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ECOLOGICAL ROLE OF HYBRIDIZATION IN ADAPTIVE RADIATIONS: A CASE STUDY IN THE *DUBAUTIA ARBOREA*–*DUBAUTIA CILIOLATA* (ASTERACEAE) COMPLEX

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Premise of research. Hybridization is a mechanism frequently invoked to account for the spectacular radiations observed in oceanic islands, but, surprisingly, there is little empirical support for its ecological role in island plant radiations. Theory predicts that hybridization should provide individuals with the phenotypic novelty required for habitat shifts, thus promoting conditions for subsequent speciation. In this article, we studied the first stages of this process using two hybridizing species of *Dubautia* (Asteraceae), the most diversified genus of the Hawaiian silversword radiation.

Methodology. Phenotypic and habitat differentiation were investigated in two recently derived species (*Dubautia arborea* and *Dubautia ciliolata*) and a hybrid zone. Morphological trait expression and physiological trait expression under field and common garden conditions and microclimatic variation were analyzed to address the patterns of differentiation among parental species and hybrids.

Pivotal results. Our analyses showed that parental species occupied contrasting habitats and represented the extremes of phenotypic variation. Conversely, hybrids displayed novel phenotypes outside parental ranges, generating a continuum of intermediate phenotypes in the study system. We also found a strong relationship between morphological and physiological variation and plant performance, which suggests that broad phenotypic variation in hybrids could be favored by the environmental heterogeneity of the hybrid zone.

Conclusions. The initial expectations for the ecological role of hybridization in adaptive radiations are confirmed in this *Dubautia* system, in which hybrids of closely related species display novel morphological and physiological variation associated with the colonization of a new habitat. To understand the evolutionary processes creating an unusual degree of variation within particular lineages, molecular studies revealing cases of hybridization in island radiations should be complemented with ecological studies.

Keywords: habitat colonization, hybrid swarm, leaf size, phenotypic novelty, silverswords, water use efficiency.

Introduction

Hybridization is a widespread phenomenon among plant taxa that has been invoked to explain some of the extraordinary diversification frequently observed in oceanic island lineages (Seehausen 2004; Herben et al. 2005; Nolte and Tautz 2010). According to this view, speciation mediated by hybridization occurs in a stepwise fashion (reviewed in Seehausen 2004). As an initial step, genetic exchange between related species creates hybrid entities (i.e., hybrid swarms) with novel phenotypic adaptations, which may allow colonization of novel habitats. If hybrids find vacant niches where they perform better than progenitors, habitat shifts and phenotypic differentiation would eventually lead to speciation (Seehausen 2004; Nolte and Tautz 2010). Oceanic islands are thus scenarios where this process could have been favored due to high

habitat diversity and reduced competition (Schluter 2000; Gavrillets and Losos 2009).

In plant species, most of the support for the pervasive role of hybridization in island radiations comes from the increasing number of molecular studies addressing phylogenetic relationships in highly diversified lineages (Baldwin et al. 1991; Francisco-Ortega et al. 1996; Howarth and Baum 2005; Bacon et al. 2011). However, hypotheses concerning the interplay between environment and hybrid phenotypes have been largely ignored in the context of adaptive radiations. Limitations of such studies are the evolutionary inferences that can be made from ecological data. For instance, studies including completely differentiated hybrid species allow inference on the processes affecting the last steps of speciation (Gavrillets and Losos 2009) but at the expense of a less accurate inference of the factors that caused early differentiation, since environments may have changed since the speciation event occurred (Donovan et al. 2010). Conversely, studies on recent hybridization under incomplete speciation provide valuable insights into the phenotypic adjustments associated with recent habitat shifts (Nolte and Tautz 2010) but do not provide conclusive evidence

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to support hybridization itself as a speciation mechanism. Despite these limitations, ecological studies on the role of hybridization in plant radiations are needed to complement molecular information, and each study system can provide information on particular stages of the process (Seehausen 2004; Nolte and Tautz 2010).

Previous ecological literature on hybridization in noninsular lineages has shown that genetic exchange between closely related taxa typically results in two patterns of phenotypic expression: transgressive segregation, i.e., phenotypic values that exceed the phenotypic range expressed by each of the parental species (reviewed in Rieseberg et al. 1999; Stelkens and Seehausen 2009), and intermediate values to those observed in progenitors (Rosenthal et al. 2002; Wu and Campbell 2006). Regardless of the pattern of variation with respect to parental ranges, expression of phenotypic novelty in hybridizing species would support the role of hybridization in lineage diversification, as this mechanism may satisfactorily explain the colonization of habitats not accessible to progenitors in a short evolutionary timescale (Lexer et al. 2003), which is consistent with the concept of adaptive radiation (Schluter 2000).

In this article, we aim to study the ecological role of hybridization in two recently derived species of the genus *Dubautia* Gaudich., the most diversified group in the Hawaiian silversword radiation (Baldwin et al. 1991). Species within the genus show weak barriers to gene flow, which supports the hypothesis that hybridization may have been a relevant evolutionary mechanism for lineage diversification (Carr 1985; Baldwin et al. 1991). As woody perennial plants, this group also constitutes an interesting study case because previous literature on hybridization has basically focused on annual or short-lived perennial species (Lexer et al. 2003; Caruso et al. 2005; Donovan et al. 2007).

The two species selected for this study (*Dubautia arborea* and *Dubautia ciliolata*) are the only *Dubautia* taxa endemic to the island of Hawai'i. They occur in parapatric locations on the recent substrates of the Mauna Kea volcano, although they hybridize in a few contact zones (Friar et al. 2007; Remington and Robichaux 2007). By comparing the patterns of habitat and phenotypic variation among parental and hybrid zones, we aimed to test whether, beyond morphological variation, hybridization provided novel phenotypic traits associated with habitat shifts, as enunciated by the hybridization theory of adaptive radiation (Seehausen 2004; Gavrillets and Losos 2009). We specifically addressed the following questions: (i) Do parental and hybrid habitats differ in microenvironmental conditions? (ii) Do hybrid phenotypes represent novel variation within the *D. arborea*–*D. ciliolata* complex? (iii) Do morphologically variant individuals within the *D. arborea*–*D. ciliolata* complex also display contrasting physiology and performance?

