role at the scales of trait variability studied here (see below) and because most of the traits measured are highly heritable, the conclusions of this study are not contingent on knowing the genetic composition of the individual plants studied.

At each site we randomly established seven 10 × 10 meter plots located in the center of the primary zone of hybridization. Due to the narrowness of the undisturbed ecotones, these plots included a range of phenotypes and habitats. The only exception was the BA site, which was too small for more than one plot. At the disturbed hybrid zones we placed plots in the geographic center of the area of greatest hybrid and parental plant density while at the undisturbed hybrid zones we established plots parallel to the linear ecotone separating dune and desert habitats. In each plot we measured leaf traits from every plant by sampling between three and seven turgid, mature, undamaged leaves and averaging trait measurements per plant in all subsequent analyses. Ten morphological traits were measured for each plant. We chose morphological traits shown in close relatives to be adaptive (Ehleringer and Björkman 1978, Ehleringer 1988) as well as some that have no putative adaptive function. Not all traits are independent; some traits are calculated from each other and many traits are likely related through developmental constraints and/or epistasis and pleiotropy. Thus, phenotypic trait correlations reflect the totality of processes affecting trait variation and covariation between two populations (Armbruster and Schwaegerle 1996). Photographs were taken of all leaves under constant illumination. Leaf area, shape, width and length were measured using Image-J (http://imagej.nih.gov/ij/). Leaf color was measured in Photoshop (Adobe Systems Inc.) by measuring the pixel intensity off of the center of the lamina in an eight-bit converted greyscale image of each leaf adjusted to a color scale included in the photographs. Leaf lamina and petiole thickness were measured with digital calipers. Leaf mass was measured by weighing leaves that had been dried for one week in a low-humidity environment, with a number of leaves dried additionally in a kitchen oven to verify that all samples were fully desiccated. Specific leaf area (SLA) was calculated by dividing the area by the mass. Petiole:leaf lamina thickness and leaf length:width ratios were calculated from data, and while they are not independent from other traits they may reflect alternative aspects of ecological strategies and so were included in the analysis.

An important caveat when interpreting phenotypic data from plants grown under a range of conditions is the degree to which variation in phenotypes is due to phenotypic plasticity (Schlichting 1986). Data from a reciprocal transplant experiment performed with the same taxa at the Bahía Asunción site suggests that univariate phenotypic plasticity within taxa is a minor component of phenotypic variation at hybrid zones relative to the differences between taxa (Chapter 1). The variation among taxa within sites is always much larger than the variation within taxa among sites, sometimes by several orders of magnitude for the same traits measured in this study. Furthermore, the direction of plasticity was frequently counter-gradient (Eckhart et al. 2004) such that variation within taxa was opposite in sign to the direction among taxa; for some traits taxa actually became more phenotypically divergent when grown in the same habitat. Together these results suggest that phenotypic plasticity is unlikely to be responsible for the range of phenotypes expressed at these hybrid zones. Future research is warranted in order to determine the magnitude of phenotypic plasticity over a larger range of environmental conditions. Additionally, the magnitude of ontogenetic variability is much smaller than the difference between populations (Chapter 1), indicating that leaves of young plants are morphometrically very similar to the leaves of old plants unlike in many plants where marked differences between new versus old leaves exist.

Data analysis

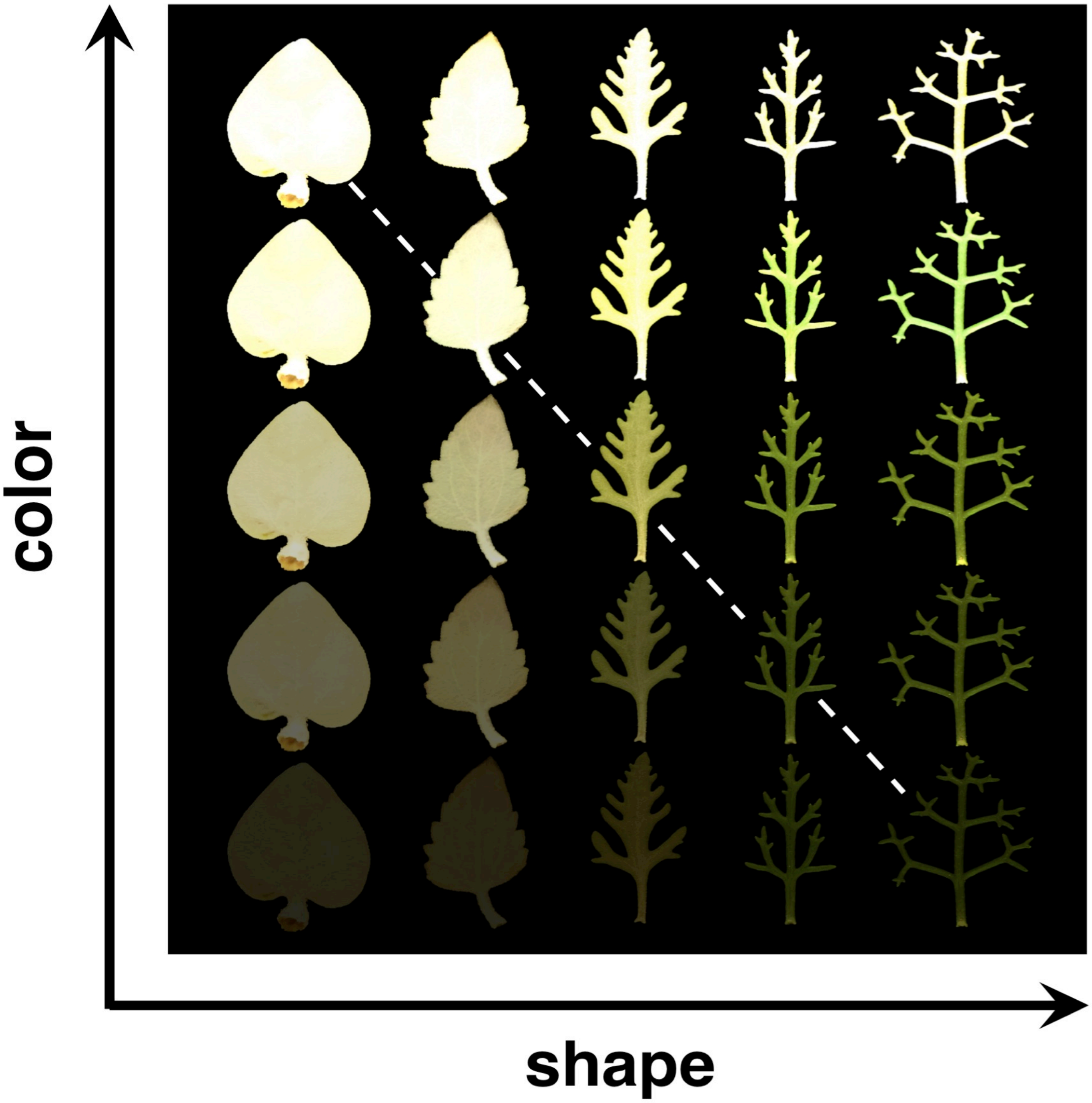
To test for differences in trait variation among sites we first examined pairwise trait correlations in disturbed versus undisturbed sites and at juvenile and mature life stages. Figure 4 illustrates the method of using trait correlations to infer the relative degree of putative recombination between taxa for two traits (Riley 1938, Anderson 1939). Parental phenotypes are represented in the upper left (*E. palmeri*) and lower right (*E. ventorum*) with lines connecting intermediate phenotypes representing perfectly covarying combinations of the two traits. In contrast, phenotypes in the upper right and lower left corners represent decoupling of one trait from the other, reducing the covariance. The presence of recombinant-like phenotypes in the population indicates that advanced generation hybridization and backcrossing is likely occurring. Importantly, for traits that are tightly linked or that are the result of pleiotropy these recombinant phenotypes would not be possible illustrating a potential unknown genetic constraint on the range of phenotypes possible (Arnold 1992).

The greater the degree of recombination the lower the average correlation coefficient should be since novel combinations of traits represent deviations from the multivariate axis maximally separating parental taxa. In Figure 1 only two dimensions are shown whereas in reality this trait space would be n-dimensional and the major axis represented by the white line would be equivalent to the first principal component of the data and to the primary eigenvector of the phenotypic variance-covariance matrix (Lynch and Walsh 1998). A low average correlation coefficient likewise corresponds to a low primary eigenvalue indicating a breakup of trait correlations presumably due to recombination.

A number of visualization techniques were used to explore the hypothesis that trait covariance breaks down in predispersal progeny and at disturbed hybrid zones. We first calculated the mean pairwise correlation coefficient (Pigliucci 2003) for disturbed-site, undisturbed-site and juvenile undisturbed progeny and rarefied the data by resampling with replacement up to the original sample size to estimate confidence intervals and to determine the sensitivity of the correlation coefficient to sample size. To do this we first estimated the mean correlation coefficient and standard error for each of the disturbed, undisturbed, and juvenile categories on the full datasets. We then sequentially reduced the sample size and calculated the mean and standard error for each of 1000 random draws from the original data and plotted the mean and standard error as a function of sample size. We also examined individual pairwise trait correlations to determine whether there is an expanded trait space occupied by juvenile plants and plants from disturbed habitats, indicating that recombinant forms are being produced and persisting. To do this pairs of traits were plotted against each other and convex hulls drawn around points from disturbed versus undisturbed habitats, and juvenile versus adult stages.

The prediction is that the juvenile plants and plants from disturbed habitats will exhibit lower covariance without necessarily exhibiting greater trait variance, indicating that phenotypes with combinations of traits missing in the undisturbed sites are persisting. Comparison of variances for individual traits were analyzed using a Fligner-Killeen test for homogeneity of variances (Sokal and Rohlf 2012). Finally, we constructed correlograms in order to visualize multivariate patterns of trait correlations (Tedin 1925, Anderson and Anderson 1954, Clausen and Hiesey 1960). To do this lines were drawn connecting traits with the thickness of the line proportional to the correlation coefficient.

Figure 4: Hypothetical data illustrating the various phenotypes created by independently varying two leaf functional traits. Phenotypes in the upper left and lower right corners represent parental phenotypes. Hybrids connected with lines represent the major axis of phenotypic variation between both parental taxa. Phenotypes in the upper right and lower left corners represent putative recombinant phenotypes.



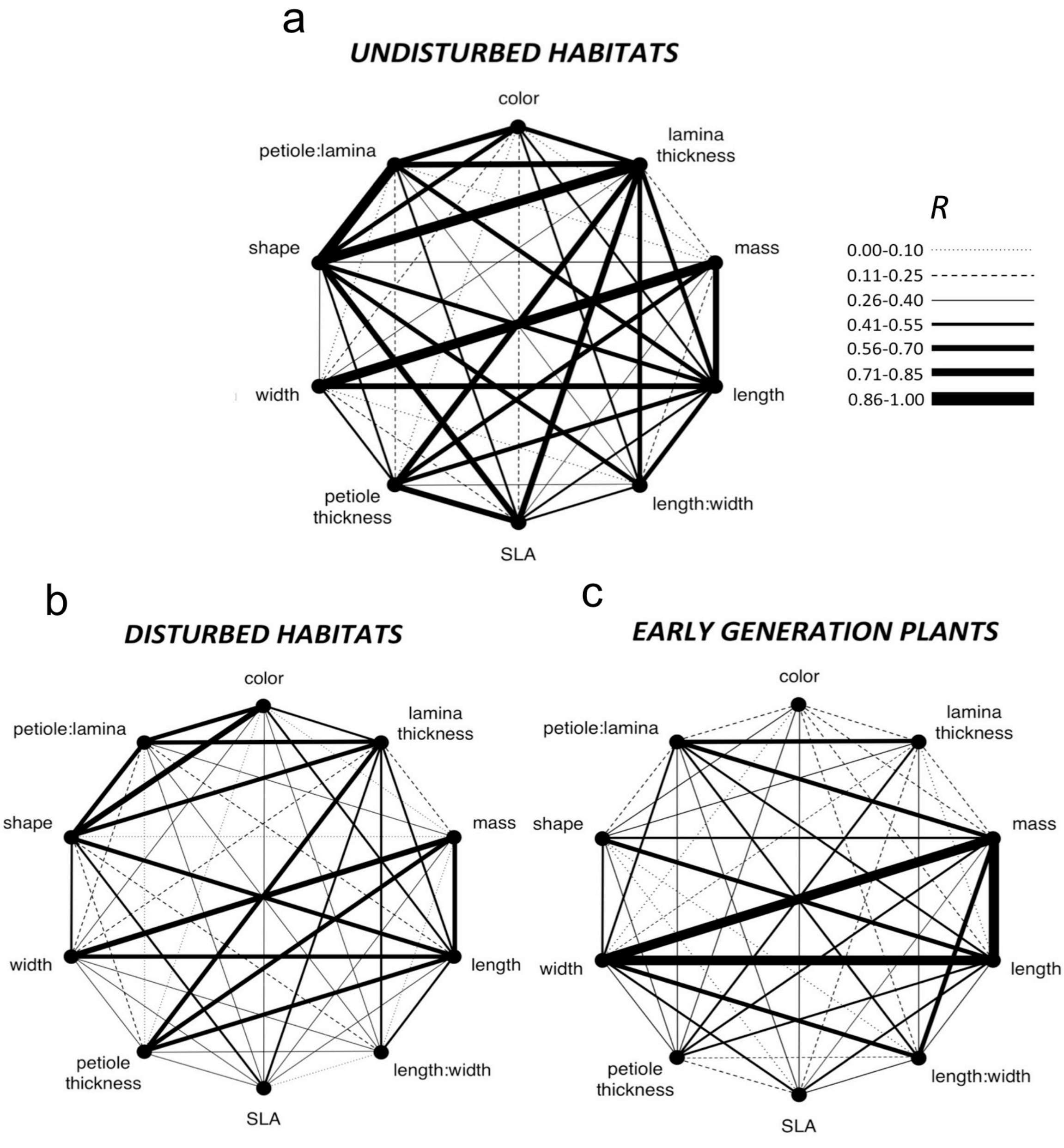
Results

Site observations suggest that all three disturbed sites were also locations of higher resource availability compared to adjacent undisturbed sites. These observations are significant because distinguishing between Andersonian versus neutral hybrid swarms requires knowledge of whether increased resource availability is associated with or caused by the disturbance under study. In all three disturbed hybrid zones plants were larger and were continuing to flower even when most other plants in undisturbed habitats had stopped flowering. The proximity of water to the surface in the wash habitat was indicated by standing pools of fresh water at the lowest elevations, indicating that the wash bottom is a site of increased water availability relative to the surrounding dune and desert habitats that are between one and five meters higher in elevation. A periodic reduction in competition may also increase resource availability. The population near the roadway likely experienced increased water inputs due to runoff from the pavement, a common pattern in plants from arid environments. Finally, the population in the graded site likely experienced increased water availability due to reduced competition and inputs of subterranean water from surrounding residential developments.

Overall, trait correlations were significantly stronger in undisturbed hybrid zones than in disturbed hybrid zones, and in adult than in juvenile plants (Figure 5). This pattern also held when comparisons were limited to geographically co-located populations at Arroyo Polvoso and San Roque (Supplemental Figure 1). Correlograms revealed the same pattern (Figure 6) and provide additional information about which traits are contributing to the overall pattern. For mature plants growing at undisturbed hybrid zones strong correlations were found between traits that are diagnostic for parental taxa. Lamina thickness, the ratio of petiole to lamina thickness, leaf shape, and leaf color were all strongly correlated and these same three traits perfectly separate out parental taxa in a principal components analysis (Supplemental Figure 2). In contrast, trait correlations in juvenile plants are much weaker, with most traits effectively decoupled from each other. Importantly, although the juvenile plants had a lower sample size, simulations show that this would bias correlation coefficients towards higher values thus the reduction in trait correlations is expected to be conservative. Furthermore, four juvenile plants expressed phenotypes outside the range of what is present at mature undisturbed sites in two different directions even despite the small sample size.

The strongest correlations and the only correlations that were substantially stronger in the juvenile plants were the correlations between leaf length, width and mass. These three traits are linked through development, thus recombination potentially would not be as effective in breaking up correlations among these traits. A similar reduction in overall trait correlation strength was found in plants from the disturbed site, whereby nearly every correlation decreased in strength compared with the undisturbed site. The strongest correlations at the disturbed sites were between leaf mass, length and area as mentioned previously and also between leaf color and shape although the strength of these correlations was still weak.

Figure 6: Correlogram illustrating the magnitude of pairwise Pearson's correlation coefficients for mature plants at disturbed hybrid zones (a), mature plants at undisturbed hybrid zones (b) and juvenile plants at an undisturbed hybrid zone (c).



Plotting pairwise combinations of traits allowed examination of the individual patterns of variation (Figure 7a-e). Due to practical limitations all possible pairwise combinations are not shown. The choice of which traits to plot does not affect analysis of trait variance but will affect which covariances are considered. Drawing convex hulls showed results consistent with the idea that novel phenotypes were more prevalent among juvenile plants and in disturbed habitats compared to undisturbed habitats (Cornwell et al. 2006). The trait combinations found in mature plants growing in undisturbed habitats are generally a subset of those found in disturbed habitats or early life history stages from undisturbed habitats, although substantial variation existed among traits. The traits most diagnostic for parental taxa are leaf thickness, shape and color (Supplemental Figure 2) and these traits generally showed the clearest patterns indicating an expansion of trait space occupied by the taxa in disturbed habitats (Figure 4a-b).

For leaf shape and thickness (Figure 7a) plants growing in disturbed and undisturbed habitats had a similar trait variance (Fligner test $p>0.05$) but had reduced covariance in the disturbed habitats. For juvenile versus adult plants, the trait variance was decreased in juvenile plants (Fligner test $p<0.05$), although this may have been due to the small sample size of juvenile plants available. Despite the small sample size, four individuals expressed phenotypes outside the bounds of what was represented in the adult population reducing the covariance below that of the mature plants. For leaf color and the ratio of the thickness of the leaf petiole to leaf lamina (Figure 7b), an excess of highly reflective leaves with low petiole:lamina thickness ratios were observed in mature plants from disturbed sites and among juvenile plants compared with plants from undisturbed habitats and in both cases the trait covariance decreases. However, there were also a large number of phenotypes found only among the mature plants from undisturbed habitats, including an abundance of plants with high petiole:lamina thickness ratios and low reflectivity (i.e. green rather than white leaves).

For leaf mass and length (Figure 7c) traits were highly correlated in all comparisons. Despite this, disturbed-site and juvenile plants exhibited a large expansion of the trait variance toward larger leaves (Fligner test, $p<0.01$) reflecting the plants' larger overall size. Likewise, the smallest leaves were found only in plants from undisturbed habitats. Despite the overall strong trait correlations, trait covariance was slightly reduced in the disturbed habitats versus undisturbed habitats while it did not change between juvenile and adult plants. For the ratio of leaf length:width versus petiole thickness, no clear patterns emerged (Figure 7d). Trait variance increased in plants from disturbed versus undisturbed sites, however trait variance decreased from juvenile to mature plants. In disturbed habitats there was an excess of plants with thick petioles compared to undisturbed habitats, whereas in mature plants there was an excess of thin petioles compared to juvenile plants. Trait covariance was similar in all comparisons. For specific leaf area versus leaf width, disturbed sites showed an increase in trait variance (Fligner test, $p<0.05$) although no change in covariance relative to undisturbed sites. For juvenile plants, variance in leaf width was higher than in mature plants (Fligner test, $p<0.05$) while the variance in SLA was lower (Fligner test, $p<0.05$). However the trait covariance was the same.

Figure 7a: Correlation between leaf shape and leaf thickness for disturbed versus undisturbed sites (left panel) and juvenile versus mature plants (right panel).

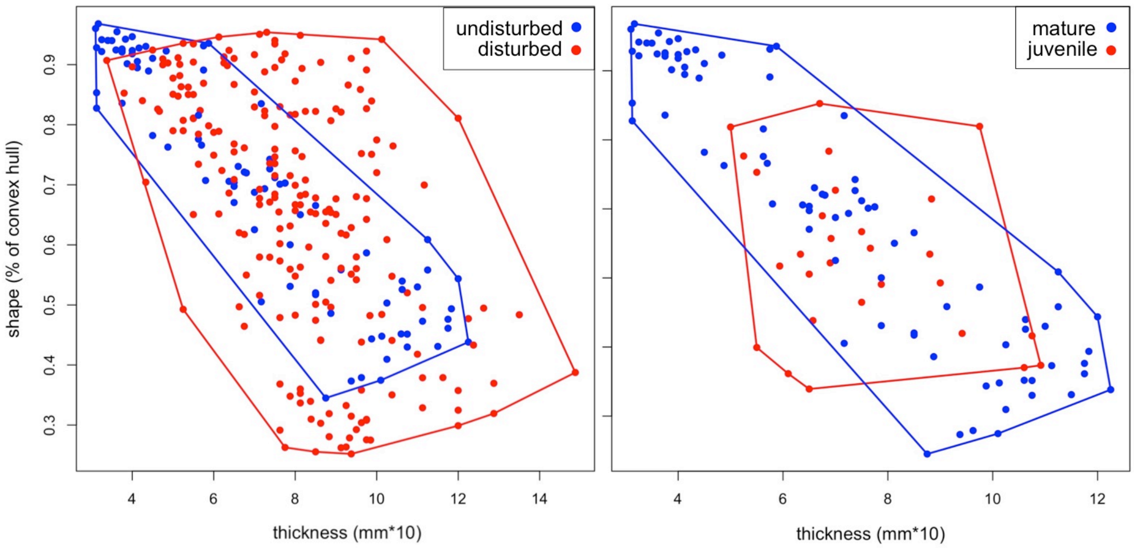


Figure 7b: Correlation between leaf color and petiole:lamina thickness for disturbed versus undisturbed sites (left panel) and juvenile versus mature plants (right panel).

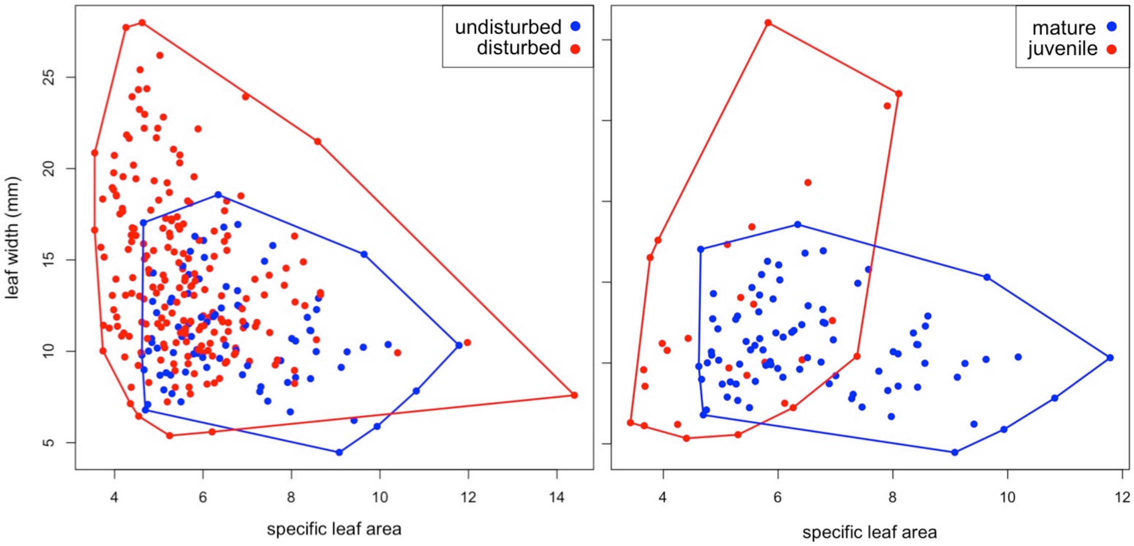


Figure 7c: Correlation between petiole thickness and leaf length:width for disturbed versus undisturbed sites (left panel) and juvenile versus mature plants (right panel).

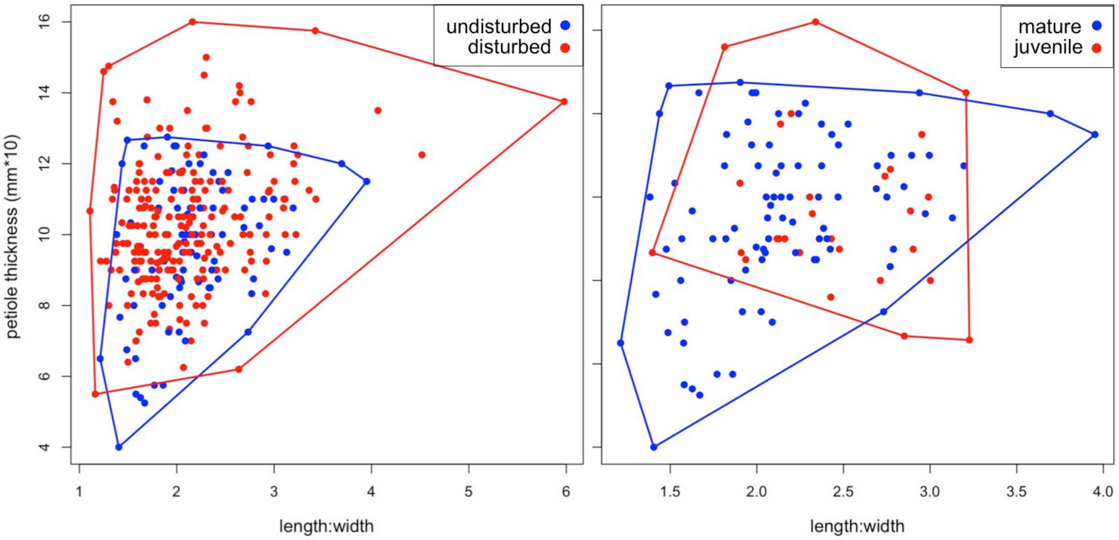


Figure 7d: Correlation between leaf length and mass for disturbed versus undisturbed sites (left panel) and juvenile versus mature plants (right panel).

