

Research Article

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Running Title: Abiotic factors isolate tree varieties across a sharp ecotone

Varieties of the highly dispersible and hypervariable tree, *Metrosideros polymorpha*, differ in response to mechanical stress and light across a sharp ecotone

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Premise The drivers of isolation between sympatric populations of long-lived and highly dispersible conspecific plants are not well understood. In the Hawaiian Islands, the landscape-dominant tree, *Metrosideros polymorpha*, displays extraordinary phenotypic differences among sympatric varieties despite high dispersibility of its pollen and seeds, thereby presenting a unique opportunity to investigate how disruptive selection alone can maintain incipient forms. Stenophyllous *M. polymorpha* var. *newellii* is a recently evolved tree endemic to the waterways of eastern Hawai'i Island that shows striking neutral genetic differentiation from its ancestor, wet-forest *M. polymorpha* var. *glaberrima*, despite sympatry of these forms. We looked for evidence for, and drivers of, differential local adaptation of these varieties across the range of *M. polymorpha* var. *newellii*.

Methods For paired populations of these varieties, we compared seedling performance under contrasting light conditions and a strong water current characteristic of the riparian zone. In a reciprocal transplant experiment, adult leaf anatomy of the two varieties was contrasted.

Results Results suggest that the riparian zone is harsh and that selection involving the mechanical stress of rushing water, and secondarily, light, led to significant reciprocal immigrant inviability in adjacent forest and riparian environments. The strongest adaptive divergence between varieties was seen in leaves and seedlings from the site with the sharpest ecotone, coincident with the strongest genetic isolation of *M. polymorpha* var. *newellii* observed previously.

Conclusions These findings suggest that disruptive selection across a sharp ecotone contributes to the maintenance of an incipient riparian ecotype from within a continuous population of a long-lived and highly dispersible tree species.

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Keywords

flooding; incipient speciation; Myrtaceae; seedling experiments; woody rheophyte;

Persistent disruptive selection across environmental gradients or ecotones can maintain plant ecotypes (Turesson, 1922) in close proximity in spite of gene flow. Co-occurring ecotypes of the same species are usually associated with variation in edaphic conditions (reviewed by Rajakaruna, 2004; Wright et al., 2006), but ecotypes associated with water availability (e.g., Aronson et al., 1992), salinity (e.g., Blits and Gallagher, 1991), and disturbance regime (e.g., Masuda and Washitani, 1992) are also known.

While studies of disruptive selection across heterogeneous environments have shed light on the maintenance of co-occurring ecotypes of short-lived plants with limited dispersal, the drivers of divergence between sympatric tree populations are less well known. Different patterns are expected for trees, in part because ecotypes of the same tree species are more commonly spread across broader geographic scales coincident with changes in photoperiod or temperature (e.g., Pauley and Perry, 1954; Vaartaja, 1954, 1959) or water availability (e.g., Abrams et al., 1992; Peuke et al., 2002; Zhang et al., 2005). Nonetheless, while the high rates of outcrossing and gene flow characteristic of trees should hamper local adaptation over fine spatial scales, ecotypes with contrasting water-use efficiencies (Kubiske and Abrams, 1992) and responses to herbivory (Sork et al., 1993) have been found in nearby (1.6 km distance) and adjacent stands of red oak (*Quercus rubra*), respectively. Similarly, a dwarf ecotype of *Eucalyptus globulus* occurs in a maritime habitat, separated from tall *E. globulus* by just 200–300 m through most of its range (Foster et al., 2007). In these cases, strong diverging selection with modest physical separation of ecotypes may contribute to their maintenance through reduced gene flow.

Metrosideros polymorpha var. *newellii* (henceforth, *newellii*), is a tree endemic to rivers and streams on eastern Hawai'i Island that affords a unique opportunity to characterize how disruptive selection maintains an incipient tree ecotype in spite of gene flow. Restriction of *newellii* to waterways despite the predominance of *M. polymorpha* throughout the island (wherever rainfall exceeds 50 mm annually; Stemmermann and Ihsle, 1993) suggests that *newellii* is an ecotype adapted to the marginal conditions of the riparian zone. Chloroplast and nuclear microsatellite data viewed in the context of the island's age suggest that *newellii* arose within the past 500,000 yr on Hawai'i Island's oldest volcano from the more abundant wet-forest variety, *M. polymorpha* var. *glaberrima* (henceforth, *glaberrima*; Fig. 1; Stacy et al.,

2014). Although yet to be confirmed, a prior allopatric phase between *newellii* and *glaberrima* seems unlikely (Stacy et al., 2014), given that *M. polymorpha* is the first tree species to colonize new lava (stocking barren flows within 20 yr and thus populating new islands within a comparable time frame; Mueller-Dombois and Loope, 1990; Drake, 1992), and given its dominance and near-continuous distribution on Hawai'i Island from sea level to ~2500 m a.s.l. wherever rainfall allows. These two glabrous (hairless) varieties are distinguished by vegetative characters; *newellii* has a stouter, sometimes prostrate, growth form and dark narrow leaves, and *glaberrima* is a larger tree with rounder, less pigmented leaves (Dawson and Stemmermann, 1990). Both forms are highly dispersible with tiny wind-borne seeds and brightly colored "shaving-brush" inflorescences pollinated by birds and insects (Carpenter, 1976; Corn, 1979; Drake, 1993) and are presumably long-lived; on the same island, individuals of *M. polymorpha* can live at least ca. 650 yr (Hart, 2010). Whereas *glaberrima* is broadly distributed, being the only *Metrosideros* taxon to occur on all of the main Hawaiian Islands, *newellii* is confined to narrow riparian zones (rarely wider than the breadth of a single tree) that flank Hawai'i Island's waterways, where it is embedded in a continuous canopy of *glaberrima* wherever natural forest remains. Along these corridors, mating opportunities are presumably random, and occasional morphologically intermediate trees are found. Thus, *newellii* and *glaberrima* fit a strict definition of sympatry (Barraclough and Vogler, 2000; Gavrillets, 2003; Butlin et al., 2008; Fitzpatrick et al., 2008, 2009; Mallet et al., 2009).