Material and Methods

Study Species and Zone Description

Dubautia arborea (A. Gray) Keck is a large shrub or small tree that grows up to 6 m tall and has leaves 3–9 cm long (Carr 1985). It occurs in subalpine communities (2125–3050 m a.s.l.) on the Mauna Kea and Hualalai volcanos of the island

of Hawai'i. *Dubautia ciliolata* ssp. *glutinosa* G. Carr, in contrast, is a small shrub with smaller leaves (0.4–3 cm long) than *D. arborea* and occupies a wider altitude range on Mauna Kea, occurring on subalpine habitats up to 3200 m a.s.l. (Hartt and Neal 1940; Carr 1985). Morphological differentiation between these two species has been typically interpreted as adaptation to contrasting habitats (Carr 1985; Friar et al. 2007). Indeed, anatomical studies describing marked leaf and stem differentiation between both species (Carlquist 1959) and earlier ecophysiological work conducted on *D. ciliolata* (Robichaux 1984) suggest that *D. ciliolata* has greater resistance to drought than *D. arborea*, which could be beneficial in the high-elevation communities where it occurs. Nevertheless, no research has specifically addressed physiological differences between these species or the performance of their hybrids.

For this study, we considered three zones located on the eastern slope of Mauna Kea, where *Dubautia* progenitors and hybrids can be found in close proximity: two zones were occupied by each *Dubautia* progenitor (*D. arborea* = “arborea” zone; *D. ciliolata* = “ciliolata” zone), and a gulch located between the arborea and ciliolata zones represented the hybrid zone (Waipahoehoe gulch; fig. 1a). Individuals displaying extreme phenotypes in the Waipahoehoe gulch, albeit rare, have been related to migrants from parental zones based on AFLP analyses (Remington and Robichaux 2007), but morphological and molecular data strongly support a hybrid origin for the great majority of individuals (Kirchoff et al. 2004; Remington and Robichaux 2007; J. M. Cruse-Sanders and E. A. Friar, unpublished data). For this study, one transect of ~250 m throughout the core of the hybrid population was utilized for sampling purposes.

Microenvironmental Characterization in the Study Zones

The aim of microclimatic characterization was to investigate environmental differences (i) among study zones and (ii) between phenotypically contrasting hybrids within the gulch. A set of sensors located at each zone allowed continuous monitoring of environmental variables throughout five consecutive months (February–July). All sensors were set to record a point measurement every hour. Two paired soil moisture sensors in each zone (ECH₂O EC-10, Onset Computer, Bourne, MA) were buried at ~5 cm from the surface, but each of the two sensors was placed next to a different *Dubautia* individual. All sensors were almost in contact with the parent bedrock, thus providing integrated values of soil moisture detected across 20 cm in depth. In the case of the shallow volcanic substrates found in this area (Scowcroft and Giffin 1983), these measurements likely provide a good estimation of soil water availability for plants (see also García-Verdugo et al. 2010b). In each parental zone, two *D. ciliolata* or *D. arborea* individuals were monitored. In the hybrid zone, in contrast, one soil moisture sensor was placed next to one individual displaying an arborea-like leaf phenotype, whereas the other sensor was placed next to a neighbor ciliolata-like plant. The aim of this design was to characterize the soil moisture conditions of phenotypically different individuals. Soil-specific calibrations were obtained from soil samples from each population following the recommendations provided by the manufacturer and previous studies (García-Verdugo et al. 2010b). In addition, pho-