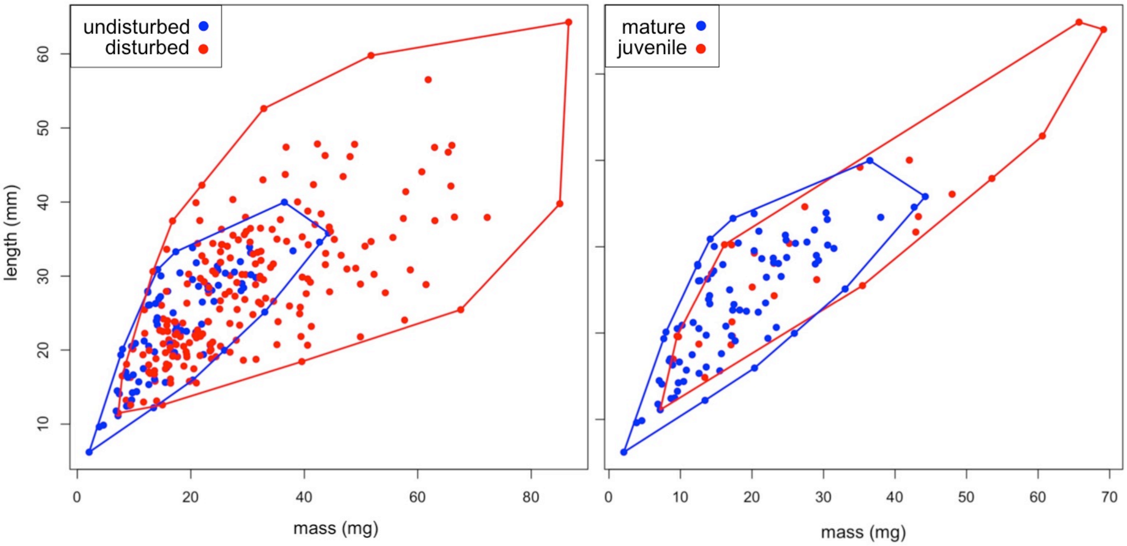


Figure 7e: Correlation between specific leaf area and leaf width for disturbed versus undisturbed sites (left panel) and juvenile versus mature plants (right panel).

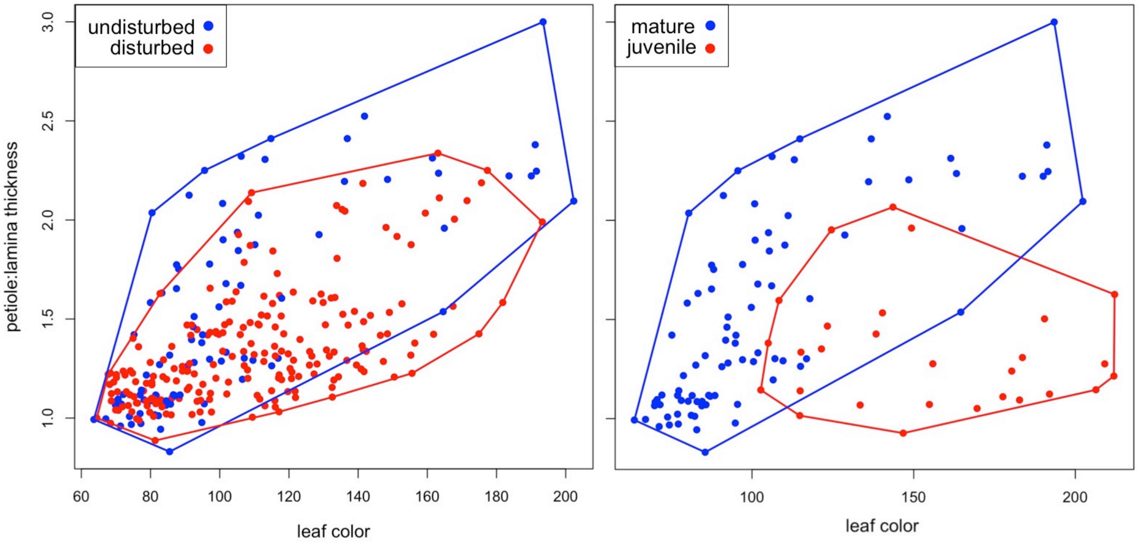
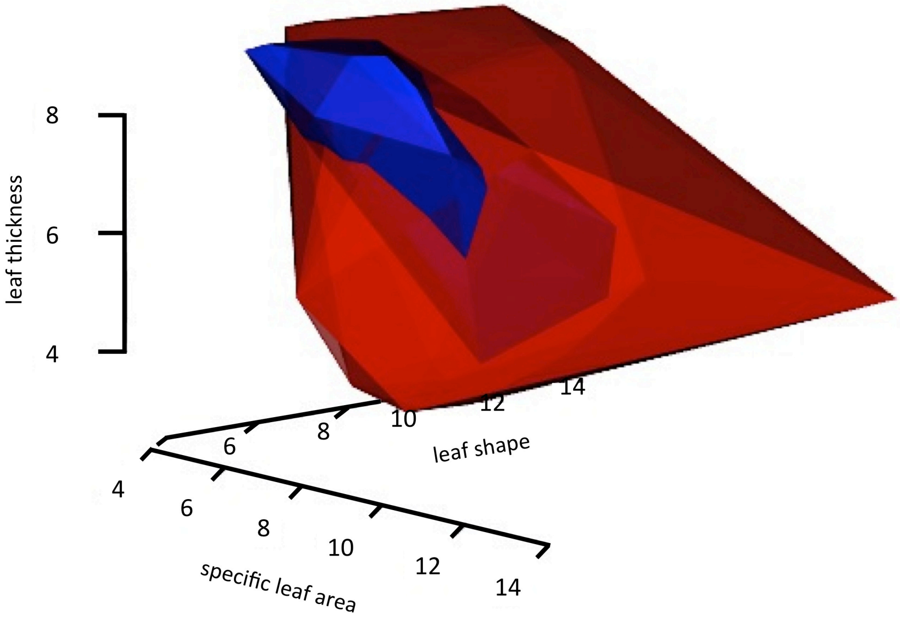


Figure 8: Three-dimensional graph of trait space for all pooled disturbed and undisturbed sites showing the almost complete envelopment of the trait space from undisturbed habitats (blue) by that of disturbed habitats (red). Leaf thickness (mm×10), specific leaf area (mm^2/mg), leaf shape (% of the smallest convex hull enclosing the leaf not occupied by leaf lamina).



Discussion

The findings of this study indicate that phenotypes suggestive of recombination appear to be produced but eliminated before maturity in undisturbed hybrid zones as evidenced by a decoupling of trait correlations in juvenile progeny and recovery of trait correlations in adults at undisturbed hybrid zones. In contrast, adult plants at disturbed hybrid zones exhibited a wide range of phenotypes spanning the range of parental trait combinations and also including combinations that were not present in the undisturbed sites. These results suggest that the recombinant phenotypes being produced persist into adulthood in disturbed sites, likely due to an alteration or relaxation of extrinsic selection. These results are also consistent with previous experimental work indicating a primary role for postzygotic extrinsic isolation in maintaining divergence between *Encelia palmeri* and *E. ventorum*.

While selection appeared to reinforce correlations among mature plants at undisturbed sites for some traits, other traits showed no difference in disturbed versus undisturbed sites or in juvenile versus mature plants. This is likely because trait correlations reflect a number of different processes in addition to selection. Foremost, trait correlations may reflect underlying structural, developmental or genetic constraints that prevent certain combinations of traits from ever being expressed (Arnold 1992, Armbruster and Schwaegerle 1996). This can include combinations of traits that are impossible due to trade-offs in resource use and allocation (e.g. photosynthetic rate versus stomatal conductance) as well as allometric constraints (e.g. leaf area versus leaf mass). Among the possible combinations of phenotypes, natural selection can further modify which phenotypes exist in a given environment (Armbruster and Schwaegerle 1996). Postzygotic selection may further reduce trait variance or may increase or decrease trait covariance by allowing a subset of the total possible phenotypes to exist. Finally, the overall shape of trait correlations may also be influenced by the amount of standing genetic variation for certain traits (Barrett and Schluter 2008). Even if a trait combination is physically possible and favored by selection there still may not be sufficient variation in the population.Although these data are observational, the patterns observed here strongly suggest that postzygotic selection is a strong force shaping patterns of phenotypic variance and covariance at hybrid zones and maintaining the discreteness of parental phenotypes (Supplemental Figure 3). These findings are consistent with those of Badyaev and Hill (2000) and other studies that found that selection was the primary cause of variation in P-matrix structure among populations. In cases such as these where selection significantly modifies the phenotypic space occupied, genotypic and phenotypic variance-covariance matrices should be the least similar, providing guidance as to when G- and P-matrices can be used interchangeably (Cheverud 1988, Arnold et al. 2008).

Several observations indicate that these hybrid swarms are due to increased resource availability consistent with Grant's (1971) relaxed selection hypothesis, as opposed to increased environmental heterogeneity and creation of novel microhabitats, as hypothesized by Anderson (1948). First, traits such as leaf mass that may be considered fitness proxies showed strong increases in overall growth in disturbed habitats (Figure 7C). Second, observational evidence strongly indicates that water availability is higher in the disturbed habitats. Finally, examination of the habitats in which hybrids were growing revealed no obvious increase in habitat diversity and likely represented a decrease in habitat diversity since all soil types in the bottom of the wash are well mixed (Figure 3). Plants with radically divergent phenotypes were found growing within meters of each other, thus it is difficult to imagine that plants were responding to precisely delineated niches on the spatial scale studied. While experimentation is required to conclusively test relaxed selection as a mechanism of hybrid swarm formation, all lines of evidence point to increased resource availability as the cause of these hybrid swarms.

Several observations also support the existence of a hybrid swarm as opposed to species fusion. While the two processes have a similar mechanistic basis, hybrid swarms are generally thought of as leading to an increase in phenotypic diversity without a reduction in phenotypic variance, whereas species fusion should to a decrease in phenotypic diversity and may or may not lead to a decrease in trait variance. For functional traits diagnostic for parental taxa, no decrease in trait variance was observed while an increase in putatively recombinant phenotypes did occur, inflating the overall trait space occupied by plants in disturbed habitats. By plotting three of the most strongly diagnostic traits and drawing convex hulls around plants from disturbed versus undisturbed sites (Figure 8) it is clear that the trait space of plants from the disturbed sites almost completely envelops that of mature plants from undisturbed sites. This inflation of phenotypic variance and weakening of phenotypic covariance supports a hybrid swarm rather than a species fusion scenario.

The divergence-with-gene-flow debate traditionally centers around whether natural selection is strong enough to maintain locally adapted gene complexes despite the tendency of gene flow to homogenize differences between populations. Most definitions of gene flow involve two steps, initial hybridization and subsequent introgression of foreign alleles into a population through repeated backcrossing. Epling (1947) distinguished between these two steps by calling the amount of hybridization "potential gene flow" and the amount of introgression "actual gene flow." Epling's distinction was motivated by the observation that in many plant species hybrids are frequently produced yet phenotypic fusion does not occur. In each case, strong extrinsic postzygotic selection was invoked to explain the maintenance of discrete phenotypic entities through the selective elimination of recombinant hybrids. Strong selective filters can thus maintain trait-habitat correlations despite a homogenization of the neutral genome, as is the case of taxa with porous species boundaries (Benson et al. 1967, Pereira and Wake 2009, Rius and Darling 2014). While we do not presently know the rate of gene flow between *Encelia* species in this study, this study and previous work strongly indicate that these taxa represent an example of phenotypic divergence maintained in the presence of very high potential gene flow but low actual gene flow due in this case almost exclusively to strong extrinsic postzygotic natural selection resulting directly from adaptation to different habitats.

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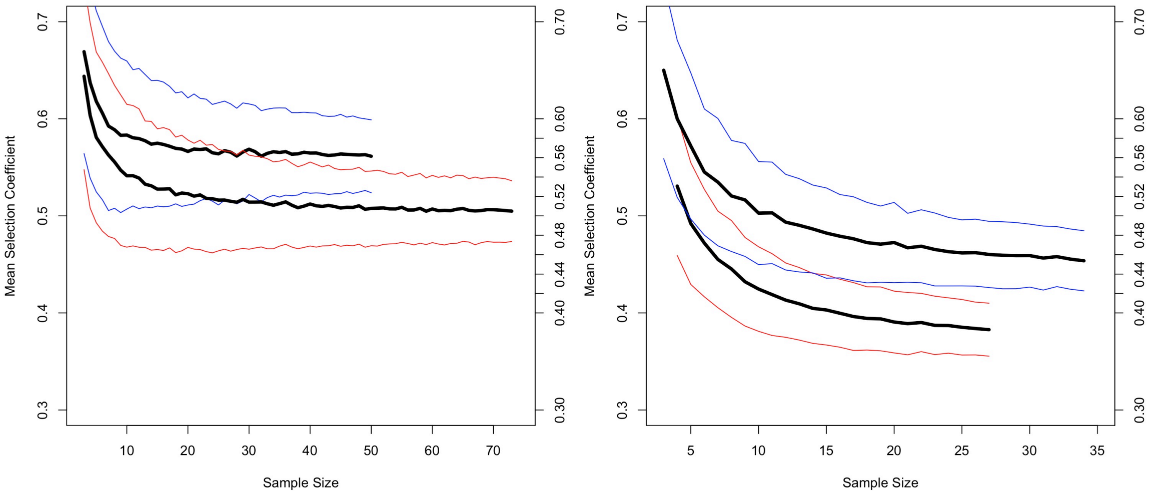
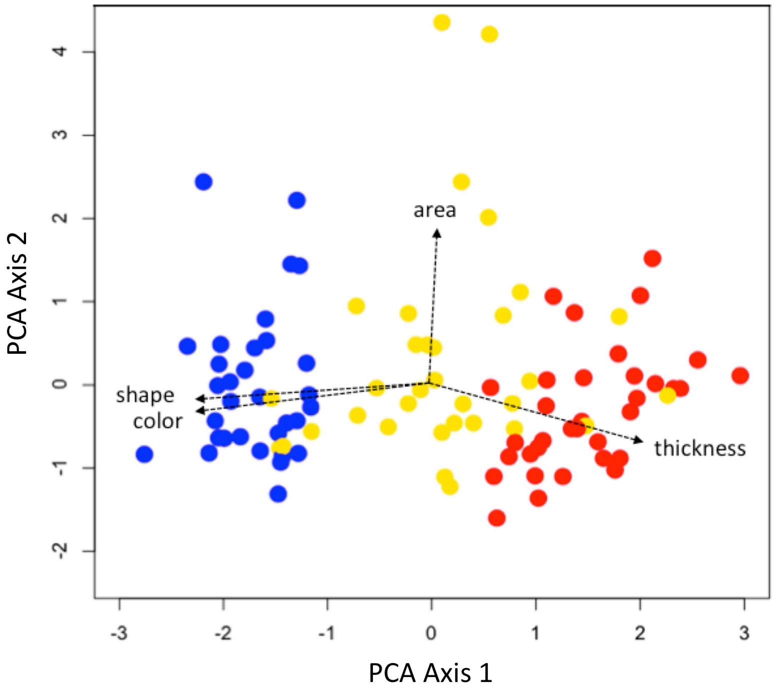
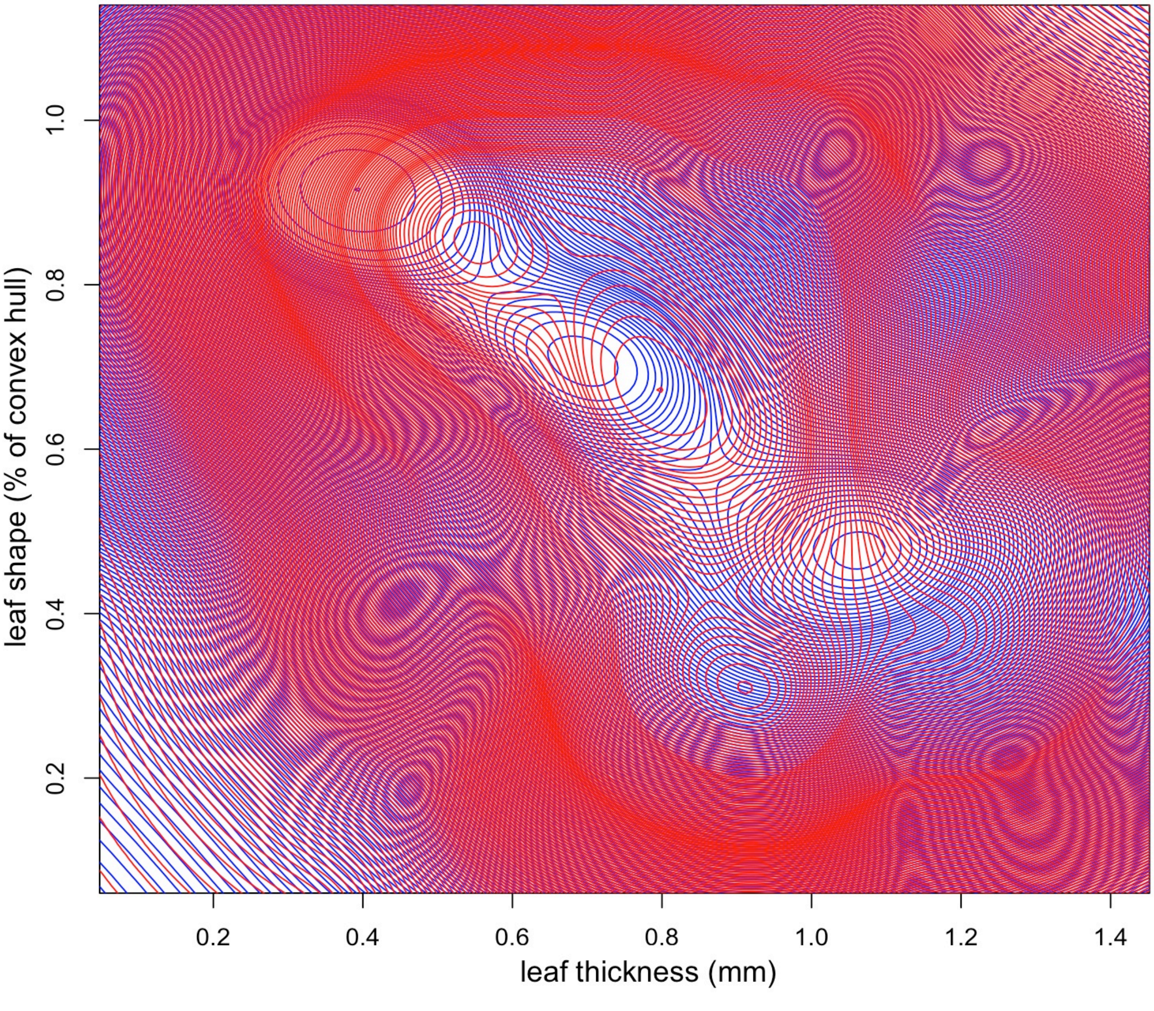
Supplemental Figure 1: Rarefied mean pairwise correlation coefficients and bootstrapped standard errors for plants in disturbed (red) versus undisturbed (blue) hybrid zones at Arroyo Polvoso (left panel), and among juvenile (red) versus mature (blue) life history stages at San Roque (right panel).



Supplemental Figure 2: Principal components analysis of leaf area, shape, color and thickness showing that the latter three traits strongly differentiate parental taxa, while leaf area shows no relationship with taxon and instead is an indicator of overall plant size.



Supplemental Figure 3: Empirical bivariate probability density contours of leaf shape and thickness for undisturbed sites (blue) and disturbed sites (red), directly comparable to the hypothesized results in Figure 4. The three discrete peaks among undisturbed sites (blue) are visible trending from the upper left (*E. palmeri*-like) to the lower right (*E. ventorum*-like) along the major axis with a discrete mode in the middle for putatively hybrid phenotypes. By comparison, the distribution of phenotypes from disturbed sites (red) exhibit less well differentiated peaks that are closer together with shallower intervening saddles and a more amorphous, less linearly correlated overall distribution. If the distribution of phenotypes among mature plants in nature is reflective of patterns of phenotypic selection, then the data shown here are empirical estimates of fitness landscapes for disturbed and undisturbed sites. Contours represent 0.1% increments.



CHAPTER 4

"Hybridization of the habitat" revisited: hybrid swarms, species fusion and ecological mechanisms of species formation

Abstract

Hybrid swarms and species fusion are often associated with habitat disturbances, suggesting that extrinsic barriers to gene flow are important for maintaining species boundaries. This is a significant observation since extrinsic barriers are at the core of ecological models of speciation. In order to gauge support for different mechanisms of species fusion and hybrid swarm formation I reviewed published studies of hybridization in response to changes in external forcing factors and organized the results into a framework based on Anderson's (1948) and Grant's (1971) hypotheses of hybrid swarm formation. The results reveal marked differences in the barriers isolating animals versus plants, with prezygotic barriers more common in animals and postzygotic barriers more common in plants. Within taxa isolated primarily by postzygotic barriers there was substantial support for Anderson's strong selection hypothesis invoking the creation of novel habitats, although there was an equal amount of support for Grant's relaxed selection hypothesis. These examples of extrinsic control over species boundaries support ecological or Darwinian models of speciation and illustrate the diversity of mechanisms via which lineages may fuse or differentiate in response to changing environmental conditions.

Key words: hybridization, disturbance, hybrid swarm, species fusion, species collapse, extrinsic selection

Introduction

Biologists have long noted that hybridization is often associated with habitat disturbance (Kölreuter 1761, Focke 1881, Kerner von Marilaun 1888, Roberts 1929, Wiegand 1935, Anderson 1948, Grant 1971, Arnold 1997, Rieseberg and Carney 1998) and that some species are prone to fusion or collapse due to changes in the external environment (Rhymer and Simberloff 1996, Seehausen et al. 1997, Gow et al. 2006, Hendry et al. 2006, Taylor et al. 2006, Gilman and Behm 2011). Aside from the obvious conservation implications, studies of the different pathways leading to hybrid swarms and species fusion are important because they provide windows into the extrinsic or ecological mechanisms maintaining phenotypic and genetic diversity at hybrid zones. Disturbances can be seen as natural experiments that directly test the importance of extrinsic factors in reproductive isolation, a process that is at the core of ecological or Darwinian mechanisms of speciation (Schluter 2000, Coyne and Orr 2004). If species begin to fuse or recombine following disturbance, determining which barrier or barriers were overcome can answer questions about the role of adaptation in speciation as well as provide insight into which barriers may have caused initial divergence (Rius and Darling 2014).

Both hybrid swarms and species fusion may result from alteration of the physical environment and can provide a conduit for gene flow between populations. Hybrid swarms are hybrid zones where recombination in advanced generation hybrids and backcrosses produces a wide variety of phenotypes that coexist spatially and are characterized by an increase in phenotypic diversity (Anderson 1948, Grant 1971). Species fusion is similar to hybrid swarm formation in that it involves the production of advanced generation hybrids and backcrosses, although species fusion is typically thought of as eventually leading to a reduction in phenotypic diversity (e.g. Kleindorfer et al. 2014) whereas hybrid swarm typically connotes an increase in phenotypic diversity.

Hybrid swarms can also be viewed as intermediate stages in the process of species fusion. Higher rates of gene flow can lead to the complete fusion of two or more lineages or such that gaps or discontinuities in phenotypes or genetic variation cease to exist and taxa become operationally indistinguishable, although during this process phenotypic diversity is expected to temporarily increase as early generation hybrid and backcrosses coexist with parental phenotypes. While the term species fusion is sometimes thought of as occurring more slowly than species collapse (e.g. Kraak et al. 2001, Taylor et al. 2006, Behm et al. 2010, Kleindorfer et al. 2014), the processes involved are the same and I will use species fusion for the rest of this article in order to avoid the negative connotations of the term "collapse."

Anderson (1948) proposed the first detailed hypothesis about the mechanism behind the formation of hybrid swarms in response to disturbance. Anderson's hypothesis invoked strong selection and the creation of novel habitats through a "hybridization of the habitat". In Anderson's model, recombinant phenotypes of segregating hybrids closely fit the novel combinations of environmental parameters created by disturbance providing hybrids with a niche of their own, separate from that of the parental taxa. Interestingly, a parallel can be drawn between Anderson's local-scale mechanism of hybrid swarm formation and the geographic-scale formation of hybrid species, that are often observed expanding into novel environments from those of their parents (Grant 1971, Arnold 1997, Rieseberg 1997, Rieseberg and Carney 1998, Rieseberg et al. 2003, Rieseberg 2006). In order to enforce the close association of new phenotypes with novel environmental niche space, Anderson's mechanism requires strong selection:

> "*It is only where man has hybridized the natural environments ... that nature can find an appropriate lodging place for the hybrids she has created.*"
>
> *Anderson (1948)*

> *"... they form a multi-dimensional jig-saw puzzle the pieces of which fit tightly together."*
>
> *Anderson and Anderson (1954)*

Although Anderson focused on the proliferation of new phenotypes in hybrid swarms, the mechanism proposed provides an equally robust mechanism for species fusion. If disturbances or other alterations of the figurative or literal adaptive landscape result in fewer habitats or resource niches rather than more, then species fusion should occur because of a "homogenization of the habitat" rather than a "hybridization of the habitat." Although the mechanism is the same, whether a hybrid swarm or species fusion is the ultimate outcome provides insights into the selective processes maintaining or suppressing phenotypic diversity at hybrid zones (Clausen and Hiesey 1960).

An alternative to the Andersonian hybrid swarm was proposed by Grant (1971) who argued that a relaxation of the selective regime could lead to similar results via invasion of competitively neutral phenotypes that otherwise could not coexist due to strong postzygotic natural selection. Relaxed selection might be caused directly by adding limiting resources or indirectly through the removal of competitors or consumers. In this framework relaxed and strong selection mechanisms are not mutually exclusive; selection can be relaxed but still strong, maintaining ecotypes in gene flow-selection balance by partially enforcing phenotype-habitat correlations among hybrids and parental taxa (Grant 1979).

Importantly, hybrid swarms have also been viewed as transitional stages on the way to species fusion, as opposed to being stable endpoints due to more niches or relaxed selection. Divergent lineages that experience an increase in hybridization are expected to increase in phenotypic diversity temporarily while parental taxa coexist with newly formed hybrid taxa. For the purposes of this review I use a mechanistic definition of the term hybrid swarm to refer more narrowly to "equilibrium" scenarios thus excluding hybrid swarms that are transitional phenomena resulting from time lags in selection and population turnover. However, while the definition is mechanistic, distinguishing between hybrid swarms and species fusion is inherently problematic since equilibrium conditions cannot be determined or do not exist in most systems (Connell and Sousa 1983) and hybrid swarms are diagnosed operationally as increases in phenotypic diversity. The practical effect on this review is that some cases of putative hybrid swarms may in fact be transitional stages on the way to species fusion and not a reflection of increased niches or relaxed selection. While the distinction between hybrid swarms and species fusion in this review is a mechanistic one, the potential existence of time lags presents a practical problem for diagnosing hybrid swarms versus species fusion.

