

Plasticity of phenotype and heteroblasty in contrasting populations of *Acacia koa*

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Received: 1 March 2019 Returned for revision: 27 April 2019 Editorial decision: 9 May 2019 Accepted: 13 May 2019
Published electronically 21 June 2019

- **Background and Aims** Heteroblastic plant species, whose morphology or growth habit changes suddenly during development, offer unique opportunities to investigate the role of selection in canalizing development or increasing the adaptive importance of plasticity. Leaf forms of the Hawaiian tree *Acacia koa* (koa) change morphologically and physiologically during the first year of growth, providing time to study abiotic factors influencing transition rates relative to other *Acacia* species.
- **Methods** The roles of light and water availability in triggering transition to the mature leaf form in contrasting (wet/dry) ecotypes of koa were investigated using a novel modelling technique to distinguish between chronological and ontogenetic controls in triggering transition. A light quality treatment was included to test interactions of heterophylly (the presence of multiple leaf forms) with heteroblastic processes on the resulting phenotype at transition.
- **Key Results** Increased light intensity increased transition rates, but reduced red to far-red light (R:FR) ratios did not affect transition rates, solidifying the current paradigm of heteroblasty. However, evidence was found for earlier transition ontogenetically under water stress, which is not part of the current paradigm and could differentiate the role of heteroblasty in some *Acacia* species versus other heteroblastic species. Ecotypic responses also indicate that plasticity of development could vary across koa's range and the adaptive significance of heteroblasty could be marginalized or amplified dependent on the disparate selective pressures present across koa's range.
- **Conclusions** The use of novel survival functions and a species with an elongated transition time helped to elucidate abiotic modifiers of ontogenetic trajectories. Differences in ontogenetic trajectories between contrasting ecotypes suggest that ongoing climate and land use change will have non-uniform effects on koa regeneration and establishment dynamics across its range.

Key words: *Acacia koa*, drought, heteroblasty, heterophylly, irradiance, light quality, leaves, morphology, ontogeny, ontogenetic trajectory, phenotype, plasticity.

INTRODUCTION

Heteroblasty in plants refers to a sudden morphological transition during development (Zotz *et al.*, 2011), classically exemplified by the transition of *Hedera helix* leaves from lobed to entire (Bauer and Bauer, 1980), of some bromeliad species from juvenile atmospheric leaves to water-holding tank forms (Zotz *et al.*, 2004), and the elongation of leaves in many *Eucalyptus* species (Jaya *et al.*, 2010). In contrast to heteroblasty at the leaf level, which refers to the transition of leaf form associated with age-related development (Zotz *et al.*, 2011), heterophylly refers to the presence of two leaf forms or the ability to revert to a previous leaf form (Pigliucci, 2001).

Phenotypic plasticity is the variation in the physical form of a plant in response to biotic and abiotic factors (Stearns, 1989). Thus, the observed range of variation of heteroblastic timing in response to abiotic stressors is an example of phenotypic plasticity. Traditionally, researchers have focused on phenotypic plasticity at the level of the genotype in response to environmental gradients (Pigliucci, 2005) and this should be viewed as

the term's definition *sensu stricto*. In answering developmental or mechanistic questions about processes among individuals within a species, studies should be designed to assess genotypic variation. However, questions regarding how plasticity of traits within populations or species affect fitness or survival in changing or contrasting environments must allow for average plasticity across a set of genotypes (Gianoli and Valladares, 2012). Plasticity can be viewed both as an adaptation to heterogeneity across space and time (Gianoli and Valladares, 2012; Valladares *et al.*, 2006) and as an intrinsic property of genotypes, populations, species, genera and life-forms that enable them to succeed in their environment and adapt to changes in that environment (Balaguer *et al.*, 2001; Pohlman *et al.*, 2005; Sánchez-Gómez *et al.*, 2008).

Misleading results in studies examining phenotypic plasticity can come from failing to control for ontogenetic drift (Wright and McConnaughay, 2002), especially when looking at resource partitioning traits, such as plasticity in root-to-shoot ratio. This is because plants naturally vary resource allocation during

development: small plants tend to have allocation patterns that differ from those of large plants of the same species. Because most species experience ontogenetic drift and will differ in growth rates when exposed to different environmental conditions, comparisons between individuals may conflate passive plasticity (Pigliucci and Hayden, 2001) with ontogenetic plasticity, which occurs when a trait's ontogenetic trajectories are affected by the environment (Wright and McConaughay, 2002). Controlling for ontogeny may help elucidate how variation in accumulated biomass, in response to environmental variation, results in a quicker or slower transition to mature morphology.