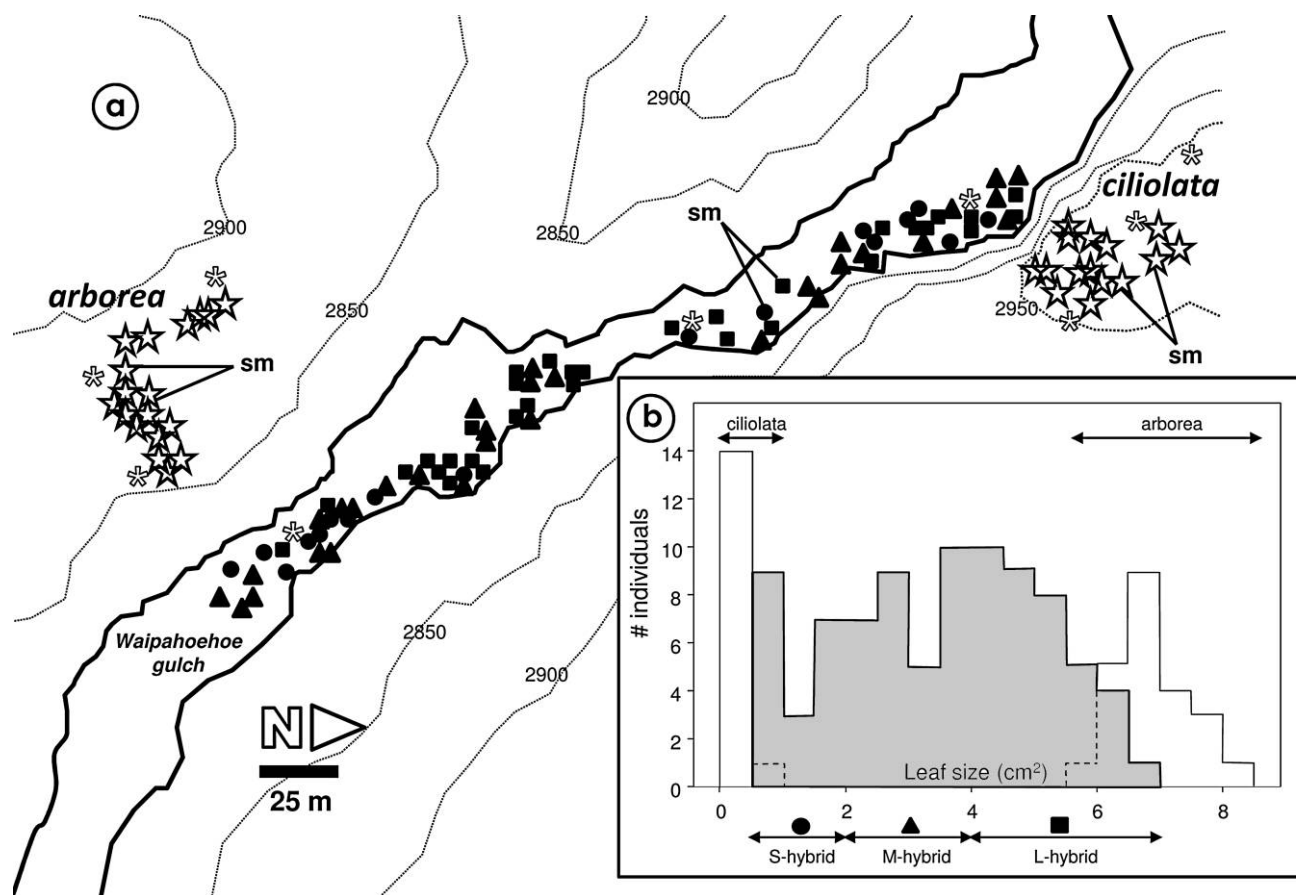


Fig. 1 Distribution of parental individuals (stars) and hybrids (filled symbols based on leaf size classes) sampled under field conditions (a) and histogram of leaf size variation (b). Location of relative humidity/temperature (asterisks) and soil moisture sensors (sm) is indicated in the map. Arrows in b represent the interval of leaf variation for each parental/hybrid class (S-hybrid, $< 2\text{ cm}^2$; M-hybrid, $2\text{--}4\text{ cm}^2$; L-hybrid, $> 4\text{ cm}^2$).

tosynthetically active radiation (PAR) was recorded with a PAR sensor (S-LIA-M003, Onset Computer). Light and soil moisture sensors were coupled with an U12 data logger (Onset Computer), which was located in a representative area of each study zone. In addition, three randomly distributed HOBO sensors (RH/T H8, Onset Computer) recorded air temperature and relative water humidity in each area.

Phenotypic Measurements under Field Conditions

We used leaf size to determine phenotypic differentiation between hybrids and parental individuals in the field. This trait is easy to measure, it is typically associated with relevant physiological traits (Donovan et al. 2007; Lambrecht and Dawson 2007), and previous studies on *Dubautia* hypothesized an important role of leaf morphology for hybrid performance (see above). All individuals intercepted along the 250-m transect ($N = 88$), as well as a sample of each parental species ($N = 15$ for *D. ciliolata* and $N = 20$ for *D. arborea*), were georeferenced with a GPS, and three leaves from each individual were taken for leaf size measurements (i) to compare the range of phenotypes expressed by hybrids with that observed in the parental species and (ii) to study the spatial distribution of leaf size phenotypes in the gulch. Because *Dubautia* species are

heteroblastic and leaf morphology can be affected by ontogeny, we followed the sampling procedure described by Kirchoff et al. (2004). Leaves were transported to the laboratory under high-moisture conditions in plastic bags and scanned. Leaf size was then calculated from scanned images with ImageJ v1.44 software (National Institutes of Health, Bethesda, MD).

Common Garden Experiment

A set of traits related to CO_2 assimilation and resource use efficiency was measured in *Dubautia* plants grown under common garden conditions. These traits can be good indicators of whole-plant performance and thus represent key components that ultimately affect individual fitness (Farris and Lechowicz 1990; Caruso et al. 2005). However, phenotypic correlations with fine-grained environmental variation may obscure differences between individuals and modify relationships among traits (García-Verdugo et al. 2009). By growing plants of hybrid and parental origin under common garden conditions, we aimed to minimize the effect of environmental variation and investigated the correlations among morphology, physiology, and plant performance (Wu and Campbell 2006).

In June 2005, a set of seeds were collected from 20 open-pollinated mother plants distributed throughout the core of

the hybrid zone. Plants were randomly selected, although extreme phenotypes resembling parental characteristics were avoided because of potential nonhybrid constitution (Remington and Robichaux 2007). For comparative purposes, seeds were also collected from 10 individuals of each parental zone. Seeds were transported to the facilities of Rancho Santa Ana Botanic Garden (RSABG; Claremont, CA), where they were germinated. Between 20 and 40 seeds from each mother plant were soaked overnight in separated petri dishes containing 0.5% gibberellic acid (10^{-6} M). The following day, seeds were placed on agar plates and kept in germination chambers for 3–5 wk. Germination was checked on a weekly basis, and once seedlings reached a height of ~5 cm, they were transplanted and transferred to a mist bench in the greenhouse. Seedlings were allowed to grow in pots filled with a mixture of sand and commercial potting soil (Pro-mix BX, Premier Horticulture, Red Hill, PA; 2 : 1) for 4–6 wk before they were transported to the common garden. As a final step, seedlings were planted in 5-L pots with a substrate comprising 35% coarse perlite, 35% cinder lava rock, 20% peat moss, 10% sand, and 10 g Micromax micronutrients (Scotts, Marysville, OH). Pots were randomly assigned to one of five outdoor benches set in the facilities of RSABG. Watering was provided as needed, typically three times per week during summers and two times per week for the rest of the year. Seedlings were supplemented twice a year with commercial fertilizer (NPK; 7 : 5 : 6) and once a year with 10 g Micromax micronutrients. Positions of the seedlings were randomized throughout the duration of the experiment (a minimum of two times per year, at the beginning of spring and winter) by moving pots from one bench to the next one, which prevented plants from acclimation to potential microenvironments within the common garden. All seedlings were allowed to grow under common garden conditions for 4 yr, which ensured that all were woody, mature individuals when phenotypic measurements were performed.

Physiological Measurements under Common Garden Conditions

Physiological characterization was performed in March 2011. To represent morphological variation in hybrids, sampling of common garden plants was stratified with respect to leaf size, based on three interval classes: S-hybrids, individuals with leaf area smaller than 2 cm²; M-hybrids, individuals within the 2–4-cm² interval; and L-hybrids, individuals with leaf size larger than 4 cm² (typically, within the 4–6-cm² interval). One individual from each family was randomly selected for phenotypic measurements until six plants for each phenotypic class (S, M, and L) were obtained. By sampling only one individual from each family, we aimed to minimize genetic relatedness among individuals (avoiding full sibs) in the measurements. Only a few individuals of each parental species were available at the end of the experiment ($N = 3$ for *D. ciliolata* and $N = 4$ for *D. arborea*) but were included for comparative purposes. A total of 25 plants were considered for physiological characterization. Because our main goal was to investigate the potential relationship between morphology, physiology, and plant performance, this sample size provides a reasonable statistical power for phenotypic correlations. To ensure that individuals experienced similar water status during

data collection, plants were watered to field capacity the evening prior to measurements.