A third alternative is that prezygotic rather than postzygotic mechanisms are responsible for the breakdown of isolation leading to hybrid swarms or species fusion. This hypothesis is supported by observations of the fusion of species isolated primarily by sexual selection when assortative mating cues becomes impossible to detect (Seehausen et al. 1997) or when allopatric populations are brought into secondary contact. Importantly, the creation of habitat corridors and the homogenization of assortative mating cues do not directly invoke adaptation in reducing gene flow. Selection against hybrids is the most direct way that adaptation can reduce gene flow (Schluter 2000).

The goal of this study is to conduct a literature review and document mechanisms of hybridization in response to disturbance and other environmental changes, and to organize mechanisms into a conceptual framework based on the level of selection and variation in fitness landscapes to test the alternative hypotheses of Anderson and Grant about the relationship between disturbance and hybridization.

Methods

Literature review

For the literature review studies were collected in which hybridization rates or the presence of putatively recombinant progeny were positively or negatively associated with natural or anthropogenic disturbances or other changes in environmental conditions. Other changes include perturbations to and gradual alteration of external forcing factors not fitting the strict definition of disturbance (sensu Sousa 1984) such as creation of habitat corridors and changes in nutrient loading or land use. Cases such as these were, however, limited to those that included a control site either in space or time that was not affected by the environmental change. Following Sousa (1984), disturbances and gradual changes in external forcing factors are two ends of a continuum of environmental perturbations. Both disturbances and gradual changes to external forcing factors have the potential to change the outcome of ecological or evolutionary processes, thus the scope of this review includes both sets of processes, although it is acknowledged that in many cases it will be difficult or impossible to identify a background environmental condition against which to measure deviations from (Connell and Sousa 1983).

Literature searches were conducted in Google Scholar and involved exhaustive examination of results using the search terms "disturbance + hybrid", "disturbance + hybridization", "disturbance + speciation", "hybrid swarm", "species collapse" and "species fusion" as well as a less exhaustive search using the terms "hybridization" and "hybrid zone." The literature cited sections of Stebbins (1940), Mayr (1942), Anderson (1949), Clausen (1967), Grant (1981), Rhymer and Simberloff (1996), Arnold (1997), Rieseberg and Carney (1998), **Levin (1999)**, Schluter (2000), Coyne and Orr (2004) and Nosil (2012) were also examined. Each paper was examined for explicit or implicit evidence that rate of hybridization was altered in any way by disturbance or changing environmental conditions.

Studies of introduced species were included if they met one of three criteria: (a) introduced species coexisted with native species for some time before an environmental change led to hybridization, (b) hybridization between introduced and native species was compared in paired disturbed and undisturbed sites, or (c) the exotic species is used as a resource (such as an oviposition host) and not a partner in the hybridization itself. The remaining cases–those where human introductions lead immediately to hybridization with native species–have been discussed in depth in previous reviews (Rhymer and Simberloff 1996, Arnold 1997) and represent boundary conditions where the perturbation is the addition of another species to the community itself rather than an alteration of the underlying abiotic selective environment. The overarching goal of this study is to use disturbances to probe the ecological mechanisms that may be maintaining species in nature rather than to estimate the prevalence of hybridization in response to disturbance, which would include a vast number of examples of human species introductions that may swamp other patterns in the data that are more informative for addressing questions about divergence mechanisms operating in natural systems.

Framework for comparison of mechanisms

When a causal mechanism was directly tested or could be inferred, studies were first classified according to the putative importance of intrinsic versus extrinsic reproductive isolation (Coyne and Orr 2004). Extrinsic isolation was further categorized according to whether prezygotic or postzygotic factors were more important. Within prezygotic and postzygotic factors several distinctions were made between mechanisms based on the strength of natural or sexual selection and the shape of the underlying gradients in resources, the distribution of habitats in space, and the transmission of assortative mating cues.

Figure 1 illustrates the simplest case, that of a one-dimensional niche axis, although in reality species would occupy an *n*-dimensional niche space with *n* reflecting the number of functional environmental gradients. The center panels illustrate geographic isolation (Figure 1a), mating cue transmission (Figure 1b) and habitat disturbance (Figure 1c), and can be thought of as the range of selective and/or geographic regimes experienced by a dividing lineage. In Figure 1a and 1c the functional gradient is some limiting resource distributed as a step function (center boxes) with the predicted phenotypic distributions shown to the right and left under strong and relaxed selective regimes, respectively. Figure 1a adds the further complexity of separation in space while Figure 1c adds an intermediate habitat condition. In Figure 1b sexual selection is illustrated with the functional gradients in this case determined by different assortative mating cues present in an environment. The predicted phenotype is under strong and relaxed sexual selection regimes are shown in the right and left panels, respectively.

<u>Postzygotic isolation</u> In a two-habitat scenario with strong postzygotic selection against hybrids and no intermediate habitat there are two favored phenotypes (Figure 1c, upper row, right panel). Creation of novel a intermediate habitat under a strong selective regime alters the adaptive landscape such that more than two phenotypes are favored (Figure 1c,

middle row, right panel). This case is analogous to two parental habitats separated by a discrete ecotone habitat, and although Anderson's (1948) model implied a proliferation of novel phenotypes even one additional phenotype resulting from the formation of a novel habitat would fit this definition because selection is matching phenotypes with habitats. This mechanism does not necessarily increase the variance of traits observed between parental taxa although it will decrease the covariance among traits by promoting recombination. Importantly, one shortcoming of illustrating the framework using only one phenotype dimension (functional trait) is that the hybrid phenotype is necessarily shown as intermediate to the parental types (Figure 1c, middle row, right panel); however, most natural systems will have many functional niche dimension, meaning that a hybrid does not have to be strictly intermediate to the parental phenotypes (see Chapter 3, Figure 4 for the two trait scenario).

This framework also accommodates the case of species fusion if disturbance leads to a homogenization rather than a hybridization of the underlying niche axes (Figure 1c, bottom row, right panel). If under a strong selective regime habitat heterogeneity goes down or a previously bimodal resource base turns into a unimodal one then the number of discrete phenotypes is predicted to go down. This is the mechanism cited in the collapse of the Enos Lake threespine stickleback as well as in the fusion of the large- and small-billed ecotypes of the Galapagos finches in response to a potential human-induced alteration of the distribution of food resources (Hendry et al. 2006, Kleindorfer et al. 2014). As with Andersonian hybrid swarms, these mechanisms of species fusion invoke strong selection in matching phenotypes with functional niche axes.

However, as noted by Grant (1971), phenomenologically similar results may be obtained simply by relaxing selection in the absence of an altered habitat (Fig. 1c, left panels). In the case of an increase in habitat heterogeneity (Fig. 1c, middle row, left panel), relaxed selection is predicted to lead to an invasion of phenotypes that otherwise would have occupied valleys in the fitness landscape. Despite this, if selection was still present to some degree, then ecotypic variation would likely be maintained in gene flow-selection balance. This type of competitively neutral hybrid swarm may be created when an increase in resource availability ameliorates resource limitation or when competitors or consumers are removed, freeing resources, and should be evidenced by a greater density and/or size of individuals in the disturbed habitats and a reduction in the discreteness of phenotypes.

Finally, a homogenization of the resource base (Fig. 1c, bottom row) should lead to species fusion regardless of the level of selection. The only difference is that in the strong selection scenario stabilizing selection reduces the trait variance (Fig. 1c, bottom row, right panel) whereas in the relaxed selection scenario, a full range of phenotypes spanning the range of variability between parental taxa is permitted (Fig. 1c, bottom row, left panel). In both cases the number of discrete phenotypes is reduced with strong selection only reducing the phenotypic variance.

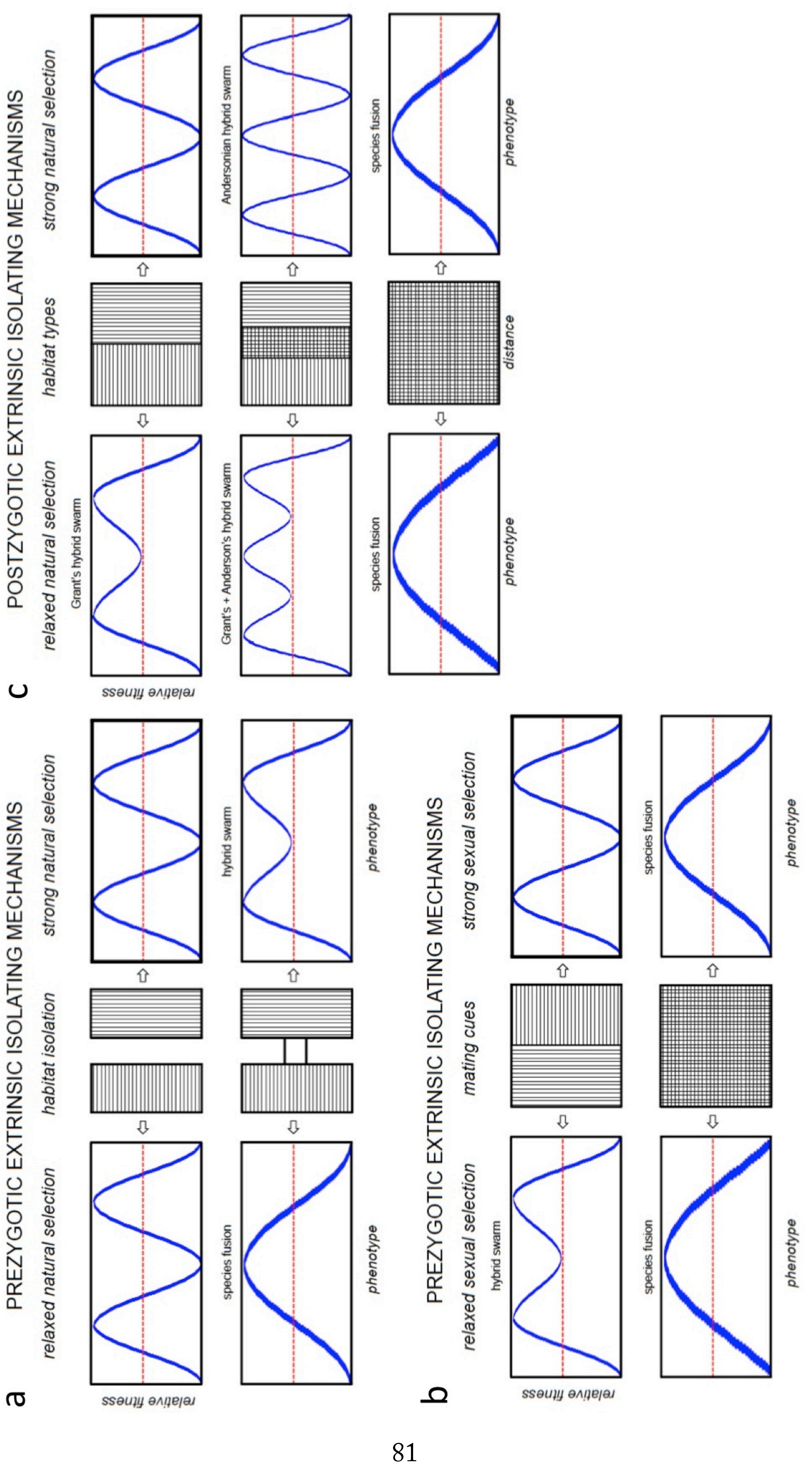
Prezygotic isolation   Relevant types of prezygotic isolation can be divided into sexual selection and geographic isolation or allopatry. Geographic isolation or allopatry can be shown as a gap between habitats representing an impermeable barrier to gene flow (Fig. 1a, top row). Postzygotic isolation due to habitat differences can also be included since identical habitats are rare in nature. In this framework, the ecological component of geographic isolation is treated as an additional postzygotic filter since differences between allopatric habitats would directly affect gene flow only if hybrids were produced and selected against. In this framework the importance of habitat differences can be modulated by changing the strength of natural selection since the evolutionary importance of habitat differences depends on how selection translates those differences into fitness variation. Selection strength can range from strong (Fig. 1a, top row, right panel) to relaxed (Fig. 1a, top row, left panel) to nonexistent (not shown). Importantly, in the completely allopatric scenario changing selection does not directly affect the number of predicted phenotypes because hybrid progeny are never produced (Fig. 1a, top row). The only difference between the relaxed and strong selection scenarios (Fig, 1a, top row, left vs. right panels) are that the location of the peaks is controlled to an increasing degree by genetic drift as selection is relaxed, evidenced by weak or nonexistent trait-habitat correlations among hybrids. In the extreme case of no selection (not shown) the location of the peaks is determined entirely by genetic drift in the two allopatric populations; however, two phenotypes are still predicted to differ unless they coincidentally happen to overlap.

Connecting allopatric habitats by allowing nonzero migration rates changes the predictions in ways that begin to depend on the strength of natural selection (Fig. 1a, bottom row). In the case of strong postzygotic selection (Fig. 1a, bottom row, right panel) the distribution of phenotypes represents the outcome of the balance between gene flow and selection and may span the range from fully discrete phenotypes to a hybrid swarm depending on the rate of migration. In the relaxed selection scenario (Fig, 1a, bottom row, left panel) phenotypes may span the range from ecotypes (not shown) to species fusion (shown) if weak or nonexistent selection is overwhelmed by gene flow.

Sexual selection presents a unique case in that the "habitats" that organisms are "adapted" to are the sexual cues provided by potential mates (Fig. 1b, top row), which if transmitted successfully maintain assortative mating between lineages (Coyne and Orr 2004). Transmission of a signal, whether noise or scent or color, is necessarily through some environmental medium such as air or water, the information content of which can be lost through an obfuscation of the signal due to decreased water clarity or noise pollution thus warranting classification of these mechanisms as extrinsic sensu Schluter (2000) even though the cue is not a consumable resource and is thus not strictly adaptive.

In this framework, instead of illustrating two habitats the shaded boxes represent two different sets of sexual cues that are analogous to a "sexual environment" (Fig. 1b, top row, center panel). While sexual selection is not equivalent to selection on mating systems in plants, for the purposes of this review their effects may be similar so they will

Figure 1 (following page): Conceptual framework illustrating hybrid swarm and species fusion. A one dimensional niche axis is shown as the simplest possible case, although in reality species would occupy an *n*-dimensional niche space with *n* reflecting the number of functional environmental gradients. One disadvantage is that in cases showing more than two phenotypes (modes), in nature the high dimensionality of environmental parameters means the hybrid does not have to be strictly intermediate to the parental phenotypes. The center panels illustrate one axis of niche space as well as the geographic separation of taxa. The specific cases correspond to geographic separation (Fig. 1a), mating cue transmission (Fig. 1b) and habitat disturbance (Fig. 1c), and can be thought of as the range of selective regimes experienced by a dividing lineage. In Figure 1a and 1c the niches are some limiting resource gradient (center boxes) showing the predicted phenotypic distribution under strong and relaxed selective regimes (right and left panels, respectively). Figure 1a adds the further complexity of separation in space while Fig. 1c adds an intermediate habitat condition. In Figure 1b sexual selection is illustrated with the niches in this case determined by different sexual selection cues present in an environment. The phenotype that is predicted in this case is likely to be some form of mate choice and the predicted phenotype distributions are shown as a function of strong and relaxed sexual selection (right and left panels, respectively). See *Methods: Framework for comparison of mechanisms* for further explanation.


a
PREZYGOTIC EXTRINSIC ISOLATING MECHANISMS
relaxed natural selection
habitat isolation
strong natural selection
relative fitness
species fusion
hybrid swarm
phenotype
phenotype
b
PREZYGOTIC EXTRINSIC ISOLATING MECHANISMS
relaxed sexual selection
mating cues
strong sexual selection
relative fitness
hybrid swarm
species fusion
species fusion
phenotype
phenotype
c
POSTZYGOTIC EXTRINSIC ISOLATING MECHANISMS
relaxed natural selection
habitat types
strong natural selection
relative fitness
Grant's hybrid swarm
Grant's + Anderson's hybrid swarm
Andersonian hybrid swarm
species fusion
species fusion
distance
phenotype
phenotype

be considered together. Assortative mating relies on successful transmission of mating cues, and disturbances or environmental changes that disrupt the ability of signals to be faithfully transmitted may lead to increased rates of hybridization, as is the case in cichlid fishes fusing in response to increases in lake turbidity (Seehausen 1997). The predictions for altering sexual selection are otherwise the same as for natural selection. Strong sexual selection can result in two discrete phenotypes (Fig. 1b, top row, right panel) that can begin to fuse into ecotypes or a hybrid swarm through a relaxation of sexual selection (Fig. 1b, top row, left panel). Homogenization of the sexual cues (Fig. 1b, bottom row) will, however, likely result in species fusion under any selective regime with the only difference being that the trait variance will be reduced in the strong selection scenario (Fig. 1b, bottom row, right panel) compared to the relaxed selection scenario (Fig. 1b, bottom row, left panel) or unless other dimensions of the resource base provide alternative axes of differentiation as may be the case with some instances of apparent incipient divergence in birds due to song (Uy et al. 2009).

Comparing components of extrinsic isolation

Comparing the relative strengths of different isolating barriers is the goal of many speciation studies, but doing so is not straightforward and existing approaches have serious weaknesses. The multiplicative approach (Ramsey et al. 2003, Nosil et al. 2005, Lowry et al. 2008) is appealing in that it is analogous to robust methods for calculating total fitness from individual fitness components or population growth rate from life stage transition probabilities (e.g. Caswell 2000). However, this analogy may not be appropriate for systems in which the measured components of reproductive isolation are not independent or essential steps in the process of gene flow (Ramsey et al. 2003, Sobel et al. 2009, Harrison 2012). For example, using selection against parental migrants in calculations of total reproductive isolation is problematic particularly for plants because this stage can be bypassed by a mobile pollen phase. While selection against parental migrants reduces the likelihood of interspecific encounters (Nosil et al. 2005), gene flow may proceed despite absolute selection against parental migrants, a scenario that is not permissible using a multiplicative framework.

Another apparent problem with comparing different components of reproductive isolation is disentangling the causes and consequences of isolation by distance (IBD). For most hybrid zone studies, selection and gene flow are measured at the point of contact or within "cruising range" of the hybrid zone. Since dispersal declines with distance, geographic separation provides a strong form of prezygotic isolation that does not invoke any direct action of selection and may be absolute in the center of a species' range, far from any zones of contact. Thus, the individuals at the centers of populations may be effectively allopatric while individuals at the periphery may be effectively parapatric or sympatric. The amount of IBD caused by local dispersal is quantifiable using existing theoretical frameworks (e.g. Slatkin 1993); thus, modeling distance as an effect should be straightforward, although it means that estimation of reproductive isolation should probably be spatially explicit.

A related question that is the subject of recent research is disentangling the *causes* of IBD rather than measuring the overall *effect* of IBD in reducing gene flow (Bradburd et al. 2013, Shafer and Wolf 2013, Yang et al. 2013), spurred in part by the goal of trying to quantify the total contribution of ecological versus non-ecological factors to divergence (Schluter 2000). The importance of habitat differences was emphasized in early models of allopatric speciation with proponents noting that even weak selection can cause rapid evolution in the absence of gene flow (Mayr 1947). Unfortunately, while simple in theory, in practice disentangling the causes of IBD is difficult and dependent on the spatial arrangement of habitats, accurate estimation of dispersal, and establishment of a non-adaptive baseline from which to compare the additional effect of adaptation, among other things (Bradburd et al. 2013, Shafer and Wolf 2013, Yang et al. 2013). Fortunately, although knowing causality is critical for predictability and reconstructing divergence history, the cause of IBD does not change the effect of IBD on current rates of gene flow for cases where prezygotic isolation is absolute (complete allopatry) because no hybrid progeny are produced to be selected against. This is illustrated in Fig. 1a (top row), where altering the level of selection does not affect the number of phenotypes because allopatry is the more proximate barrier.

Another hurdle in assessing the importance of different isolating barriers is the proliferation of terminology related to isolation and isolating barriers (Harrison 2012). For example, "ecological isolation" is a term used extensively in the biosystematic literature to refer to what Coyne and Orr (2004) call extrinsic isolation. Thus, the term "isolation by ecology" may be confusing as currently used to refer much more narrowly to the proportion of prezygotic IBD caused by adaptation (Shafer and Wolf 2013). Due to the broad connotations of the term "ecology" in general, it may be most useful to reserve this term for isolation relating to the totality of extrinsic factors (Schluter 2000). "Isolation by adaptation" may be similarly confusing. Isolation by adaptation is often equated with isolation by ecology, however use of the term "adaptive" in the sense of an isolating barrier traditionally connotes postzygotic selection against hybrids, the only mechanism of adaptation that directly prevents gene flow. Moreover, isolation by adaptation implies that information is known about the process that generated the isolation, when in reality it is rarely known whether isolation is caused by adaptation or exaptation.

Qualitative assessments of barrier strength

In light of uncertainties in quantitative estimates of relative barrier strength, the method that was chosen was a multiple working hypotheses framework in which the preponderance of the evidence was assessed for each mechanism by weighing the investigators' original conclusions including reconciling claims from different studies, detailed readings of field site descriptions and reanalysis of experimental results. While this qualitative approach cannot provide precise estimates of barrier strength, it is advantageous for precisely this reason; it does not provide false confidence in overly precise estimates of barrier strength. Development of a method that can provide accurate, quantitative estimates of relative barrier strength remains a major goal in speciation research.

In all cases, determination was first made whether intrinsic or extrinsic barriers were more responsible for maintaining species boundaries, noting if equal support was given to both categories. Intrinsic isolation was evidenced by low hybrid fertility or viability in controlled crosses or evidence of environment-independent assortative mating or pollen-pistil incompatibilities, among other mechanisms reviewed in detail in Coyne and Orr (2004). Extrinsic isolation was inferred if changing environmental conditions in space or time led to an increase in hybridization. Within extrinsic isolating mechanisms, determination was made whether prezygotic or postzygotic factors were more important, noting whether equal support was given to both classes of mechanisms. Prezygotic isolation was determined by noting the presence of allopatry, published reports of assortative mating in undisturbed settings, or experimental evidence of pollinator discrimination, among other mechanisms. Postzygotic isolation was inferred if hybrid progeny were produced and persisted in the disturbed habitats but were not represented as adults in the undisturbed habitats, and if parental taxa exhibited phenotype-habitat correlations (Schluter 2000).

Finally, of the cases of hybrid swarms and species fusion where isolation was attributable to postzygotic extrinsic factors, determination was made whether there was support for Anderson's (1948) strong selection hypothesis, Grant's (1971) relaxed selection hypothesis or both. If evidence was found of relaxed selection, then additional distinction was made where possible between direct addition of resources and reduction of competition or predation. An Andersonian hybrid swarm was inferred if habitat diversity increased, if phenotypic variance did not go down and if phenotype-habitat correlations were present among hybrids. Similarly, a neutral hybrid swarm of the type described by Grant was evidenced by an increase in resource availability and/or reduction in competition, an increase in the density and/or growth rate of individuals, an increase in the phenotypic variance without a concomitant increase in habitat heterogeneity and/or lack of correlation between hybrid phenotypes and environmental gradients.

In many cases determination of causal mechanisms was unambiguous and all sources of inference agreed. In other cases there was insufficient information or conflicting information such that a judgment about the operation of a particular mechanism or the relative importance of various mechanisms was not possible. These cases were noted in the data as not containing sufficient information to make a judgment.

Methodological biases

First, the studies examined for this review were limited to cases where external forcing was determined to be important; thus this survey cannot provide an estimate of the relative importance of extrinsic versus intrinsic factors in maintaining divergence. However, these data are informative about whether extrinsically maintained lineages exist

and can be used to test the relative importance of different extrinsic mechanisms. Second, this study is likely biased toward those taxa that are prone to species fusion in the first place, that is those that were already isolated by weak or unstable barriers prior to disturbance. If divergence processes in lineages with weak barriers are different than those in lineages with strong barriers then inferences about the processes involved in extrinsic isolation in general may be biased. However, this could potentially be seen as an advantage in that it favors consideration of lineages early in the speciation process before many different intrinsic and extrinsic isolating barriers have had time to develop.
An additional source of bias is that studies examined for this review needed to provide extra information in order to establish whether a mechanism was or was not operating, thus potentially biasing the data in favor of negative results. To quantify this bias evidence for or against each mechanism was classified as either supportive, not supportive, or no information provided. Many studies lacked the necessary background information or site descriptions that could have provided insight into potential mechanisms, thus the bias in this study would be towards not detecting a mechanism making conclusions related to the importance of different mechanisms conservative. Confirmation bias may also be a problem. At the level of this study all references were read in their entirety, and most references were originally collected for different purposes. Confirmation bias, however, may however be a problem at a deeper level in the data, since Anderson himself was personally involved in a large number of the studies performed on plants, and few of the investigations performed during that era used strong inference. This "Anderson factor" is denoted in the data table with an asterisk.

Results

Of the studies that met the search criteria (Figure 2) a roughly equal number were found for plants (~60%) and animals (~40%), with the average age of the botanical publications older than that of the zoological publications by ~20 years. All taxa were isolated strongly by extrinsic factors, with a minority equally strongly isolated by intrinsic factors (~15%) with no difference in the proportion of taxa isolated by intrinsic factors between plants and animals (chi square $p \geq 0.05$).

Within extrinsic mechanisms, plants were isolated primarily by postzygotic barriers while animals were isolated primarily by prezygotic barriers (chi square $p < 0.05$). Despite this general pattern, a substantial number of taxa (~15%) broke this trend and were either equally isolated by prezygotic and postzygotic barriers or were isolated more by the atypical barrier for that group. Interestingly, some of the most notable speciation study systems fell in this category including the Galapagos finches and threespine stickleback, which are strongly isolated by postzygotic barriers and *Helianthus* sunflowers and *Aquilegia* columbines, which are strongly isolated by prezygotic barriers.