Among several *Acacia* species clearly showing heteroblasty, *Acacia koa* (koa) has been less studied. Koa is a large tree (>25 m in height) endemic to the Hawaiian Islands, USA (Hall, 1904), which is closely related to Australian and other South Pacific *Acacia* species, such as *A. melanoxylon* in Australia (Robinson and Harris, 2000). Koa occupies a dominant or co-dominant canopy position in forests from near sea level to >2100 m and where precipitation ranges from <1000 to >4000 mm year⁻¹ (Little and Skolmen, 1989; Baker et al., 2009). The genetic structure of koa populations is strongly associated with annual rainfall (Gugger et al., 2018). In contrast to some other heteroblastic genera and species, such as *Eucalyptus occidentalis*, in which the vegetative phase change consists of an increase in the leaf length-to-width ratio (Jaya et al., 2010), koa and other phyllodinous *Acacia* species undergo a transition in what constitutes a 'leaf'. Koa leaves transition from bipinnately compound, horizontally oriented true leaves to vertically oriented phyllodes. Although a gradient is still present through a series of transitional leaf forms in which leaves show a mix of juvenile and mature morphology, the clear distinction between early-form and later-form foliage allows easier disentangling of ontogenetic drift, ontogenetic trajectory, and phenotypic plasticity in response to abiotic stressors. Because of the wide climatic range of koa and the longer transitional period relative to other heteroblastic *Acacia* species, such as *A. implexa* (Forster and Bonser, 2009a), koa represents an ideal tree species for investigating the adaptive role of heteroblasty across a range of climate types. Koa transitions usually within the first 3–5 months of growth after germination (Walters and Bartholomew, 1990), but the transition can be delayed for years when irradiance is low (e.g. the tree is overtopped by competing vegetation). The roles and functional advantage conferred on koa by each leaf form have been studied over the last 30 years (Walters and Bartholomew, 1984, 1990; Hansen, 1986, 1996; Craven et al., 2010; Pasquet-Kok et al., 2010), but the triggers of the change itself have not been addressed in previous work.

Koa's bipinnately compound true leaves have been proposed as functionally important for ensuring fast early growth in forest gaps, where light is limiting, at a low carbon cost. It has been argued that phyllodes are important for drought resistance and as an adaptation to high-light environments because of increased stomatal responsiveness to reduced vapour pressure deficit and ability to respond to desiccation and rehydration (Walters and Bartholomew, 1984, 1990; Hansen, 1986, 1996; Craven et al., 2010; Pasquet-Kok et al., 2010). Walters and Bartholomew (1984) found that true leaves and phyllodes had similar photosynthetic responses to light. The findings of Pasquet-Kok et al. (2010) and Craven et al. (2010) were

restricted to single populations from restricted ecotypes within koa's range, but we would expect the relative importance of true leaves versus phyllodes for regeneration and establishment success to be different across koa's wide climatic range. Because of this, studies that characterize differences between leaf types without considering the rate of change might be missing an important adaptation allowing establishment on harsh sites in different ecotypes across koa's range. The importance of plasticity in timing might be more important for a population from a wet ecotype, where maintenance of light-capturing true leaves allows fast growth in canopy gaps, whereas a quick transition for a dry ecotype allows greater resistance to drought in areas where droughts occur randomly.

Although true leaves have some traits of shade leaves and phyllodes have some traits of sun leaves (Walters and Bartholomew, 1990), the phasic component during the first year of growth could be more important for survival on harsh sites across koa's climatic range. The crowns of mature, healthy trees are almost always entirely composed of phyllodes. The true leaves indeed have shade leaf characteristics compared with phyllodes (Pasquet-Kok et al., 2010) and are often present in the lower portion of crowns, but the developmental trajectory suggests that these are not the defining characteristics over the life of the tree. There are no documented cases of koa producing fruit before phyllodes. Thus, phyllode production and the mature, reproductive phase are at least concurrent, if not related, although phyllode production typically occurs years before reproduction.

If true leaves are an adaptation to shaded conditions present during regeneration and establishment in gaps (Mueller-Dombois, 1981), the timing of transition may be delayed by shaded conditions (Hansen, 1996). The heteroblastic trigger, therefore, could be connected to the shade avoidance syndrome (SAS) response (Tao et al., 2008), a plant response that initiates morphological modifications that promote apical growth at the expense of lateral and below-ground growth. In another phyllodinous *Acacia* species, Forster and Bonser (2009a) found a decrease in the rate of transition under reduced red (660–670 nm) and blue (400–500 nm) light. This result contrasts, however, with the most recent paradigm for heteroblasty and heterophylly, which has separated the processes at the molecular level (Chitwood and Sinha, 2016). Whereas heteroblasty in *Acacia* species, *Hedera helix* and *Quercus acutissima* is controlled by light availability, sugar gradients and photosynthetic nutritional status, heterophylly has been linked to a low ratio of red to far-red (R:FR) light and to temperature (Chitwood and Sinha, 2016).

In forests with uniform climatic conditions throughout the year, transition could be a function of body size (ontogeny). In forests where drought is frequent and/or unpredictable, transition could be canalized and fast, regardless of weather conditions. Therefore, the timescale used for evaluating transition triggers could also be important. Despite the hypothesized controls on heteroblastic timing, if the timing of change from true leaves to phyllodes is heritable in koa, selection will probably depend on both light and water availability because selection pressures during the regeneration and establishment phases vary across koa's range. These pressures likely result from differences in water availability and stand density. This

can lead to population-level differences in the plasticity of transition timing. Because of this, studies examining plasticity of heteroblastic timing should include contrasting ecotypes that can help elucidate the importance of the timing of phase change relative to the importance of the different leaf types themselves.

We aimed to determine whether the timing of transition in koa is a plastic trait in response to light and water availability. Further, we tested contrasting (wet/dry) populations of koa to observe whether variation in ontogenetic trajectories in response to resource availability suggests that the timing of transition is an adaptive response to disparate selective pressures across koa's range. Although we were interested primarily in the plasticity of ontogenetic trajectories, we also aimed to understand the effect of abiotic stressors on traits associated with the multivariate phenotype hypothesized by the SAS at the point of transition. We predicted (1) a plastic transition rate in response to light and water availability; (2) a faster transition rate for the dry-site population regardless of resource availability; and (3) changes in the multivariate phenotype at the time of transition, with no effect on the rate of transition, as a result of differences in R:FR light.