Physiological data were obtained from two sets of measurements. First, photosynthetic light response curves were performed for each individual in order to obtain physiological traits related to photosynthesis. Because of the nature of our common garden experiment and the randomization of pots during the study, any variation in photosynthetic characteristics among taxa was interpreted as genetic variation in allocation to maximum carbon gain capacity rather than phenotypic variation in response to light. Photosynthetic light curves characterize an important physiological process for plant performance and are highly conserved among plant species, which means that even small sample sizes allow detection of consistent patterns (Potvin et al. 1990; Granado-Yela et al. 2011; for a similar comparison of parental and hybrid physiology, see Wu and Campbell 2006). Photosynthetic light curve measurements were taken with a portable infrared gas analyzer (LI-6400, Li-Cor Biosciences, Lincoln, NE) on current-year, fully expanded leaves following standard protocols (Granado-Yela et al. 2011). Light-acclimated leaves were initially placed in the cuvette at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density (PPFD). Irradiance was then set to different light levels for which CO₂ assimilation rates were recorded: 2000, 1500, 1000, 800, 500, 400, 200, 100, 50, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Air temperature (20°C) and CO₂ concentration (400 $\mu\text{mol CO}_2 \text{ mol air}^{-1}$) were kept constant within the cuvette. Following photosynthetic measurements, leaf area inside the cuvette was carefully cut out with a blade and scanned for size calculation with ImageJ v1.44 software, as indicated above, and then dried for 48 h at 65°C and weighed.

The second set of measurements was the characterization of water use efficiency (WUE) in the same plants, as this trait has been shown to be subject to strong selection in habitats with variable water availability and is often tightly linked to plant fitness (Caruso et al. 2005; Donovan et al. 2007). We calculated intrinsic WUE as the ratio of photosynthetic CO₂ assimilation per leaf area (A_{area}) and stomatal conductance (g_s) as A_{area}/g_s , following the protocol described in Caruso et al. (2005) with slight modifications. Maximum steady state leaf gas exchange was measured above saturating irradiance (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with the LI-6400 (Li-Cor Biosciences), but in order to avoid potential photoinhibition, leaves were initially induced at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD for 2–3 min before increasing irradiance to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. To improve accuracy, WUE was calculated from measurements taken on the same plant every 2–3 d until three leaves per plant were analyzed. Current-year, fully expanded leaves were randomly selected within each individual, but the replicates were taken from different shoots. All measurements were performed in a short time interval each day (0900–1100 hours), to maintain air temperature and leaf-to-air vapor pressure deficit among measurements within restricted ranges of variation (22°–28°C and 1.02–1.32 kPa, respectively). To investigate the potential link between physiology and plant performance, plant height was measured in all common garden plants used for physiological measurements, as this trait proves to be a good estimate of the overall size of *Dubautia* plants (Kirchoff et al. 2004).

Table 1
Description of Study Zones Based on Field Observations and Data Recorded with Environmental Sensors

	Soil ^a	Total radiation (mol d ⁻¹ m ⁻²)	Air temperature (°C)	Air temperature variation (%)	Air relative humidity (%)	Air relative humidity variation (%)	Soil moisture (%)	Soil moisture variation (%)
Arborea	B/H	42.5 ± 1.2 ^A	10.0 ± .2 ^A	3.5	53.0 ± 2.2 ^A	2.6	18.3 ± .3 ^A	13.6
Hybrid	B/H/Cl/Co	45.6 ± 1.2 ^B	8.6 ± .2 ^B	7.9	56.9 ± 2.2 ^B	5.6	17.4 ± .3 ^B	46.5
Ciliolata	Ci	53.1 ± 1.4 ^C	9.0 ± .2 ^B	6.4	53.4 ± 2.2 ^A	5.9	15.1 ± .4 ^C	20.5

Note. Different superscript capital letters denote significant differences among groups for that trait according to Tukey post hoc tests. Repeated-measures ANOVA (RM; $F_{2,282}$) compared differences among months recorded with sensors (mean ± SE values shown), whereas Friedman tests (χ^2 , df = 2) compared the range of variation among sensors within zones with respect to mean values (%). For total radiation, RM = 137.2. For air temperature, RM = 0.62 (based on epsilon values of Huynh-Feldt corrections). For air temperature variation, χ^2 = 77.8. For air relative humidity, RM = 0.88 (based on epsilon values of Huynh-Feldt corrections). For air relative humidity variation, χ^2 = 78.1. For soil moisture, RM = 75.9. For soil moisture variation, χ^2 = 171.2. All tests were significant at $P < 0.001$.

^a Based on field observations and data from Scowcroft and Giffin (1983). H = humus; B = bedrock; Cl = clay; Co = colluvial; Ci = cinder.

Data Analysis

All field environmental variables were averaged daily, prior to statistical analyses, and data obtained from the same type of sensors were averaged per zone. Differences in mean values for environmental variables were investigated separately with repeated-measures ANOVAs with zone as the within-subject factor. Normality in the distribution of data was checked with normal probability plots and Shapiro-Wilk tests. Some variables showed a small deviation from the expected sphericity patterns for the covariance matrices (Mauchly test, P values in the range 0.03–0.01), and significance was therefore adjusted by the Huynh-Feldt correction in those cases. Differences in measurements among sensors of each zone were also compared with Friedman tests for those variables for which more than one sensor was available (air temperature, air relative humidity, and soil moisture). To analyze the spatial distribution of phenotypes in the hybrid zone, a Mantel test (Mantel 1967) was performed with 999 permutations using phenotypic and geographic distances. Phenotypic distance between each pair of individuals was calculated as the absolute difference of leaf size values (Burstin and Charcosset 1997), and geographic distance was calculated on the basis of GPS coordinates.