Fig. 1 Adults of *Metrosideros polymorpha* vars. *glaberrima* and *newellii* (left and right, respectively) in their natural habitats (top) with their leaves shown side by side (bottom); var. *newellii* has floral buds.

In spite of their tight sympatry and shared chloroplast haplotypes, *newellii* shows relatively strong isolation from *glaberrima* at neutral nuclear loci. Mean F_{ST} between *newellii* and *glaberrima* populations sampled island-wide ($F_{ST} = 0.094$) is greater than that observed among the three common varieties on Hawai'i Island ($F_{ST} = 0.054$), with the largest *newellii* population along the Wailuku River especially isolated from adjacent *glaberrima* ($F_{ST} = 0.16$; Stacy et al., 2014). This degree of isolation is more in line with differentiation observed among conspecific tree populations at regional scales (Hamrick et al., 1992) rather than local scales, and it points to strong disruptive selection in the maintenance of these forms. The riparian zones of east Hawai'i Island are characterized by higher light than the adjacent forest understory and are periodically flooded with fast-flowing water after prolonged heavy rain. Such extreme conditions are expected to drive divergence in plants (Van Steenis, 1981; Lexer and Fay, 2005; Mitsui et al., 2011) and have led to the formation of novel, riparian-adapted perennial herbs on Okinawa Island within possibly as little as 9000 yr (*Ainsliaea oblonga*; Mitsui and Setoguchi, 2012a, 2012b). *Newellii* on Hawai'i Island appears to be a case of incipient speciation in trees driven by intense disruptive selection at the forest–riparian ecotone, and it offers an unprecedented opportunity to examine how extreme environments maintain a novel ecotype within a highly dispersible, continuously distributed, and long-lived species.

We carried out a reciprocal transplant experiment and examined responses of *newellii* and *glaberrima* seedlings from multiple sympatric populations (Appendix S1, see the Supplementary Data with this article) to two abiotic factors that are likely to maintain population divergence across the forest–riparian ecotone. Because divergence of this progenitor-derivative taxon pair is infraspecific and recent (<0.5 Myr) relative to the extended speciation times expected for trees (Petit and Hampe, 2006), we posited that the selective agents that drove the initial emergence of *newellii* are likely still important today (Crawford, 2010). We focused on seedlings because selection acting on early life stages is likely most important for determining adult distributions in trees (Petit and Hampe, 2006; Poorter, 2007). Because leaf venation traits influence adaptation to light (Sack and Holbrook, 2006; Sack and Scoffoni, 2013), we also compared adult leaf morphology and venation of these forms and predicted that relative to *glaberrima*, *newellii* will show foliar adaptations to, and greater tolerance of, the riparian environment.

MATERIALS AND METHODS

Field sites and seed collection

From November 2012 to May 2013, we collected ripe, open-pollinated fruits from populations of *M. polymorpha* on the windward slopes of eastern Hawai'i Island (Appendices S1 and S2), including four *newellii* populations and three sympatrically occurring *glaberrima* populations. Sympatric populations of *glaberrima* were limited or unavailable at two of the four *newellii* sites due to deforestation, and sampling of *newellii* in general is constrained by its scarcity in nature (IUCN, 2013). As such, the populations in this study, although limited, represent a good approximation of the diversity of extant *newellii* and sympatric *glaberrima*. Outside of the four *newellii* populations sampled, only scattered individuals can be found along windward Hawai'i Island streams. The two sites with the highest densities of both varieties, Wailuku and Kalohehewa, were also used for the reciprocal outplanting experiment and measures of adult leaf morphology and venation. Fruits were stored in coin envelopes in an air-conditioned lab for 2–7 wk, so that all seeds could be sown simultaneously. For each maternal tree, seeds from four fruits were sown in a communal well atop packed, well-drained soil (a 1:2:3 ratio of super-coarse perlite, sifted cinder, and Sunshine Mix #1; Sun Gro Horticulture, Agawam, MA, USA) covered with a thin layer of black sand to prevent seed loss (Drake, 1993). Seed trays were misted for 5 s every 10 min during daylight hours for 10 wk. Seedlings from all families were evenly distributed among the light, mechanical stress, and reciprocal transplant experiments.