MATERIALS AND METHODS

Treatments and experimental design

A split-plot design was used with three replicate blocks and three factors: light (whole-plot, four levels), water (sub-plot, two levels) and population (two levels). We used two contrasting populations of koa from the Hawaii Island of Hawaii: Honomalino (19.43°N, 155.81°W) and Umikoa (19.93°N, 155.35°W). The dry (leeward) population was composed of seeds from 20 half-sib families collected in 2012 from native forest in the Honomalino area with an average annual rainfall of 900 mm, estimated from the nearby Haupuu weather station, located at 1370 m above sea level (m.a.s.l.; Giambelluca *et al.*, 2013). The wet (windward) population, from windward Hawaii Island, includes 24 half-sib families collected in 2007 from a 9-year-old progeny trial of trees from natural stands in Umikoa at 1570 m.a.s.l. It has an average annual rainfall of 2018 mm (Giambelluca *et al.*, 2013). Families within each population were bulked.

Photosynthetically active radiation (PAR) and R:FR were quantified using a LI-250A light meter (Li-Cor, Lincoln, NE, USA) and a Spectroradiometer PS-100 (Apogee, Logan, UT, USA), respectively, at noon on 11 August 2013 (Table 1). The R:FR ratios were calculated as the ratio of light falling between

650–680 nm (red) and 710–740 nm (far red). The Full treatment utilized no shade cloth or plastic film. The 70Film treatment utilized a neutral density filter with an estimated transmission rate of 69.3 % (298 0.15 ND; LEE Filters, Andover, UK). The 25Film treatment utilized a neutral density filter with an estimated transmission rate of 23.5 % (210 0.6 ND; LEE Filters, Andover, Hampshire, UK). The 25Cloth treatment was used to achieve an average PAR of approximately the same intensity as the 25Film treatment while providing an R:FR equal to that of the Full treatment. This facilitated the decoupling of irradiance- and light quality-mediated processes. Each light treatment, including the Full treatment, was surrounded by a 1.83 m × 1.22 m structure made of PVC piping. The 70Full and 25Film treatments were covered on top (1.83 m × 1.22 m) and the sides (1.83 m × 0.3 m) with a neutral density filter. Although the original height of the structures was 1.3 m, the structures were raised 0.5 m three times to accommodate the seedlings when the tallest seedling of a block reached the top of the structure. This ensured that the apical meristem received consistent light quality and light intensity treatment conditions during the study.

The water availability effects were evaluated under two conditions: well-watered (WW) and water-stressed (WS). The WW seedlings were drip-irrigated to saturation each morning during the study. The media for the WS seedlings dried to 60 % of field capacity (FC). Soil moisture was estimated using a HydroSense II (Campbell Scientific, Logan, UT, USA) with 20-cm probes. The volumetric water content (VWC) of the soil for all the WS seedlings was measured every 3 d during the study and pots were watered up to saturation when the soils were at or below 60 % FC (using the average of two sub-samples). Watering occurred, on average, between 56.6 and 56.8 % FC [10.25 and 10.38 % (v/v) VWC]. A total of 144 seedlings constituted the study (3 seedlings per treatment combination in a block).

Propagation

Seeds were scarified using a nail clipper and soaked overnight in warm water before sowing the next day (20 May 2013) in single-cell trays for germination under mist. Plants were selected for uniform size and transplanted (6 d post-germination) to 9.63-L pots (TP818; Stuewe & Sons Tangent, OR, USA) with Farfard Professional Potting Mix with Resilience (Sun Gro Horticulture, Agawam, MA, USA). The potting mix was amended at planting (incorporated into media) and the beginning of September (top-dressed) with 15 g of

TABLE 1. Description of light treatments used in study

Treatment	Red light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Far-red light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Ratio (F:FR)	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
25Cloth	19.66 ± 8.7	13.46 ± 6.0	1.46 ± 0.01	643 (±265)
25Film	14.68 ± 6.1	21.25 ± 8.6	0.68 ± 0.01	463 (±180)
70Film	43.06 ± 17.0	33.66 ± 13.2	1.28 ± 0.01	1468 (±540)
Full	71.78 ± 3.8	48.54 ± 1.9	1.48 ± 0.02	2432 (±157)

Values are mean ± s.e. ($n = 3$).

Each treatment combination within each block was sampled three times.

Measurements were taken to calculate the ratio of red light (wavelength 660–670 nm) to far-red light (725–735 nm) and total PAR within each treatment.

Osmocote 15-9-12 (Scott's MiracleGro, Marysville, OH, USA) with a release rate of 3–4 months.

Seedlings were randomly assigned to groups of 12 (six from each population). Seedlings were stabilized and arranged to provide ~0.19 m² of space for each seedling. Eleven days post-germination, seedlings were assigned randomly to light and water availability treatments. Light treatments began between 28 and 32 d post-germination when at least 50 % of the seedlings in a block were >10 cm in height. Water availability treatments were initiated 62 d post-germination.