Within postzygotic extrinsic mechanisms, there was substantial support for both Grant's (1971) and Anderson's (1948) hypotheses of hybrid zone structure. A large fraction of studies (~60%) found evidence for increased habitat heterogeneity and matching of hybrid phenotypes with novel habitats indicating an Andersonian hybrid swarm. A nearly equal fraction of studies (~60%) also found support for Grant's neutral hybrid swarm hypothesis by observing an increase in phenotypic diversity without corresponding increase in habitat heterogeneity, although in some cases it was difficult to determine the effect of disturbance on habitat heterogeneity or to know whether observed increases in heterogeneity are of the sort that lead to increased recruitment opportunities for plants. Of those studies that found evidence for relaxed selection, an approximately equal proportion found evidence of direct increases in resources (~50%) and reduction in competition (~50%). Interestingly, a high fraction (~35%) presented findings consistent with both Anderson's strong selection hypothesis and Grant's relaxed selection hypothesis to be occurring simultaneously.

Within prezygotic mechanisms, the most common reason for the breakdown of reproductive isolation was the connection of previously allopatric populations (~75%). Within this category is the creation of corridors as well as allopatric populations that were brought into contact because habitat destruction or low population sizes forced animals to disperse in order to find food or mates. Breakdown of sexual isolation was also important (~25%) and primarily involved disruption of assortative mating due to decreased visibility of mate choice cues.

Discussion

> *"The scanty evidence that is available indicates that speciation by geographical segregation without isolation is rare; if it occurs at all."*
>
> *Mayr (1947, p.268)*

> *"Mayr was thinking mostly of animal species, else he must immediately have seen in the more sedentary plant species a partial refutation of his argument."*
>
> *Muller (1952, p.160)*

The available evidence indicates that cases of isolation primarily by extrinsic mechanisms are not uncommon and are found among both plants and animals, refuting the claim that *"Cases where species remain distinct only because of postzygotic isolation are extremely rare, if they exist at all,"* (Kirkpatrick and Ravigné 2002, p.23). Interestingly, there are marked differences in the types of extrinsic mechanisms operating in plants versus animals (Figure 2). Animals were found to be predominantly isolated by prezygotic factors including geographic isolation and sexual selection, while plants were predominantly isolated by postzygotic selection, presumably against hybrids as predicted by ecological speciation theory (Schluter 2000). Although there were many exceptions to these patterns, the difference is highly significant (chi-square $p<0.01$) and suggests either that

Figure 2 (following page): Table of results of the literature review organizing studies by the different types of barriers present. Barrier importance was assessed qualitatively using all available evidence. Barriers were split first into whether intrinsic versus extrinsic barriers were more important or if they were equally important (denoted in grey). Within extrinsic barriers, red boxes denote whether prezygotic or postzygotic barriers were inferred to be stronger. Text in the "prezygotic" and "postzygotic" columns denotes where a secondary source of isolation was reported. Within postzygotic barriers, blue boxes indicate studies that found evidence for an Andersonian hybrid swarm (dark blue) and two types of evidence for Grant's relaxed selection hybrid swarms (light blue). Studies that Edgar Anderson was personally involved in are denoted with an "X" in the "Andersonian hybrid swarm" column. Orange boxes denote whether ecological differences such as diet or habitat are known between the species prior to an increase in hybridization. Beige boxes indicate whether disturbances were attributable at least in part to anthropogenic sources. The mechanisms of disturbance as well as the citations are also provided. If disturbance was natural as well as anthropogenic this was denoted with an "X" in the "anthropogenic" column.

taxon		kingdom	intrinsic	extrinsic	prezygotic	postzygotic	Andersonian hybrid swarm	Grant's relaxed selection	Grant's reduced competition	ecological differences	anthropogenic	mechanism	references
Ainsliaea		plant		▨		🟥				🟧	🟧 X	stream bed, development	Mitsui et al. 2010
Aquilegia	columbine	plant		▨	🟥 phenology	yes	🟦 X			🟧		erosion	Grant 1952, Chase & Raven 1975, Hodges & Arnold 1994
Arctostaphylos	manzanita	plant	▨	▨		🟥	🟦	🟦	🟦		🟧 X	logging, volcanic eruptions	Kruckeberg 1977
Balsamorhiza		plant		▨		🟥	🟦 X		🟦	🟧		stream bed, landslide	Ownbey & Weber 1943
Banksia		plant		▨	🟥 phenology	yes		🟦	🟦	🟧	🟧	roadcut	Lamont et al. 1989, Lamont et al. 1994, Lamont et al. 2003
Crataegus		plant		▨		🟥	🟦	🟦		🟧	🟧	development, agriculture	Byatt 1975
Encelia		plant		▨		🟥		🟦	🟦	🟧	🟧 X	stream bed, development	Kyhos et al. 1981; DiVittorio unpub data
Helianthus	sunflower	plant	▨	▨	🟥 allopatry	yes		🟦	🟦	🟧	🟧	roadcuts	Heiser 1947, Heiser 1951
Iris		plant		▨		🟥	🟦 X		🟦	🟧	🟧 X	pasturing, stream beds	Riley 1938
Iris		plant		▨	allopatry	🟥	🟦	🟦		🟧	🟧	logging	Clarkson 1959
Liatris		plant		▨	phenology	🟥	🟦	🟦	🟦	🟧	🟧	development	Hadley & Levin 1967
Populus	cottonwood	plant		▨		🟥		🟦	🟦		🟧	development	Spies & Barnes 1982
Primula		plant	▨	▨	allopatry	🟥		🟦	🟦	🟧	🟧	deforestation	Woodell 1969
Quercus	oak	plant		▨		🟥			🟦	🟧	🟧 X	fire breaks, stream beds	Nason et al. 1992
Quercus	oak	plant		▨		🟥	🟦			🟧		erosion	Muller 1952
Ranunculus		plant		▨		🟥	🟦		🟦	🟧	🟧 X	flooding, development	Briggs 1962
Rhododendron		plant		▨	phenology	🟥				🟧	🟧	deforestation	Zha et al. 2008, Zha et al. 2010
Rorippa		plant		▨		🟥		🟦	🟦	🟧	🟧 X	stream bed, development	Bleeker & Hurka 2001
Salvia	sage	plant	▨	▨	🟥	🟥	🟦 X			🟧	🟧	development, roadcut	Epling 1947, Anderson & Anderson 1954
Shorea		plant		▨	phenology	🟥		🟦		🟧	🟧	fragmentation, unclear	Kamiya et al. 2011
Tradescantia		plant		▨		🟥	🟦 X			🟧	🟧 X	erosion, development	Anderson 1936
Viola		plant		▨		🟥	🟦 X	🟦	🟦	🟧	🟧	ditches, grading	Anderson 1954
Alouatta	monkey	animal		▨	🟥 allopatry					🟧	🟧	fragmentation	Dias et al. 2013
Bolitoglossa	salamander	animal		▨	🟥 allopatry					🟧	🟧	roadcuts	Wake & Lynch 1976, Wake et al. 1980
Bufo	toad	animal		▨	🟥 allopatry					🟧	🟧	development	Malmos et al. 2001, Schwaner & Sullivan 2009
Cercopithecus	monkey	animal		▨	🟥 allopatry						🟧	deforestation	Dandelot 1959, Kingdon 1971, Chapman & Chapman 1996
cichlids	fish	animal		▨	🟥 sexual					🟧	🟧	turbidity, invasive species	Seehausen et al. 1997
Cyprinella	fish	animal		▨	🟥 sexual						🟧	turbidity	Page & Smith 1970
Daphnia	water flea	animal		▨	🟥	🟥					🟧	eutrophication	Keller et al. 2008
Drosophila	insect	animal		▨	🟥 sexual					🟧	🟧 X	lava flows, development	Carson et al. 1989
Gasterosteus	fish	animal		▨		🟥 yes				🟧	🟧	turbidity, invasive species	Kraak et al 2001, Taylor et al. 2006, Behm et al 2010
Geospiza	bird	animal		▨		🟥 yes	🟦 fewer	🟦		🟧	🟧	development	Hendry et al. 2006
Hyla	frog	animal	▨	▨	🟥 allopatry						🟧	pond building	Mecham 1960, Schlefer et al. 1986
Oncorhynchus	fish	animal		▨	🟥 allopatry						🟧	logging, development	Heath et al. 2009
Papilio	insect	animal		▨	🟥 allopatry	yes	🟦			🟧	🟧	climate warming	Mercader et al. 2009
Papio	monkey	animal		▨	🟥 allopatry						🟧	development	Detwiler et al. 2005
Podarcis	lizard	animal	▨	▨	🟥 allopatry						🟧	development	Capula 1993
Rhagoletis	insect	animal	▨	▨	🟥 allopatry		🟦			🟧	🟧	introduced species	Feder et al. 1994, Schwarz & McPheron 2007
Stegastes	fish	animal		▨	🟥 allopatry		🟦 fewer			🟧	🟧 X	hurricanes	Mullen et al. 2012
Xenopus	frog	animal		▨	🟥 allopatry	yes	🟦			🟧	🟧	altered hydrology	Picker 1985

plants and animals respond differently to different types of disturbances, or that the mechanisms causing speciation in the early stages of divergence are different for plants and animals. Surprisingly, this review found 22 cases where taxa were isolated predominantly by extrinsic postzygotic natural selection such that disrupting postzygotic barriers resulted in species fusion or hybrid swarm formation.

Does this mean that plants undergo ecological speciation more frequently than do animals? Review of the available literature found 18 pairs of plant taxa (~70%) in which gene flow is blocked primarily by ecologically based postzygotic selection, whereas only four pairs of animals (~20%) were isolated primarily by postzygotic extrinsic selection. While there are many different pathways by which speciation may be considered adaptive, selection against hybrids is the only direct mechanism for adaptation to reduce gene flow (Coyne and Orr 2004) and is considered the hallmark of ecological or Darwinian speciation. Despite this, several arguments suggest that Darwinian speciation may be more common in animals than these data suggest. First, these studies are biased in favor of small-scale disturbances that are noticeable to an observer and amenable to study. While this review attempted to cover all spatial scales of environmental perturbations, it is possible that animals and plants sense the spatial scale of environmental variability differently and that studies are biased in favor of small-scale disturbances. Plants are less mobile in their adult phases than non-sessile animals and cannot exercise much habitat choice once they are established. In contrast, habitat choice by animals partially decouples them from small-scale edaphic or climatic gradients that may be strong selective filters for a plant. Thus, plants may be more sensitive to small-scale environmental heterogeneity than many animal taxa, in which case a bias against studies of large-scale disturbances would result in an underestimation of the importance of postzygotic isolation for animals.

Potential support for this explanation comes from the few cases of large-scale environmental perturbations such as the eutrophication of entire lakes that have led to the collapse of fish taxa where disturbance has caused a homogenization of the resource base and where there is a lack of spatial refuges from environmental change (Gow et al. 2006, Taylor et al. 2006). Regardless of the cause, the difference in perceived isolating barriers between animals and plants is consistent with the division among botanists and zoologists for nearly 100 years over species definitions. If these results are general it is easy to imagine that zoologists would be comfortable more often than not drawing the line for species at prezygotic isolation, while botanists have argued for a long time that drawing the line at zygote formation fails to capture much of the variability among plants.

Mechanisms of prezygotic isolation

The two most common sources of prezygotic isolation were allopatry and sexual selection (Figure 2). In animals, sexual selection most often took the form of mate choice while in plants prezygotic selection on mating systems most often took the form of pollinator fidelity. In the allopatric scenario the most common source of the breakdown of prezygotic isolation was through the creation of habitat corridors, and while disturbances are frequently the cause of corridors, in the sense used here corridors themselves are the analogous process to disturbance. The altered environmental condition in this case is connectivity, as opposed to habitat properties or sexual signals. Geographic isolation was more often than not found to correlate with ecological differences, but in some cases hybridizing taxa occupied nearly identical habitats. In many of these cases, removal of the prezygotic barrier resulted in phenotypic fusion within several generations indicating that adaptation to different habitats, if it exists, is not strong enough to prevent species fusion. While the distinction between creating a corridor and physically moving an organism is subtle, cases of direct introduction of organisms by humans was not included in this review (see *Methods*). A secondary mechanism of overcoming allopatry was via reductions in population size or resource availability due to habitat destruction or a reduction in resources which often led to increased dispersal distances to find mates and/or food. While this mechanism does not explicitly invoke adaptation or natural selection, as mentioned previously most allopatric populations are likely subject to some degree of natural selection and are also allopatric partly because of their history of selection.

Sexual selection was invoked in maintaining species boundaries in the remaining ~20% of cases. Sexual selection in animals most often took the form of disruption of mating cue signal transmission and most often led to species fusion. In plants, pollinator fidelity was shown to maintain species boundaries via selection against intermediate pollinator syndromes (Grant 1952, Chase and Raven 1975). This represents an analogous process to sexual selection in animals although it does not fit the strict definition of sexual selection. One complication of classifying selection on mating systems in plants is that it is unclear whether selection on mating systems is an extrinsic mechanism relying on pollinator behavior and availability or an intrinsic mechanism relying on genetically determined specialized floral morphologies, pointing out a potential weakness in using the extrinsic versus intrinsic dichotomy (Coyne and Orr 2004). Among plants differences in flowering time also imparted substantial isolation. This is effectively a form of assortative mating, and like pollinator fidelity may have extrinsically and intrinsically determined components. An increase in resource availability changing flowering time such that species now overlap in phenology is a mechanism of hybrid swarm formation in *Banksia* and illustrates a case of relaxed selection disrupting prezygotic isolation in plants (Lamont et al. 2003).

Mechanisms of postzygotic isolation

Evidence was found in support of both Anderson's (1948) and Grant's (1971) hypotheses of hybrid swarm formation and in many cases they worked in tandem. In at least 60% of cases where postzygotic isolation was implicated the mechanism was at least partially attributed to an increase in novel habitats due to disturbance thus supporting an Andersonian hybrid swarm. However, ~60% of studies also supported a neutral hybrid swarm as envisioned by Grant, where a proliferation of putatively recombinant phenotypes is due to relaxed selection. Of those cases where relaxed selection was inferred, ~40% of studies reported direct inputs of limiting resources while ~40% of studies reported a reduction in competition or predation. Interestingly, in the majority of cases both strong and relaxed selection mechanisms jointly operated to explain the proliferation of hybrid phenotypes (Figure 2)

In contrast, we found few cases in plants where disturbance caused a reduction in phenotypic variance indicating species fusion. In plants, the effect of disturbance was usually to increase or maintain the same number of coexisting phenotypes, although the discreteness of phenotypes was typically eroded. This is in contrast to the case in animals, where little evidence of proliferation of recombinant phenotypes was found associated with environmental change, with most cases categorized as species fusion and evidenced by an unambiguous reduction in phenotypic diversity and/or variance. Why disturbances appear to have diversity-promoting effects in plants and diversity-inhibiting effects in animals is potentially related to the scale of disturbances examined (as discussed above) or differences in mobility.

Interestingly, the vast majority of studies involved anthropogenic disturbances, begging the question of whether hybridization in "natural" systems is as important a process as the literature suggests. In one sense, Anderson was undermining his claim of the importance of hybridization in nature by pointing out repeatedly that each instance of hybridization was more often than not accompanied by some kind of disturbance. While natural disturbances are frequent in the literature and in Anderson's writings as well, it is a valid question to ask whether natural hybridization is truly omnipresent or whether anthropogenic disturbance is currently omnipresent, which in turn causes breakdown of unstable ecological barriers.

Despite this, the impact of hybridization on plant and animal evolution is likely to be profound even though natural hybridization may not be readily apparent. Extant interfertile lineages can be thought of as the "ghosts of speciation past" such that they represent the lineages remaining after those with weak or unstable barriers already fused. It follows that in the remaining lineages there should be a range of strengths of isolation exhibited from very strong to very weak, with those taxa exhibiting weak barriers "on the brink" such that small changes in the underlying adaptive landscape may qualitatively alter the evolutionary outcome of divergence versus fusion. This is most likely in species with shallow fitness landscapes indicating weak or relaxed selection. In contrast, species at the peaks of very rugged adaptive landscapes such as in serpentine soil or hypersaline coastal habitats may be relatively buffered to invasion by foreign alleles.

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CHAPTER 5

Climatic, morphological and physiological divergence within and among species in *Encelia*

Abstract

Correlations between phenotypes and habitats provide strong evidence for adaptive evolution. However, the degree to which observed trait-environment correlations are due to heritable differences between taxa versus phenotypic plasticity is often not known. Here I use a common garden to examine divergence in morphology, physiology and climate both within and between taxa for eight species and hybrids in the genus *Encelia*, a genus known for its high diversity of leaf morphologies and physiological strategies. I additionally used species occurrence data to test whether patterns of trait variation can be predicted by climatic variables. Although dramatic differences in morphology, physiology and climate were observed among taxa when grown in a common environment, no single trait was strongly correlated with any single climate variable, and the direction of variation within species was often different than among species. These results indicate that divergence in climatic niche, morphology and physiology is frequent but that compensatory variation among traits can generate multiple ecologically equivalent strategies, weakening univariate correlations between traits and climate. Although candidate key traits can be identified for specific taxa, no one trait explains divergence genus-wide, and ecological strategies appear to diverge along multiple orthogonal axes of physiological and morphological differentiation.

Key words: common garden, ecotype, parallel evolution, drought, adaptive radiation, ecologically equivalent strategy, leaf economics

Introduction

Correlations between climate and phenotype are often interpreted as signatures of natural selection. Likewise, correlations that appear multiple times within a rapidly multiplying lineage are seen as strong evidence of adaptive radiation. Thus, characterizing the magnitude and nature of divergence in phenotype and habitat is a necessary first step in investigating putative cases of adaptive radiation. While it is often difficult to predict the direction of evolution, bouts of adaptive radiation frequently follow the creation of ecological opportunity such as the appearance of novel climate zones following climate change or the creation of new habitats during orogeny or the appearance of oceanic islands (Simpson 1944, Clausen 1951, Baldwin et al. 1990, Schluter 2000, Gillespie and Roderick 2002). Studies of adaptive radiations in response to environmental changes have shown that evolution can be extremely rapid and frequently acts to fill newly available dimensions of niche or climate space, subject to the limitations imposed by genetic constraints (Arnold 1992, Schluter 1996), available phenotypic variation (Armbruster and Schwaegerle 1996) and the depth of intervening fitness minima (e.g. selection against intermediate phenotypes; Schluter 2000, Coyne and Orr 2004). Determining which traits are strongly shaped by the environment and which are shaped by developmental or genetic constraints is important for understanding the sources of phenotypic variation which in turn is critical for understanding the processes of adaptation and adaptive radiation.

For plants, the leaf economic spectrum (Reich et al. 2003, Wright et al. 2004, Donovan et al. 2011) and related frameworks (Chapin 1980, Chapin et al. 1993) provide a context to organize ecological strategies into suites of covarying traits according to trade-offs in development and resource allocation that roughly ordinate species along a "fast" to "slow" axis. This axis is composed of traits such as leaf life span, maximum photosynthetic rate and leaf nutrient concentration that are important indicators of rates of nutrient turnover and metabolic and photosynthetic activity which can then scale up to affect population demography, growth rates and eventually, evolutionary trajectories. The leaf economic spectrum focuses on patterns among traits at a global scale. At smaller spatial scales empirical studies within single communities that quantify a broader set of functional traits have yielded important insights into the ecological assembly processes generating patterns of functional diversity across habitat gradients (Ackerly 2004, Cavender-Bares et al. 2004, Cornwell et al. 2006, Cornwell and Ackerly 2009). Among the insights of studies at both large and small scales is that covarying suites of key traits explain a disproportionate amount of the variation among taxa even when accounting for phylogenetic conservatism (Lambers et al. 2008).

However, despite some success in the search for key integrating traits and climate variables, much variation remains to be explained both within single communities and across biomes (Wright et al. 2004, Cornwell and Ackerly 2009, Kraft and Ackerly 2010). Compensatory variation among coupled functional traits can lead to the existence of multiple ecologically equivalent strategies analogous to evolutionarily stable strategies (Smith 1982), alternative adaptations (West-Eberhard 1986), phenotypic lines of least resistance (Schluter 1996) and demographically neutral dynamics (Hubbell 2001). These mechanisms can produce evolutionarily equivalent dynamics among highly phenotypically divergent lineages even under uniform environmental conditions. These interacting suites of correlated traits can lead to strikingly different solutions to the same ecological problems, introducing noise into univariate or low-dimensional correlations of traits on climate. In this scenario, the absence of a linear correlation between trait and environment does not imply selective neutrality but instead indicates that the trait in question is one dimension of a multivariate strategy, with the adaptive value of that trait depending on the states of the other functional traits (Lande 1979, Lande and Arnold 1983).

Encelia is an ideal system for addressing questions about the mechanisms of ecological diversification because nearly all 22 minimum rank taxa in the genus are sympatric with at least one other species and are considered habitat specialists (Ehleringer and Clark 1988, Clark 1988). This and other observations indicate that *Encelia* is likely a classic case of adaptive radiation in that all of the criteria described by Schluter (2000) appear to be met. First, the genus is monophyletic and exhibits a burst of lineage splitting relative to its sister groups roughly coincident with the onset of the desert climate and the formation of the Baja California Peninsula beginning approximately ten million years ago and accelerating greatly approximately four million years ago (Axelrod 1950, Holt et al. 2000, Oskin et al. 2001, Oskin and Stock 2003, Fehlberg and Ranker 2007). Second, experimental and observational evidence indicates that divergent natural selection is strong, due to trade-offs resulting from adaptation to habitat gradients and is sufficient to maintaining species boundaries despite high amounts of potential gene flow at hybrid zones (Chapter 1). Third, classic ecophysiological studies clearly demonstrated the adaptive value of several functional traits including leaf reflectance, conductance and water use efficiency (Mooney et al. 1966, Ehleringer et al. 1976, Ehleringer 1993, Sandquist and Ehleringer 2003).

The fourth criterion is the existence of phenotype-environment correlations. Ehleringer and Clark (1988) and Clark (1998) provided much evidence suggesting that species of *Encelia* occupy distinct climatic niches, and Ehleringer et al. (1981) showed that this variation in climate is associated with variation in traits, namely leaf reflectance. However, the coexistence of glabrous and farinose species of *Encelia* growing in sympatry in the Mojave Desert suggests that the story may be more complex. As mentioned above, Ehleringer (1988) and Ehleringer and Cook (1990) revisited the work and showed that the ecological importance of leaf color is modulated by the state of a number of other traits including conductance, water use efficiency and leaf life span. However, no genus-wide systematic survey relating climate, morphology and physiology has been performed across multiple functional traits, and few of the previous measurements were made under common garden conditions thus the contribution of environmental plasticity to species differences is unknown.

The goals of this study are to determine whether there is evidence for phenotype-environment correlations and whether there is evidence for phenotypic evolution within and among taxa in the genus *Encelia*. By comparing multiple populations within each species we are able to address these questions at multiple phylogenetic scales. We first used herbarium occurrence data to determine whether the climatic niche of *Encelia* and its closest outgroup species are consistent with a hypothesis of broad-scale niche partitioning (Harvey and Pagel 1991). We then used the results from a common garden including both within and among species comparisons to determine whether traits were consistently related to climate and whether patterns of trait variation within species were the same as those observed among species. If the patterns of heritable trait variation within and among species are the same this suggests that the selective pressures acting early in divergence are the same as those acting later in divergence (Coyne and Orr 2004, Geber and Eckhart 2005). Alternatively, if patterns of trait variation within species are not related to or are negatively related to patterns of trait variation among species then evolutionary constraints may be limiting evolvability at a macroevolutionary scale (Arnold 1992, Armbruster and Schwaegerle 1996, Schluter 1996). I sought to answer the following specific questions. (1) Are the distribution of species throughout California consistent with partitioning of climate niches? (2) Do traits among species vary in the directions predicted by the leaf economic theory? (3) Do traits within species vary in the directions predicted by the leaf economic theory? (4) Are patterns of variation within species the same as among species? (5) Is taxon or climate a better predictor of trait values? Our initial predictions for the directions of change are presented in Table 2 and are based primarily on the leaf economics spectrum and on the morphological and hydraulic axes of differentiation previously described for *Encelia*.

Methods

Common garden experiment

Plants were obtained by collecting seed from two source populations per species when possible and were pooled across a minimum of 20 maternal plants per population. The locations of all populations are shown in Table 1. Seeds were sown in greenhouse trays filled with equal parts pumice, sand and vermiculite and watered daily for 8-12 weeks or until germination stopped. Overall rates of germination were very low; approximately 5% of sown seeds germinated which is consistent with other studies (Padgett et al. 1999). Plants were grown in the University of California Jane Gray Greenhouse in Berkeley, California until they were five to ten centimeters tall or until they had four foliage leaves. Beginning in May 2011 plants were transferred to the University of California Agricultural Operations Station in Riverside, California where they were planted into randomized blocks and watered twice per week via drip irrigation system for approximately six more months to simulate the conditions that would occur during a wet recruitment year. Between August and November 2011 water was turned off to each block in the order they were planted. Leaf and canopy measurements were made February-April 2012 and water potential measurements were made at the end of the dry season in August 2012. Conductance measurements were made in May 2013.

Trait measurements

Leaf shape, area and color were assessed by analyzing images of between 3 and 7 fully expanded sun leaves per plant harvested from plants in the common garden. Photographs were taken of all leaves under constant illumination. Leaf area, shape (measured as solidity, the proportion of the smallest convex hull enclosing the leaf that contains leaf lamina), leaf width and length were measured using Image-J (http://imagej.nih.gov/ij/). Leaf color was measured in Photoshop (Adobe Systems Inc.) by measuring the pixel intensity off of the center of the lamina in an eight-bit converted greyscale image of each

Table 1: Localities of populations of *Encelia* used in the common garden experiment.

taxon	population	locality	elevation (m)
actoni	high elevation	9 Mile Canyon Road, Inyo Co., CA	2,060
actoni	low elevation	Anza Borrego State Park, CA	1,810
asperifolia	North	Hwy1 30km SE of El Rosario, BC	550
asperifolia	South	back dunes near Villa Jesus Maria, BC	5
californica	North	San Clemente State Beach, CA	15
californica	South	coastal plain, Costa Rica, BC	15
canescens	--	Mollebaya, Arequipa, Peru	2,513
farinosa	mesic	University of California, Riverside, CA	430
farinosa	xeric	Hidden Hills Mine, Kelso, CA	1,115
frutescens	high elevation	Granite Mountain, Kelso, CA	830
frutescens	low elevation	Route 66, Chambliss, CA	330
palmeri	North	Villa Jesus Maria, BCS	5
palmeri	South	San Roque, Bahía Asunción, BCS	5
ventorum	North	Punta Lobos, Santa Rosalillita, BC	15
ventorum	South	Arroyo Polvoso, Bahía Asunción, BCS	5
hybrids	--	Arroyo Polvoso, Bahía Asunción, BCS	5

Table 2: Predicted directions of trait variation based on leaf economic theory showing the direction of variation predicted for each putative functional trait based on increasing temperature or precipitation.

trait	increasing temperature	increasing precipitation
canopy architecture (length:width)	--	+
canopy volume (cm^3)	--	+
stem hydraulic conductance ($kg/s/m^2/Mpa$)	--	+
flowering effort (# flowers)	--	+
flowering phenology (% open flowers)	+	--
leaf area (mm^2	--	+
leaf color (0-255)	+	--
leaf thickness (mm)	+	--
specific leaf area (mm^2/mg)	--	+
mid-day water potential (bar)	--	+

leaf adjusted to a color scale included in the photographs. Leaf lamina and petiole thickness were measured with digital calipers. Leaf mass was measured by weighing leaves that had been dried for one week in a low-humidity environment. Specific leaf area (SLA) was calculated by dividing the area by the mass.

