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Trait convergence and diversification arising from a complex evolutionary history in Hawaiian species of *Scaevola*

Athena D. McKown[1] · Michelle Elmore Akamine[2] · Lawren Sack[3]

Abstract Species variation in functional traits may reflect diversification relating to convergence and/or divergence depending on environmental pressures and phylogenetic history. We tested trait-environment relationships and their basis in finer-scale evolutionary processes among nine extant Hawaiian species of *Scaevola* L. (Goodeniaceae), a taxon with a complex history of three independent colonizations by different phylogenetic lineages, parallel ecological specialization, and homoploid hybridization events in Hawai'i. Using a wild population for each species, we evaluated traits related to plant function (morphology, leaf and wood anatomy, nutrient and carbon isotope composition). Hawaiian *Scaevola* species were distributed across coastal, dry forest and wet forest environments; multivariate environmental analysis using abiotic and biotic factors further showed that species from distantly related lineages inhabited similar environments. Many traits correlated with environment (based on the multivariate environmental analysis), considering both distantly related species and more closely related species. *Scaevola* species within shared habitats generally showed trait convergence across distantly related lineages, particularly among wet forest species. Furthermore, trait diversification through divergence was extensive among closely related *Scaevola* species that radiated into novel environments, especially in plant morphology and traits affecting water relations. Homoploid hybrid-origin species were "intermediate" compared to their ancestral parent species, and possessed trait combinations relevant for their current habitat. The diversity in functional traits reflected strong influences of both ecology and evolutionary history in native Hawaiian *Scaevola* species, and trait correspondence with environment was due to the combination of multiple processes within the taxon: trait pre-adaptation and filtering, evolutionary convergence, divergence, and hybridization.

Keywords Adaptive radiation · Divergence · Homoploid hybridization · Island evolution · Functional traits

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Introduction

Combinations of traits that influence how a plant gains or uses resources (i.e., plant functional traits) can be similar among diverse and unrelated species where abiotic and/or biotic factors within the environment provide a strong, overarching selection pressure (Weiher and Keddy 1995; Reich et al. 2003; Kraft et al. 2015). Accordingly, common relationships between functional traits and environmental factors have been established across diverse plant species within functional groups (Reich et al. 2003; Santiago and Wright 2007), within communities (Kraft and Ackerly 2010; Baraloto et al. 2012), across landscapes (Cornwell and Ackerly 2009; Kooyman et al. 2010), and at the global scale (Wright et al. 2004, 2005a, b; Díaz et al. 2004; Freschet et al. 2011). Distantly related species with

Fig. 1 Phylogenetic history of Hawaiian *Scaevola* species, adapted from Howarth and Baum (2005). Lineages *A–C* indicate the three independent colonizations of *Scaevola* taxa in Hawai'i. Within lineage C, "wet" clade and "dry" clade designations are indicated, and homoploid hybridization is illustrated with *thinner branches* spanning both clades. Reprinted with permission from *Evolution* (Wiley-Blackwell)
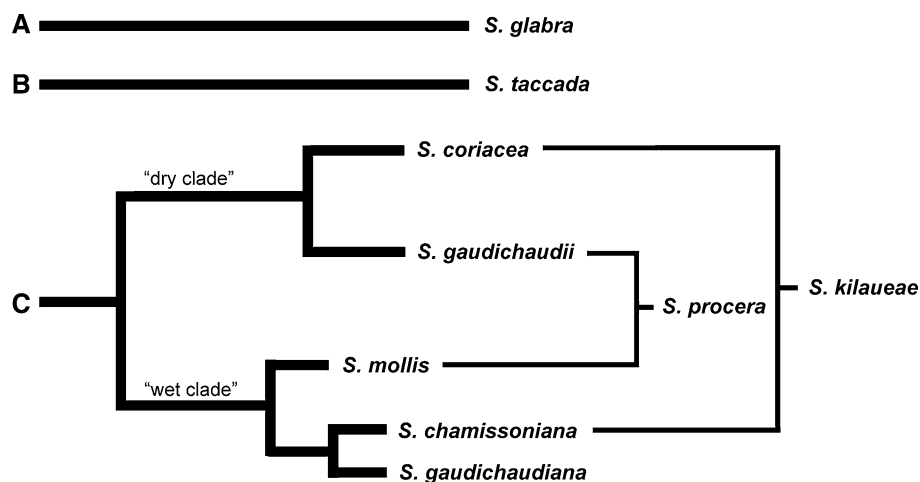
such trait-environment patterns imply trait convergence: for example, larger leaves and taller stature tend to be associated with mesic forest habitats across phylogenetically diverse species (Kooyman et al. 2010). Nevertheless, divergence in traits among coexisting taxa can arise due to competition for resources and niche differentiation (Grubb 1977; Grime 2006). For instance, species adapted to high light gaps within forests tend to have traits associated with greater gas exchange capacity, such as high nitrogen concentrations and stomatal pore area, compared to co-occurring taxa under more dense canopies (Sack et al. 2003; Givnish et al. 2004). In addition, trait variation among closely related or recently derived species may not reflect the larger-scale trends between functional traits and environment (Edwards et al. 2014). This can be due to the strong influences of recent phylogenetic and demographic histories and/or limited potential for trait diversification (Reich et al. 2003; Ackerly 2009; Blonder et al. 2015).

Studies of trait coordination with environment and the influence of phylogeny on this process have often focused on endemic lineages in Hawai'i, which originated in relatively recent colonization and/or speciation events (<10 Ma) (Carlquist 1980; Baldwin and Wagner 2010). Hawaiian radiations have shown that relationships of functional traits to environment (especially leaf traits) can arise rapidly and/or change as species within given lineages diversify across environments suggesting lability in functional traits (Robichaux and Pearcy 1984; Cordell et al. 1999; Givnish et al. 2004, 2009; Cornwell et al. 2007; Montgomery and Givnish 2008; Dunbar-Co et al. 2009; Havran et al. 2009; Creese et al. 2011; Scoffoni et al. 2015; Blonder et al. 2015). In this study, we aimed to evaluate the variation and coordination of a wide range of functional traits to environment using *Scaevola* L. (Goodeniaceae), a taxon with a particularly complex evolutionary history in Hawai'i.

Hawaiian *Scaevola* species, also known as naupaka, are shrubby in habit, varying from low plants in coastal areas and exposed volcanic rock shrub lands to small trees in high-elevation rainforests (Carlquist 1980; Patterson 1990, 1995). Native Hawaiians noted this ecological diversity in the legend of *Naupaka*, attributing the "half-flower" morphology of *Scaevola* to the separation of two ill-fated lovers into coastal and mountaintop locations (Degener 1945). Studies using multiple genetic markers indicated that Hawaiian *Scaevola* species arose from three independent colonizations of relatively distantly related taxa within the genus (lineages A–C, Fig. 1; Table 1; Howarth et al. 2003). These lineages originated through dispersal events from differing global localities and have distinct island distributions, habitat preferences, and species diversification. Lineage A, represented by *Scaevola glabra*, originated from Australia and occurs exclusively in high-elevation rainforest habitats on two islands, while lineage B, represented by *Scaevola taccada* (syn. *Scaevola sericea*), is a common and widespread coastal species of Polynesian origin (Fig. 1; Table 1; Howarth et al. 2003). In Hawai'i, *S. glabra* and *S. taccada* remain single-species lineages, existing within environments similar to those of their progenitor taxa and are not known to have spread into novel habitat types. By contrast, Lineage C likely arrived from the Americas, and the colonizing taxon gave rise to multiple endemic Hawaiian species through adaptive radiation (Howarth et al. 2003). Clades within lineage C reflect species diversification into dryland ("dry" clade = *Scaevola coriacea*, *Scaevola gaudichaudii*) vs. mesic environments ("wet" clade = *Scaevola chamissoniana*, *Scaevola gaudichaudiana*, *Scaevola mollis*) (Fig. 1; Carlquist 1974; Patterson 1995; Howarth et al. 2003; Howarth and Baum 2005). In addition, two endemic Hawaiian *Scaevola* species within lineage C resulted from homoploid hybridization of the dry and wet clades (Fig. 1; Howarth and Baum 2005). Both hybrid-origin species, *Scaevola kilaueae* (from dry clade *S. coriacea* and wet clade *S. chamissoniana*) and *Scaevola procera* (from dry clade *S. gaudichaudii* and wet clade *S. mollis*), have relatively divergent morphologies

Table 1 Hawaiian Island distributions, evolutionary history and conservation status of *Scaevola* species in Hawai'i

Scaevola species	Lineage[a]	Species status[b]	Hawaiian Island distributions[b]					
			H	K	L	MA	MO	O
Scaevola glabra Hook. and Arnott	A	Endemic		x				x
Scaevola taccada (Gaertn) Roxb.[c]	B	Indigenous	x	x	x	x	x	x
Scaevola chamissoniana Gaud.	C	Endemic	x		x	x	x	
Scaevola coriacea Nutt.	C	Endemic, endangered	FA	FA	FA	x	x	FA
Scaevola gaudichaudiana Cham.	C	Endemic		x				x
Scaevola gaudichaudii Hook. and Arnott	C	Endemic	x	x	x	x	x	x
Scaevola hobdyi Wagner	C	Endemic, extinct				FA		
Scaevola kilaueae Degener	C[d]	Endemic, Rare	x					
Scaevola mollis Hook. and Arnott	C	Endemic		x			x	x
Scaevola procera Hillebr.	C[d]	Endemic		x			x	

H Hawai'i; *K* Kaua'i; *L* Lāna'i; *MA* Maui; *MO* Moloka'i; *O* O'ahu; *FA* former area, locally extinct

[a] Colonization events: *A* Australia, *B* Polynesia, *C* Americas (Howarth et al. 2003; Howarth and Baum 2005)

[b] Patterson (1990, 1995)

[c] Also referred to as *Scaevola sericea* (Shannon et al. 1997)

[d] Species with homoploid hybridization origin (cf. Howarth and Baum 2005)

from their ancestral parent species and are considered to be reproductively isolated.