Mechanical stress experiment

To simulate the effects of a storm event on seedlings established in the rocky substrate characteristic of riparian habitats, we filled two 10-cm-diameter, 4-m-long PVC pipe halves (cut longitudinally) with gravel secured with 2.5-cm steel grating. Four months after germination, 108 seedlings from 30 *newellii* families and 32 *glaberrima* families were transplanted into the pipe troughs using a thin layer of Sunshine Mix #1 and compost to cover the seedlings. Seedlings were spaced 7 cm apart in random order within each trough with 15 cm left unplanted at the ends of each trough. Seedlings were left to establish in the rocky substrate under 19% natural sunlight in the cold frame. In April 2014, after 9 mo of growth and just after a significant storm event, each trough was suspended in the Wailuku River from a bridge for 60 min, which subjected the completely submerged seedlings to a 2 m s^{-1} current (as a reference, Honoli'i Stream flows an average of 0.30 m s^{-1} , but achieved velocities averaging 8.05 m s^{-1} during a 10-yr storm; Ries et al., 2008). Water velocity was estimated using the float method with 10 lemons (Gordon et al., 2004). Total stem length and number of leaves were recorded for each seedling just before exposure, and initial mortality (i.e., loss from the trough), stem length lost, and number of leaves lost were recorded immediately for each seedling after troughs were pulled from the current. The troughs were then transported back to the cold frame where mortality was tracked for five additional weeks. Based on mortality initially and at 5 wk, coefficients of selection (S) were calculated.

Light experiment

We exposed 285 seedlings from 38 *newellii* and 36 *glaberrima* families to contrasting light levels in a cold frame (ca. two subsamples per family per treatment). At 4 mo post-germination, seedlings (~5 mm tall) were transplanted individually to 20-cm dibble tubes, which were then divided evenly between high-light and low-light treatments (58% and 3% natural sunlight transmittance, respectively, measured with a LI-250A light meter (LiCor, Lincoln, NE, USA) with seedlings randomly distributed among trays within treatments. The light levels used were consistent with levels reported previously (Burton and Mueller-Dombois, 1984; Morrison and Stacy, 2014) and with our own measurements in the field (36–100% irradiance, mean = 69% in riparian zones; and 1–17%, mean = 10% in the wet-forest understory; Appendix S3).

During the 18-mo experiment, seedling trays were rotated every 2 wk, provided 20-10-30 (N-P-K) fertilizer monthly, and treated with fungicide as needed. Each seedling was measured at both the start and end of the experiment for shoot height, total stem length, and length of the largest leaf, and shoot meristems ≥ 0.2 cm long (branch count), and leaves were counted. Mortality was monitored weekly for the first 12 mo, and mean relative growth rate (MRGR) was calculated at 18 mo for height, total stem length, leaf length, and leaf count using the following formula where T_1 is the time a seedling was first placed in the treatment when the initial measurement (M_1) was taken, and T_2 is the time the final measurement (M_2) was taken at the end of the experiment: $\text{MRGR} = (\ln M_2 - \ln M_1) / (T_2 - T_1)$. Also at 18 mo, root length and final seedling mass were measured, ratios of root to shoot dry mass and length to width were calculated, and the number of branches was recorded as an index of branching morphology. Based on mortality in each light treatment, a coefficient of selection (S) was calculated for the relatively less-fit variety using the following equation: $S = 1 - w$, where w is the relative fitness of that form (McGraw and Antonovics, 1983). S varies from zero (for the more fit of the two varieties) to one (indicating zero fitness for that variety).

Reciprocal transplant experiment

In November 2013, we outplanted 238 9-mo-old seedlings from 26 *newellii* families and 28 *glaberrima* families in six replicate reciprocal common gardens (six riparian and six forest plots) split between the Wailuku and Kalohewahewa sites. In most cases, one seedling per family was assigned a haphazard position within any of the three plots in each site-habitat combination, with individual plots containing even proportions of each variety. Outplanted seedlings were bare-rooted, wrapped in sphagnum moss and paper towels saturated with a 1:128 solution of Vitamin B1 Plant Starter (Lilly Miller, Walnut Creek, CA,

USA) and water and planted 10 cm apart (~10 seedlings/variety/plot). Individual seedling mortality was recorded monthly for 1 year, and shoot height, number of branches, total stem length, leaf count, and the length and width of the largest leaf were recorded at the time of outplanting and at 12 mo. MRGR at 12 mo was calculated (as above) for each of seedling height, total stem length, length of the largest leaf, and leaf count. Irradiance at each outplanting site was estimated using LI-250A light meters (Appendix S3).

Leaf morphology and venation measures

We sampled a single sun leaf from each of nine *newellii* and 13 *glaberrima* adults from the Wailuku or Kalohewahewa sites to examine vascular networks. Leaves were cleared via chemical clearing methods (see Appendix S4 for details), then mounted and photographed at 5× with a dissecting scope (Leica EZ4, Wetzlar, Germany) to obtain comparable leaf images, which were scaled and converted to binary images contrasting the veins and areoles using LEAF GUI software (Price et al., 2011). Summary statistics for leaf dimensions, vein density, vein connectivity, and areole dimensions were extracted using LEAF GUI. The leaf length to width ratio was calculated for each leaf. Specific leaf area (SLA: leaf area /leaf dry mass) was estimated also using five sun leaves from each of 14–15 adults of each taxon from each of the same two sites. Whole leaves were processed through a LI-3100 leaf area meter, then dried in a Lindberg/Blue M oven (Thermo Fisher Scientific, Waltham, MA, USA) at 80°C for 48 h before weighing dry mass. To compare adult leaf morphologies in natural populations with those of seedlings grown under controlled conditions, we measured the length and width, of the largest leaf of each seedling surviving the light experiment, calculated the length to width ratio, and determined the SLA using five leaves per seedling.