Mid-day and pre-dawn water potentials were measured on plants from 12:00-4:00 PM and 2:00-6:00 AM, respectively. Water potentials were measured on healthy ten centimeter long shoots with a Scholander-type pressure bomb (PMS Model 1000) on one to two shoots per plant in August 2012 at the peak of the summer dry season. The mid-day water potential is used as a measure of the minimum water potential a plant will maintain before closing stomata, while the difference in pre-dawn and mid-day water potentials is a measurement of the amount of adjustment a plant experiences from a hydrated baseline before closing stomata (Lambers et al. 2008). Due to the small size of some leaf petioles only shoot water potential was possible to measure across all taxa.

Potential stem hydraulic conductance to water was measured using a portable vacuum chamber apparatus (Figure 6). Healthy 20-30 centimeter long shoots were obtained and the area of all leaves measured by photographing excised leaves and analyzing images with Image-J as described above. Each shoot was placed in a vacuum chamber made of PVC, with the end of the branch attached to a tube that was connected to a flask on a balance. The flask and tube were filled with buffer solution and a vacuum placed in the chamber with a pump. The vacuum in the chamber was read off a gauge and the volume of water passing through the stem measured as the rate of mass loss in the beaker. The vacuum is stepped up sequentially with each step lasting approximately one minute. The rate of water loss per square meter of leaf area per second per Mpa is the measurement of potential hydraulic conductance to water and reflects the total resistance to water passage through a stem relativized by the leaf area available to conduct it.

Flowering effort and flowering phenology were measured by counting the number of open and unopened floral buds (unopened heads) on each plant in February 2012 (Figure 7). Large numbers of flowering heads indicate a large reproductive effort, while a high proportion of open flowers indicates an earlier flowering time. Canopy architecture and volume were estimated by measuring the widest diameter and the diameter of the canopy orthogonal to the widest diameter as well as the height of the tallest stem above the ground. Canopy volume was calculated as a half ellipsoid and canopy architecture was measured as the ratio of the height to the width.

Data analysis

Specimens of all Californian *Encelia* taxa and close relatives in the genera *Enceliopsis*, *Geraea*, *Bahiopsis* and *Viguiera* available on the California Consortium of Herbaria webpage (http://ucjeps.berkeley.edu/consortium/) were downloaded and their latitude and longitude coordinates used to query locality-specific thirty second resolution climate

Figure 1: Geographical distribution of *Encelia* in California (a) and nearest outgroup genera (b). Top panel: *E. actoni* (purple), *E. californica* (green), *E. farinosa* (white), *E. frutescens* (red), *E. virginensis* (blue). Bottom panel: *Enceliopsis* (yellow circles), *Geraea* (blue circles), *Bahiopsis* (white diamonds) and *Viguiera* (white squares).

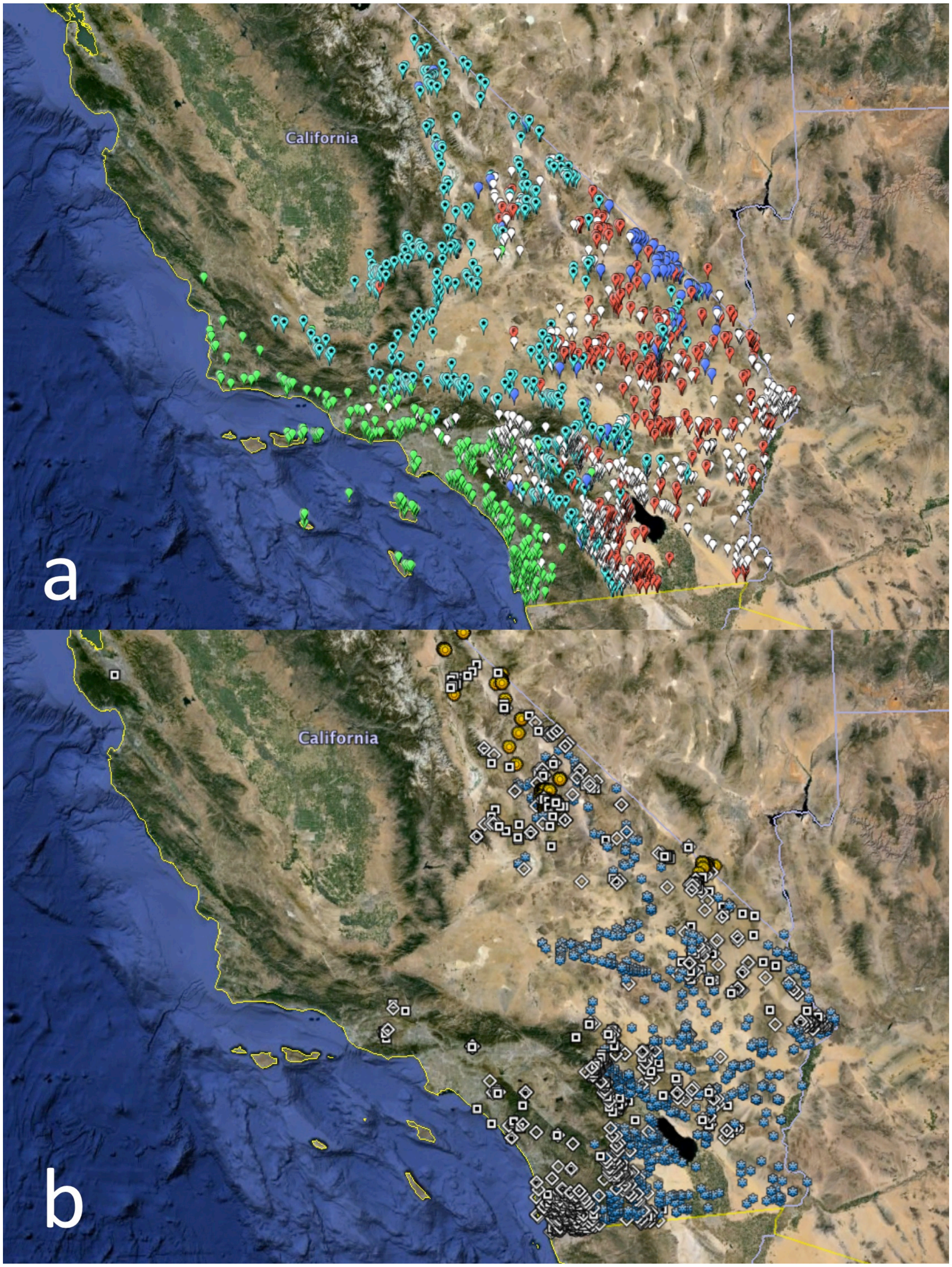
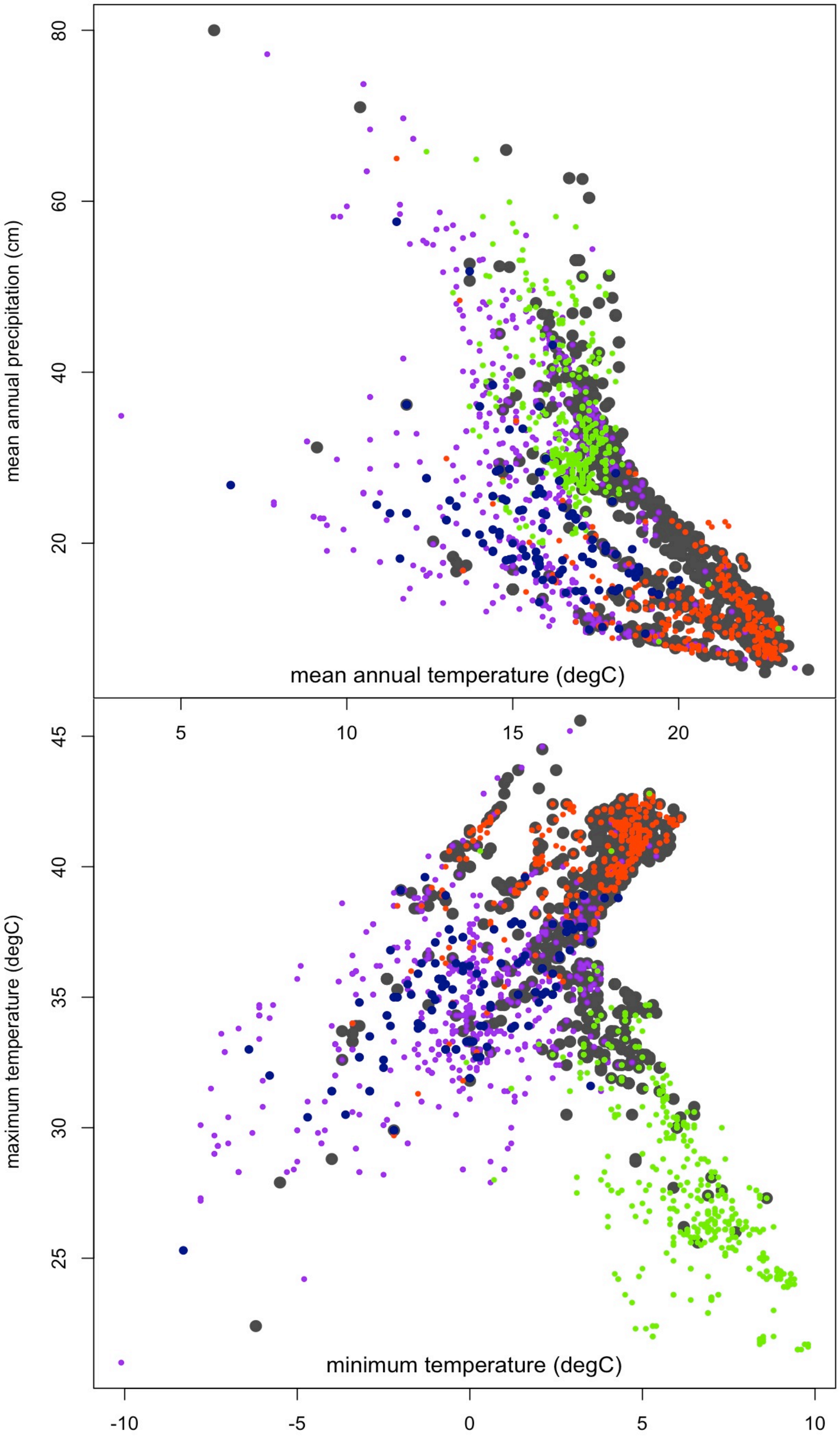


Figure 2: Climate space of all Californian *Encelia* taxa plotted by mean annual temperature and mean annual precipitation (a), and average minimum and maximum temperatures of the coldest and hottest months (b). Dot size for visual clarity.



data from Worldclim (www.worldclim.org) (Figure 1). A total of 2,749 georeferenced specimens were obtained (625 for *E. actoni*, 500 *E. californica*, 1038 *E. farinosa* including all varieties, 450 *E. frutescens* and 136 *E. virginensis*). One specimen labeled *E. frutescens* var. *actoni* was treated as *E. frutescens* and nine specimens categorized as *E. virginensis* ssp. *actoni* were classified as *E. virginensis* even though *E. virginensis* is likely itself a homoploid hybrid species with *E. actoni* and *E. frutescens* as the progenitor taxa (Allan et al. 1997). Worldclim climate point data associated with the latitude and longitude coordinates of the locations of the source populations from which common garden plants were grown was also retrieved and used in all subsequent analyses. For comparison of within versus between species variability for individual traits a two-way analysis of variance (ANOVA) was used. For comparison of the predictive power of different climate variables on phenotypic traits measured in common garden plants a multiple regression was used. For comparison of whether taxon or climate parameters better predict trait variability analysis of covariance (ANCOVA) was used. All statistical and graphical analyses were implemented in R (R Core Development Team 2012).

Results

Climatic divergence

All California taxa of *Encelia* showed differences in their geographic distributions (Figure 1) and climatic niches (Figure 2) although there was substantial sympatry and also substantial overlap in climate space. The plot of maximum versus minimum temperature tended to maximize the variance between different species' climate niches (Figure 3 versus 4) with most species tending to occupy one extreme or another of the climate space. One axis of differentiation is clearly visible with the chartreuse colored *E. californica* shown occupying coastal habitats with low maximum and high minimum temperatures. Another axis of differentiation is visible with the light purple colored *E. actoni* occupying regions of low maximum and low minimum temperatures that correspond to the western mountains bordering the Mojave and Sonoran deserts as well as high elevation regions near Death Valley. These habitats experience frequent freezing temperatures and *E. actoni* appears to possess a unique capacity to tolerate freezing. *Encelia frutescens* and *E. farinosa* occupy the hottest regions of the climate space. *Encelia farinosa* is an exception to the general rule of climate specialization in that it is much more widespread geographically and climatically and strongly overlaps in distribution with most other species.

Evidence of geographic and climatic range expansion comes from *E. actoni* inhabiting cold, high elevation sites in the western mountains including the Transverse Ranges and Death Valley, and *E. californica* inhabiting coastal habitats from northern Baja California to Santa Barbara. Both of these taxa exhibit climatic and geographic niches that are outside the range of other taxa in *Encelia* as well as the closest four sister genera to *Encelia* (Figure 1b). Plotting the average minimum and maximum temperatures of the coldest

Figure 3: Climate spaces of individual *Encelia* species plotted by minimum and maximum temperatures, on top of the combined climate space of all *Encelia* taxa in California. *Encelia frutescens* (red), *E. farinosa* var. *farinosa* (blue), *E. californica* (green), *E. farinosa* var. *phenicodonta* (orange), *E. virginensis* (dark blue) and *E. actoni* (purple).

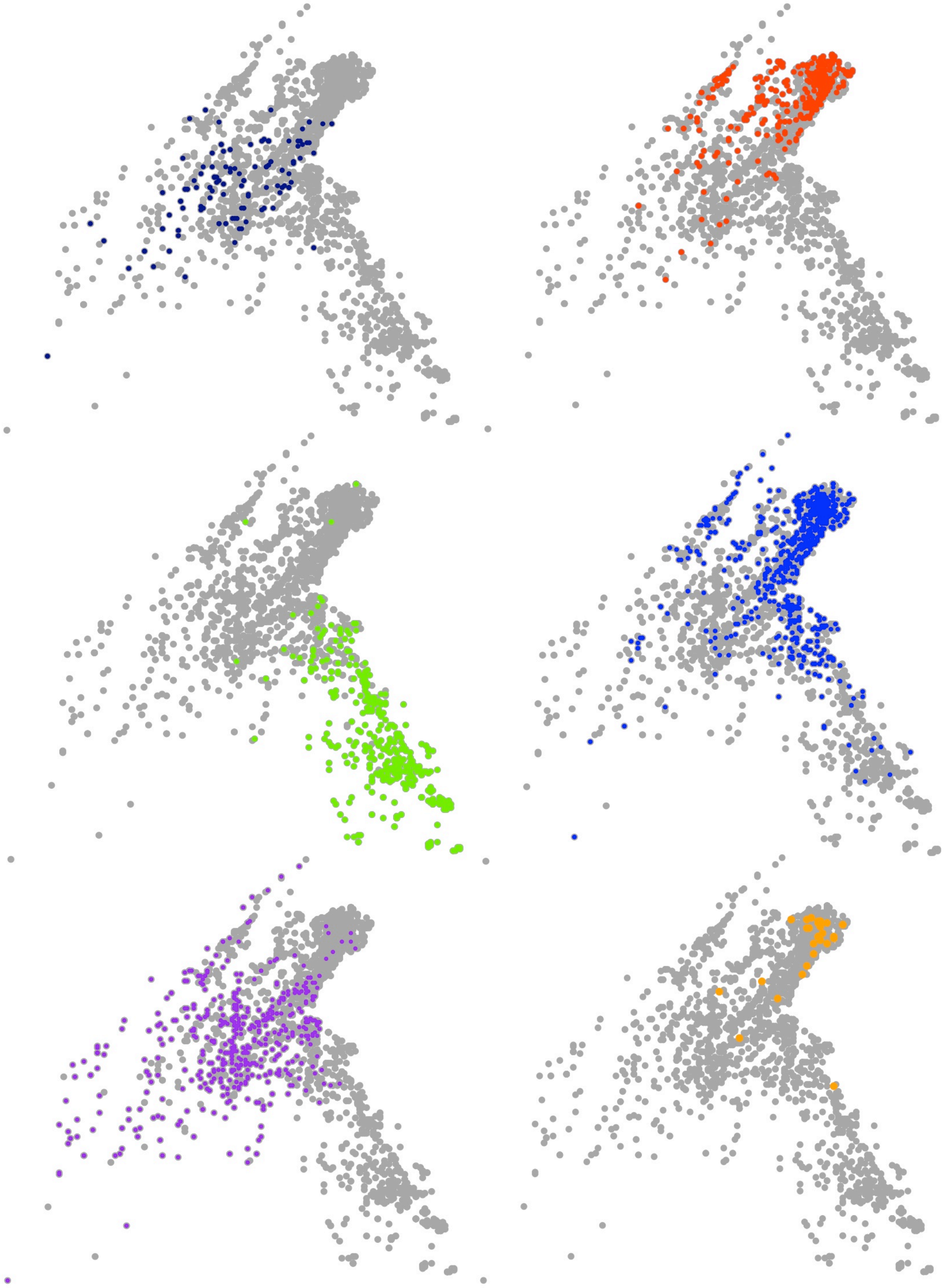
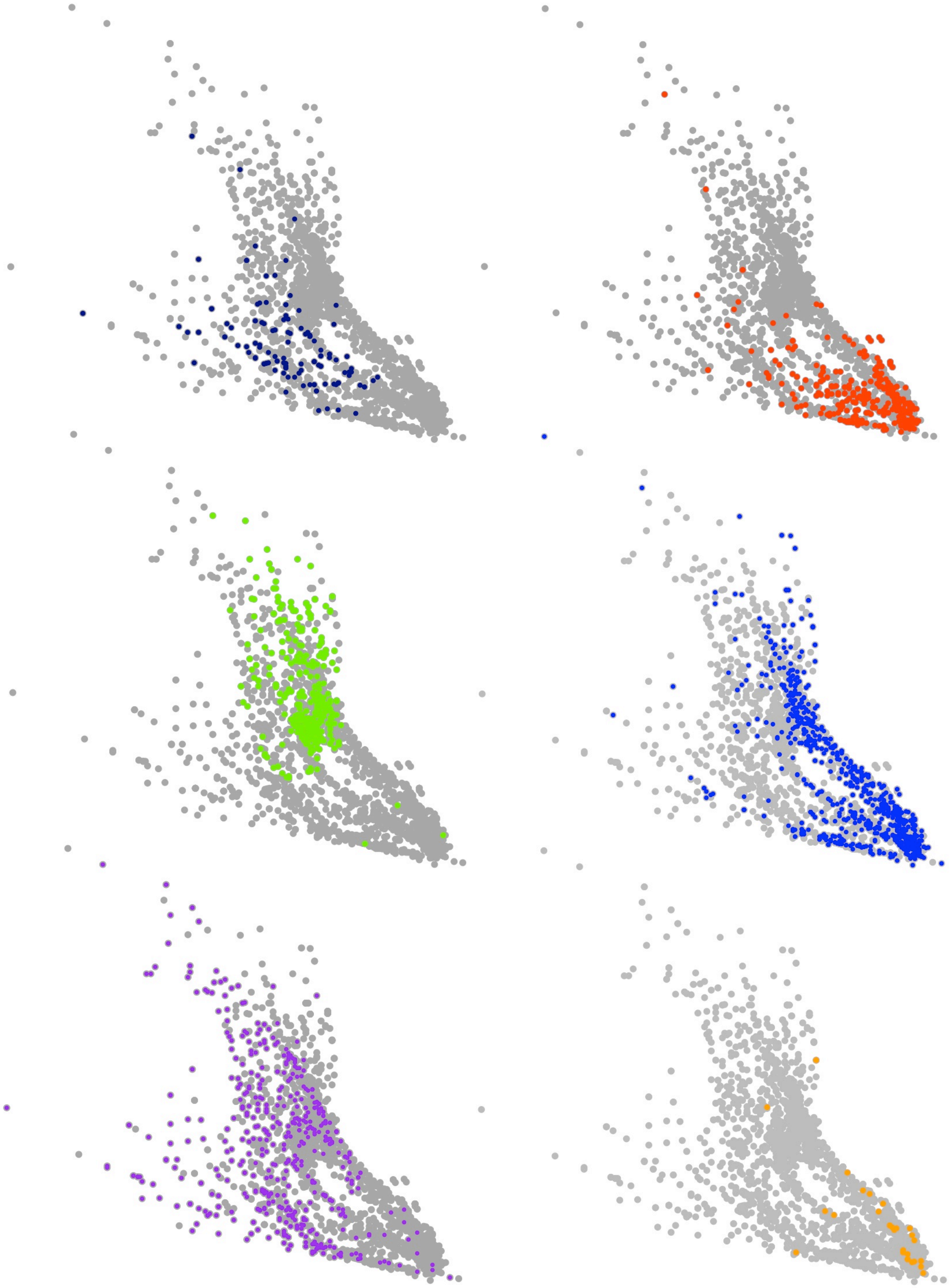


Figure 4: Climate spaces of individual *Encelia* species plotted by average temperature and precipitation, on top of the combined climate space of all *Encelia* taxa in California. *Encelia frutescens* (red), *E. farinosa* var. *farinosa* (blue), *E. californica* (green), *E. farinosa* var. *phenicodonta* (orange), *E. virginensis* (dark blue) and *E. actoni* (purple).



and hottest months, respectively (Figure 3) shows that *E. actoni* (purple dots) exists in habitats with low maximum temperatures and minimum temperatures indicating a potential shift in limiting factors from high summer temperature to low winter temperature. For *E. actoni*, 6% of the localities exhibit average minimum winter temperatures below -5degC.

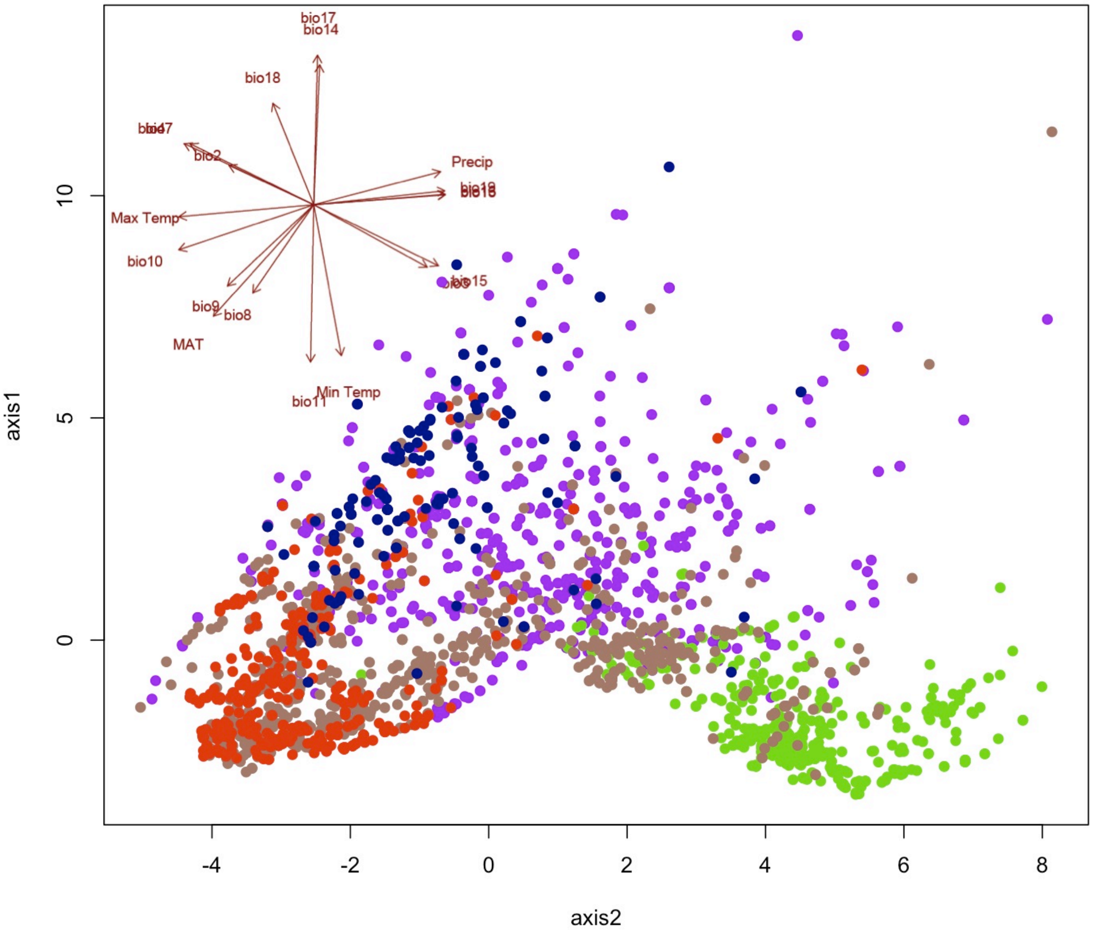
Two "leaks" of populations of *E. actoni* can even be found extending into the Central Valley and Coast Range Mountains (Figure 1). Contiguous populations can be observed from Inyokern in the Mojave Desert up the Highway 178 corridor and through the Kern River Gorge to Lake Isabella spilling out into the foothills above Bakersfield. A second leak appears to extend west along the north and south margins of the Transverse Ranges, meeting in the vicinity of Tejon Pass and continuing as far west as the Carrizo Plain and Sierra Madre Mountains in eastern Santa Barbara County. The Central Valley and Coast Range Mountains provide what appear to be climatically suitable habitats for a number of other taxa in *Encelia* in addition to *E. actoni* based on their distributions in the Mojave Desert, however the freezing conditions that exist in the southern Sierra Nevada and Transverse Ranges may present a climatic barrier to dispersal due to freezing temperatures. If this is the case, *E. actoni* may represent a taxon whose range is able to expand due to adaptation overcoming a barrier to dispersal. An alternative explanation is that populations of these taxa including *E. actoni* did exist but changing land use extirpated these outlying populations such as is likely with any taxa that previously inhabited the floor of the Central Valley which has been entirely converted to agriculture, or the foothill grasslands that have experience heavy grazing pressure for over a century.