Measurements and harvest

Seedlings were evaluated every 2 d and destructively harvested after the development of at least one fully expanded phyllode. At the time of harvest, phyllode biomass (g), which included both fully and partially formed phyllodes, accounted for 2.79 % (1.37 ± 0.66 g) of total biomass. Transition to phyllodes began 88 d post-germination and continued until 171 d post-germination, when the study was concluded. At the time of harvest, morphological measurements were taken and the seedling was harvested and separated into parts for biomass and area calculations. Counts of nodes did not include cotyledons. The following morphological parameters were measured at this time: height, basal diameter, number of nodes to transition (the node that included the phyllode), total number of nodes, height of node with phyllode, number of branches to node with phyllode, total number of branches, height of first branch, diameter of first branch (measured at base of branch after taper), height of the branch with largest diameter, diameter of the largest branch, and height of the first phyllode (see Table 2 for definitions and abbreviations of traits). The number of nodes to transition and total nodes were used with the height of the transitional node and total height to calculate the internode average to transition and total internode average, respectively. In order to calculate leaf area, the true leaves were clipped to include the rachis and placed flat on a white 1-m² piece of cardboard with a ruler. A photograph was taken of the leaves and leaf area for each photo was calculated using ImageJ v. 1.48 (Schneider et al., 2012). All phyllodes and transitional leaf sections were also harvested and leaf area was calculated in the same manner. Above-ground stems (cut at the soil line), roots (washed with water to remove media), leaves and phyllodes were bagged

individually, dried at 65 °C to a constant weight, and weighed to calculate above-ground biomass, leaf biomass, below-ground biomass, and phyllode biomass.

Statistical analyses

Three seedlings were dropped from the study because they were mutants, in two cases never producing chlorophyll and in one case producing many nodes but with limited cell expansion. Cox proportional hazards models [using the Survival package in R from Therneau and Grambsch, (2000)] were used to test the effect of treatments on rates of transition over chronological (days since germination) and ontogenetic timescales. These models show the cumulative transition of a treatment (e.g. 100 % cumulative transition means that all individuals within a treatment transitioned). The significance of effects was tested with likelihood ratio tests, where terms were successively added to the model, running χ^2 tests to evaluate the significance of the effect. Tukey's HSD tests were used for *post hoc* comparisons within significant fixed-effect factors. Because of limited sample size, interaction effects were not tested. The illustrations in this article showing the effects were produced using models run with only the relevant factor.

The multivariate response to fixed factors was tested using a multivariate ANOVA (MANOVA). The fixed factor effects of light, water and ecotype were tested for the multivariate effect on the following set of traits: specific leaf area, height of first branch, diameter of first branch, slenderness, stem mass ratio, root mass ratio, leaf mass ratio and average internode length (see Table 2 for definitions and abbreviations of traits). These traits were selected because they are associated responses seen in the SAS (Tao et al., 2008). Because it was significant ($\alpha = 0.05$), the MANOVA was followed by a linear discriminant analysis. This was used to test the ability to predict the light treatment assigned to a seedling from a known set of trait responses. Traits used in this model were those listed above, except mean internode length, which was non-significant in other tests. In order to determine the effect of light quality on the phenotype, two linear discriminant analysis models were fitted, one including the 25Cloth light level and one not including it. The increased accuracy of the model fitted without 25Cloth was used as an approximation of the importance of light quality in affecting the phenotype of *koa* at low light intensity. All linear discriminant

TABLE 2. Description of traits measured, with abbreviations

Abbreviation	Trait name	Unit	Description
Slenderness	Slenderness	cm mm ⁻¹	Height divided by basal diameter
LAR	Leaf area ratio	cm ² g ⁻¹	Ratio of leaf area to total biomass
SMR	Stem mass ratio	g g ⁻¹	Ratio of stem mass to total biomass
RMR	Root mass ratio	g g ⁻¹	Ratio of root mass to total biomass
LMR	Leaf mass ratio	g g ⁻¹	Ratio of leaf mass to total biomass
TINT	Total internode average	mm	Height divided by total number of nodes
INTT	Internode average to transition	mm	Height at node with phyllode divided by nodes below
TNB	Total number of branches	count	Count of total number of branches
HFB	Height to first branch	cm	Height of first branch
DFB	Diameter of first branch	mm	Diameter of first branch
DLB	Diameter of largest branch	mm	Diameter of largest branch
SLA	Specific leaf area	cm ² g ⁻¹	Ratio of leaf area to leaf mass
HG_day	Height growth per day	cm d ⁻¹	Difference in baseline height and final height divided by number of days

analyses employed cross-validation in order to maintain independence between fitted and predicted classes.

To further elucidate light effects on the resulting phenotype, a series of linear mixed effects models were fitted (using the lme4 package in R) for each trait (Table 2) in the MANOVA analysis. For these models, block was a random effect and all fixed effects were included in the models.

RESULTS

Transition rate

One hundred and seventy-one days post-germination, 95 % of Full plants, 71 % of 70Film plants, 29 % of 25Film plants and 25 % of 25Cloth plants had transitioned to phyllodes. Of those in WW and WS water treatments, 55 and 54 %, respectively, had transitioned to phyllodes. For the Honomolino and Umikoa populations, 66 and 44 %, respectively, had transitioned to phyllodes.