Statistical analyses

Seedling growth and morphology

Principal component analyses (PCAs) were performed for initial seedling traits measured at the start of each of the light and reciprocal transplant experiments using R version 3.4.0 (R Core Team, 2017). For both the light and reciprocal transplant experiments, the assumption of equal variances was met. In both cases, PC1 had positive loadings from all traits and thus represented initial seedling size (Appendices S5 and S6 for PCA). All statistical models were tested using the lme4 package (Bates et al., 2014) in R (see Appendix S7 for R code). All seedling growth (MRGR) and morphological measures, and branch and leaf retention (reciprocal transplant) were analyzed using linear models. PC1 for initial seedling size, variety, treatment, and the interaction between variety and treatment were included as factors, and all seedling growth measures and traits as the dependent variables. In the mechanical stress experiment, stem and leaf loss were also analyzed via linear models with variety included as a factor.

Seedling mortality rates

In each seedling experiment, mortality was equal to the proportion of the initial number of individuals that did not survive the experiment, with the value of each sample weighted by the number of subsamples. Generalized linear models (GLMs) were used to analyze seedling mortality, with a binomial distribution and logit link function, and mortality as the dependent variable. PC1 for seedling size, variety, treatment, and the interaction between variety and treatment were used as factors for mortality in the light and reciprocal transplant experiments, whereas only variety was used as a factor for mortality in the mechanical stress experiment.

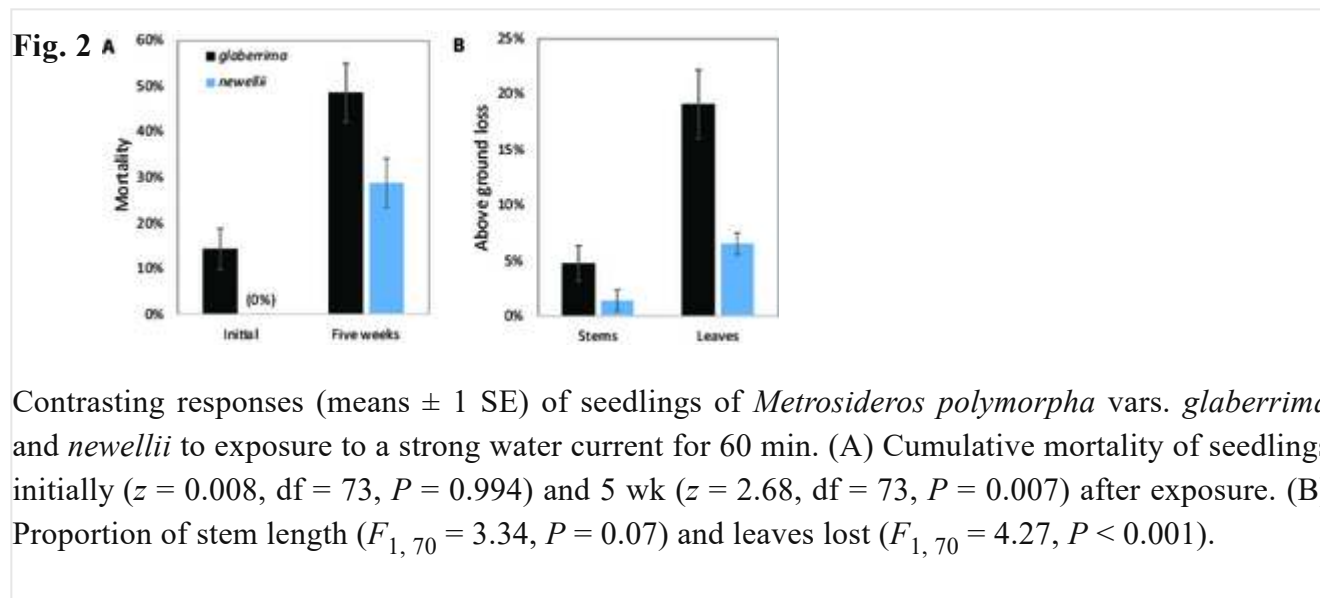
Leaf morphology and venation

We used linear mixed-effects models to compare each measure of adult leaf venation and SLA, with variety and site (nested as a random effect within variety) as factors. Adult leaf length to width ratios were analyzed using a linear model with only variety as a factor.