Interestingly, *E. virginensis*, a putative homoploid hybrid species between *E. actoni* and *E. frutescens* (Allan et al. 1997) is climatically intermediate to its likely progenitors (Figure 3 and 4) but occupies a novel geographic niche (Figure 1) having expanded into the far eastern Mojave Desert (shown) and into the Grand Canyon (not shown). Unfortunately the absence of specimens from Nevada in the CCH database limits our ability to test whether *E. virginensis* inhabits a climate intermediate to or divergent from that of it's putative progenitor taxa.

Common garden: within- versus among-species trait variation

Univariate comparisons of trait means across species generally showed strong differences among species, with all one-way ANOVAs performed detecting significant differences at $\alpha=0.01$ (Supplementary Figures 1-12). However, few comparisons within species were significant using Tukey post-hoc tests. Although the low sample size reduced the power to detect intraspecific differences in trait means, some within-species comparisons were still significant at $\alpha=0.01$. Notably, *E. farinosa* showed differentiation in the predicted directions for leaf thickness, leaf area, flowering phenology and the difference between mid-day and pre-dawn water potentials, and varied in the predicted direction, with marginal significance for all remaining measured traits: mid-day water potential, growth rate, leaf color, number of flowers, SLA and stem hydraulic conductance. Notably, the

Figure 5: Principal components analysis of all georeferenced *Encelia* specimens in California according to the climate of their collection locality, with a biplot included in the upper left corner showing the loadings of different Bioclim variables. *Encelia frutescens* (red), *E. farinosa* var. *farinosa* (blue), *E. farinosa* var. *phenicodonta* (orange), *E. virginensis* (dark blue), *E. californica* (green) and *E. actoni* (purple).



sample size is larger for *E. farinosa* than for other populations. *Encelia asperifolia* also showed intraspecific differentiation in the predicted directions more often than not with mid-day water potential, growth rate (as measured by canopy volume), number of flowers and canopy architecture differing significantly at $\alpha=0.01$.

Notably, *Encelia frutescens* had a significantly higher conductance than all other species, consistent with previous research showing this taxon utilizes evaporative cooling and exists in microsites with high water availability (Ehleringer 1988, Ehleringer and Cook 1990). Finally, for leaf thickness (Supplementary Figures 7 and 8) the presence of a highly divergent taxon (*E. ventorum*) triggered significant model-wide effects of taxon, although these effects disappeared when *E. ventorum* was removed.

The localities of the source populations for the common garden were from a range of climates. However, Figures 8 and 9 illustrate the observation that *E. farinosa* tends to exist everywhere that other species do climatically, with the exception of the previously noted extreme high elevation habitats of *E. actoni* and the northern coastal habitats of *E. californica*. Examination of the source populations show that a range of climates are inhabited but that the range of variability between sub-populations is frequently greater than that between species (Figure 9). Furthermore, principal component analysis (PCA) of all plants used in the common garden as a function of morphology (Figure 10) or physiology (Figure 11) failed to distinguish all but a few highly differentiated taxa. Importantly, the multicolored clusters of points in Figures 10 and 11 remain largely undifferentiated even when the outlier taxa are removed and the analysis repeated on the smaller dataset.

Relationship of traits to climate parameters

In order to test whether climate parameters are associated with certain trait states, I compared the trait values from the common garden plants to the climate of the population from which they were collected. For all traits analyzed individually, multiple regression revealed a number of significant correlations at the among-species level, although most traits were uncorrelated with most climate parameters. Of those traits and climate parameters that were correlated, all varied in the directions predicted by leaf economic theory. Leaf area was positively related to mean annual precipitation ($t=5.39$, $n=91$, $p<0.001$), canopy volume was positively related to minimum temperature ($t=3.11$, $n=124$, $p=0.002$), leaf color (a measure of reflectance due to white pubescence) was positively related to maximum temperature ($t=2.35$, $n=91$, $p=0.020$) and plants exhibited earlier flowering phenologies in habitats with higher amounts of precipitation ($t=-3.16$, $n=110$, $p=0.002$). Leaf thickness showed strong correlations with mean annual temperature, mean annual precipitation, maximum temperature and minimum temperature, however none of these factors were significant at the $\alpha=0.05$ level after *E. ventorum* was removed. *Encelia ventorum* has very thick, succulent leaves that are likely an adaptation to the hyper saline coastal dune environment and likely has high leverage in all intraspecific analyses of leaf thickness.

Figure 6: Apparatus for measuring stem hydraulic conductance.

Figure 7: Photograph of the common garden in February 2013 after two years of growth under ambient conditions illustrating the earlier flowering and smaller size of *Encelia farinosa* collected from the eastern Mojave Desert (xeric habitat) versus Riverside (mesic habitat).

By plotting the mean values of each population and connecting populations within a species with a line, the direction of variation within species can be compared with the direction of variation among species (Supplementary Figures 13-24). The predicted direction of variation based on leaf economic theory (red line in inset black box) for mean annual temperature as well as for mean annual precipitation is also shown to compare with the observed data both among and within taxa. For some traits, ANCOVA revealed patterns in the data that were missed by multiple regression and ANOVA. For example, leaf thickness showed no relationship to climate variables after removing the highly divergent taxon *E. ventorum* from the analysis, however ANCOVA revealed significant effects of species (F=201.61, df=6, p<0.001), MAT (F=4.69, df=1, p=0.033) and precipitation (F=6.27, df=1, p=0.014) and the strength of these effects increased rather than decreased when *E. ventorum* was removed (species: F=5.85, df=5, p<0.001; MAT: F=11.33, df=1, p=0.001; precipitation: F=20.46, df=1, p<0.001). This can be seen in Figures 14 versus 15 whereby eliminating *E. ventorum* allows the patterns among other traits to be observed more easily.

The exception to these patterns was *E. actoni* which may be more sensitive to minimum temperatures than to maximum temperatures since it appears to be expanding into high elevation habitats. A similar pattern was observed for SLA, which is likely tightly linked to leaf thickness developmentally. ANCOVA revealed significant effects of species (F=18.24, df=6, p<0.001), and precipitation (F=3.99, df=1, p=0.49) and a marginally significant effect of MAT (F=3.53, df=1, p=0.063).

Discussion

Overall these data provide substantial evidence for climatic, morphological and physiological differentiation, although much of the variation was not predictable based on leaf economic theory. There was a pronounced lack of univariate correlations of traits with climate (Figures 12-23). However, notable exceptions exist. For SLA (Figure 20) nearly all taxa responded in the same direction, the directions predicted by leaf economic theory (Wright et al. 2004). SLA increased with increased precipitation and decreased with increasing temperature consistent with an increase in sclerophyly found in arid climates worldwide. However, there was generally no pattern among species indicating that the processes generating and constraining divergence may be different at different taxonomic scales. Within taxa the environment appears to cause change in the predicted directions for most taxa, while among taxa the role of alternative adaptations may undermine strong univariate correlations of any single trait with climate. Interestingly, as with many of the other traits, the two populations of *E. actoni* were outliers for SLA and showed little intraspecific differentiation despite existing in very different temperature regimes, perhaps reflecting a reduced importance of maximum temperature and leaf energy balance in general in controlling population composition and increased importance of minimum temperature as a limiting factor concomitant with expansion into high elevation habitats.

Figure 8: Climate space of all georeferenced specimens of *Encelia farinosa* in the CCH database, plotted by mean annual temperature and precipitation and color coded by maximum temperature of the hottest month. The locations of the wet (Riverside) and dry (Mojave) populations used in the common garden are superimposed over these points illustrating where these populations fit in the overall climatic range of the species.

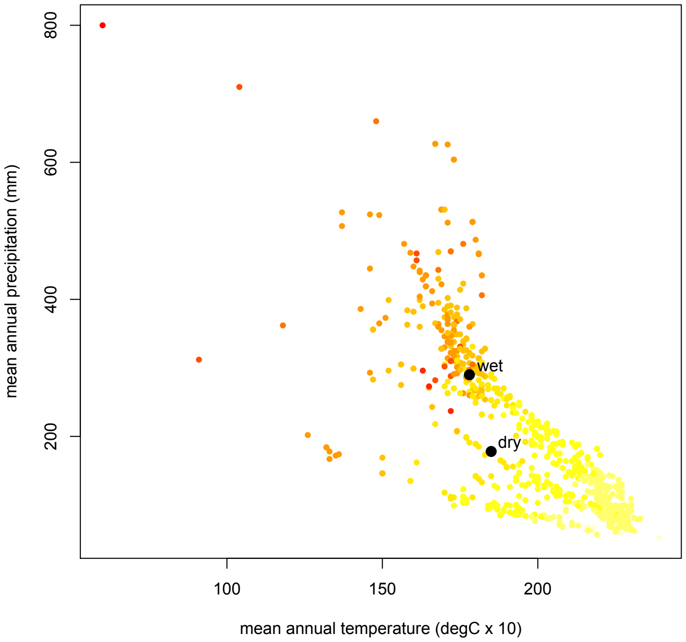


Figure 9: Climate space of all georeferenced specimens of *Encelia farinosa* as above with the locations of all other populations used in the common garden superimposed.

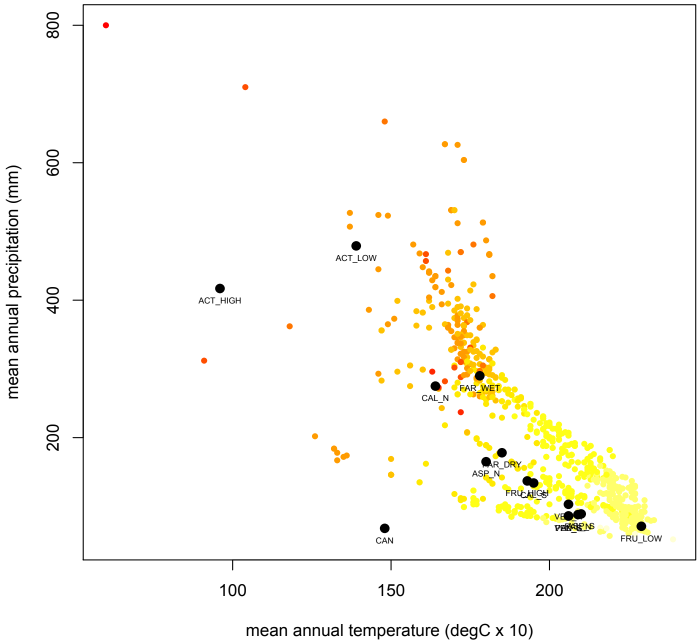


Figure 11: Leaf morphologies of the different *Encelia* taxa used in the common garden. Top row (L to R): *californica* south ecotype, *asperifolia* north ecotype, suspected *palmeri* x *ventorum* hybrid backcross to *palmeri*, *asperifolia* south ecotype, *frutescens*, *ventorum*, *palmeri*. Middle row: *californica* north ecotype, *actoni* low elevation, suspected *asperifolia* x *ventorum* hybrid, *californica* south ecotype, *farinosa* var. *farinosa* Mojave ecotype. Bottom row: suspected *palmeri* x *ventorum* hybrid backcross to *palmeri*, *ventorum*, *asperifolia* south ecotype, *farinosa* var. *farinosa* Riverside ecotype, *californica* north ecotype.

For leaf thickness the same pattern was observed as with SLA (Figures 18 and 19), though this is not surprising since leaf thickness and SLA are highly developmentally constrained with low-SLA leaves often referred to as being "small and thick." However, leaf thickness revealed a surprising innovation of *E. ventorum* that was not detected in the analysis of SLA alone–origination of succulent leaves that likely function as a key adaptation allowing it to persist in hyper-saline coastal dune habitats. While other taxa such as *E. californica* have expanded into coastal habitats, *E. ventorum* is clearly the only taxon to evolve leaf succulence, even though succulence was not measured directly and is inferred from leaf thickness.

Among species, *E. farinosa* was the only taxon to exhibit consistent variation in the directions predicted by leaf economic theory for nearly all traits examined. This is consistent with the unusually variable and widespread nature of this taxon, and suggests that divergence processes or history may be different in *E. farinosa* than in the rest of the genus. The ecotypes examined here are dramatically divergent, with differences in flowering time evident qualitatively (Figure 7). The most recent historical biogeographic analysis of the taxon infers multiple isolated Pleistocene refugia (Fehlberg and Ranker 2009) suggesting that periods of allopatry may also be responsible for observed patterns of variation. This is consistent with its highly variable phenotype, currently divided into three described varieties, and with the high degree of physiological and morphological variation apparent even within varieties (Monson et al. 1992, Schuster et al. 1994, Sandquist and Ehleringer 1997). It is not known whether the hyper-variability exhibited by *E. farinosa* is due to greater genetic variation, stronger selection pressures or time spent in allopatry, but this is a promising avenue for future research.

There was also evidence that some key traits may be strongly favored in some taxa. *Encelia ventorum* was shown to have much thicker leaves than the rest of the taxa (Figure 10), and succulence is known to be an adaptation to hyper-saline coastal dune environments (Greenway and Munns 1980). Another interesting case is provided by the extreme phenotype of *Encelia farinosa* var. *farinosa* Riverside ecotype, which exhibits very large undulating leaves with occasional dentation and very thick petioles, a phenotype that is out of sync with current treatments (Baldwin et al. 2012) and represents a novel phenotype that appears to be adapted to the relatively mesic environments of the semi-natural areas in the hills bordering the University of California, Riverside. This ecotype was studied previously for variation in canopy architecture (Housman et al. 2002) and this study showed that the comparison between it and interior eastern Mojave Desert populations were the only intraspecific comparisons that consistently exhibited trait variation in the directions predicted by leaf economic theory. These differences are consistent with the hyper-variability of this taxon found in other studies (e.g. Kyhos 1971, Monson et al. 1992, Sandquist and Ehleringer 1996, Fehlberg and Ranker 2009) and suggest a fundamentally different history of divergence for *E. farinosa* compared to the rest of the members of the genus.

Figure 10: Principal components analysis of all plants in the common garden ordinated by their leaf morphology. The only taxa to separate substantially is *E. ventorum*, bottom points, and both ecotypes of *E. farinosa*, the two right-most clouds of points.

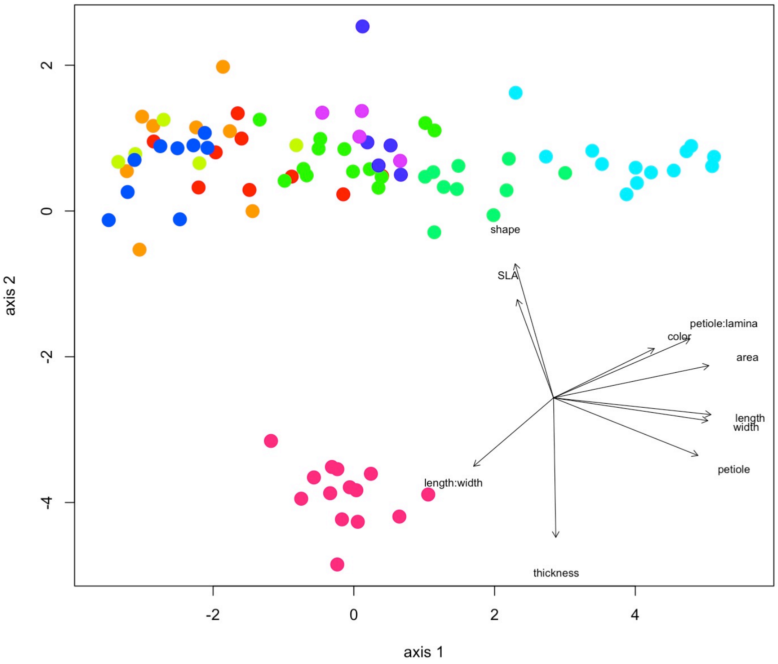
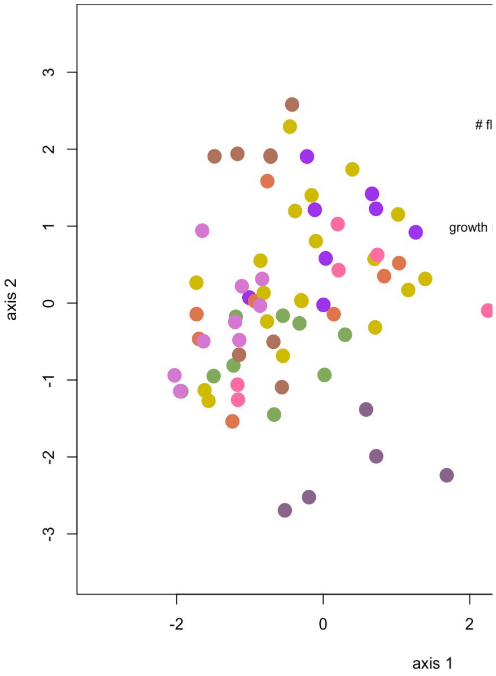


Figure 11: Principal components analysis of all plants in the common garden ordinated by their leaf morphology illustrating no clear separation of taxa by physiology, except that *E. ventorum* (dark points at bottom) exhibits low water potential adjustment (mid-day minus pre-dawn) consistent with its high water availability habitat.



The expansion of *Encelia ventorum* and *E. californica* to the coast, and *E. actoni* into high elevations represents both a geographic and a climatic departure from the rest of the genus and closest outgroups. Most taxa of *Encelia* including *E. farinsoa* var. *farinosa*, a taxon that is considered to be frost-tolerant, show nearly complete mortality at the temperatures characteristic of habitats occupied by *E. actoni* (Sandquist and Ehleringer 1996) suggesting that adaptations to freezing are driving expansion of the range of this taxon. The evolution of leaf succulence and an upright growth habit in *E. ventorum*, and the use of alternative adaptations to circumvent reliance on leaf pubescence in *E. frutescens* also represent phenotypic departures from the rest of the species in the genus and closest relatives. Thus, there is substantial evidence for climatic and phenotypic diversification in the genus consistent with an interpretation of adaptive radiation in response to the opening up of new climatic and geographical niches since the end of the Miocene (Axelrod 1950, Riddle et al. 2000, Oskin and Stock 2003, Jacobs et al. 2004, Fehlberg and Ranker 2009).

Similarly altered selection pressures may explain the expansion into and subsequent restriction to coastal habitats. In the case of *E. californica*, its glabrous leaves and high growth rate may increase its competitive ability in the mild coastal scrub climate but these same traits create a trade-off by lowering resistance to high temperatures (Ehleringer and Clark 1988, Ehleringer and Cook 1990, Knight and Ackerly 2002, Knight and Ackerly 2003). The nearest outgroup genera *Enceliopsis* and *Geraea* as well as the next most closely related clade made up of *Bahiopsis* and *Viguiera* (Figure 1b) are distributed generally south and east; they are not represented far north along the coast and are completely absent from the western high elevation mountains where temperatures drop well below freezing for a substantial portion of the year. Interestingly, *E. californica* and *E. ventorum* may represent another case of alternative adaptations since they both live in coastal habitats but have radically divergent morphologies. However, they are not sympatric and it is not known whether other selective factors may prevent one taxa from invading the habitat of the other.

For the rest of the traits, the lack of any univariate correlations with climate at the among-species level may be due to the many alternate evolutionarily equivalent strategies possible for maintaining positive population growth under a given set of environmental conditions. This multiplicity of potential solutions to a common environmental problem combined with extremely strong abiotic selection pressures found in desert habitats may explain the extremely high functional diversity of ecological strategies and morphologies found in desert habitats worldwide.

A classic example of alternative adaptations studied extensively by Jim Ehleringer and colleagues is the coexistence of *Encelia frutescens* and *E. farinosa* throughout large expanses of the eastern Mojave Desert (Ehleringer 1983, Ehleringer 1988). These perennial desert shrubs are sympatric throughout most of their ranges and inhabit the hottest, driest climatic niche of any of the Californian members of the genus (Figures 1-5). While *E. farinosa* possesses densely pubescent white leaves that reflect the majority of incident solar radiation maintaining leaves at sub-lethal temperatures (Ehleringer and Björkman 1978, Ehleringer and Mooney 1978, Ehleringer 1982, Sandquist and Ehleringer 1997), *E. frutescens* exhibits bright green leaves with only a scabrous coating of spine-like multicellular trichomes (Ehleringer and Cook 1987). In light of previous studies that showed a strong correlation of leaf pubescence with aridity across multiple spatial and taxonomic scales (Ehleringer et al. 1976, Ehleringer et al. 1981), this observation is surprising since energy balance calculations indicate that without the reflective pubescence *E. farinosa* leaves would quickly exceed lethal temperatures.

This paradox is resolved by considering variation in a third trait, conductance (Ehleringer and Cook 1990). *Encelia frutescens* has much higher transpiration rates than *E. farinosa* thus maintaining a similar leaf temperature via alternate ecologically equivalent strategies. However, a trade-off is immediately apparent. Although conductance and leaf color are partially compensating strategies for maintaining a favorable leaf energy balance, evaporative cooling requires more water (Lambers et al. 2008). Thus, for a given amount of water *E. frutescens* can photosynthesize for a shorter duration of time than can *E. farinosa*, and this is reflected in the much greater deciduousness of *E. frutescens* compared with *E. farinosa*. In the end, the shorter duration of photosynthesis may be compensated for by increased rate of photosynthesis compared to *E. farinosa*, which reflects back to the atmosphere much of the photosynthetically active radiation with its dense pubescence. Thus, different ecological strategies and corresponding morphologies developed within the constraints of resource limitations and resource trade-offs result in convergence in plant functioning toward a common leaf temperature, all of which is then reinforced or selected against at a population level if the strategy employed is too costly and results in lower fitness relative to other strategies. These alternative strategies may explain the widespread sympatry of *E. frutescens* and *E. farinosa* across vast expanses of the Mojave Desert. In this case, the absence of a correlation between leaf color and climate does not mean that leaf color is selectively neutral. Rather, it means that color is one dimension of a multivariate strategy with the adaptive value of color depending on the level of other functional traits.

As a further complication, *E. frutescens* is also frequently found in depressions or wash bottoms, microsites within zones of sympatry that have locally higher water availability. Why doesn't *E. farinosa* invade these higher resource microsites? The most likely explanation invokes yet another trade-off, this time between drought tolerance and mechanical susceptibility to disturbance (Ehleringer 1988, Ehleringer and Cook 1990). The common name of *E. farinosa* is brittlebrush which comes from the very brittle stems. The mechanical properties of these stems likely result from rigid xylem walls that help prevent cavitation at low water potentials thus increasing drought tolerance (Lambers et al. 2008). However, rigid stems may be deleterious in highly disturbed wash habitats where the flexible stems of *E. frutescens* are better able to withstand periodic flash flooding (Ehleringer 1988. Ehleringer and Clark 1988, Ehleringer and Cook 1990). As this example shows, even a single well-circumscribed selective pressure such as the need to maintain sub-lethal leaf temperatures can result in cascading feedbacks of epistatic effects involving hierarchies of traits and sub-traits including some such as mechanical resistance to flooding that initially appear to have little to do with leaf temperature. The diversity of ecologically equivalent strategies means that it may be unlikely that any one trait will show very strong correlations with habitat or climate unless that trait is required to invade certain extreme habitats or utilize hard-to-obtain resources. These layers of contingencies introduce noise into univariate correlations of traits on climate and provide a robust mechanism for generating the spectacular functional diversity found in most ecosystems.

Additional examples of ecologically equivalent strategies can be found in most ecosystems. For example, in the Central Desert of Baja California, México one frequently sees sclerophylous low-SLA taxa such as *Simmondsia* (jojoba) and *Larrea* (creosote bush) coexisting with high-SLA taxa such as *Fouquieria* (ocotillo) and *Pachycormus* (elephant tree) (Shreve and Wiggins 1964). In these cases, leaf life span is again an important contingency; habitats with the briefest periods of water availability should have the highest SLA leaves, but only if they are deciduous. Ephemeral leaves are typically built as quickly as possible with the least investment in protective and structural compounds, and short growing seasons should select for even more rapid construction of low-maintenance leaves. The epistatic nature of drought tolerance means that multiple strategies can exist to deal with the same environmental stimulus, undermining the negative correlation between SLA and drought that is otherwise predicted without knowing the states of other functional traits that are coupled to SLA either developmentally (e.g. leaf thickness), physiologically (e.g. leaf temperature) or relevant to whole-plant fitness (e.g. stem brittleness).

While drought has been shown to lead to rapid adaptation within lineages, it is also thought that drought is a particularly powerful force driving lineage splitting, and that this effect should be more pronounced in deserts compared with more mesic biomes. Stebbins (1952) and Axelrod (1972) argued that a gradient in precipitation of, for example, five centimeters per year in a habitat that averages fifteen centimeters per year is a stronger selective gradient compared to the same absolute change in precipitation in a habitat that receives 150 centimeters of precipitation per year. Thus, small absolute gradients in resource availability in arid climates represent much greater proportional changes, and for sedentary plants exposed to extremely high temperatures and prolonged drought these small gradients can be very significant ecologically and should lead to accelerated rates of evolution in deserts.