When significant for a given timescale, the WS and Honomolino treatments transitioned more quickly (Table 3). Regarding the

rate of transition over time, decreasing light intensity slowed the rate of transition (Table 3, Fig. 1), but the 25Film and 25Cloth treatments did not differ. Over time, the Honomolino population transitioned more quickly (Table 3, Fig. 2), but water availability did not affect transition rates over time (Table 3, Fig. 3).

Light and population did not affect the timing of transition when analysed from the perspective of total biomass as a time-scale; across light treatments, plants transitioned at a similar size (Table 3). Reduced water availability did result in smaller trees at the time of transition (Table 3, Fig. 4). Although no factors were significant when analysed as a function of below-ground biomass (Table 3), for the above-ground biomass (AGB), light, water availability and population affected the timing of transition in ways congruent with using the number of days since germination as the timescale. Similar to the differences seen across time for the light treatments, decreasing light intensity slowed the rate of transition for AGB, but there was no longer a difference between the 70Film treatment and the others.

This pattern and ranking were also observed for total nodes produced before transitioning, in which light (Table 3, Fig. 5),

TABLE 3. Cox proportional hazards models of survival curves for transition timing across time and ontogenetic timescales

Time scale	Factor	χ^2	d.f.	Pr(> χ)	Level	Tukey group
Days since germination	Light	64.02	3	<0.001	Full 70Film 25Film 25Cloth	a b c c
	Water	24	1	0.6235	Well-watered Water-stressed	a a
	Population	14.7	1	<0.001	Honomolino Umikoa	a b
Total biomass	Light	4.24	3	0.2363	Full 70Film 25Film 25Cloth	a a a a
	Water	8.55	1	0.0035	Well-watered Water-stressed	a b
	Population	3.27	1	0.0704	Honomolino Umikoa	a a
Above-ground biomass	Light	17.76	3	<0.001	Full 70Film 25Film 25Cloth	a ab b b
	Water	6.02	1	0.0141	Well-watered Water-stressed	a b
	Population	7.62	1	0.0058	Honomolino Umikoa	a b
Below-ground biomass	Light	4.46	3	0.216	Full 70Film 25Film 25Cloth	a a a a
	Water	1.82	1	0.177	Well-watered Water-stressed	a a
	Population	0.1	1	0.7566	Honomolino Umikoa	a a
Total nodes	Light	42.99	3	<0.001	Full 70Film 25Film 25Cloth	a b c bc
	Water	4.48	1	0.0343	Well-watered Water-stressed	a b
	Population	10.18	1	0.0014	Honomolino Umikoa	a b

Fixed effects and Tukey's HSD *post hoc* significance ($\alpha = 0.05$) are presented with letter groups.

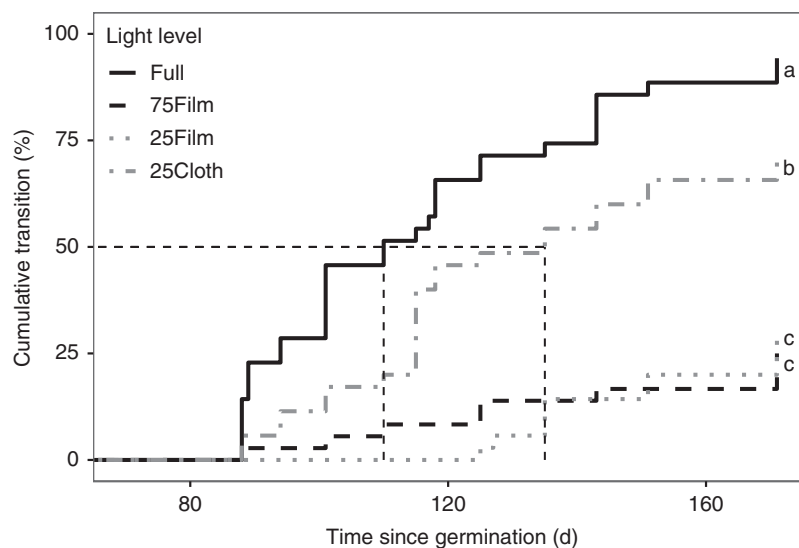


FIG. 1. Modelled cumulative event curves from Cox proportional hazards model for light over time. Letters on the right indicate Tukey's HSD groups ($\alpha = 0.05$). Vertical dotted lines indicate when a treatment reached a median transition level.

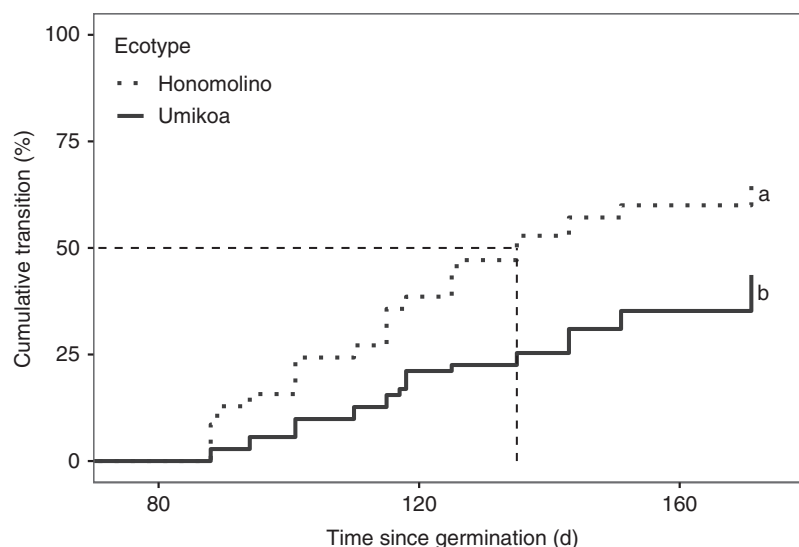


FIG. 2. Modelled cumulative event curves from Cox proportional hazards model for ecotype over time. Letters on right indicate Tukey's HSD groups ($\alpha = 0.05$). Vertical dotted lines indicate when a treatment reached a median transition level.