Given their complex evolutionary history arising from multiple colonizations of the islands and more recent radiation and hybridization events, the Hawaiian *Scaevola* species are valuable for examining the finer-scale correlative links between environment and functional traits. In this study, we characterized whole plant, leaf, and anatomical traits from a wild population of each extant Hawaiian *Scaevola* species and compared trait-environment patterns from these data with wood anatomy traits previously published for the genus (Carlquist 1969). We predicted that trait-environment relationships would be similar to those reported across phylogenetically diverse species, and hypothesized that regardless of the timing of colonization and radiation/speciation events:

1. *Scaevola* taxa within the same habitat would show similar suites of functional traits relating to that environment (i.e., trait convergence across distantly related lineages).
2. *Scaevola* taxa within different habitats would show functional trait diversification reflecting distinct environments (i.e., trait divergence among closely related species).
3. The hybrid-origin *Scaevola* species would show trait combinations aligned more closely with their environment than their ancestry, reflecting the strength of environmental selection and lability of plant functional traits.

Materials and methods

Study species and sites

Plants were sampled in their natural, native environments on the islands of Hawai'i, Kaua'i, Maui, and O'ahu over a 2-week period. While phenotypic plasticity may contribute to some variance in the observed traits, we assume that most variation included a genetic component relating to species evolutionary and demographic histories and that assessing wild plants under natural conditions better indicates differences that are achieved in the native state (Cordell et al. 1999; Givnish et al. 2004; Cornwell et al. 2007). We studied one population for each species to assess interspecific variation of traits and habitat characteristics. Study sites were selected for: (1) situation within known ranges for *Scaevola* species (cf. Patterson 1995; Howarth and Baum 2005; Elmore 2008), (2) site accessibility, (3) multiple mature individuals being present, and (4) no range overlaps [i.e., where species known to hybridize were isolated, thereby avoiding intermediate forms (cf. Gillett 1966; Elmore 2008)]. Similar sampling strategies have been applied to other taxa in Hawai'i including *Viola* (Ballard and Sytsma 2000), *Scaevola* (Robichaux and Pearcy 1984; Howarth and Baum 2005), *Plantago* (Dunbar-Co et al. 2009), lobeliads (Givnish et al. 2004; Scoffoni et al. 2015), *Euphorbia* (Robichaux and Pearcy 1984), and silverswords (Blonder et al. 2015).

At each sampling site, three locations (ranging from 50–500 m apart) were selected to describe plant community and habitat characteristics. Global positioning system (GPS) co-ordinates and elevation were recorded using a handheld GPS meter (GPSMAP; Garmin, Olathe, KS). We identified all *Scaevola* individuals at each location and the co-occurring vascular plant species (native Hawaiian, or non-native, alien species) within a 5-m radius of each *Scaevola* plant. As an index of community species richness (CSR), we determined total numbers of species excluding minor components of the understory, such as mosses and short grasses. The percentage of canopy closure (COV) was visually assessed for each *Scaevola* plant, which was previously shown to correlate strongly with percentage of transmitted diffuse irradiance (Waite and Sack 2010). Two soil horizons were described at each location by digging a shallow soil profile to assess general organic and mineral materials (as an indication of nutrient and water-holding capacities) (Hue et al. 2007; US Department of Agriculture 2010). Litter, humus and organic soil were measured together to determine the depth of the organic (O) horizon. Mineral soil was assessed for general particle size, texture, and color to categorize the upper mineral (A) horizon. Based on GPS co-ordinates, we obtained climate data for each site using nearby weather station records and climate maps (spanning 8–58 years of data collection) from the Natural Resources Conservation Service-PRISM Climate Group (http://www.prism.oregonstate.edu/), the National Oceanic and Atmospheric Administration and National Climatic Data Center (NOAA/NCDC: https://www.ncdc.noaa.gov/), and the Western Regional Climate Center (WRCC: http://www.wrcc.dri.edu/). Climate variables included mean annual temperature (MAT) and precipitation (MAP), and lowest monthly temperature (LMT) and precipitation (LMP) and highest monthly temperature (HMT) and precipitation (HMP).

Whole plant and leaf traits

At each of the three locations per site, we selected four healthy, mature plants (5–10 m apart) for functional trait assessments ($n = 12$ per species). Traits were chosen to reflect general plant growth, importance to ecophysiology, and predicted associations with environment (detailed in Table 2). Plants were assessed in situ for size traits (height, branch diameter). We collected ten to 15 mature leaves from the exposed portions of the mid- to upper canopy from two individuals per location ($n = 6$ per species) and used these for leaf trait assessments [size, nutrient and carbon (C) isotope composition, anatomy, venation architecture, and stomata]. Due to permit restrictions, federally endangered *S. coriacea* was studied only for non-destructive measurements within natural habitats. Collections of

S. coriacea leaves (and traits determined from these) were taken from five plants grown at Maui Nui Botanical Garden (Kahului, HI) located near the Waiheʻe dune site in Maui (Table 3).

Leaves were scanned and measured for morphometric traits, including leaf area, petiole diameter, leaf length (*L*) and width (*W*), *L:W* shape ratio (*L:W*), and leaf marginal teeth, using Image J software (Rasband 2007). We weighed all leaves for fresh mass per leaf area as an index of relative succulence. Five leaves were then oven-dried at 60 °C and reweighed to determine the leaf dry mass per unit area (LMA). One oven-dried leaf from each individual ($n = 6$ per species) was analyzed at the Marine Science Department, University of Hawaiʻi (Hilo, HI) for foliar C, nitrogen (N), and phosphorus (P) for calculation of nutrient stoichiometry (N:P, C:N), and mass- (N_{mass}, P_{mass}) or area-based (N_{area}, P_{area}) nutrient concentrations (methods in Online Resource 1). We also analyzed leaves for the C isotope ratio ($\delta^{13}C$) to infer integrated water-use efficiency (Farquhar et al. 1989). Typically, less negative $\delta^{13}C$ values reflect lower stomatal conductance relative to C assimilation rate within the leaf and can be interpreted as reflecting higher water-use efficiency.

From each individual, we assessed three replicate leaves fixed in 70 % formaldehyde-acetic acid–ethanol (18:1:1) (Berlyn and Miksche 1976) for anatomical traits, including trichomes, stomata, foliar sclereids (i.e., non-vascular, lignified cells within the mesophyll), and vascular architecture. Leaves were cleared using 50 % (w/v) aqueous sodium hydroxide followed by 50 % bleach (modified from Berlyn and Miksche 1976). Cleared leaves were then stained with 1 % (v/v) safranin in ethanol and counterstained with 1 % (v/v) fast green in ethanol. Whole leaves were scanned at 1200 d.p.i. (Epson Perfection 4490 Photo; Epson America, Long Beach, CA) and micrographs of central sections of the lamina were captured with light microscopy (Leitz DMRB microscope; Leica Microsystems, Bannockburn, IL) using a SPOT Insight 4Mp Mosaic 14.2 camera and SPOT software version 4.6 (Diagnostic Instruments, Sterling Heights, MI). All images were evaluated for anatomical traits using Image J.