Although the mechanisms behind these early theories of adaptive radiation were somewhat vague and various, it is clear today that deserts are hotspots of endemism and adaptive radiation (Axelrod 1950, Stebbins and Major 1965, Raven and Axelrod 1978). Genus-to-species and family-to-genus ratios are particularly low in desert habitats (Stebbins and Major 1965, Raven and Axelrod 1978, Schwarzback and Kadereit 1995, Klak et al. 2003, Rabosky et al. 2007) which despite the numerous problems with these floristic metrics does suggest increased rates of speciation in desert habitats. The enhanced effective precipitation gradient effect, combined with the history of recent climatic change (Axelrod 1950, Jacobs et al. 2004) and formation of the Baja California peninsula over the past five to ten million years (Riddle et al. 2000, Oskin and Stock 2003) created the ecological opportunity and geographic isolation that is thought to be required for adaptive radiation, likely leading to the spectacular proliferation of functional diversity that is instantly recognizable as the New World desert flora.

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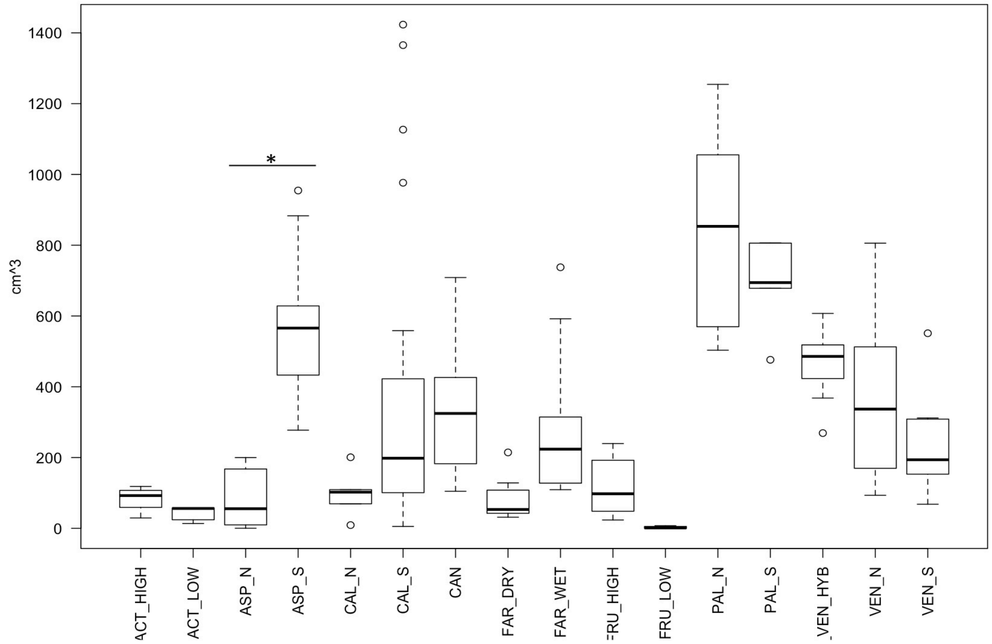
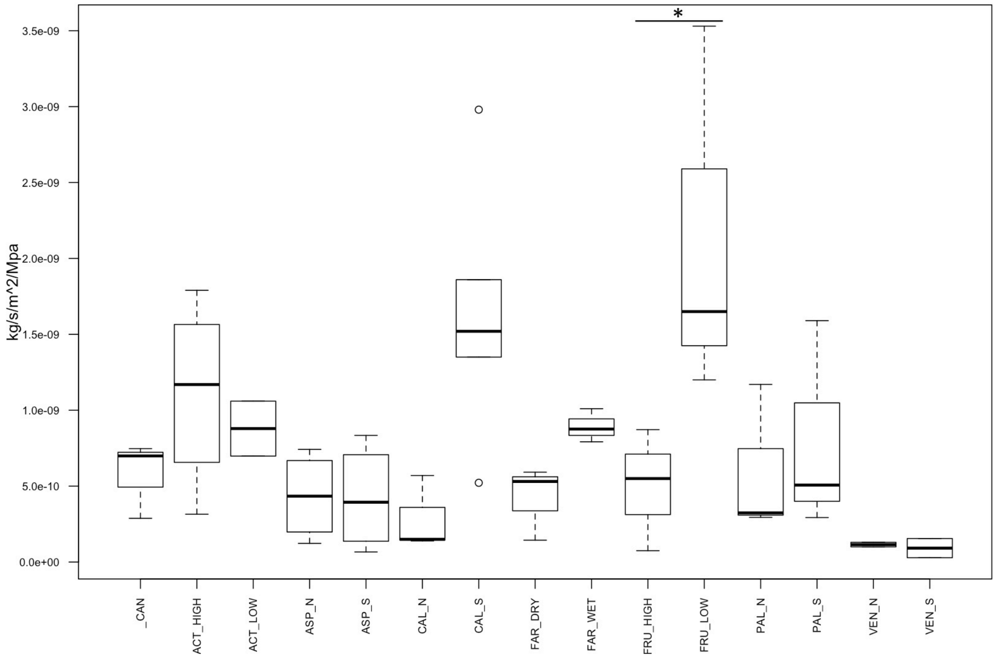
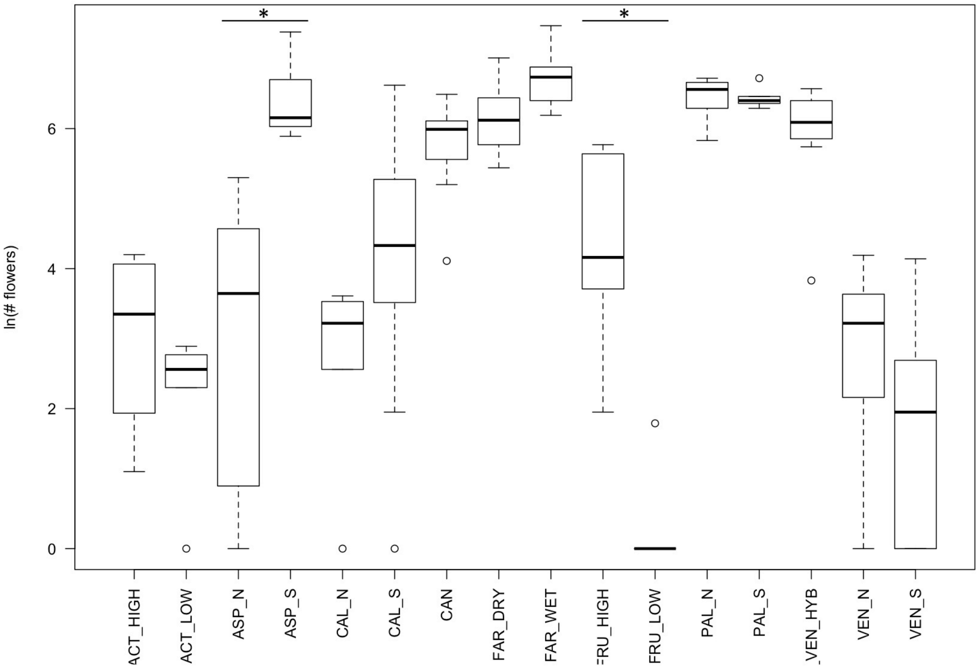
Supplemental Figure 1: Canopy volume (growth rate) as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



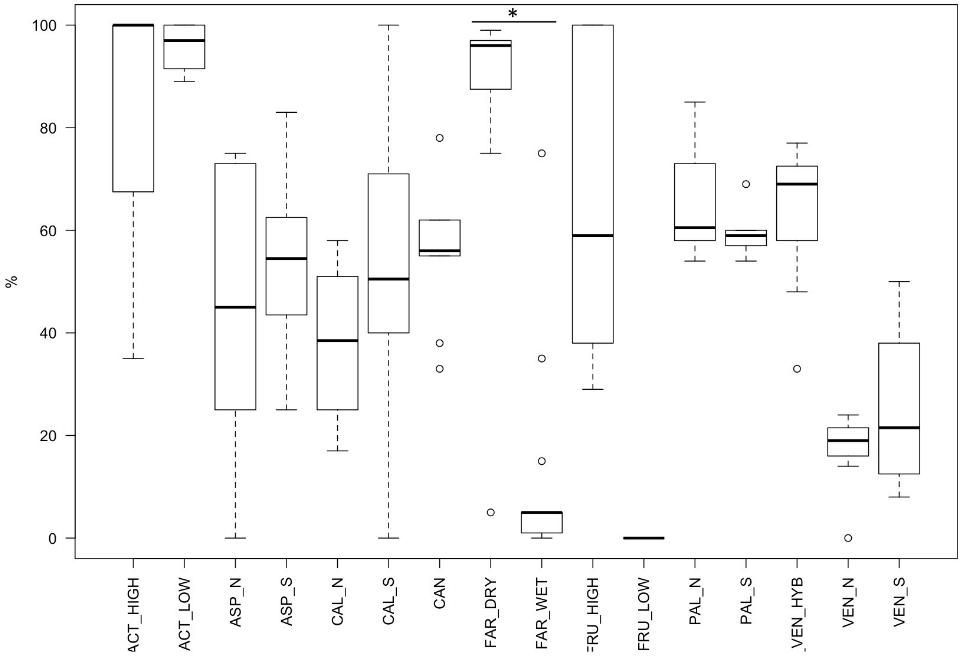
Supplemental Figure 2: Stem hydraulic conductance as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



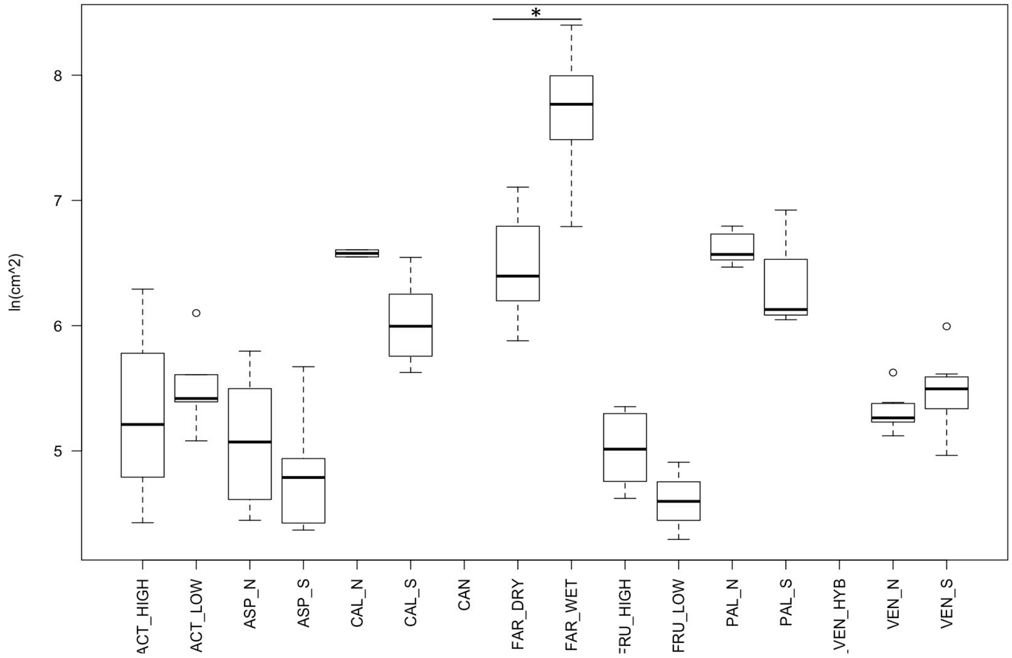
Supplemental Figure 3: Flowering effort as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



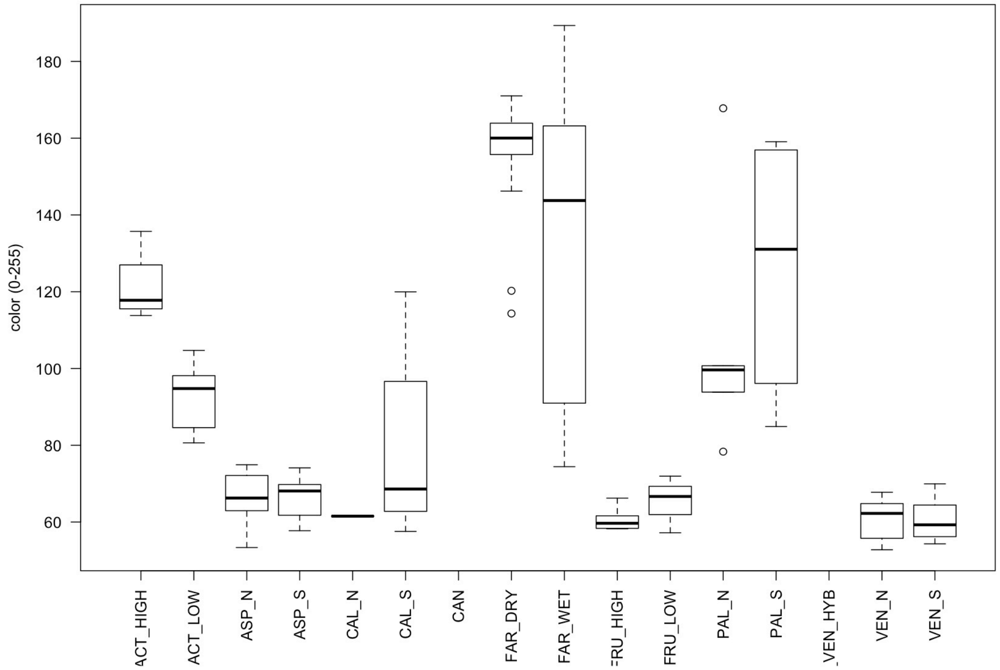
Supplemental Figure 4: Flowering phenology as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



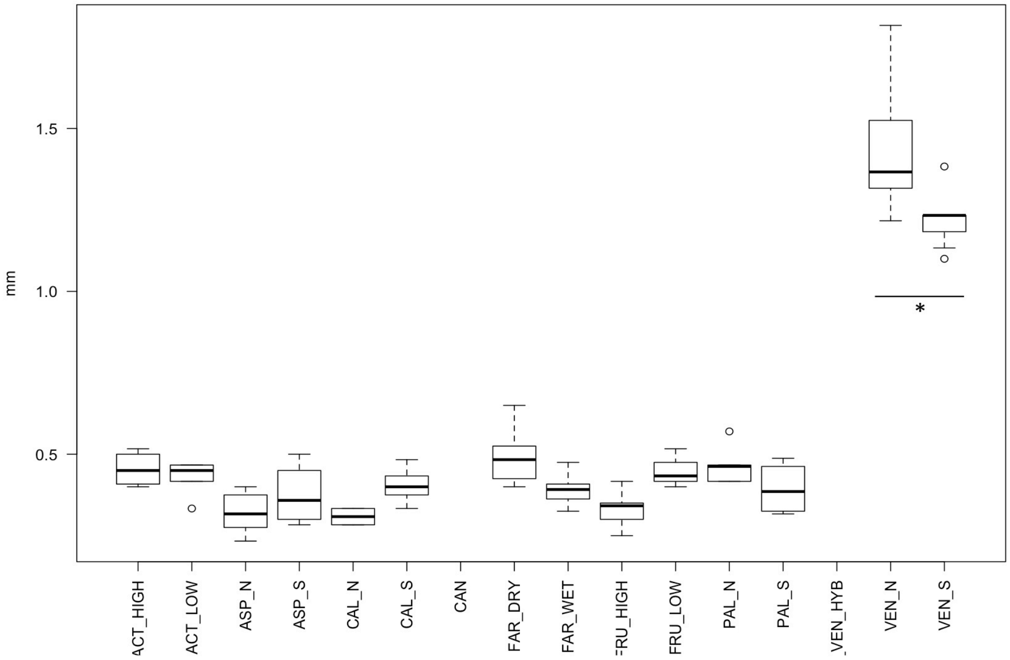
Supplemental Figure 5: Leaf area as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



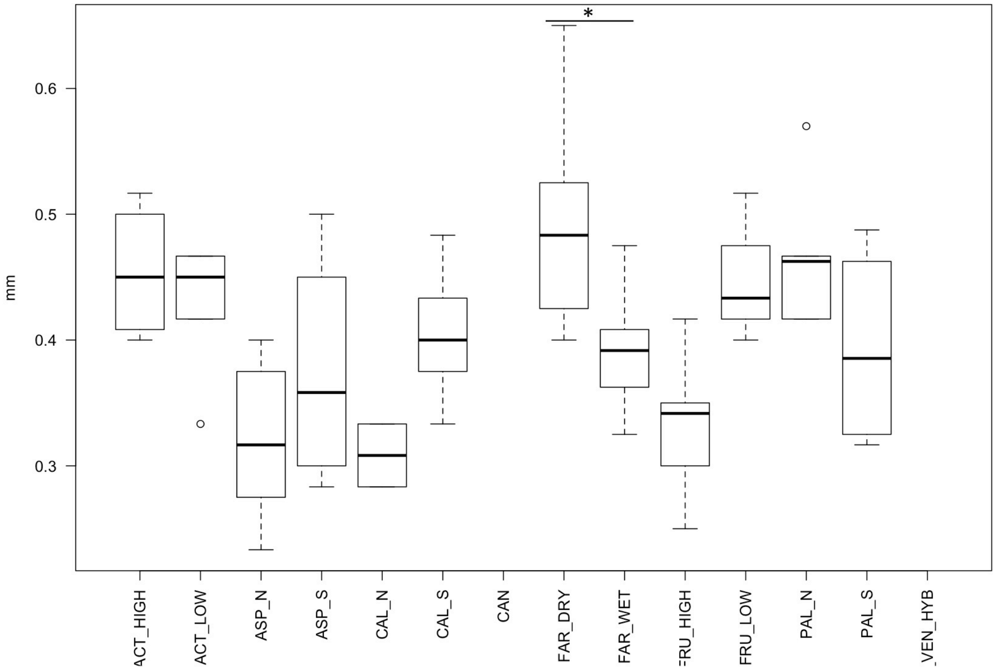
Supplemental Figure 6: Leaf color as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



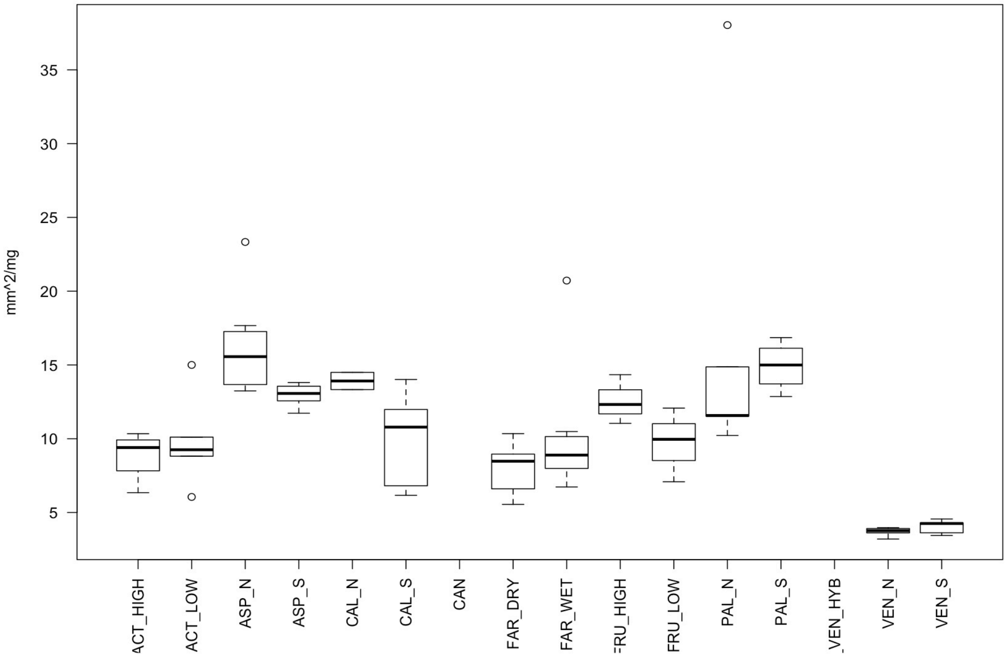
Supplemental Figure 7: Leaf thickness as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



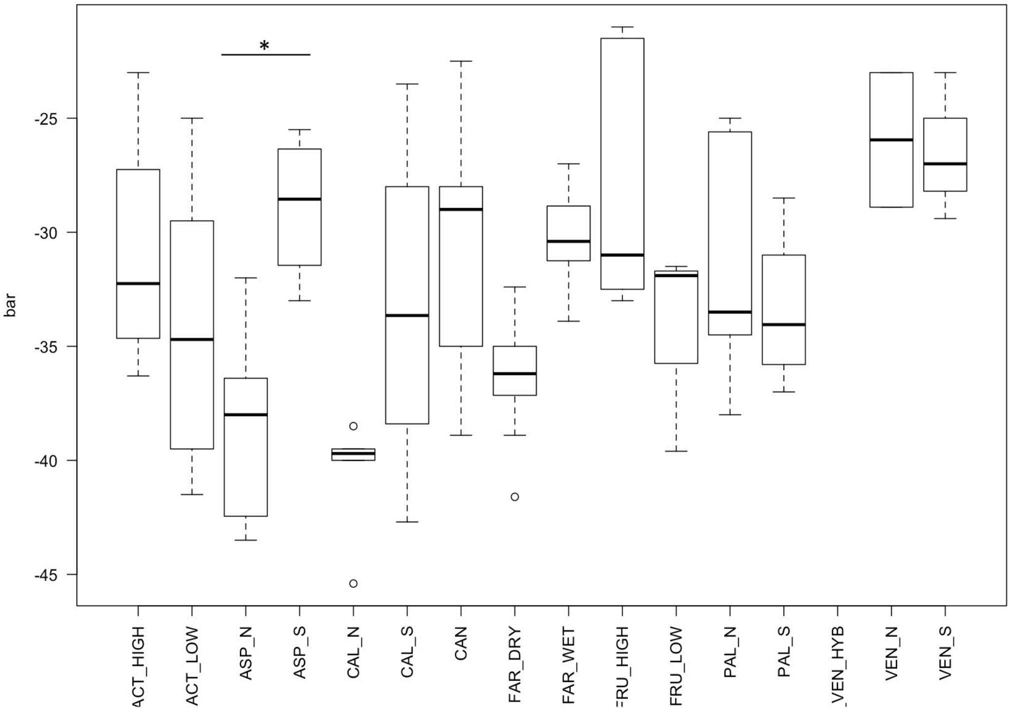
Supplemental Figure 8: Leaf thickness as a function of source population without *E.* ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).*ventorum.*



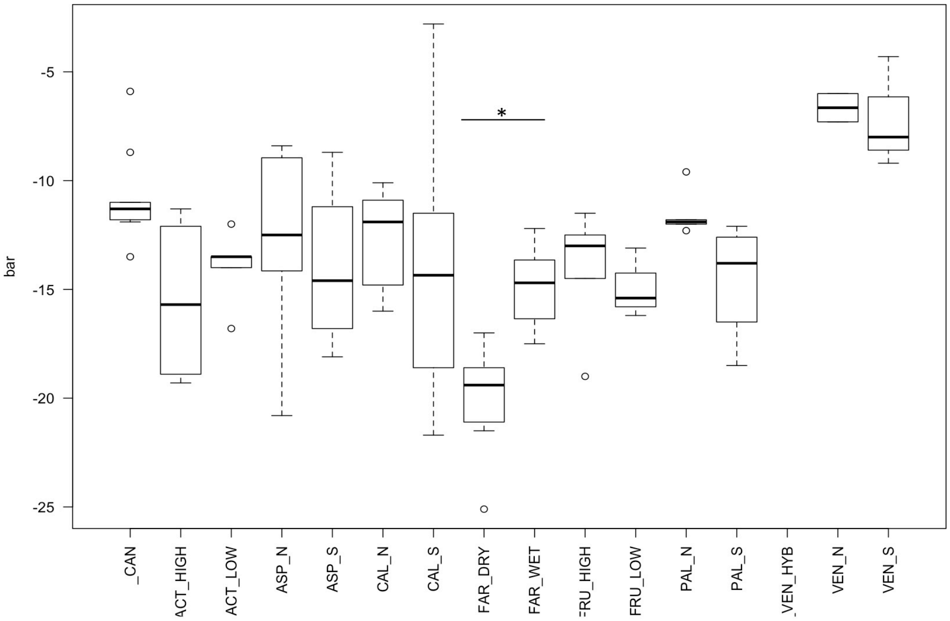
Supplemental Figure 9: Specific leaf area as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



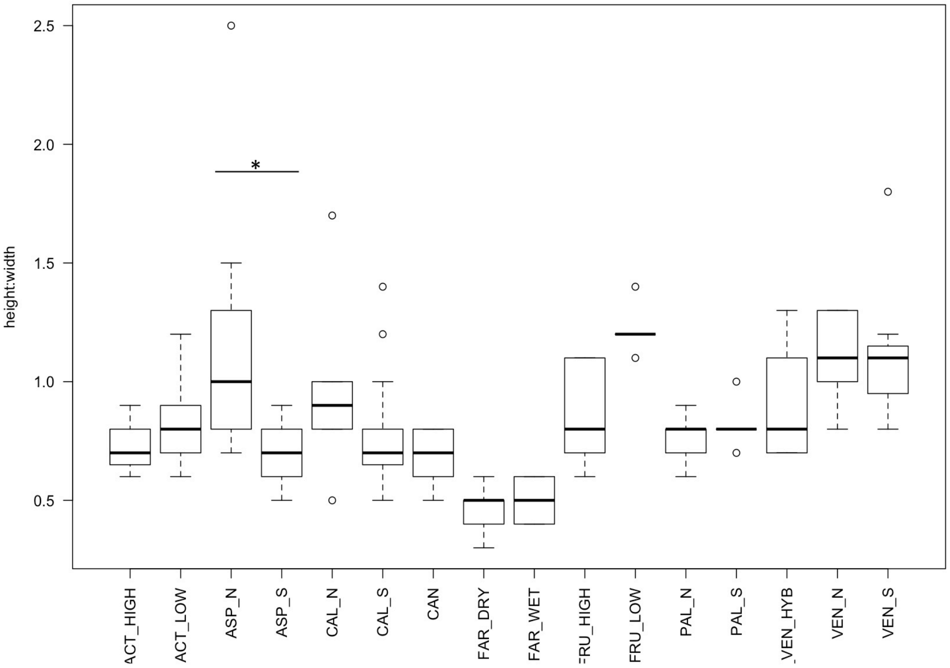
Supplemental Figure 10: Water potential as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