RESULTS

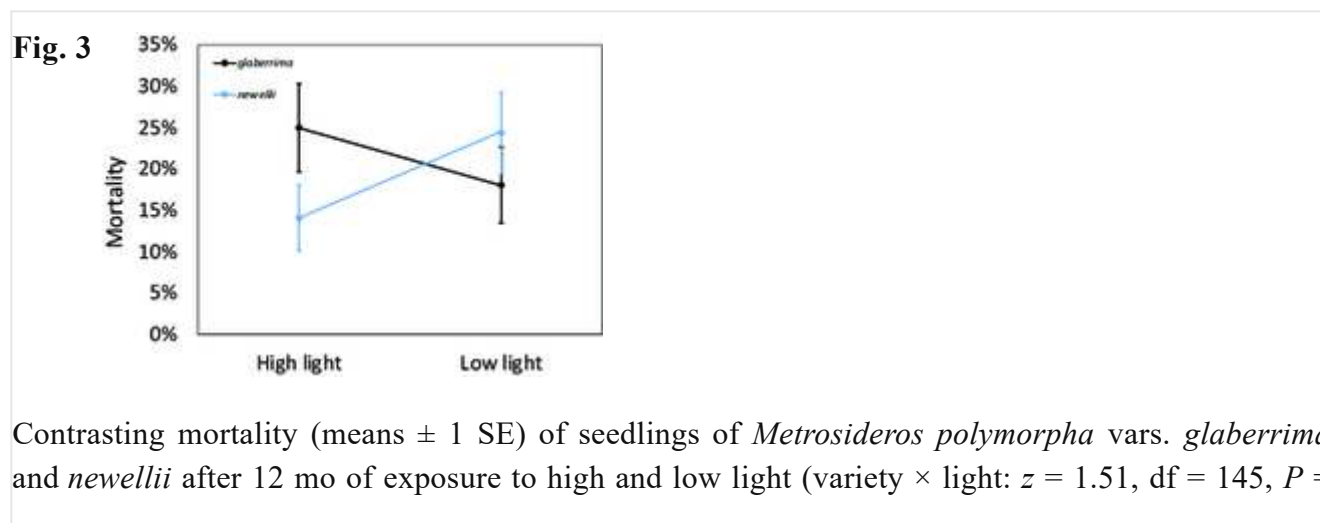
Mechanical stress experiment

Relative to seedlings of *glaberrima*, *newellii* seedlings better withstood both the acute and delayed effects of 60 continuous minutes of submersion in an intense natural water current intermediate between the average and maximum flow rates of a reference stream on Hawai'i Island (Reis et al., 2008). Immediately after exposure to the current, 14.3% of *glaberrima* seedlings had become uprooted and washed away, while none of the *newellii* seedlings were lost ($S_{\text{glab}} = 0.14 \pm 0.05$ [1 SE]; Fig. 2A). Of the remaining seedlings, those of *glaberrima* lost significantly more leaves (19.1% vs. 6.5% for *newellii*; Fig. 2B), while the difference in branch loss was lower (4.7% vs. 1.4% for *newellii*). Through 5 wk of post-exposure recovery, cumulative mortality climbed significantly higher for *glaberrima* (max 48.6%) than for *newellii* (max 28.7%; $S_{\text{glab}} = 0.28 \pm 0.06$ [1 SE]; Fig. 2A).



Light experiment

While mean mortality of *glaberrima* seedlings was greater in high light than in low light (25% and 18%, respectively), mortality of *newellii* seedlings was greater in low light (24%) than in high light (14%; consistent with a marginally significant light \times variety interaction; Fig. 3, Appendix S8). Otherwise, mortality did not differ between varieties or between light levels ($P \gg 0.05$). The selection coefficient for *newellii* (S_{new}) in low light was 0.08 ± 0.03 (1 SE), and for *glaberrima* (S_{glab}) in high light was 0.13 ± 0.002 .



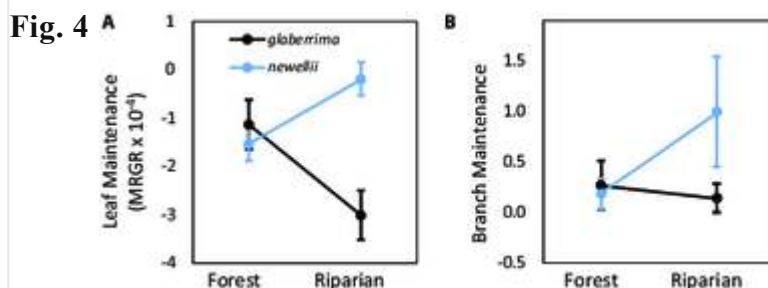
0.13).

At 18 mo, seedlings of both varieties experienced greater growth in high light. For both varieties, high light resulted in greater branching, leaf production, final root length, root to shoot dry mass and root to shoot length ratios and higher growth rates in stem length, final seedling mass, and leaf size; only seedling height was not impacted by light ($P > 0.05$; Appendix S9). In high light, mean branch count was 6.5% greater for *newellii* relative to *glaberrima*, and in low light, this measure was greater for *glaberrima* (66.4%; variety \times light interaction: $F_{4, 124} = 2.03$, $P = 0.004$). Leaf length to width ratio was significantly greater for *newellii* than for *glaberrima* in both light treatments (variety: $F_{4, 123} = 4.26$, $P < 0.001$; Appendices S9 and S10A). In contrast, SLA differed between the varieties only in the low-light treatment (13.7 m²/kg for *glaberrima* versus 11.8 m²/kg for *newellii*; variety \times light interaction: $F_{4, 97} = 3.932.01$, $P = 0.050$); SLAs of both varieties were lower in high light (mean: 6.6 m²/kg; Appendices S9 and S10B).

Reciprocal transplant experiment

At 12 mo post-outplanting, there was no significant variety \times habitat interaction effect on mortality, nor was mortality different between the varieties ($P > 0.05$). Variation in mortality between habitats, however, was striking (riparian: $90.1 \pm 0.15\%$ [1 SE] vs. $24.2 \pm 0.07\%$ in the forest plots, Appendix S8). The very high seedling mortality in the riparian plots appeared to be associated with three large storms that occurred during the experiment (Appendix S11). Following the flooding from Tropical Storm Iselle 8 months into the experiment, riparian plots on the Wailuku River were scoured of all but three outplanted seedlings; all three were *newellii* anchored in a rocky outcrop in the center of the river. One abnormally dry (Heim, 2014) month later, these three seedlings were dead. At 12 mo, only five experimental seedlings of *newellii* and six of *glaberrima* survived in the riparian zone, all restricted to a single plot at the high-water mark at Kalohehewa Stream.