water availability and population affected the timing of transition. In contrast to the time and AGB timescales, the *post hoc* Tukey's HSD test indicated that the total nodes timescale differentiated slightly between the lowest light intensity treatments, where the 25Cloth treatment was not different from the 70Film treatment ($P = 0.1716$), but the 25Film treatment was different from the 70Film treatment ($P = 0.0208$).

Phenotype

The MANOVA analysis (Table 4A) indicated that light affected the multivariate phenotype at transition, whereas water and population did not. The linear discriminant analysis including 25Cloth in the model was able to correctly predict the light class with an accuracy of 58 %. When 25Cloth was

removed from the model, the accuracy of the model improved by 14 %.

Individual linear mixed effects models showed significant trait responses to light at the time of transition (Table 4B). Light affected trait responses for slenderness, LAR, SMR, RMR, LMR, TNB, HFB, DFB and SLA, but did not affect DLB, TINT, INTT or HG_{day}. Periodic water stress reduced the diameter of the largest branch by 31.5 % (4.60 ± 0.29 mm in the saturated treatment and 3.15 ± 0.19 mm in the low water treatment) and HG_{day} by 13.3 % (1.05 ± 0.04 cm day⁻¹ in the saturated treatment and 0.91 ± 0.02 cm day⁻¹ in the low water treatment). SMR was also different by population; the Honomolono population had a lower ratio (0.46 ± 0.03) than the Umikoa population (0.54 ± 0.04). This trend was the inverse for RMR in the Honomolono and Umikoa populations; the Honomolono population had a higher (0.63 ± 0.09) RMR

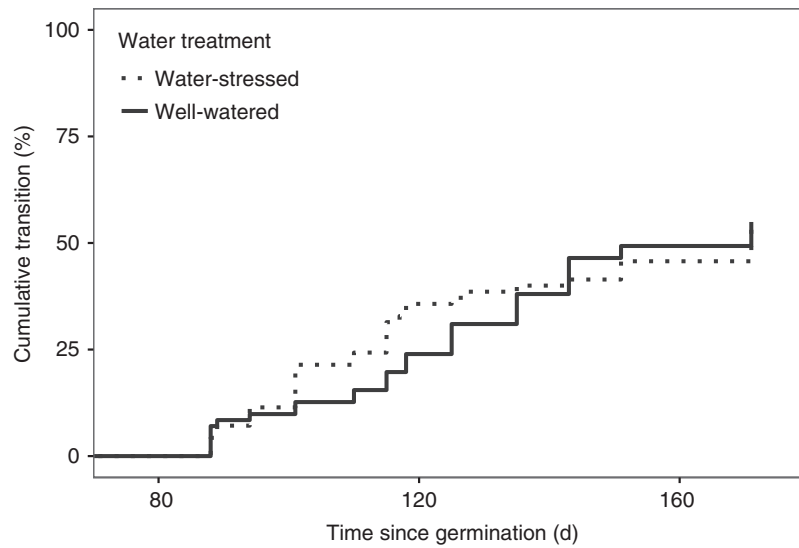


FIG. 3. Modelled cumulative event curves from Cox proportional hazards model for water over time.

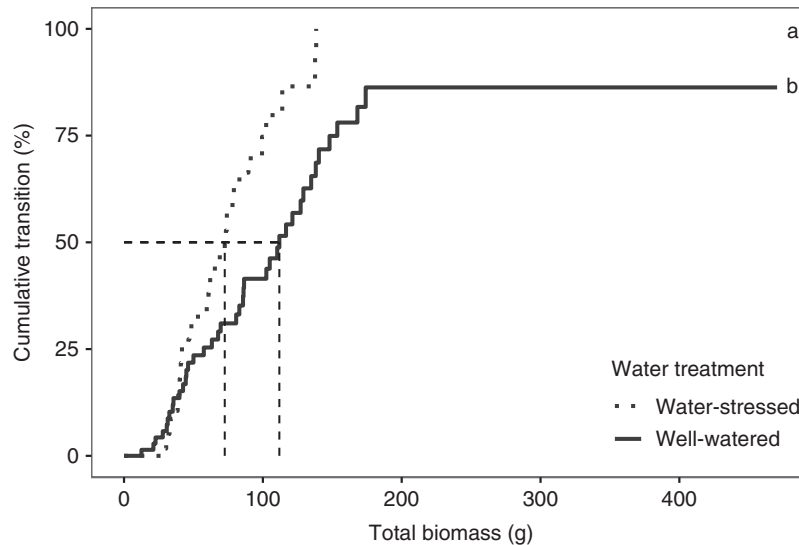


FIG. 4. Modelled cumulative event curves from Cox proportional hazards model for water over total biomass. Letters on right indicate Tukey's HSD groups ($\alpha = 0.05$). Vertical dotted lines indicate when a treatment reached a median transition level.

than the Umikoa population (0.44 ± 0.06). Populations also differed for HFB, the Honomolino population having lower branches than the Umikoa population (56.3 ± 5.2 and 66.8 ± 5.1 cm, respectively). None of the other traits was significant for either water or population, although population was only slightly non-significant for INTT and water was only slightly non-significant for HFB.