To obtain physiological traits of interest from gas exchange measurements, we analyzed the two sets of data previously generated. Assimilation data obtained from each individual in the common garden were fitted by nonlinear regression with the software SigmaPlot 12.0 (SPSS, Chicago) using a nonrectangular hyperbola (Potvin et al. 1990). Photosynthetic parameters including maximum photosynthetic CO₂ assimilation rate per area (A_{area}), quantum efficiency of photosynthesis (A_{qe}), and light compensation point (LCP) were obtained from each individual curve. Maximum CO₂ assimilation rate on a mass basis (A_{mass}) was calculated from A_{area} considering the weighed mass of the leaf area included in the cuvette for each light curve. The second set included stomatal conductance (g_s) and intrinsic WUE calculated from averaged values of the gas exchange measurements taken from three leaves of the same individual. Because of the limited sample sizes for parental groups, differences in physiological traits among hybrid classes and parental species were analyzed separately with nonpara-

metric Kruskal-Wallis tests. To investigate overall patterns of physiological differentiation, we performed a principal coordinate analysis (PCA) on the correlation data matrix of all six physiological traits. Last, associations between leaf size, physiological traits, and plant performance under common garden conditions were analyzed with linear regressions. We largely followed the recommendations provided by Warton et al. (2006) when considering linear regression over other line-fitting methods to test for an association between traits.

Results

Environmental Variation among and within Zones

Measurements of total radiation and soil moisture and field observations indicate that the three zones differed significantly, with parental zones representing extremes of environmental variation. Thus, *Dubautia ciliolata* plants experienced higher daily radiation (25% on average) and lower soil moisture (21%) than *Dubautia arborea* plants (table 1). The hybrid zone, in contrast, showed intermediate values for these latter variables and presented very heterogeneous soils along the sampling transect, presumably related to the marked differences found between soil moisture measurements at a local scale (table 1; fig. 2). Thus, the comparison of soil moisture values recorded by paired sensors revealed significant differences between L (arborea-like) and S (ciliolata-like) hybrids in the hybrid zone ($F_{1,141} = 3199.0$, $P < 0.001$), and each of these measurements was not significantly different when compared to the most similar parental phenotype (see *D. ciliolata* vs. S-hybrid and *D. arborea* vs. L-hybrid values; fig. 2b). Air temperature and relative humidity were found to be statistically different among areas, but the degree of variation of these variables did not seem sufficiently large to account for phenotypic differences among hybrids and parental species (e.g., 1°C between ciliolata and arborea zones in air temperature; table 1).

Phenotypic Variation under Field Conditions

Phenotypic frequencies of hybrids revealed that most of the variation in leaf size corresponded to values not observed in

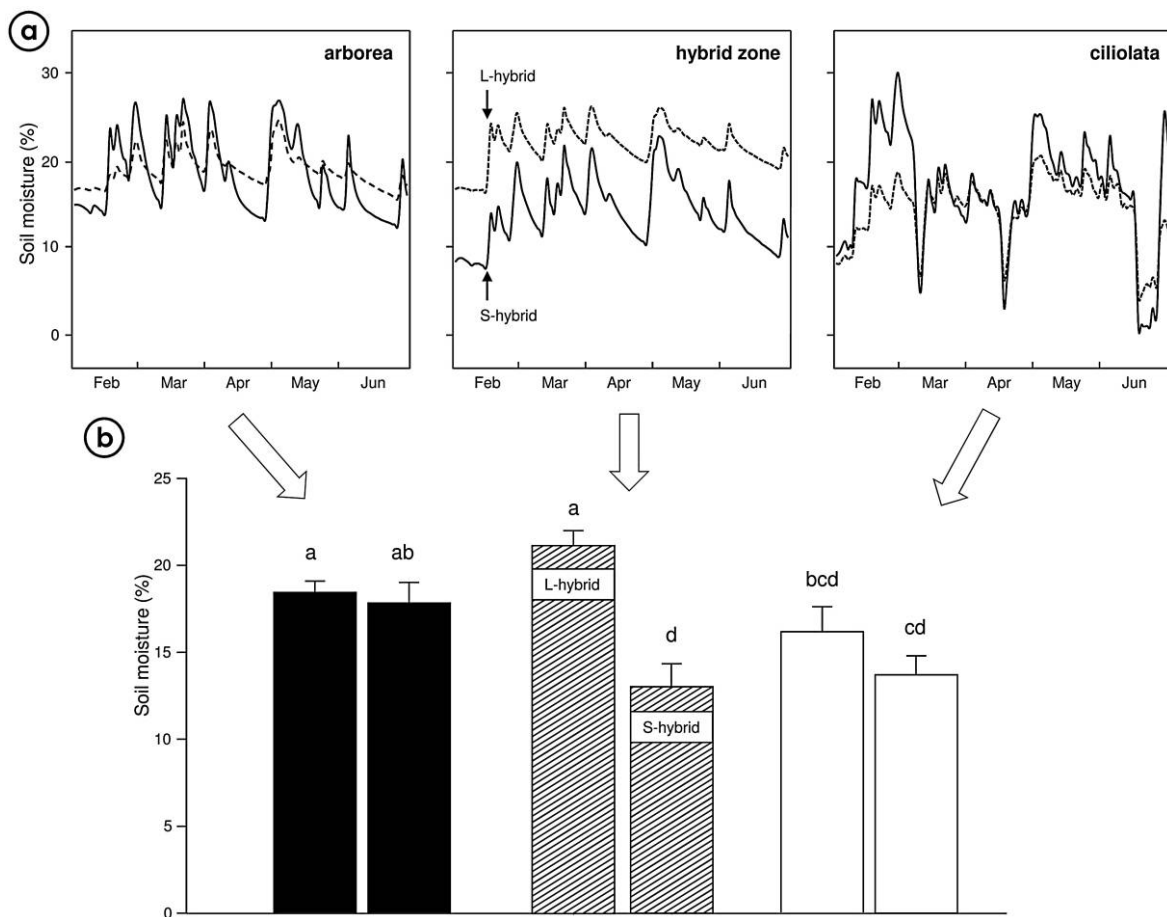


Fig. 2 Daily mean values in soil moisture throughout 5 mo of continuous monitoring of each of the study areas (a) and mean (\pm SE) values of soil moisture recorded by sensors located next to representative *Dubautia* individuals at each zone (b). Lines in a represent soil moisture measurements for the two individuals monitored at each zone. For the hybrid zone, the *Dubautia* hybrid class (L- and S-hybrid) that was monitored by each sensor is indicated. Letters over bars indicate homogeneous groups based on Tukey post hoc tests.