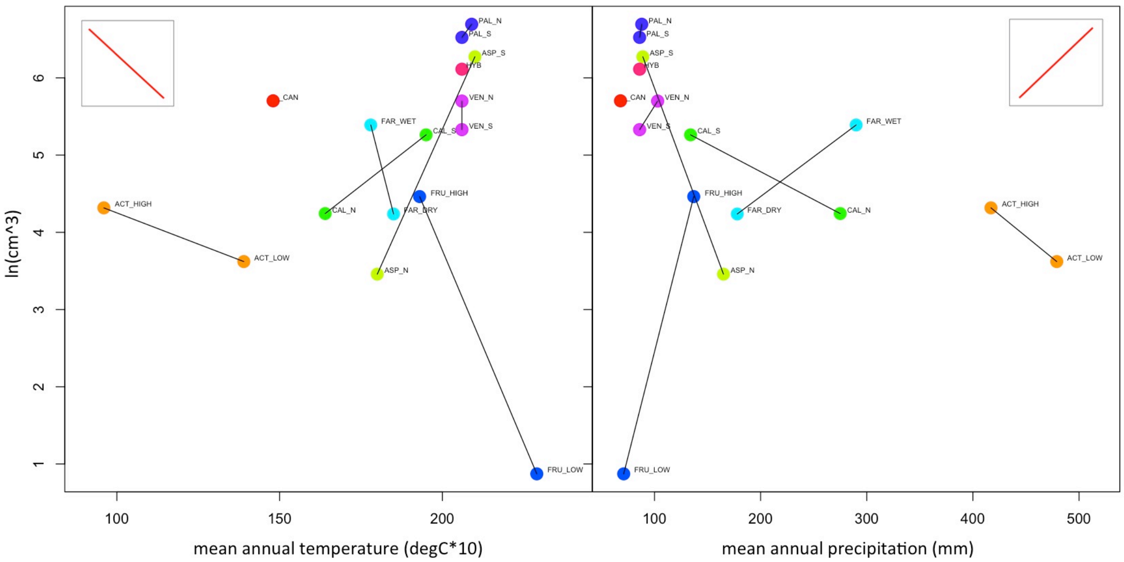
Supplemental Figure 11: Water potential difference as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



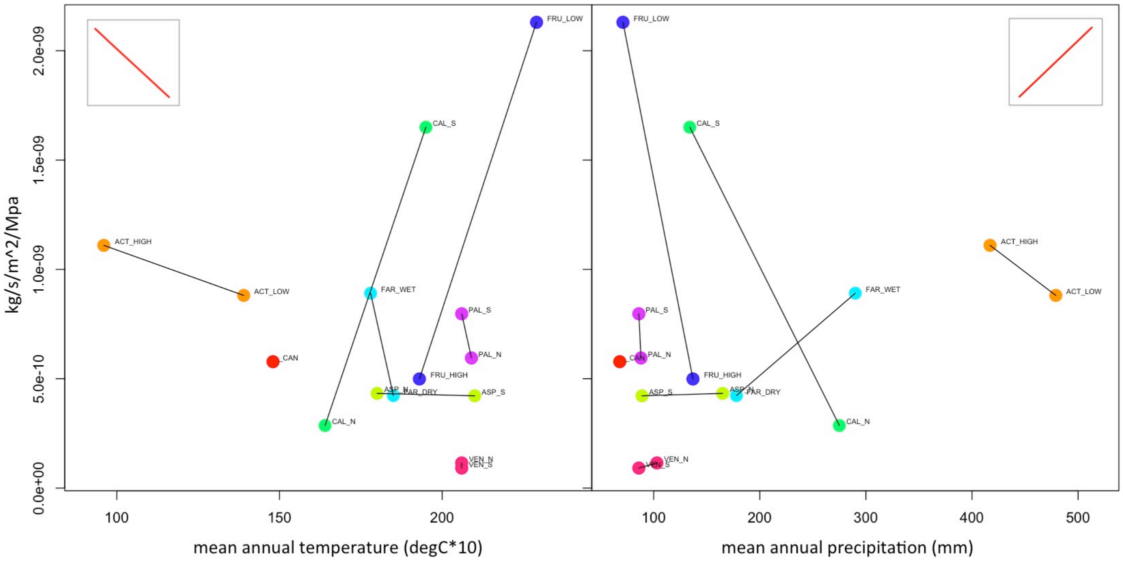
Supplemental Figure 12: Canopy architecture as a function of source population. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



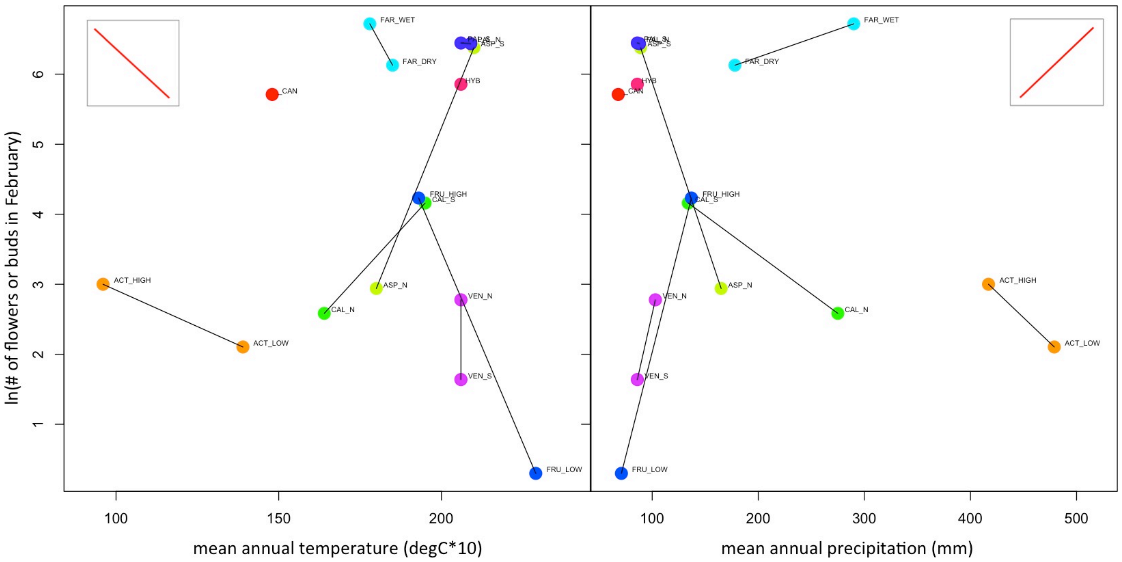
Supplementary Figure 13: Within versus among species variation in canopy volume as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



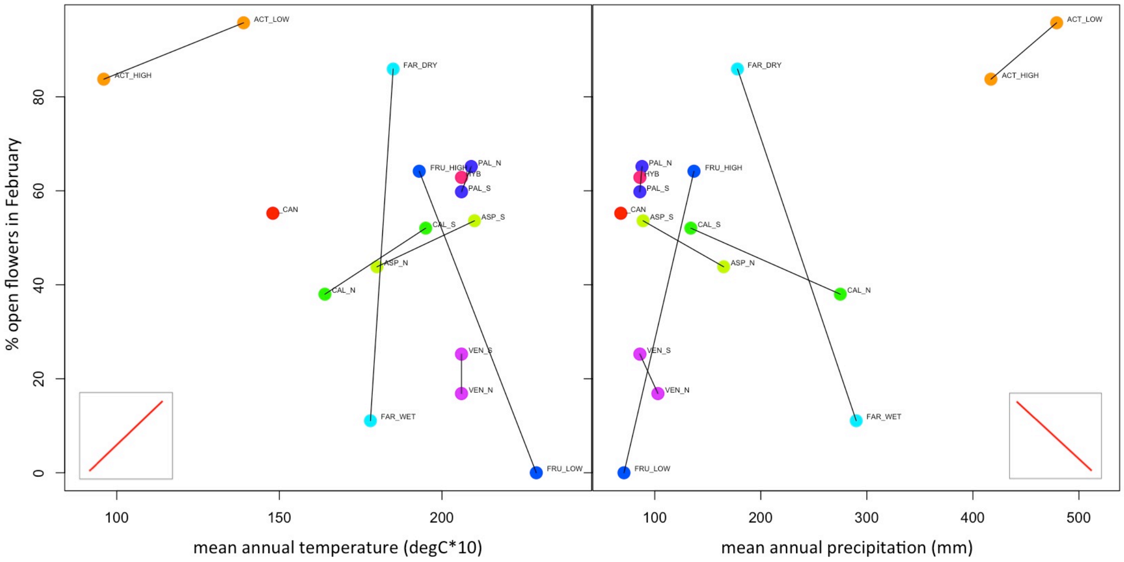
Supplementary Figure 14: Within versus among species variation in stem hydraulic conductance as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



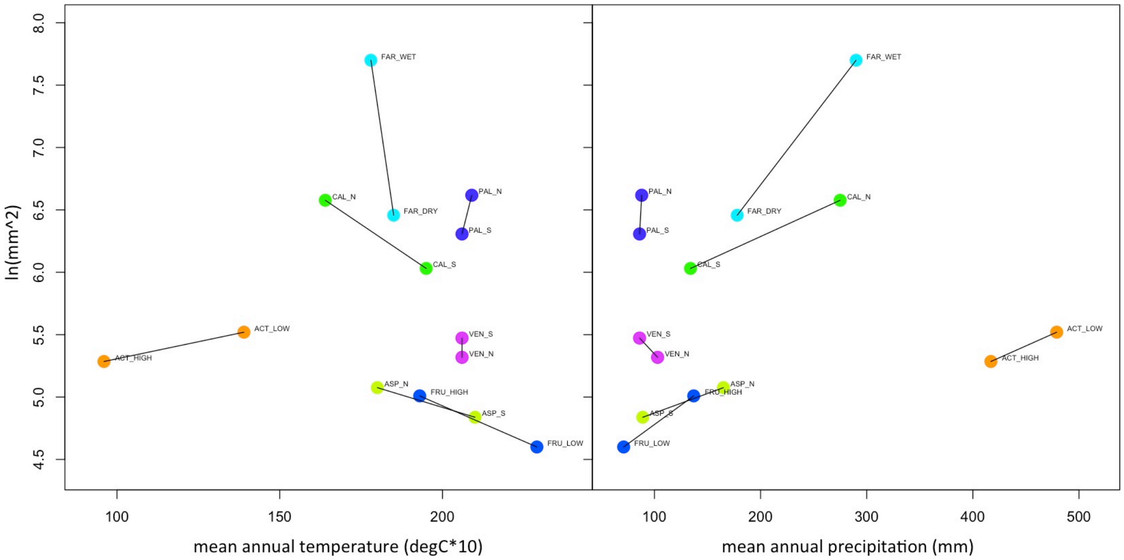
Supplementary Figure 15: Within versus among species variation in flowering effort as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



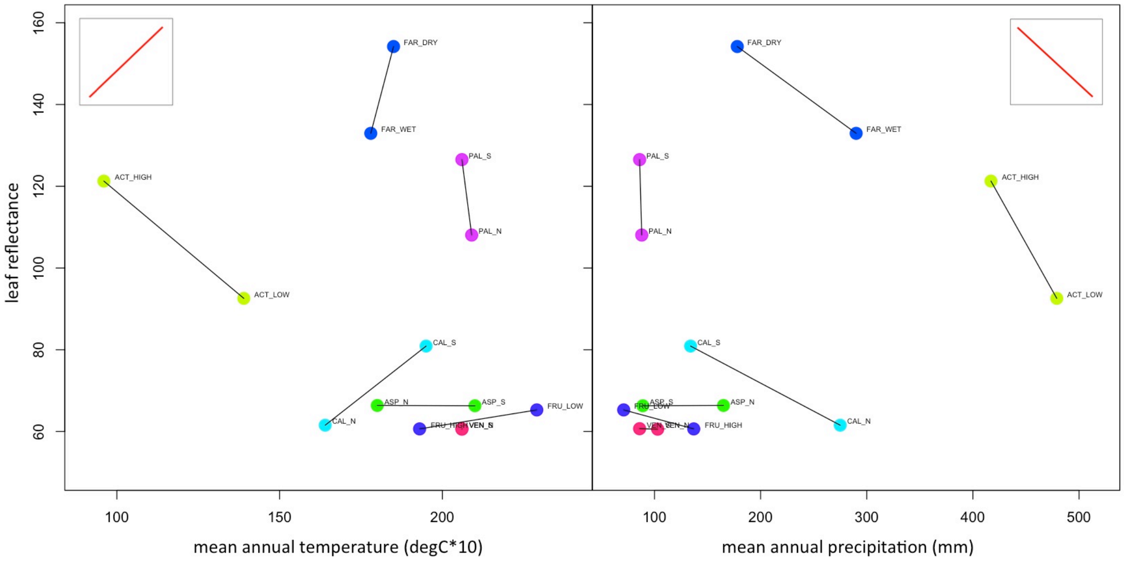
Supplementary Figure 16: Within versus among species variation in flowering phenology as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



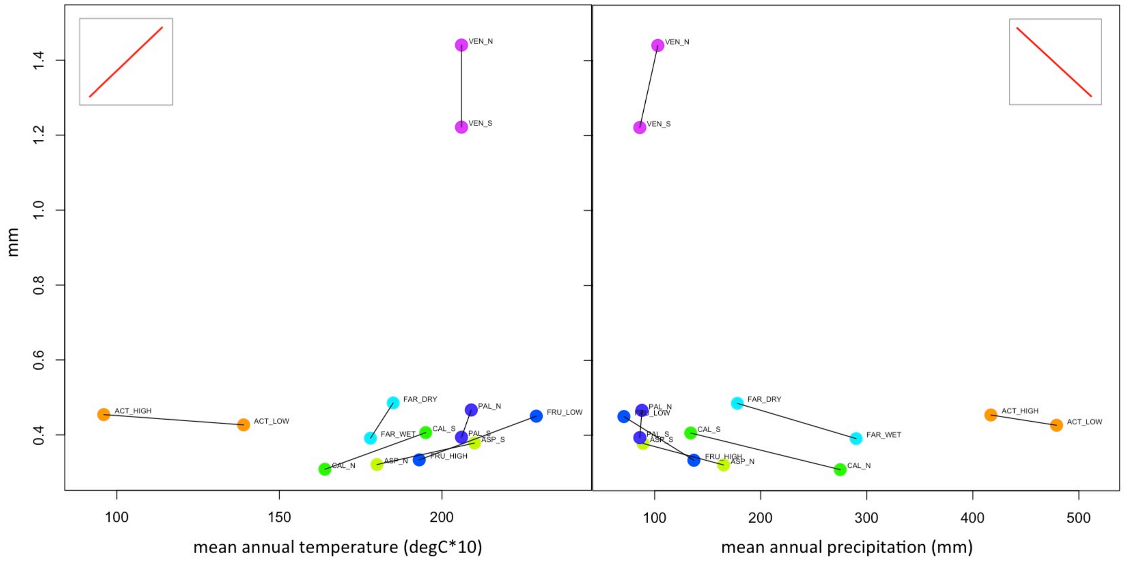
Supplementary Figure 17: Within versus among species variation in leaf area as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



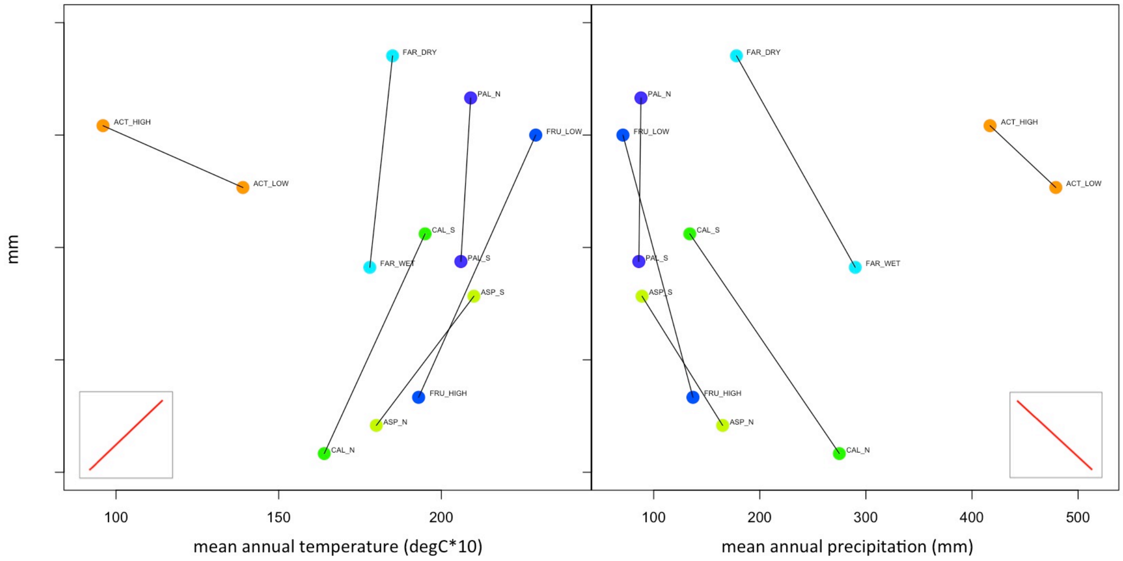
Supplementary Figure 18: Within versus among species variation in leaf color (reflectance) as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



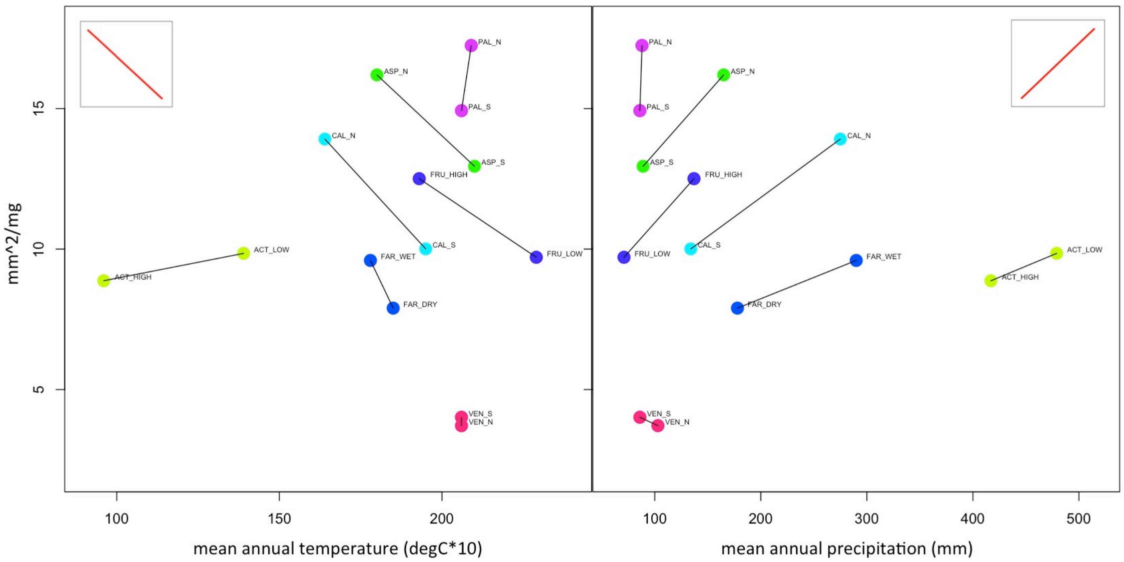
Supplementary Figure 19: Within versus among species variation in leaf thickness as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



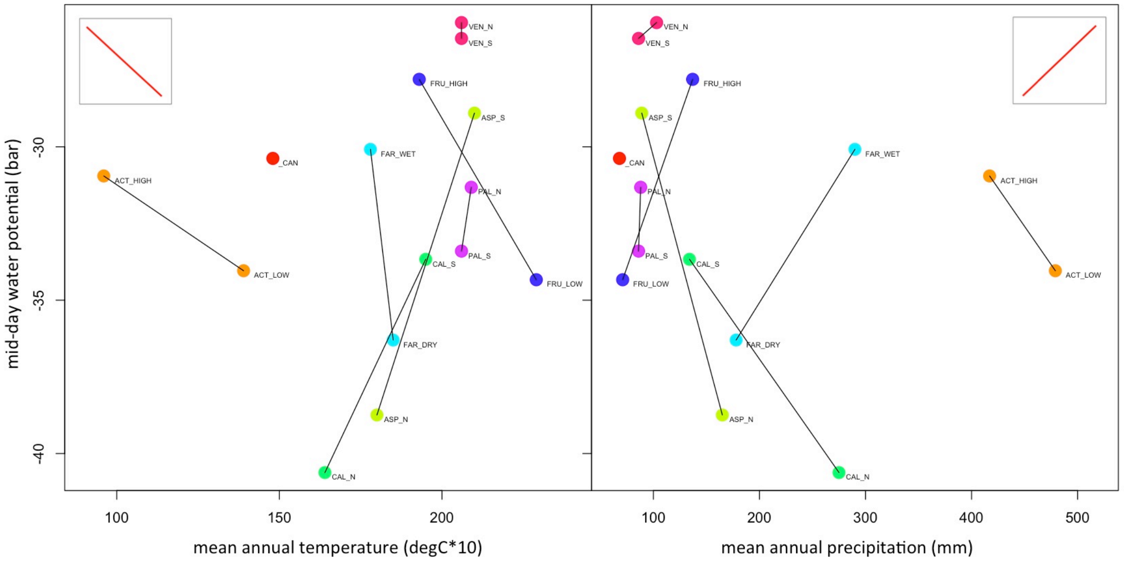
Supplementary Figure 20: Within versus among species variation in leaf thickness removing *Encelia ventorum* which has several fold greater leaf thickness than all other taxa. Trait means are plotted as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



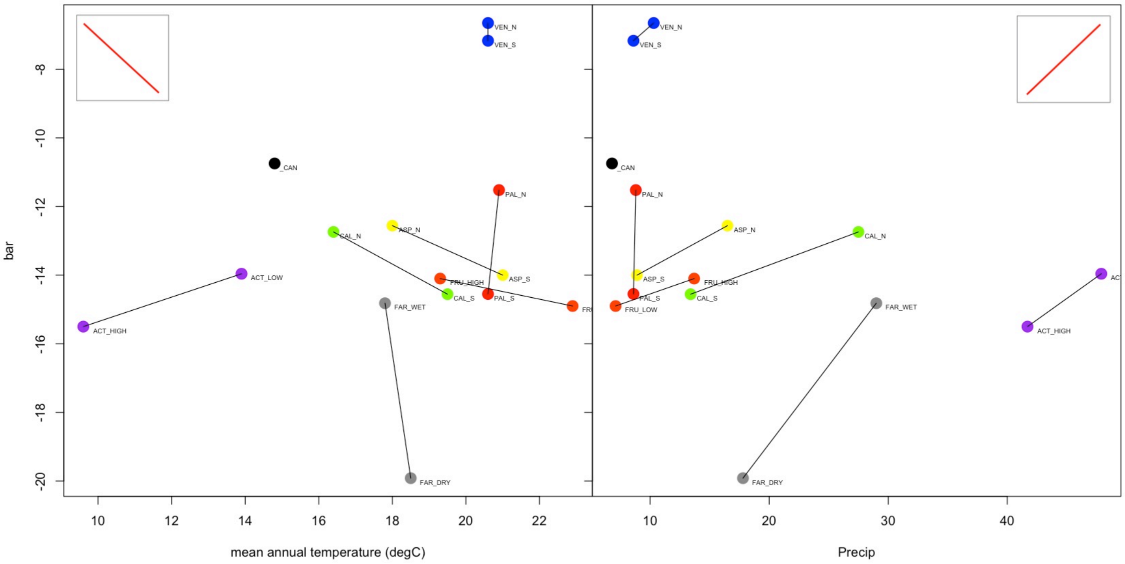
Supplementary Figure 21: Within versus among species variation in specific leaf area as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



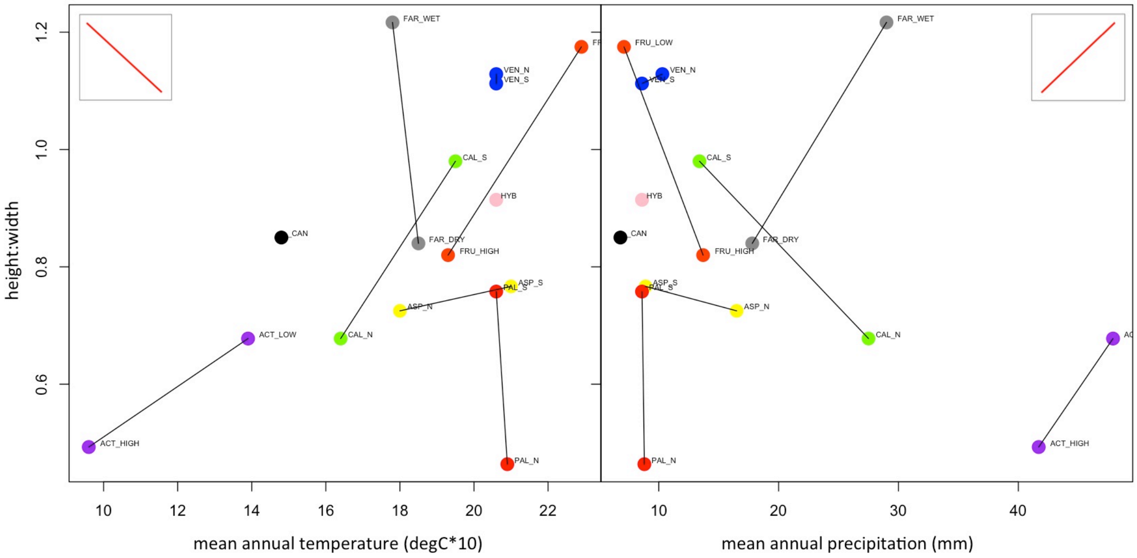
Supplementary Figure 22: Within versus among species variation in mid-day water potential as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



Supplementary Figure 23: Within versus among species variation in mid-day minus pre-dawn water potential differential as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



Supplementary Figure 24: Within versus among species variation in canopy architecture as a function of (a) mean annual temperature and (b) mean annual precipitation. ACT (*E. actoni*), ASP (*E. asperifolia*), CAL (*E. californica*), CAN (*E. canescens*), FAR (*E. farinosa* var *farinosa*), FRU (*E. frutescens*), PAL (*E. palmeri*), HYB (hybrid), VEN (*E. ventorum*).



Supplementary Figure 25: Bees on flowers of *Encelia farinosa* var. *farinosa*, Hidden Hills ecotype.