DISCUSSION

Heteroblastic response

The timing of transition from true leaves to phyllodes in koa and other *Acacia* species has typically focused on time and light availability and concluded that decreased irradiance decreased

transition rates (Walters and Bartholomew, 1984, 1990; Forster and Bonser, 2009a, b). Our study agrees with these findings, but our finding that reduced water availability increased transition rates ontogenetically differs from the only other study that incorporated water availability in a study of heteroblasty in *Acacia* (Forster et al., 2016). Walters and Bartholomew (1990) found that reduced irradiance could stimulate the production of true leaves on koa that had been producing phyllodes, but it was heterophylly, rather than heteroblasty, that was studied. Forster et al. (2016) investigated the effect of water availability on heteroblasty in *A. implexa* and concluded that reduced water availability led to more biomass allocation to roots and reduced slenderness, but did not result in a faster transition to phyllodes.

As in other studies with *A. implexa* (Forster and Bonser, 2009a, b), low light delayed transition at nodal and biomass scales. Transition rates across both chronological and

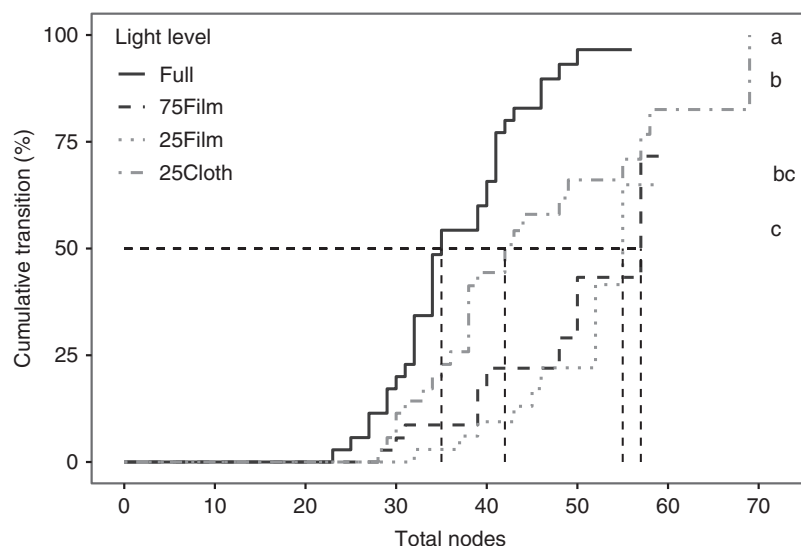


FIG. 5. Modelled cumulative event curves from Cox proportional hazards model for light over total nodes produced. Letters on right indicate Tukey's HSD groups ($\alpha = 0.05$). Vertical dotted lines indicate when a treatment reached a median transition level.

TABLE 4. Results of MANOVA for multivariate phenotype response to fixed factors and ANOVA results for individual mixed effects linear regressions of traits.

(A) MANOVA	d.f.	Pillai's trace	F value	num d.f.	den d.f.	P value
Light	3	0.699	3.52	15	174	<0.001
Water	1	0.161	2.15	5	56	0.073
Ecotype	1	0.159	2.12	5	56	0.076

(B) ANOVA	Light			Water			Ecotype		
Trait	F value	d.f.	P value	F value	d.f.	P value	F value	d.f.	P value
Total biomass	3.9	(3, 70)	0.012	7.4	(1, 70)	0.008	1.0	(1, 70)	0.318
Slenderness	14.2	(3, 69.5)	<0.001	0.2	(1, 69.4)	0.671	0.7	(1, 69.1)	0.4
LAR	11.4	(3, 61.2)	<0.001	3.3	(1, 61.3)	0.074	0.2	(1, 61.1)	0.687
SMR	11.1	(3, 68)	<0.001	5.9	(1, 68)	0.018	7.3	(1, 68)	0.009
RMR	8.3	(3, 66.5)	<0.001	0.3	(1, 66.3)	0.574	7.5	(1, 66.2)	0.008
LMR	5.4	(3, 68)	0.002	0.3	(1, 68)	0.573	0.6	(1, 68)	0.434
TINT	0.4	(3, 69.3)	0.758	0.9	(1, 69)	0.357	1.5	(1, 69.3)	0.222
INTT	0.2	(3, 43.2)	0.88	1	(1, 43.2)	0.324	3.33	(1, 43.5)	0.075
TNB	3.2	(3, 70)	0.028	2	(1, 70)	0.162	0.8	(1, 70)	0.368
HFB	12.4	(3, 68.7)	<0.001	3.5	(1, 68.8)	0.065	5.5	(1, 68)	0.022
DFB	5.8	(3, 70)	0.0498	7	(1, 70)	0.01	0.18	(1, 70)	0.673
DLB	2.6	(3, 52.7)	0.06	23.3	(1, 53.5)	<0.001	2.1	(1, 52.8)	0.154
SLA	7	(3, 60.2)	<0.001	3.7	(1, 60.1)	0.059	0.5	(1, 60.1)	0.486
HG_day	1.3	(3, 71)	0.277	10.8	(1, 71)	0.002	0.7	(1, 71)	0.419

Significant *P* values are in bold.