D. ciliolata or *D. arborea* individuals, as shown by the marginal degree of leaf size overlap between hybrids and parental plants (fig. 1b). A Mantel test strongly rejected a significant association between phenotypic distances based on leaf size and geographical distance among individuals in the hybrid zone ($R = 0.01$, $P = 0.33$). No clear spatial association was observed with respect to hybrid class when individuals were distinguished by hybrid class and georeferenced in space (fig. 1a).

Physiological Traits and Phenotypic Correlations

Light curves for each common garden plant showed a good fit to the nonrectangular hyperbola function ($R^2 > 0.95$ in all cases). The overall pattern observed for physiological traits measured under common garden conditions was similar to leaf size variation under field conditions. Thus, with the exception of A_{qe} , which did not show significant differences among groups (Kruskal-Wallis test, $K = 2.39$, $df = 2$, $P = 0.30$), all of the physiological traits of hybrids fell within the range delimited by parental species (table 2). The greatest differences among groups were again found between the two parental

lines, while the hybrids consistently showed intermediate values for physiological traits (table 2; fig. 3). Individuals of *D. arborea* exhibited higher maximum photosynthetic rates (both on an area, A_{area} , and on a mass basis, A_{mass}), higher g_s , lower LCP, and lower WUE than *D. ciliolata* plants. Results of the PCA considering all six physiological traits showed that each parental species occupied one extreme of the phenotypic range described by the first axis (fig. 4), which absorbed a high proportion of variance (66%) and was characterized mainly by A_{mass} , A_{max} , g_s , and WUE (loading factors >0.90). Hybrid individuals, in consonance with the Kruskal-Wallis results, spread along the phenotypic space limited by both progenitors (fig. 4).

Last, phenotypic regressions showed that morphology, physiology, and plant performance were strongly related. Leaf size correlated with all of the physiological traits measured in plants grown under common garden conditions except A_{qe} ($R = 0.14$, $P = 0.90$). Values of A_{mass} , WUE (fig. 5), and g_s ($R = 0.72$, $P < 0.001$) were the physiological traits showing a greater association with this morphological trait. Plant height, in addition, showed a strong association with physiological

Table 2
Mean Values (\pm SE) for Physiological Traits Measured in Plants Grown under Common Garden Conditions
(Hybrids, $N = 18$; Arborea, $N = 4$; Ciliolata, $N = 3$)

	$A_{\text{mass}}^{\text{a}}$ ($\text{nmol g}^{-1} \text{s}^{-1}$)	$A_{\text{area}}^{\text{a}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	A_{qe}^{a} (mol mol^{-1})	LCP ^a ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s^{b} ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE ^b ($\mu\text{mol mol}^{-1}$)
Arborea	179.4 \pm 19.9 ^A	15.7 \pm 1.4 ^A	.09 \pm .02 ^A	11.1 \pm 6.8 ^A	.321 \pm .010 ^A	50.2 \pm 1.0 ^A
Hybrids	104.7 \pm 11.2 ^B	10.2 \pm 1.2 ^B	.11 \pm .04 ^A	26.8 \pm 3.5 ^B	.164 \pm .011 ^B	71.1 \pm 3.4 ^B
Ciliolata	76.1 \pm 13.7 ^C	7.4 \pm .9 ^C	.06 \pm .02 ^A	49.7 \pm 2.9 ^C	.130 \pm .005 ^C	80.7 \pm 4.1 ^C

Note. Different superscript capital letters denote significant differences among groups for that trait according to Nemenyi post hoc tests. A_{mass} = maximum photosynthetic CO_2 assimilation rate per mass; A_{area} = maximum photosynthetic CO_2 assimilation rate per area; A_{qe} = quantum efficiency of photosynthesis; LCP = light compensation point; g_s = stomatal conductance; WUE = intrinsic water use efficiency.

^a Individual values obtained from light curve measurements and fitted to a nonrectangular hyperbola function.

^b Individual values obtained from the gas-exchange procedure described in Caruso et al. (2005).

traits such as A_{mass} and WUE (fig. 5), as well as leaf size ($R = 0.73$, $P < 0.001$).

Discussion

Our results provided support for the idea that hybridization between closely related species of a highly diversified island lineage is associated with habitat shifts and remarkable phenotypic novelty. Hybrids of the *Dubautia arborea*–*Dubautia ciliolata* complex occur in a gulch where environmental conditions differ from those observed in parental areas. In addition, phenotypic measurements under field and common garden conditions indicated that hybridization provides individuals with novel ranges of variation in morphological and physiological traits tightly associated with plant performance.

Hybridization in the *D. arborea*–*D. ciliolata* Complex Is Linked to Novel Variation

Our data showed that the environment experienced by each parental species was substantially different, and these differences in habitat correlated with contrasting phenotypic trait expression between species. For instance, *D. arborea* individuals occurred in areas with greater soil water-holding capacity and slower drawdown of soil water (fig. 2). Previous studies have shown that fine-grained variation in water availability causes strong phenotypic selection on physiological traits, such as WUE (Donovan et al. 2007; Rosenthal et al. 2010). In those habitats with greater water availability, as our data suggested for the *D. arborea* zone, plants typically display higher photosynthetic rates at the expense of lower WUEs (Lambrecht and Dawson 2007 and references therein). Phenotypic measurements under common garden conditions supported these expectations for *D. arborea*, whereas *D. ciliolata* individuals exhibited higher WUEs, most likely as a response to the drier conditions of their habitat (i.e., more restrictions in soil water availability and higher levels of radiation; table 1; fig. 2). In addition, the expression of a smaller leaf size under stressful conditions (Donovan et al. 2007; García-Verdugo 2011) may also represent a phenotypic advantage in the drier areas occupied by *D. ciliolata* individuals.