Despite the harshness of the riparian environment, *newellii* seedlings showed significantly higher leaf and branch counts (through greater retention) in the riparian plots than in the forest plots, while *glaberrima* seedlings showed significant leaf loss in the riparian plots, but retained more leaves in the forest plots (Fig. 4, Appendix S12). Otherwise, seedlings of the two varieties grew equally well in the forest plots, where growth rates (i.e., height and leaf length) trended nonsignificantly higher relative to those in the riparian plots.



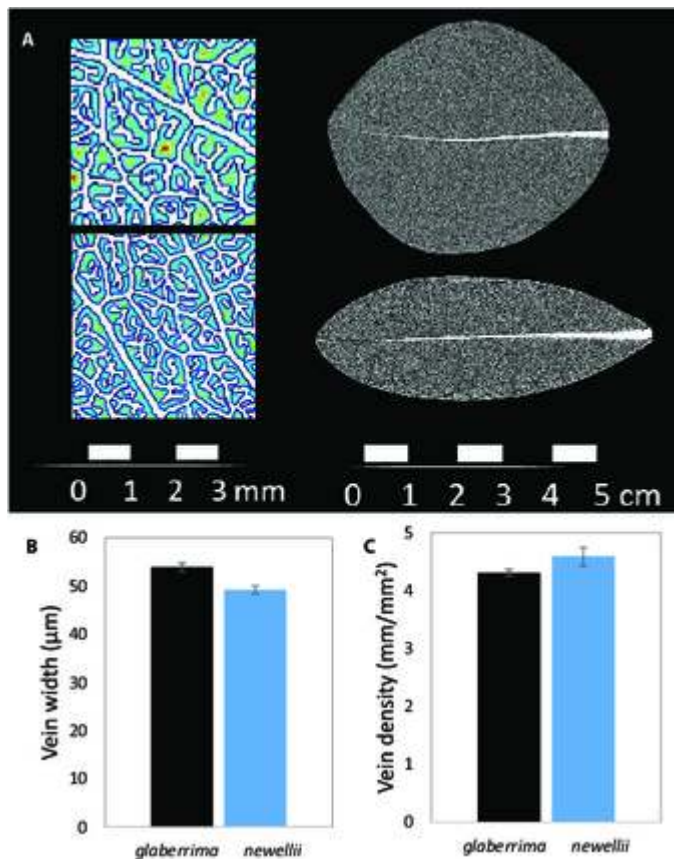
Contrasting responses of seedlings of *Metrosideros polymorpha* vars. *glaberrima* and *newellii* 12 mo after transplanting to forest and riparian habitats. Shown are mean (± 1 SE) (A) relative growth rate for leaf count and (B) branch count (variety \times habitat interaction terms: leaf retention: $F_{4, 66} = 3.06$, $P = 0.003$; branch retention: $F_{4, 66} = 2.09$, $P = 0.040$).

Leaf morphology and venation observations

Leaves collected from adult populations of *newellii* were significantly narrower than those of *glaberrima*, being on average 50% longer relative to leaf width (i.e., stenophyllous, $t = 7.50$, $P < 0.001$), but SLA of adult leaves differed only marginally between varieties, largely at one site (6.12 ± 0.28 [1 SE] m^2/kg for *glaberrima* and 5.5 ± 0.19 m^2/kg for *newellii*; $t = 1.75$, $P = 0.086$; Appendix S13).

Field-collected adult leaves of *glaberrima* were 49% larger with 40% longer vein networks on average (due to larger size), 19% wider mean areole distances (i.e., the spaces between veins, Appendix S14C), and 10% wider vein segments (Fig. 5A and Appendix S15). In contrast, veins of *newellii* leaves branched significantly more (17% more vein segments and 4% more nodes/ mm^2 , Appendix S14A, B), resulting in higher vein density (vein length per unit leaf area; Fig. 5A, C, and Appendix S15).

Fig. 5



Contrasting leaf venation observed in adults of *Metrosideros polymorpha* vars. *glaberrima* and *newellii*. (A) Left: Heat maps show smaller areoles and narrower, denser veins in riparian *newellii* (bottom) relative to *glaberrima* (top); warmer colors (green to red) indicate increasing distances from the nearest vein. Right: Binary images show veins and leaf shapes of vars. *newellii* (bottom) and *glaberrima* (top). (B) Vein width ($t = 4.03$, $P < 0.001$) and (C) vein density ($t = 2.22$, $P = 0.039$) expressed as mean \pm 1 SE.

DISCUSSION

The harshness of riparian zones

Results of our reciprocal transplant experiment indicate that riparian zones along the windward coast of Hawai'i Island are harsh, and establishment of riparian seedlings is likely episodic and restricted to periods of benign conditions. By replicating the effects of two abiotic factors on tree seedlings, we showed that disruptive selection across the sharp ecotone that separates the riparian zone from adjacent

wet forest likely contributes to the maintenance of a morphologically distinct riparian variety within a continuously distributed tree species. Our extensive field observations revealed no saplings or adults of *glaberrima* in the riparian zones; only infrequent seedlings were observed. These results suggest that invasion of the riparian environment on Hawai'i Island by the landscape-dominant tree, *Metrosideros polymorpha*, has led to the emergence of a novel genotype with adaptations to periodic strong water currents and secondarily to high light.