Whole leaf scans were used for leaf vascular architecture to compare structural/bulk flow veins (categorized as "major" were first, second and third orders) with the smaller vein network (categorized as "minor" were fourth and higher orders). Vein orders were determined based on their size and location within the leaf (Hickey 1979; Ellis et al. 2009) and densities were calculated by measuring the total lengths of veins (by order) per given leaf area. Micrographs of leaf surfaces were assessed for presence of trichomes, and densities of abaxial (lower surface) and adaxial (upper surface) stomata. Where present, stomata were further measured for guard cell pore lengths to calculate the stomatal pore area

Table 2 Predicted functional trait-environment relationships based on previous literature for trends observed within lineages, communities, and across species globally

Trait	Predicted relationships	References
Height, branch diameter	Increase with greater water availability, canopy cover or competition; decrease with higher elevation	Cornelissen et al. (2003), Dunbar-Co et al. (2009), Cornwell and Ackerly (2009), Kooyman et al. (2010)
Leaf area, size, petiole	Increase with greater water availability or canopy cover; decrease with higher elevation, irradiance, or temperature	Cordell et al. (1999), Cornelissen et al. (2003), Reich et al. (2003), Martin et al. (2007), Dunbar-Co et al. (2009), Cornwell and Ackerly (2009), Kooyman et al. (2010), Scoffoni et al. (2011), Sack et al. (2012)
Leaf teeth	Increase with greater water availability and distance from coast; decrease with higher elevation or temperature	Royer et al. (2009)
LMA, N_{area}, P_{area}	Increase with higher elevation, irradiance, or temperature; decrease with greater water availability, canopy cover, or competition	Cordell et al. (1999), Cornelissen et al. (2003), Reich et al. (2003), Givnish et al. (2004), Wright et al. (2004, 2005b) Martin et al. (2007), Cornwell and Ackerly (2009), Dunbar-Co et al. (2009)
N_{mass}, P_{mass}	Increase with greater water availability, canopy cover, or competition; decrease with higher elevation, irradiance, or temperature	Wright et al. (2005a, b), Martin et al. (2007), Cornwell and Ackerly (2009)
$\delta^{13}C$	Less negative with higher elevation or lower water availability (i.e., greater water use efficiency)	Cordell et al. (1999), Dawson et al. (2002), Dunbar-Co et al. (2009), Blonder et al. (2015)
Major, minor vein density	Increase with higher irradiance, lower water availability and/or higher temperature	Dunbar-Co et al. (2009), Brodribb et al. (2010), Scoffoni et al. (2011), Sack et al. (2012), Sack and Scoffoni (2013)
Freely ending veinlet density	Increase with greater water availability	Scoffoni et al. (2011), Sack and Scoffoni (2013)
Epidermal trichomes	Increase with higher elevation or lower water availability	Martin et al. (2007), Dunbar-Co et al. (2009)
Stomatal density, pore length, SPI	Increase with higher elevation, irradiance, or water availability; decrease with higher temperature	Sack et al. (2003), Dunbar-Co et al. (2009)
Sclereid density	Increase with higher irradiance or temperature or lower water availability; increase with shade	Karaboumiotis (1998), Brodribb et al. (2010)
Wood vessel elements and pits	Increase in length, width, density and pit diameter with greater water availability or lower elevation	Hacke et al. (2006), Cornwell and Ackerly (2009), Dunbar-Co et al. (2009)

$\delta^{13}C$ Carbon stable isotope ratio, *LMA* leaf mass per area, *N* nitrogen, *P* phosphorus, *SPI* stomatal pore index

Table 3 Site information for sampled populations of *Scaevola* species within the Hawaiian Islands

Scaevola species[a]	Location (island)[b]	Elevation (m)	General site habitat	MAT (LMT–HMT; °C)[c]	MAP (LMP–HMP; mm^3)	COV[d]	CSR (*n*)[e]
S. glabra (A)	Na-Pali-Kona Forest Reserve (K)	1226–1250	*Metrosideros* forest, bog edge	15.1 (8–22.1)	1690 (48.5–291.1)	Closed	11–14
S. taccada (B)	Ka'ena Point recreational area (O)	12–21	coastal strand, dunes	24.1 (18.0–30.2)	720 (17.0–107.4)	Open	2–4
S. chamissoniana (C)	Waihe'e Ridge trail (M)	687–726	*Metrosideros* shrubland	19.4 (13.6–25.3)	2320 (80.5–336.6)	Open	8–11
S. coriacea (C)	Dunes at Waihe'e golf course (M)	19–64	coastal strand, dunes	24.1 (17.5–30.6)	1139 (31.0–178.3)	Open	4–15
S. gaudichaudiana (C)	Lanipō Summit trail (O)	412–490	*Acacia/Metrosideros* mixed forest	20.3 (15.4–25.6)	3286 (193.5–326.9)	Mixed	6–11
S. gaudichaudii (C)	Waimea Canyon (K)	717–737	*Acacia/Eucalyptus* mixed forest	18.6 (12.2–25)	870 (19.8–147.3)	Mixed	5–7
S. kilaueae (C)	Hawai'i Volcanoes National Park (H)	996–1055	*Metrosideros/Morella* shrubland, and forest	18.1 (11.9–24.2)	1615 (48.8–208.3)	Mixed	7–8
S. mollis (C)	Mokulē'ia Forest Reserve (O)	1118–1197	*Metrosideros* mixed forest	17.8 (13.1–22.5)	1851 (45.2–266.2)	Mixed	12–14
S. procera (C)	Kokee State Park (K)	1258–1293	*Metrosideros* mixed forest	15.1 (8–22.1)	1690 (48.5–291.1)	Mixed	5–11

MAT Mean annual temperature, *LMT* lowest (coldest) month mean temperature, *HMT* highest (warmest) month mean temperature, *MAP* mean annual precipitation, *HMT* highest (warmest) month mean temperature, *HMP* highest (wettest) month mean precipitation, *LMP* lowest (driest) month mean precipitation, *COV* canopy cover, *CSR* community species richness

[a] See Table 1 for phylogenetic lineage designations and Hawaiian Island abbreviations

[b] See Online Resource 2 for sampled population positional information, soil descriptions, and co-occurring species lists

[c] Climate data from National Oceanic and Atmospheric Administration, PRISM, and Western Regional Climate Center (see Online Resource 1)

[d] Average canopy coverage: *Open* canopy shading <20 %, *Mixed* mixture of open-closed canopy, *Closed* canopy shading >50 %

[e] Species counts include predominant, sympatric vascular plant species. Non-native species reported as a proportion of total species numbers

per leaf area index (SPI; stomatal density × squared pore length), which correlates with maximum stomatal conductance (Sack et al. 2003; Franks and Beerling 2009). Micrographs of mesophyll tissues were used to determine densities of freely ending veinlets (i.e., xylem conduits ending in mesophyll) and foliar sclereids. Individual sclereids were further measured for cell length, width, and *L:W*.

We compared the suites of functional traits measured within our study to a data set of eight wood anatomical characteristics relating to water flux previously published for the Hawaiian *Scaevola* species (Carlquist 1969), including the number of vessels per group, average vessel-element length and diameter, vessel pit diameter, average tracheid/fiber length and diameter, tracheid wall thickness, and ray height. Wood anatomical data were based on field-collected plants (from one to nine plants per species) (Carlquist 1969).

Statistical analyses

To provide a general quantitative value for environmental differentiation based on more detailed habitat parameters, we used principal components (PC) analysis (PCA) to define multivariate environmental PC (Env-PC) (Feoli 1977; Nichols 1977). This analysis does not provide the limits of the habitats that given species may occupy, but simply the habitats in which our study populations were found. Climate (MAT, MAP), community characteristics (COV, CSR), and soils (A, O) were tested with PCA using NT-SYSpc version 2.1 (Exeter Software, New York). Variables were standardized (by minimum and range) and used to calculate eigenvectors determining the Env-PCs. We considered a component to be significant that explained greater than predicted variance (tested with broken-stick modeling in NT-SYSpc version 2.1). To obtain environmental values for our populations (one per species), all individuals were projected with eigenvector data. These projected values for Env-PC1 were analyzed with one-way ANOVA using the standard R package (GNU freeware; http://cran.r-project.org) and Tukey–Kramer post hoc pairwise comparison tests to assess habitat groupings among all non-hybrid-origin *Scaevola* species.

Plant functional traits were analyzed across species and lineages in a multi-step approach. For all statistical

tests where Holm-Bonferroni or other multiple testing corrections would be applied, we accepted significance at $\alpha = 0.01$ to reduce type I error rather than applying a multiple testing correction as this would increase type II error. We first used one-way ANOVA tests in the standard R package to determine trait variability across species (within the genus). While traits across evolutionary lineages are commonly tested with phylogenetically independent contrasts, this approach could not be applied to the Hawaiian species of *Scaevola*, despite the existence of nine taxa, due to insufficient contrasts (i.e., lack of common ancestors across all three lineages in Hawai'i) and the reticulated nature of the phylogenetic tree (i.e., two cross-clade hybridization events). We then evaluated trait-environment associations across all species, and correlated individual traits (excepting those without statistically significant variation across species) with Env-PC1 values using Pearson's product moment correlations in R. We repeated this approach using only species from lineage C to determine if the correlative relationships between traits and environment changed in direction and/or significance. Finally, we used nested ANOVAs in R to test trait diversification and whether (1) trait convergence exists across (relatively) distantly related species that occupy the same habitats, and (2) trait divergence exists among (relatively) closely related species that occupy different habitats. The first nested ANOVAs tested distantly related species from all three lineages that occupied "wet forest" and "coastal" habitats (see "Results"). We considered a significant habitat effect as evidence of trait convergence among species within habitats (and divergence between habitats). The second nested ANOVAs tested closely related species of lineage C within dry clade and wet clade phylogenetic branches. We considered a significant clade effect as evidence of trait divergence among closely related species that occupy distinct habitats.