Measurements under field and common garden conditions showed that hybrids displayed phenotypic intermediacy when compared to their *Dubautia* progenitors (figs. 3, 4). None of

the traits showed a pattern congruent with transgressive segregation (Rieseberg et al. 1999), most likely because the marked phenotypic differentiation observed between progenitors in all traits hinders extreme phenotypes in hybrids (Stelkens and Seehausen 2009). Morphological and physiological traits in hybrids, however, were novel in the sense that most of their phenotypic values were not observed in either parental species. Relatively few parental individuals were considered for physiological measurements and therefore small sample sizes may have underestimated the ranges of physiological variation for the *Dubautia* progenitors. Nevertheless, the pattern of phenotypic intermediacy in hybrids described here is supported by two pieces of evidence despite this sample size limitation in the physiological characterization of parental species. First, the morphological intermediacy of hybrids is clearly supported by leaf size variation under field and common garden conditions (figs. 1, 5), and previous studies using higher sample sizes obtained a very similar pattern in other morphological measurements (Kirchoff et al. 2004; Friar et al. 2007). Second, the strong relationship found between leaf size variation and physiological traits (fig. 5) suggests that parental variation in

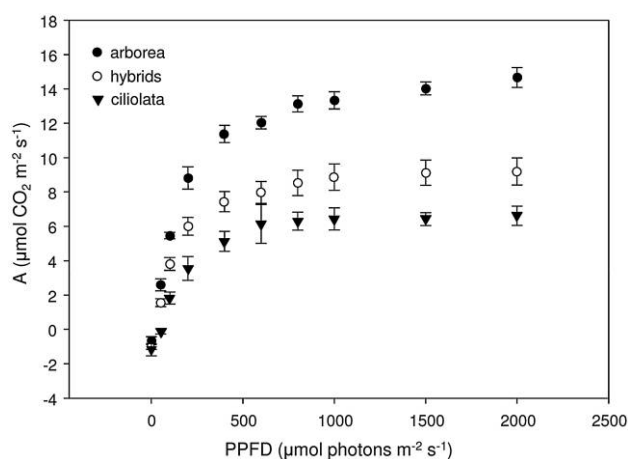


Fig. 3 Mean (\pm SE) rates of net photosynthetic CO_2 assimilation per leaf area (A) as a function of light irradiance (expressed as photon flux density [PPFD]). The figure shows mean values for parental (*Dubautia arborea*, $N = 4$; *Dubautia ciliolata*, $N = 3$) and hybrid ($N = 18$) plants grown under common garden conditions.

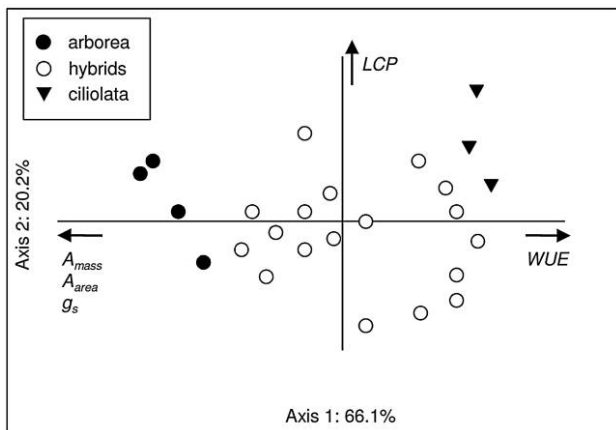


Fig. 4 Principal components analysis of six physiological traits (A_{mass} , A_{area} , A_{ge} , LCP , g_s and WUE ; see “Material and Methods” for details) measured in plants grown under common garden conditions. The percentage of variance explained by each component is shown next to the axis. Traits showing high loading factors (>0.90) are shown in italics.

physiology with a more extensive sampling would follow a pattern similar to that observed in morphology (fig. 1*b*), with hybrid phenotypes consistently showing intermediate values and parental individuals representing the extremes of variation.

The Hybrid Zone Favors the Expression of Phenotypic Polymorphism

Hybrid phenotypes were not related to a clear spatial pattern of distribution across the gulch (Mantel test results; see also fig. 1*a*). A significant correlation between phenotypic classes and spatial distribution would be expected in a hybrid zone showing cline variation, i.e., a gradient in phenotypic change throughout space (Barton and Hewitt 1985). What are the factors accounting for such a seemingly random distribution of hybrid phenotypes within the gulch?

The two main mechanisms that have the potential to maintain hybrids in contact zones between parental areas are either a selection/gene flow balance or the effects of selection alone (Barton and Hewitt 1985; Martin and Cruzan 1999). Although the distribution of hybrids could be related to gene flow limitations (Martin and Cruzan 1999), this seems unlikely in our study system because of the geographical proximity of parental and hybrid zones and the dispersal-pollination syndrome displayed by *Dubautia* species. In the absence of interspecific barriers to gene flow (Carr 1985), extensive gene flow in these air-dispersed, insect-pollinated plants would lead to weak population structure (García-Verdugo et al. 2010*a*). Pollen limitation is not supported by previous studies (Carr 1985), and the presence of insect species potentially acting as pollinators is similar between the hybrid and the *Dubautia* parental zones (Drew and Roderick 2005). On the other hand, seeds from parental areas appear to reach the hybrid zone (Remington and Robichaux 2007), but the marginal representation of parental-like phenotypes in this zone (fig. 1; Remington and

Robichaux 2007) is suggestive of recruitment limitation rather than dispersal limitation.

Our results further suggest that the distribution and maintenance of hybrids in the contact zone could be explained by selective patterns related to the environmental conditions found within the gulch. The high floristic diversity and the complex topography typically observed in these gulches represent remarkable microsite variation (Hartt and Neal 1940; Hodges 1994). Comparisons between soil water measurements performed in phenotypically different but spatially adjacent plants (fig. 2) and field observations also suggested a considerable degree of spatial heterogeneity within the gulch (table 1). Because phenotypic variation of hybrids has a significant association with plant performance (fig. 5), plants displaying phenotypes that fit microsite conditions throughout the gulch would maximize their growth and reproduction, and thus phenotypic polymorphism in the hybrid zone could be maintained by spatial heterogeneity (Kawecki and Ebert 2004). Measurements of selection under field conditions (Lexer et al. 2003; Donovan et al. 2007) would be needed to test this hypothesis and provide more conclusive evidence of the strength and type of selection. However, even when a deeper understanding of the selective forces acting on the hybrid zone would require fine-grained analyses, our data demonstrate through common garden experiments that phenotypic differences among progenitors and hybrids are largely genetic and the directional variation of traits is consistent with the contrasting habitat conditions experienced at each zone (table 2).