Newellii seedlings withstood the immediate and delayed effects of simulated flooding significantly better than *glaberrima* seedlings in the mechanical stress experiment. After 9 mo of growth in a rocky, riparian-like substrate and subsequent exposure to a strong water current, *newellii* seedlings had greater survivorship and physical resistance to the current, retaining significantly more leaves than *glaberrima* seedlings. The greater tolerance of *newellii* to strong water current was mirrored in the reciprocal transplant experiment; while branch and leaf counts of outplanted seedlings were low and often declined for both varieties across all forest and riparian plots, within the sole riparian plot that contained surviving seedlings after 1 year, branch and leaf retention was significantly higher for *newellii* (Fig. 4). Greater retention of stenophyllous leaves under mechanical stress is one of many observed adaptations that allow riparian plants to tolerate periodic inundation by strong water currents (Van Steenis, 1981; Tsukaya, 2002; Nomura et al., 2006; Mitsui et al., 2011). In the weeks following exposure, the delayed effects of the stress were apparent on both varieties with overall mean mortality rising from 7% to 38% at 5 wk. Nonetheless, mortality of *newellii* seedlings (24%) remained about half that of *glaberrima* (48%).

Eight months after seedlings were reciprocally outplanted in forest and riparian environments, Tropical Storm Iselle (7 August 2014) dropped up to 35 cm of rain on eastern Hawai'i Island (Lang, 2014), resulting in extreme, but not unusual, conditions in the island's riparian zones. The high water levels felled canopy trees and removed soil, grass, shrubs, and smaller trees, pushing the vegetation back several meters in some riparian areas and exposing rock. One *newellii* tree that was prostrated by this event persisted in a prone state, with no detectable damage to its shoots 4 mo later. Iselle exacerbated the already-high mortality of the riparian-habitat seedlings in our reciprocal transplant experiment, with mortality climbing from 60% to 86% within days, underscoring the intense selection that shapes Hawai'i Island's riparian vegetation and the importance of space and time for seedling establishment in the riparian zone (i.e., landing where anchoring is possible, several months before a major rain event). Although the high mortality in the riparian-zone seedling plots affected the two varieties evenly, notably all 11 seedlings that survived the full 12 mo were restricted to a single plot situated at the relatively benign high-water mark; otherwise, the longest-surviving seedlings were three *newellii* seedlings in a low rocky outcrop in the river's center that survived Iselle only to succumb 1 month later during a drought. Across the mechanical stress and reciprocal transplant experiments *newellii* outperformed *glaberrima* in branch retention and leaf retention in its "home" treatment, maintaining a more branched morphology in riparian habitats and a greater portion of its total stem length in rushing water. Increased branching—a morphological trait common to woody rheophytes (Van Steenis, 1981)—may be a strategy for positioning branches in a way that is more favorable to maintaining leaves in riparian conditions.

Differential adaptation to either side of the forest–riparian ecotone was also evident in leaf anatomy and morphology. *Newellii* is recognized by its stenophyllous leaves, and stenophyllously persisted in seedlings regardless of the conditions under which they were grown. For the widespread and highly variable *glaberrima*, in contrast, the relationship between leaf length and width was more variable. The fixed leaf shape of *newellii* may be consistent with persistent directional selection in an extreme environment (Hansen, 2006), analogous to the fixed leaf shape of *M. polymorpha* var. *incana*, the colonizer of barren, new lava flows on Hawai'i Island (Stacy et al., 2016). Further, the tendency of *newellii* seedlings and adults to have relatively lower SLA values, which tend to correlate with thicker leaves (Reich et al., 2003), may be another adaptation to mechanical stress (Nomura et al., 2006). Combined, these traits may help to explain the superior leaf retention of *newellii* seedlings exposed to current. *Newellii* showed

smaller vein conduits and denser vein networks, which may reflect drought tolerance and/or adaptation to the higher light in the riparian zone (Reich et al., 2003; Sack and Hollbrook, 2006; Brodribb et al., 2007; Sack and Scoffoni, 2013). The greater concentration of branching nodes in *newellii* leaves (26% greater than that of *glaberrima*) reduces the distance between xylem and evaporation sites within the leaf, thus allowing greater photosynthetic capacity (Feild et al., 2011).

AQ3

Disruptive selection by light across a forest–riparian ecotone

The light experiment revealed contrasting responses of the two varieties, with *newellii* having relatively greater responsiveness to light. While the observed interaction between variety and light was not within the conventional bounds of statistical significance for mortality, it was as predicted; mortality was greater for *newellii* in the low light characteristic of the forest understory and greater for *glaberrima* in the high light characteristic of the riparian zone. Both varieties experienced greater above- and (especially) belowground growth under high light. The combination of a high root to shoot ratio and greater survivorship of *newellii* under high light may be a key interaction in riparian habitats, with *newellii* thriving in high-light conditions that promote root development and therefore improve anchoring of seedlings that can withstand such conditions. The marked increase in branching displayed by *newellii* only in the high-light treatment was consistent with its highly branched morphology in nature (J. M. Ekar, personal observation). These results add to the evidence (Morrison and Stacy, 2014) that variation in light availability across Hawai'i Island's heterogeneous landscape imposes variety-differentiating selection on seedlings of Hawai'i's dominant tree species.

It should be noted that some of the variation observed in our seedling studies may well be explained by maternal effects of wild-collected seed. While we attempted to correct for maternal effects to some extent by using seed from the same individuals across contrasting treatments, maternal effects cannot be fully eliminated without growing a second generation in controlled conditions, a particular challenge when studying long-lived organisms.