To evaluate functional trait combinations among *Scaevola* species, we used PCA as described above to calculate significant components (Trait-PC) using all trait data, excepting traits without statistically significant variation across species. An additional PCA was conducted for the independent data set of wood traits (Wood-PC). We focused on the highest loading traits for each significant component as the strongest signal of coordinated trait variation (in that axis) among *Scaevola* species. We then plotted each Trait- or Wood-PC against Env-PC1 values for coordination in suites of traits with environment across *Scaevola* species (cf. Díaz et al. 2004). Using the same testing design (see above), we assessed these multivariate trait combinations for correlation to environment, convergence among species within habitats (wet forest vs. coastal) and divergence among closely related species within clades of lineage C.

We evaluated the relative distinction of trait combinations between homoploid hybrid-origin *Scaevola* species, their respective ancestral parent species (see "Introduction") and species within similar environments (see "Results") using Mantel tests (Mantel 1967) in NT-SYSpc version 2.1. Using standardized trait data, we calculated distance (dissimilarity) matrices for each species. Distance matrices were compared using Mantel tests with 10,000 randomizations to calculate correlation coefficients (r) between the hybrid and ancestral or ecologically similar species.

Results

Environmental distinction and species associations

In Hawai'i, individual *Scaevola* populations ranged from low-elevation coastal dune to high-elevation rainforest communities, and from very exposed areas to enclosed forest canopies (Table 3; Online Resource 2, Table S1). Species-richness (as assessed from 5-m-radius plots around each *Scaevola* plant) was variable, with number of co-occurring species varying from two to 14. Organic soils ranged from no O horizon (i.e., bare soil, sand or rock) to 2 cm of organic soil and litter. Upper mineral (A) horizons were unconsolidated, undeveloped sand and/or loam (entisols) in lower elevation sites or dense volcanic silty-clay (andisols) in high-elevation sites (Online Resource 2, Table S1). PCA of these biotic and abiotic environmental variables resulted in one significant PC explaining 52 % of data variance (Fig. 2; Online Resource 3, Fig. S1). MAP, species richness, and O soil depth were positive drivers of this axis whereas MAT was negatively associated with Env-PC1 (Online Resource 2, Table S2). The Env-PC1 axis was significant across the genus (based on one-way ANOVA applied to Env-PC1 values), and correlated significantly with elevation ($r = 0.50$, $P < 0.0001$). Distributions of *Scaevola* along this main axis showed clear environmental sorting from negative values to positive values (coastal strand, dry forest, wet forest, respectively) (Fig. 2). Furthermore, distantly related species were grouped together (based on Tukey–Kramer comparisons) in low-elevation coastal habitats (lineage-B *S. taccada* and lineage-C *S. coriacea*) and higher elevation wet forest habitats (lineage-A *S. glabra* with lineage-C *S. gaudichaudiana* and *S. mollis*) (Fig. 2; Online Resource, Table S3). Notably, the hybrid-origin species (*S. kilaueae* and *S. procera*) appeared to occupy a more "intermediate" type of environment, based on our analysis, compared with their respective ancestral parent species.
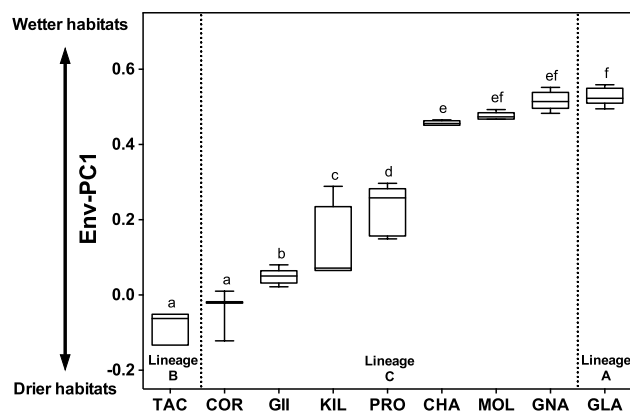
Fig. 2 Interquartile ranges of the primary environmental principal component (*Env-PC1*) values for each *Scaevola* species indicating environmental sorting and habitat similarities between species across phylogenetic lineages. *Letters* indicate species groupings using one-way ANOVA and Tukey–Kramer comparisons on Env-PC1 values. *CHA Scaevola chamissoniana, COR Scaevola coriacea, GII Scaevola gaudichaudii, GLA Scaevola glabra, GNA Scaevola gaudichaudiana, KIL Scaevola kilaueae, MOL Scaevola mollis, PRO Scaevola procera, TAC Scaevola taccada*

Trait variability across environments and evolutionary lineages

Most quantitative plant traits (30 out of 33 functional traits) varied significantly among *Scaevola* species (trait details in Online Resource 2, Tables S4-S5; Online Resource 3, Fig. S2; one-way ANOVA test details in Online Resource 2, Tables S6). Species ranged in height from sprawling shrubs as low as 10 cm (e.g., *S. coriacea*) to short-stature trees up to 4 m (e.g., *S. procera*). Leaf morphology and anatomy were also highly variable across lineages and species (Fig. 3). Both *S. taccada* and *S. coriacea* had orbicular leaves while other *Scaevola* species had short to elongated/lanceolate leaves. Leaf marginal teeth ranged from none (e.g., *S. coriacea*) to numerous (e.g., *S. chamissoniana* and *S. mollis*). Foliar sclereids were observed embedded in mesophyll tissues of all *Scaevola* species, except sister-species pair *S. chamissoniana* and *S. gaudichaudiana*. Dense trichomes were found consistently on the leaf surfaces of coastal *S. taccada* and forest *S. mollis*, whereas other species had sparse trichomes or glabrous leaves (Online Resource 2, Table S4). Stomatal distribution varied by lineage, and both *S. glabra* and *S. taccada* were hypostomatous (i.e., abaxial stomata only), while all the lineage-C species were amphistomatous (i.e., stomata on both leaf surfaces).

We analyzed correlations between functional traits and the multivariate environmental component (Env-PC1) (Table 4; Online Resource 2, Table S7). Many traits were significantly correlated with environment (at $\alpha = 0.01$) across all Hawaiian *Scaevola* species. Traits positively correlated with Env-PC1 (i.e., increasing trait values in wet
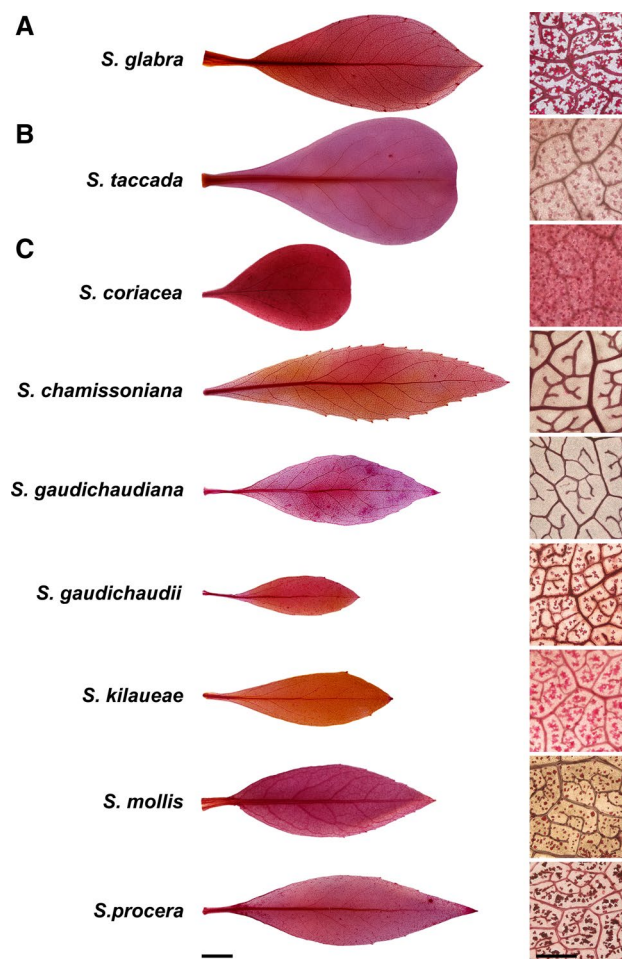
Fig. 3 Variation in leaf morphology and anatomy of Hawaiian *Scaevola* species. *Left-hand images* of cleared and stained leaves show contrasting whole leaf morphology, leaf marginal teeth, and major vein patterning among *Scaevola* species. *Scale bar* 1 cm. *Right-hand images* show corresponding variation in minor veins and foliar sclereids in each *Scaevola* species. *Scale bar* 100 μm. *A–C* Lineages of Hawaiian *Scaevola* species. Color version of this figure is available online

forested environments) included plant height, leaf area, length and shape (*L:W*), leaf marginal teeth, and abaxial stomata (density, SPI). Traits negatively correlated with Env-PC1 (i.e., decreasing trait values in wet forested environments) included nutrient concentrations (N_{area}, P_{area}, P_{mass}), water-use efficiency (i.e., more negative $\delta^{13}C$), leaf succulence, second- and third-order vein densities, and sclereid density. We then tested trait-environment relationships within lineage C *Scaevola* species. The direction of significant correlations (positive or negative) did not change but additional significant trait-environment relationships were found. Within lineage C, traits positively correlated with Env-PC1 also included branch diameter, petiole diameter and leaf width, while traits negatively correlated with Env-PC1 also included LMA, densities of first-order