Hybridization as a Mechanism Driving Lineage Diversification on Oceanic Islands

Contrasting patterns of selection among closely related species are a frequent scenario within radiating lineages (Francisco-Ortega et al. 1996; Bacon et al. 2011), which are favored by the high habitat diversification typically observed at local scales in oceanic islands (Dunbar-Co et al. 2009; Santiago and Kim 2009; García-Verdugo et al. 2010*b*). In some of these cases, hybridization may have acted as the triggering mechanism that provided species at early stages of differentiation with the phenotypic novelties necessary for exploitation of novel habitats, initiating a process of differentiation between parental and hybrid progenies. The lack of empirical studies addressing the ecology of hybrids of insular plant lineages hinders general conclusions. For example, questions regarding the importance of transgressive segregation in highly diversified island lineages (Rieseberg et al. 1999) and whether sympatric differentiation mediated by hybridization is a frequent phenomenon on these lineages (Seehausen et al. 2004) remain unanswered.

Oceanic islands provide an ideal scenario for testing future hypotheses on the ecological role of hybridization in lineage diversification. In the Hawaiian islands, for instance, 10% of the flora is thought to experience natural events of hybridization (Whitney et al. 2010). Molecular and morphological data have already identified cases of hybridization within highly diversified lineages, such as *Scalesia* in the Galápagos Islands (Nielsen et al. 2003) or *Argyranthemum* (Brochmann et al. 2000) and *Convolvulus* (Carine et al. 2007) in the Canary

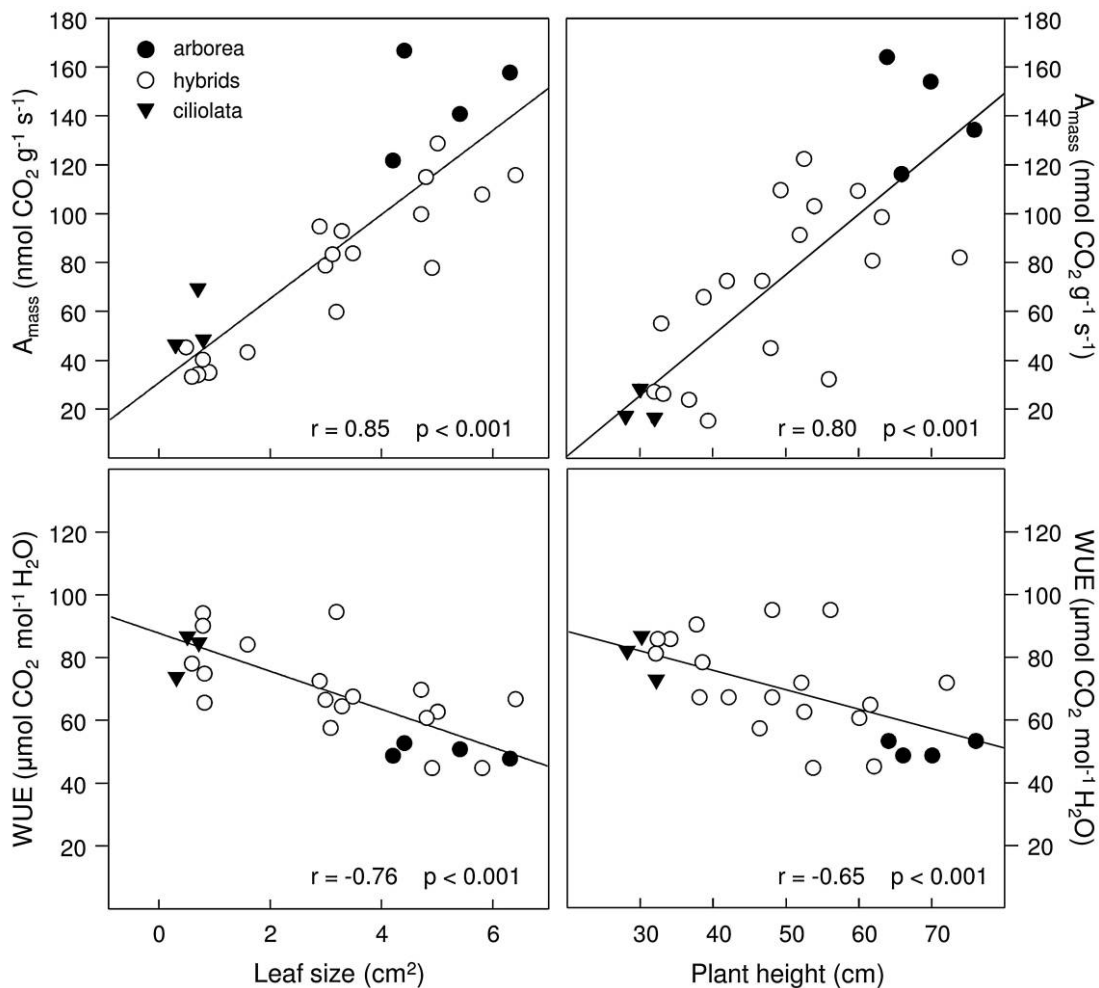


Fig. 5 Bivariate plots of leaf size (*left*) and plant height (*right*; X-axis) and physiological traits (Y-axis) obtained from plants grown under common garden conditions. Lines represent linear regression axes. Correlation coefficients (r) and significances (P) are provided for each pair of traits.

Islands. The proliferation of molecular-based studies is providing incessant evidence for the occurrence of hybridization events in island radiations, but if we rely solely on this approach, we will fail to fully understand the interplay between ecological and evolutionary processes creating such an unusual degree of variation within particular lineages. These latter examples may represent valuable study cases for analyzing the ecological role of hybridization at different stages of the process of speciation in radiating lineages.

Conclusions

This study provides empirical evidence to support the significant role of hybridization at initial stages of differentiation within a radiating lineage. Our study suggests that hybridization between recently derived species occurring in contrasting habitats can result in highly differentiated, novel phenotypes. The morphological and physiological novelties derived from this phenomenon allow phenotypic adjustment in habi-

tats essentially different from those experienced by parental species. In summary, this study supports the idea that hybridization between species at early stages of differentiation can be a mechanism that explains dramatic phenotypic differentiation and frequent habitat shifts in a short evolutionary timescale, which is congruent with the rapid events of speciation described in island adaptive radiations.

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