Incipient riparian ecotype maintained by selection

While *newellii* as a whole appears to tolerate riparian conditions significantly better than *glaberrima*, the degree of adaptive phenotypic divergence between these sympatric varieties varied across forest–riparian sites. In both the seedling experiments and adult leaf observations, we noted variation across populations within varieties that appears consistent with the strength of disruptive selection across the forest–riparian ecotone. The greatest contrasts in seedling mortality and adult leaf characters between co-occurring *newellii* and *glaberrima* were seen at the Wailuku site. Similarly, *newellii* seedlings from Wailuku showed the strongest responsiveness to light with mortality 21% lower in high light relative to that under shade. In contrast, mortality in high light averaged across all other *newellii* populations was only 9% lower than that under shade. Notably, the Wailuku site hosted both the darkest forest (~3% irradiance) and the harshest riparian zone (Juvik et al., 1998) of all sites examined, creating a sharper contrast between adjacent environments. Notably, the greater seedling-stage divergence and sharper ecotone at Wailuku coincide with the strongest neutral genetic differentiation observed between sympatric *newellii* and *glaberrima* populations on Hawai'i Island. Pairwise $F_{ST} = 0.16$ between sympatric *newellii* and *glaberrima* at Wailuku is considerably greater than that averaged across population pairs of these varieties sampled island-wide ($F_{ST} = 0.094$; within their respective ranges; Stacy et al., 2014), with some pairs showing relatively little divergence (e.g., $F_{ST} = 0.035$; data of Stacy et al. 2014).

The parallels between functional and neutral genetic divergence between these varieties and the strength of disruptive selection across the forest–riparian ecotone suggest that *newellii* may be maintained at an early stage of divergence through selection alone (Nosil, 2008), without the aid of significant intrinsic isolating barriers. The selection coefficients estimated through our experiments ($S = 0.08$ to 0.28)

involved only a portion of the life cycle, yet may be sufficient to drive the evolution of phenotypic differences between these varieties given genetic variation for these traits, which is expected (Stacy et al., 2016). The severe water current and, to a lesser degree, high light that are characteristic of the riparian zone may be sufficient to explain the lack of saplings and adults of *glaberrima* there, and the abrupt transition to this habitat may be similarly sufficient to thwart the establishment of a *glaberrima*–*newellii* hybrid zone. For populations that traverse the riparian–forest ecotone, selection against migrants and maladapted hybrids before sexual maturity alone could be sufficient to drive morphological, anatomical, and physiological changes that may over time result in speciation (i.e., ecological speciation; Lenssen et al., 2004; Nosil et al., 2005; Mitsui and Setoguchi, 2012b, 2012c). Such selection would essentially bar gene flow from outside the riparian-adapted community to a degree proportional to the severity of the riparian zone (Engelbrecht et al., 2005; Poorter, 2007; Stella et al., 2010; Mitsui et al., 2011). At sites where disruptive selection is weaker (e.g., slow-moving streams embedded in forests with relatively open canopies), hybridization and introgression would lead to lower adaptive differentiation and neutral genetic divergence between sympatric populations, as was inferred for forest–riparian sister species of the perennial herb *Ainsliaea* on Yakushima Island (Mitsui et al., 2011). In fact, strong disruptive selection on parental phenotypes with only modest selection against hybrids may provide a likely scenario for the evolution of polymorphism across an ecotone, assuming a polygenic basis for the traits involved in local adaptation (Kawecki and Ebert, 2004). Additional studies are needed to determine the strengths and stages of reproductive isolating barriers separating *newellii* and *glaberrima* beyond immigrant inviability at the seedling stage.

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AUTHOR CONTRIBUTIONS

J.M.E. and E.A.S. conceived of the study, designed the study, and drafted the manuscript; E.A.S. and D.K.P. obtained funding; J.M.E. collected field, controlled light experiment, and leaf venation data; M.A.J. prepared leaf venation images; J.M.E., E.A.S. and D.K.P. carried out statistical analyses. All authors assisted with final drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.0fr4158> (Ekar et al., 2019).

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Supplementary Material

APPENDIX S1. Map of eastern Hawai'i Island waterways and six *M. polymorpha* sampling sites.

APPENDIX S2. Descriptions of six *M. polymorpha* sampling sites.

APPENDIX S3. Mean irradiance in greenhouse light treatments and among *M. polymorpha* forest and riparian habitats.

APPENDIX S4. Leaf clearing methods.

APPENDIX S5. Principal component analyses of initial *M. polymorpha* seedling size in the light experiment.

APPENDIX S6. Principal component analyses of initial *M. polymorpha* seedling size in the reciprocal transplant experiment.

APPENDIX S7. R code for all mortality, growth, morphology, and anatomy statistics.

APPENDIX S8. GLM results for mortality in the light and reciprocal transplant experiments.

APPENDIX S9. Linear model results for light, variety, and their interaction in light experiment.

APPENDIX S10. Contrasting SLA and stenophylly of *M. polymorpha* seedlings in high and low light.

APPENDIX S11. Survivorship of outplanted *M. polymorpha* seedlings in forest and riparian habitats.

APPENDIX S12. Linear model results for light, variety, and their interaction in reciprocal transplant experiment.

APPENDIX S13. Contrasting SLA and stenophylly within varieties of adult *M. polymorpha*.

APPENDIX S14. Contrasting leaf venation within varieties of adult *M. polymorpha*.

APPENDIX S15. Linear mixed-effects analysis of leaf venation traits in adult *M. polymorpha*.

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