Table 4 Plant functional trait correlations to environment, convergence across Hawaiian *Scaevola* species (lineages A–C), divergence among lineage-C *Scaevola* species, and trait contributions to multivariate trait axes

Trait[a]	Trait-environment correlation (r)[b]		Traits across lineages A–C within shared habitats[c]		Traits across "dry" and "wet" clades within lineage C[d]		Contribution to PC[e]
	All species	Lineage C	Habitat	Species (Habitat)	Clade	Species (Clade)	Component
Plant size traits							
Height	0.53***	0.34**	***	NS	***	***	Trait-PC2
Branch diameter	−0.06	0.57***	***	***	***	NS	Trait-PC1
Nutrient and isotope traits							
N_{mass}	−0.18	0.01	**	NS	NS	NS	Trait-PC3
N_{area}	−0.47***	−0.70***	***	***	***	***	Trait-PC1
P_{mass}	−0.55***	−0.43**	***	NS	***	***	Trait-PC2
P_{area}	−0.56***	−0.64***	***	***	***	***	Trait-PC2
N:P	0.21	0.05	***	***	NS	***	–
C:N	0.23	0	***	NS	**	NS	Trait-PC3
$\delta^{13}C$	−0.34**	−0.12	**	***	NS	**	–
Leaf morphometric traits							
Area	0.30**	0.71***	NS	***	***	***	Trait-PC1
Length (*L*)	0.60***	0.78***	***	***	***	***	Trait-PC1
Width (*W*)	0	0.65***	***	***	***	***	Trait-PC1
L:W	0.64***	0.52***	***	NS	***	***	Trait-PC2
Leaf teeth	0.60***	0.62***	***	NS	***	NS	–
Petiole diameter	−0.02	0.54***	**	NS	***	NS	Trait-PC1
LMA	−0.11	−0.40***	NS	***	NS	***	Trait-PC3
Succulence	−0.52***	−0.53***	***	***	***	***	Trait-PC2
Leaf vasculature							
Total vein density	−0.10	−0.31	**	***	***	***	NA
First-order vein density	−0.27	−0.62***	**	***	***	***	Trait-PC1
Second-order vein density	−0.39**	−0.73***	NS	***	***	NS	Trait-PC1
Third-order vein density	−0.44**	−0.65***	NS	NS	***	***	Trait-PC1
Minor vein density	0.02	−0.08	***	***	**	***	Trait-PC2
FEV density	0.05	0.10	NS	NS	NS	NS	–
Leaf stomatal traits							
Adaxial stomatal density	−0.30	−0.63**	***	***	***	NS	Trait-PC1
Abaxial stomatal density	0.68***	0.78***	***	NS	***	NS	Trait-PC2
Abaxial pore length	−0.34	−0.42	***	***	***	***	Trait-PC2
Abaxial SPI	0.46**	0.61**	NS	NS	***	NS	–
Leaf sclereid traits							
Sclereid density	−0.41**	−0.53***	NS	***	**	***	Trait-PC3
Sclereid length	0.12	−0.50	NS	***	**	**	Trait-PC3
Sclereid *L:W*	0.24	−0.31	**	***	NS	**	Trait-PC3
Wood traits							
Vessel numbers	–	–	NS	NS	NS	NS	–
Vessel-element length	–	–	***	NS	***	NS	Wood-PC1
Vessel-element diameter	–	–	***	NS	NS	NS	Wood-PC2
Pit diameter	–	–	NS	**	NS	NS	Wood-PC1
Tracheid length	–	–	NS	NS	***	NS	Wood-PC1
Tracheid diameter	–	–	NS	**	NS	NS	Wood-PC1
Tracheid wall thickness	–	–	***	***	NS	NS	Wood-PC2
Ray height	–	–	NS	***	NS	NS	Wood-PC1

Table 4 continued

Trait[a]	Trait-environment correlation (r)[b]		Traits across lineages A–C within shared habitats[c]		Traits across "dry" and "wet" clades within lineage C[d]		Contribution to PC[e]
	All species	Lineage C	Habitat	Species (Habitat)	Clade	Species (Clade)	Component
Multivariate trait PC							
Trait-PC1	0.33***	0.73***	NS	***	***	NS	–
Trait-PC2	0.56***	0.42***	***	***	***	***	–
Trait-PC3	−0.08	−0.35**	NS	***	NS	NS	–
Wood-PC1	–	–	**	***	***	NS	–

Trait-environment relationships tested using correlations between the multivariate environmental values and plant traits considering lineages A–C or lineage C only ($\alpha = 0.01$). Convergence tested among distantly related species within the same environment (wet forest species in lineage A vs. C or coastal species in lineage B vs. C) using nested ANOVAs for habitat. Divergence tested among closely related species (dry and wet clades within lineage C) using nested ANOVAs for clade. Contributions of traits to a multivariate trait component (*Trait-PC1* to *Trait-PC3*, *Wood-PC1* to *Wood-PC2*) indicated

FEV Freely ending veinlets, *PC* principal component; for other abbreviations, see Table 2

** $P < 0.01$, *** $P < 0.001$, *NA* not applicable, *NS* not significant

[a] See Online Resource 2, Table S4 for trait data, units, and sample sizes

[b] See Online Resource 2, Table S7 for r- and p-values

[c] See Online Resource 2, Table S8 for statistical details

[d] See Online Resource 2, Tables S9, S10 for loading scores for each multivariate trait component

veins, and adaxial stomata. Only $\delta^{13}C$ was statistically independent of Env-PC1 within lineage C (but significantly correlated to Env-PC1 when considering all lineages).

The numerous significant trait correlations with Env-PC1 across all *Scaevola* species suggested trait commonalities across species (i.e., trait convergence) within similar environments. We found strong evidence of trait convergence for distantly related species *within* given habitats using nested ANOVAs (species nested within habitat). Here, we compared individual traits (including wood traits) across species from different lineages in coastal or wet forest habitats (Table 4; Online Resource 2, Table S8). Most functional traits in our study varied significantly by habitat (22 out of 30 traits), while this was less apparent among wood traits (three out of eight traits). Furthermore, a number of traits were equivalent across species within each habitat, including plant height, nutrients (N_{mass}, P_{mass}, C:N), leaf morphology (L:W, teeth, petiole), abaxial stomatal density, and vessel-element length and diameter. Most of these traits were also strongly correlated with environment individually (using Env-PC1 values; Table 4).

We found strong evidence of functional trait divergence *across* environments for closely related species using nested ANOVAs (species nested within clade). Here, we compared individual traits of species within lineage C (Table 4; Online Resource 2, Table S8). Most functional traits (24 out of 30 traits) showed differences corresponding to clade and ecological radiation (i.e., trait diversification following dry vs. wet clades in lineage C). By comparison, fewer wood traits were significantly different (two out of eight traits) across species within each clade. Many traits

were equivalent among species within each clade, including branch and petiole diameters, C:N, leaf teeth, second-order major vein density, stomata (adaxial and abaxial stomatal densities, SPI), and vessel-element/tracheid lengths. We noted that shared traits within clades also tended to correlate with environment (using Env-PC1 values; Table 4).

We tested trait coordination (considering that many traits will be functionally linked) and assessed distributions of functional trait combinations between closely and distantly related *Scaevola* species using 29 traits in PCA. Multivariate analysis resulted in three significant PC (Trait-PC) explaining 35, 18, and 13 %, respectively, of data variance (Fig. 4a; Online Resource 2, Table S9; Online Resource 3, Figs. S3–5). In general, all wet forest species tended to have smaller ranges in "trait space" along any multivariate trait component, whereas coastal and dry forest species showed much wider multivariate trait distributions (Fig. 4a; Online Resource 3, Figs. S3-5). Combining Trait-PC1 and 2 (encompassing 53 % of trait variation) resulted in evident groupings of wet forest species (across all lineages) while coastal and dry forest species were much more dispersed. Placement of the hybrid species also revealed "intermediacy" in trait combinations compared with their ancestral parent species.

Trait-PC1 was positively correlated with environment (considering all species or only lineage-C species) and represented mainly canopy-related traits where higher values indicated that plants had larger branch diameters, larger leaf size (area, length, width, petiole), lower major vein density, lower N_{area}, and fewer adaxial stomata (Table 4). Trait-PC2 was a combination of traits relating to plant size
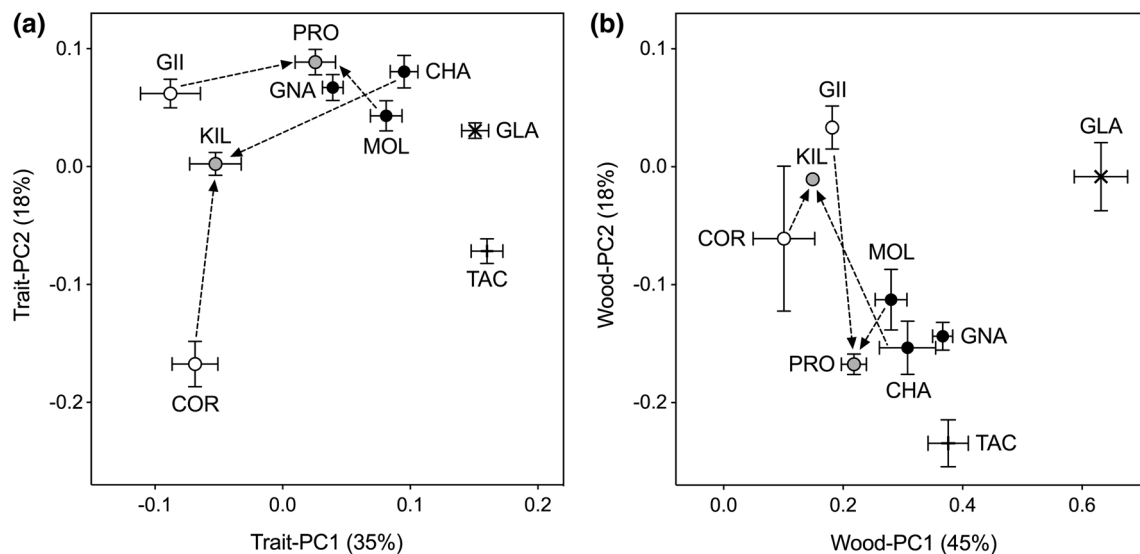
Fig. 4 Multivariate principal components (PC) analysis (PCA) of quantitative plant traits for Hawaiian *Scaevola* species. Trait PCs illustrate the two-dimensional "trait space" occupied by Hawaiian *Scaevola* lineages. **a** Mean values (±SE) for species along the first two trait PCs (*Trait-PC*) comprising 53 % of trait data variance. **b** Mean values (±SE) for species along wood trait PCs (*Wood-PC*) comprising 63 % of data variance (data from Carlquist 1969). *Arrows* show homoploid hybrids and ancestral parent species. Lineages are represented by *different symbols* (*star* lineage A, *cross* lineage B, *circles* lineage C). Within lineage C, *white* indicates dry clade species, *black* indicates wet clade species, and *gray* indicates homoploid hybrids (cf. Howarth and Baum 2005). For other abbreviations, see Fig. 2

and other leaf traits, and was also positively correlated with environment (considering all lineages or only lineage-C species). Higher values indicated plants had greater height, lower leaf succulence, higher minor vein density, greater abaxial stomatal density, smaller stomatal pore lengths, and lower P_{mass}/P_{area}. Trait-PC3 was negatively correlated with environment (lineage-C species only) and higher values indicated greater LMA, lower FEV density, larger/denser foliar sclereids, lower nitrogen (N_{mass}), and higher C:N.

We assessed the variability of wood anatomy traits across Hawaiian *Scaevola* species (Online Resource 2, Table S5) with an independent multivariate analysis. PCA yielded one significant component explaining 45 % of data variance (Fig. 4b; Online Resource 2, Table S10; Online Resource 3, Fig. S6). Higher values in Wood-PC1 primarily indicated longer xylem cell lengths (vessel elements, tracheids, fibres), larger pit diameters, and greater ray height (Table 4). Although not significant, Wood-PC2 explained 18 % of data variance and was associated with vessel-element diameters and tracheid wall thickness. *Scaevola* species followed a distinct spread by combining Wood-PC1 and 2, both by phylogenetic lineage and environment (Fig. 4b). Notably, *S. glabra* was clearly divergent to all other species, with exceptionally large vessel elements, tracheids and pit diameters (Online Resource 2, Table S5). Likewise, coastal *S. taccada* was divergent to ecologically similar *S. coriacea*, and vessel elements in *S. taccada* were ~1.5 times longer and wider with 1.7 times greater

diameter pits than *S. coriacea* (Online Resource 2, Table S5). Wood trait differentiation was evident across clades of lineage C, where wet clade species had ~1.5 times longer vessel elements and tracheids, wider xylem cells with thinner walls, and greater diameter pits than dry clade species (Online Resource 2, Table S5). We noted that hybrid-origin species had a very strong resemblance to one parent species compared to the other.

Evaluation of hybrid-origin *Scaevola* species and their ancestors

Our analyses suggested that the hybrid-origin *Scaevola* species occupied environments dissimilar to their ancestral parent species (Fig. 2) and had trait combinations comparable to more distantly related species within lineage C (Figs. 3, 4). We contrasted the suites of measured traits between hybrids and their ancestral parent species or ecologically similar species: (1) *S. kilaueae* with parents *S. chamissoniana*/*S. coriacea* and dry forest species *S. gaudichaudii*, and (2) *S. procera* with parents *S. gaudichaudii*/*S. mollis* and wet forest species *S. gaudichaudiana*. Mantel tests of distance matrices determined that *S. kilaueae* was equally distant to each parent, *S. chamissoniana* ($r = 0.02$, ns) and *S. coriacea* ($r = -0.08$, ns), and more comparable to dry forest species *S. gaudichaudii* ($r = 0.17$, $P = 0.022$) (Fig. 5a). By comparison, wood traits indicated that hybrid *S. kilaueae* was closer to both *S. coriacea* and
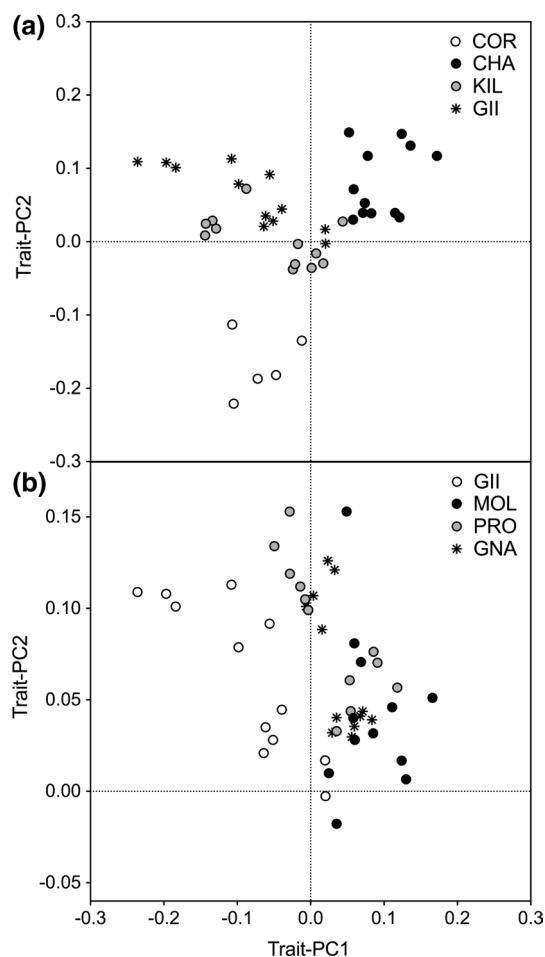
Fig. 5 Trait-PC1 and 2 illustrate the two-dimensional spread of homoploid hybrid and ancestral parents species with ecologically similar species in Hawaiian *Scaevola* lineage C. **a** Hybrid *S. kilaueae* with ancestral parents *S. chamissoniana* and *S. coriacea* and dry forest species *S. gaudichaudii* along the first and second trait axes. **b** Hybrid *S. procera* with ancestral parents *S. gaudichaudii* and *S. mollis* and wet forest species *S. gaudichaudiana* along the first and second trait axes. *White* indicates dry clade species, *black* indicates wet clade species, *gray* indicates homoploid hybrids, and *stars* indicate ecologically similar species. For abbreviations, see Figs. 2 and 4

S. gaudichaudii than *S. chamissoniana* (Fig. 4b, arrows). Mantel tests determined that the other hybrid-origin species, *S. procera*, had greater trait similarity to one parent, *S. mollis* ($r = 0.18$, $P = 0.0099$) than the other, *S. gaudichaudii* ($r = 0.14$, $P = 0.044$), but was most comparable to wet forest species *S. gaudichaudiana* ($r = 0.37$, $P = 0.0001$) (Fig. 5b). Wood traits also reflected this assessment, as *S. procera* corresponded more with *S. gaudichaudiana* and *S. mollis* than *S. gaudichaudii* (Fig. 4b, arrows). Comparatively, overall trait differentiation from both parent species was greater in *S. kilaueae* than *S. procera*, apparent by the correlation coefficients (r) from the Mantel tests and the spread of each hybrid and ancestral parent species along Trait-PCs 1 and 2 (Figs. 4, 5).

Functional trait combinations across environments

Multivariate trait components across lineages of Hawaiian *Scaevola* were largely consistent with a directional environment, particularly among lineage-C species (Fig. 6; nested ANOVA results, Table 4). The highest loading traits for Trait-PC1 and 2 tended to correlate with the environmental component (Env-PC1), and while there was some dispersion among lineages, many traits followed the predicted global trait-environment relationships (Table 5). Trait-PC1 indicated that *Scaevola* species in warmer, xeric habitats generally coordinated canopy traits such as smaller leaves with denser major venation and higher N_{area} compared with species in cooler, mesic habitats. Trait-PC2 mainly discriminated coastal vs. inland forest species and specified that *Scaevola* species in coastal environments tended to be smaller in stature and have increased P (P_{area}, P_{mass}). Trait-PC3 had a less obvious relationship with the environmental axis and greater dispersion among species (Fig. 6). Nevertheless, individual traits loading this axis tended to follow predicted global trends (Table 5). By comparison, Wood-PC1 was clearly related to Trait-PC1 (Fig. 6) and followed global trait-environment predictions, where species in warmer, xeric environments had shorter vessel elements, shorter and narrower tracheids, and smaller pits and rays compared to species in mesic environments, except *S. taccada* (Table 5). Notably, more adaxial stomata were observed in drier environments (lineage C only), opposite to the predicted trend. Other traits (e.g., N_{mass}, minor and FEV vein density, epidermal trichomes, stomatal pore length) did not follow global trait-environment predictions either.

Discussion

We tested trait-environment relationships in combination with evolutionary history in Hawaiian *Scaevola* to discern the processes of finer-scale evolution and the extent that phylogenetic history impacts trait diversification. Considering functional traits individually or in combination indicated that trait-environment correlations arose from all possible processes—colonization of habitats by single-species lineages, evolutionary processes of convergence (among distantly related species) and divergence (among closely related species), and hybridization.

Ecological preferences among *Scaevola* species

Species radiations and diversifications often show adaptation to environmental factors, with trait changes associated with selective pressures (including both abiotic and biotic factors) (Price and Wagner 2004; Rundell and Price 2009;

Fig. 6 Hawaiian *Scaevola* species distribution using mean values (±SE) for multivariate trait (Trait-PC1 to Trait-PC3) and wood anatomy combinations (Wood-PC1) along a directional environment (Env-PC1). Variables with the highest loadings for each component are listed along each axis. Lineages are represented by *different symbols* (*star* lineage A, *cross* lineage B, *circles* lineage C). Within lineage C, *white* indicates dry clade species, *black* indicates wet clade species, and *gray* indicates homoploid hybrids (cf. Howarth and Baum 2005). *MAT* Mean annual temperature, *MAP* mean annual precipitation; for other abbreviations, see Figs. 2 and 4
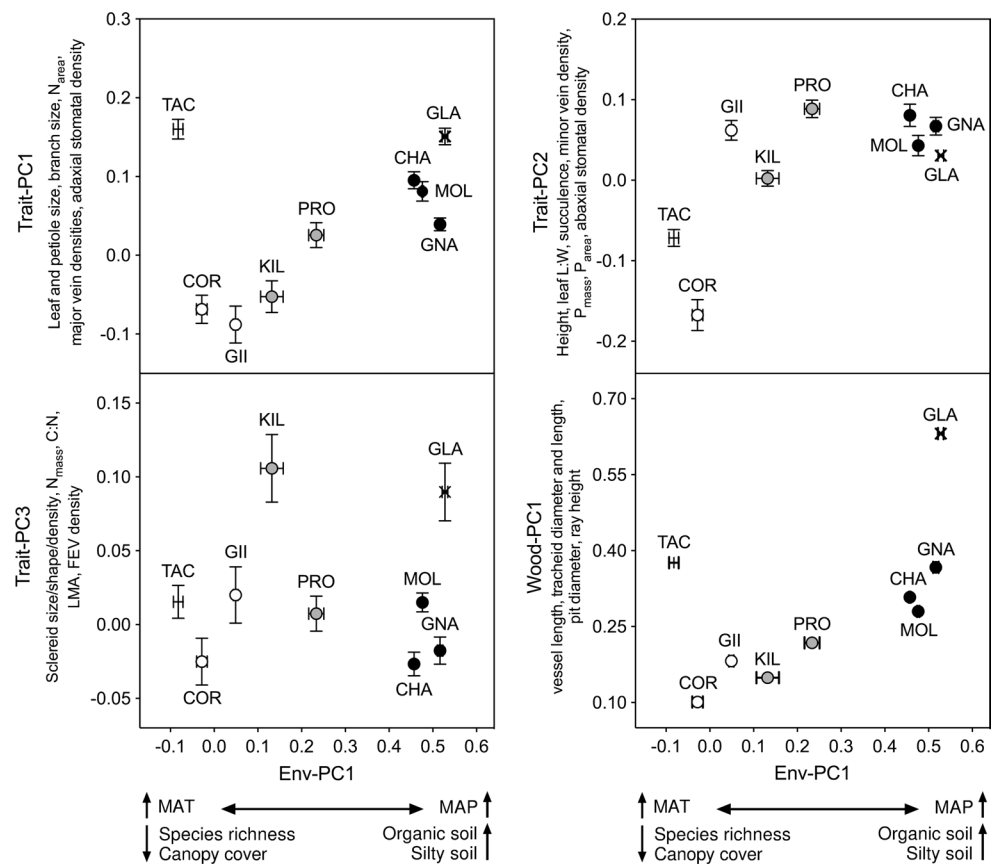
Kraft et al. 2015). Previous studies of *Scaevola* ecological distributions have tended to focus on elevation (Patterson 1990; Howarth and Baum 2005). In our study, a combination of factors presented a clear gradient from coastal and dry shrub forest sites with high temperature, low precipitation, and high drainage soils to rainforest habitats with greater resource availability, but also potentially stronger competition for space and light. Coastal strand and dry forest habitats had high exposure and soils tended to be coarse and lacking organic horizons. By comparison, wet forest habitats had fine volcanic silt or clays with organic horizons (indicating higher nutrient status and water-holding capacity). Canopy cover and species richness were higher in wet forest sites, and we noted that plants were not found in areas where the canopy shading was consistently >50 %, except in the rainforest species *S. glabra*, suggesting a genus-wide preference for higher light environments.

Trait diversification due to convergence and divergence among Hawaiian *Scaevola* species

We found evidence for convergence in functional traits (and suites of traits) among distantly related Hawaiian *Scaevola* species occupying comparable habitats with indications of stronger trait convergence among wet forest species than

dry habitat species. This was apparent in Trait PC-1 for wet forest species and Trait-PC2 for coastal species. The rainforest species *S. glabra* (lineage A) was found in environments characterized by relatively lower temperatures, higher precipitation, and greater canopy cover, most similar to the wet forests occupied by lineage-C *Scaevola* species (Table 3). Many traits (and combinations of traits) indicated convergence between these lineages, such as plant size and development of long leaves with low major vein densities, are features consistent with cooler environments and greater water availability (Kooyman et al. 2010). Nevertheless, some traits in *S. glabra* differed from those of wet forest lineage-C species, including thicker leathery leaves (with higher LMA), lower minor vein density, and long foliar sclereids, which may contribute to improved performance in shade and high water availability (Karabourniotis 1998; Scoffoni et al. 2011; Pivovaroff et al. 2014). Wood characteristics in *S. glabra* were also consistent with strong adaptation to cool, moist conditions (Carlquist 1969). Functionally, the larger vessel elements with greater sized pits would contribute to high hydraulic conductance but with a higher risk of embolism and water transport failure under dry episodes (Hacke et al. 2006).

Functional traits in the coastal species also suggested that particular characteristics were shared relating to that

Table 5 Trait directionality agreement among Hawaiian *Scaevola* species with predicted global trait-environment relationships

Multivariate component	Trait	Patterns among Hawaiian *Scaevola*	Agreement with predicted global relationships (across diverse phylogenetic species)
Trait-PC1	Branch diameter	Lineage C species	Increase with greater water availability, canopy cover
Trait-PC1	Leaf area	All species	Increase with greater water availability or canopy cover; decrease with irradiance and temperature
Trait-PC1	Petiole	Lineage C species	Increase with greater water availability or canopy cover; decrease with irradiance and temperature
Trait-PC1	N_{area}	All species	Increase with irradiance or temperature; decrease with greater water availability or canopy cover
Trait-PC1	Major vein density	All species	Increase with higher irradiance, lower water availability and/or higher temperature
Trait-PC1	Stomatal density (adaxial)	Lineage C species	Opposite relationships observed
Trait-PC2	Height	All species	Increase with greater water availability, canopy cover
Trait-PC2	P_{mass}	All species	Decrease with higher elevation
Trait-PC2	P_{area}	All species	Increase with irradiance or temperature; decrease with greater water availability or canopy cover
Trait-PC2	Minor vein density	All species	No relationship observed
Trait-PC2	Stomatal density (abaxial)	All species	Increase with higher elevation or water availability; decrease with higher temperature
Trait-PC2	Stomatal pore length	All species	No relationship observed
Trait-PC3	LMA	Lineage C species	Increase with irradiance or temperature; decrease with greater water availability or canopy cover
Trait-PC3	N_{mass}	All species	No relationship observed
Trait-PC3	Sclereid density	All species	Increase with higher irradiance or temperature or lower water availability
Wood-PC1, 2	Vessel elements, pits	All species	Increase in length, width, and pit diameter with greater water availability
–	$\delta^{13}C$	All species	Less negative with lower water availability (i.e., greater water use efficiency)
–	Leaf teeth	All species	Increase with greater water availability and distance from coast
–	Epidermal trichomes	All species	No relationship observed
–	FEV density	All species	No relationship observed
–	SPI	All species	Increase with higher elevation and water availability; decrease with higher temperature

For abbreviations, see Tables 2 and 4

habitat. Both *S. taccada* (lineage B) and *S. coriacea* (lineage C) existed within a narrow coastal band typified by higher MAT (up to 30 °C) and relatively low precipitation (Table 3); these species can be found coexisting (Online Resource 2, Table S1). Hydraulic stress from saltwater spray and sandy soils with high drainage is substantial in these coastal environments (Alpha et al. 1996), and *S. taccada* and *S. coriacea* shared particular traits, such as low habit, orbicular and succulent leaves, and relatively few stomata, that would limit effects of salt received on the surface area of their leaves relative to narrower leaves (cf. Alpha et al. 1996). However, *S. taccada* differed strongly from *S. coriacea* in other traits (Fig. 6), consistent with drought tolerance arising from alternative suites of coordinated traits (Grubb 1998). In particular, *S. taccada* had

much larger leaves with numerous trichomes, smaller stomatal pores, and lower vessel density than *S. coriacea*, a suite of traits consistent with minimizing water loss after stomatal closure (Hacke et al. 2006; Scoffoni et al. 2011). By comparison, *S. coriacea* had leaves with higher major vein and stomatal densities, and wood with shorter vessel elements and tracheids with small pits indicating hydraulic "safety." This suite of traits is consistent with high gas exchange when water is available and/or to maintain transpiration as water supply decreases (Hacke et al. 2006; Brodribb et al. 2010; Scoffoni et al. 2011).

Equally, we found strong evidence of trait diversification through divergence among closely related *Scaevola* species within lineage C (i.e., along dry and wet clades). The phylogenetic history of lineage-C species outlined that

radiation occurred from a colonizing progenitor similar to *Scaevola plumieri* (a species morphologically comparable to *S. coriacea*) and from coastal to inland habitats (Carlquist 1980; Howarth et al. 2003). All inland species shared the development of shrubby-arborescent features and elongated leaves, while other traits followed water or competition stresses, with evident divergence of trait combinations according to dry or wet clades, particularly in Trait-PC1 and Wood-PC1 (Fig. 6; see below).

Trait-environment correlations arise from trait filtering, trait evolution and hybridization

While there exist no immediate ancestors of *S. glabra* (lineage A) or *S. taccada* (lineage B) in Hawai'i for comparative analyses, we infer that the matches between individual traits and coastal or forest environments support some "trait filtering" where species were pre-adapted for colonizing the environments present in Hawai'i (cf. Reich et al. 2003). *S. taccada* is widely distributed and a major component of Hawaiian coastal communities, but has remained a single species lineage (Carlquist 1980; Alpha et al. 1996). As the floating fruits of *S. taccada* are highly adapted to widespread oceanic distribution (cf. Carlquist 1980), a lack of speciation or radiation in *S. taccada* may relate to high, homogenizing gene flow that reduces or precludes population isolation and subsequent speciation. By comparison, functional traits in *S. glabra* suggest high sensitivity to water stress, which may limit its spread into more arid and/or exposed environments. The $\delta^{13}C$ values for *S. glabra* in our study supported this assessment, and indicated the lowest water-use efficiency among the Hawaiian *Scaevola* species (cf. Farquhar et al. 1989).

The dry clade species within lineage C encompass coastal and dry shrub forest habitats, both of which are characterized by exposed habitats with low precipitation and bare mineral soils. We found some functional traits shared between both environments (invoking trait filtering and conservation from the putative coastal ancestral species), including small leaves with few leaf teeth, low stomatal densities and wood traits. However, following radiation into dry shrub forest habitats, most traits appeared to be divergent (invoking trait evolution). Dry forest species *S. gaudichaudii* showed substantial trait differentiation compared to its coastal sister species, *S. coriacea*, with narrower leaves (increasing leaf *L:W*, doubling vein density), lower leaf succulence, smaller stomatal pores, and elongated foliar sclereids. A dense leaf venation and a smaller pore area generally reflect adaptation for limited water availability (Scoffoni et al. 2011), while foliar sclereids in these environments may improve conductance and water flux within the leaf under highly exposed conditions (Brodribb et al. 2010). Additionally, *S. gaudichaudii* had

hydraulically "safer" wood traits (i.e., lower vessel density, narrower xylem cells, smaller pits) relative to its canopy size. This trait combination was also observed in the dry forest hybrid species *S. kilaueae* (see below). This trait divergence among dry clade species likely reflects strong physiological differentiation. Lower stomatal pore index and LMA values in *S. gaudichaudii* (compared to *S. coriacea*) were consistent with lower stomatal conductance to CO_2 but higher net photosynthesis (per unit leaf dry mass) and water-use efficiency than *S. coriacea*, as reported by Robichaux and Pearcy (1984) for plants grown under uniform conditions.

The wet clade species of lineage C (*S. chamissoniana*, *S. gaudichaudiana*, *S. mollis*) occurred in forest habitats characterized by high rainfall and potentially greater resource competition. These species showed strong trait differentiation (i.e., trait evolution) from the ancestral condition (~ *S. coriacea*) but shared a large number of similar traits affecting water relations due to trait conservation and/or further convergence (Fig. 6). Plants tended to be taller with greater canopies and larger leaves, and possessed wood traits contributing to increased hydraulic capacity (i.e., longer vessel elements and tracheids, larger pits). Despite these similarities, the wet forest species are physiologically differentiated. In our study, *S. mollis* had higher LMA than *S. gaudichaudiana*, consistent with findings from a previous study (using plants grown in common conditions) where *S. mollis* had lower net photosynthetic rate (per mass) than *S. gaudichaudiana* (Robichaux and Pearcy 1984).

The two homoploid hybrid-origin species, *S. kilaueae* and *S. procera*, demonstrated the potential for rapid reorganization of trait combinations given the recent nature of these hybrids (cf. Howarth and Baum 2005). Patterson (1995) described the morphology of *S. kilaueae* as somewhat resembling that of *S. chamissoniana*, while *S. procera* was considered most comparable to *S. mollis*. In our study, both species had a mixture of functional traits retained from both ancestral parent species, and suites of traits in both species were consistent with their current environment (i.e., trait combination similarities to ecologically similar species). *S. kilaueae* was found at mid-elevations on exposed lava beds in open scrub forests, unlike either of its ancestral parent species (Fig. 2). The distinct combination of characters in the hybrid resulted in greater water-use efficiency than in either parent species (Online Resource 2, Table S4), and an overall combination of traits equivalent to that of the more distantly related dry forest species *S. gaudichaudii* (Figs. 5, 6). *S. procera* was found in high-elevation, open forest habitats similar to the wet forest parent species *S. mollis* (Fig. 2). The hybrid retained a suite of traits common to all wet forest *Scaevola* species, including plant height, leaf size, venation, and wood anatomy (Figs. 4, 5, 6), highlighting the strong filtering and conservation of

traits following this environment. In general, endemism in Hawai'i coupled with significant habitat specialization is not without risk and species can be especially sensitive to environmental change (Rundell and Price 2009). Nevertheless, the homoploid hybrids (and other *Scaevola* species within lineage C) underscored that many functional traits are also reasonably labile following speciation events and diversify according to environment, particularly for traits related to water flux.

Author contribution statement A. D. M. and L. S. designed and conceptualized the study. M. E. A. provided *Scaevola* sampling locations, plant identification, and expertise on Hawaiian flora and ecology. A. D. M. and M. E. A. collected the field data. A. D. M. led the data synthesis and analysis. A. D. M. and L. S. wrote the manuscript with contributions from M. E. A.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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