Values of d.f. in Table 4B represent approximate values of d.f. from Satterthwaite's method.

The numerator degrees of freedom (num d.f.) and denominator degrees of freedom (den d.f.) correspond to the F-ratio for the Pillai's trace test where num d.f. is degrees of freedom for the model and the den d.f. is the degrees of freedom for the model errors.

ontogenetic timescales increased with increased light intensity, indicating complex developmental plasticity (Wright and McConnaughay, 2002) in response to light, where growth rates and ontogenetic trajectories were both affected (Table 3). Heteroblastic differences were not the result of ontogenetic drift, but of changes in the ontogenetic trajectory; under lower light conditions, plants were taller with less total biomass at the time of transition (Table 4). The response to light contrasts with koa's developmental response to water availability, in which reduced water availability induced a quicker transition along

ontogenetic timescales alone; growth rate increased with water availability and more nodes were produced by the median time of transition, but transition timing as a function of days since germination did not differ between watering treatments.

Although a transition to phyllodes earlier in ontogeny could be advantageous in water-stressed conditions (Pasquet-Kok et al., 2010), especially under high-light conditions (Craven et al., 2010), no previous studies have found a modification in the ontogenetic trajectory in koa or other *Acacia* species in response to decreased water availability. Recent work by Forster

et al. (2016) has shown decreased growth rates in *A. implexa* in water-stressed seedlings relative to non-stressed seedlings, but this did not affect the ontogenetic timing of heteroblasty. Our work, in contrast, showed no change in the ontogenetic trajectory in response to water availability over time since germination, but did show changes over total biomass, above-ground biomass and nodal accumulation timescales. In contrast to the strong heteroblastic canalization demonstrated for *A. implexa* in response to water availability, koa exhibits plasticity in the ontogenetic programme, in which plants are smaller at the time of transition in water-stressed environments. Moreover, our water-stress treatment was probably less extreme than that of Forster and Bonser (2009a) and lower than that found in koa's native range (Baker *et al.* 2009) or the treatment used in Craven *et al.* (2010), suggesting that the direction of the response detected in our study would remain consistent with our findings when tested in the field.

Plasticity of the ontogenetic trajectory in koa in response to water availability, relative to *A. implexa*, may be more relevant or detectable because of the length of the transition period in koa. Whereas in Forster *et al.* (2016) transition to phyllodes in *A. implexa* occurred after nine nodes, phyllodes developed on WS koa at 42 nodes (median) and on WW koa at 49 nodes (median). This effect of a seven-node delay in transition in response to water availability means a window exists during establishment that could be selective in favour of increased plasticity in ontogenetic trajectories (Pigliucci, 1997). The finding of changes in the ontogenetic trajectory in response to water availability represents an potential mechanistic explanation of heteroblasty additional to those described by Chitwood and Sinha (2016).

Consistent with Chitwood and Sinha (2016), in which the processes and stimuli governing heteroblasty and heterophylly were disparate, we did not see differences in the timing of transition between 25Cloth and 25Film. This result contrasts with that of Forster and Bonser (2009a), where low blue light and low red light delayed transition at nodal scales. In our study, the trees in the 25Film treatment had fewer nodes than the Full and 70Film treatments according to nodes produced, but the 25Cloth treatment was not different from the 70Film treatment. This might suggest that, in a larger study space, transition rates could have differed between the 25Cloth and 25Film treatments. Only about 10 % of the trees in each low-light treatment transitioned and it is difficult to extrapolate to median transition rates between these treatments.

Ecotypic responses

The differences in ontogenetic trajectories between contrasting populations in this study support the hypothesis that populations from dry areas would transition to phyllodes faster than populations from wet areas because of the advantage phyllodes have over true leaves in periods of drought stress. In this study, the wet site population transitioned more slowly over time, with a 69.5 % larger above-ground biomass and 34.1 % increase in nodes produced at transition. This pattern has been shown for *A. melanoxylon*, in which populations from areas with lower rainfall experienced a faster transition to phyllodes compared with those from more mesic areas (Farrell and

Ashton, 1978). True leaves represent a lower carbon investment per unit of carbon assimilation, allowing faster growth rates at a lower carbon cost but at the cost of reduced tolerance to and recovery from drought (Pasquet-Kok *et al.*, 2010). Our finding that the dry-site population transitioned faster and allocated more resources to root biomass, regardless of resource availability, fits well with the hypothesis of a growth–tolerance trade-off (Grime, 2006).

Because genetic structure in koa is strongly associated with annual rainfall, projected changes in rainfall distribution are expected to produce genetic offsets that could destabilize populations, especially in transitional rainfall zones (Gugger *et al.*, 2018). Changes to the climate across koa's range on Hawaii Island are not forecast to be uniform (Elison Timm, 2017). Traits associated with tolerance of wet-site and dry-site pressures, whether biotic or abiotic, during regeneration and recruitment could therefore be maladapted to the new environment. Heteroblastic timing, and the plasticity of that timing, represent one trait that could aid in continued regeneration and recruitment.

Phenotypic plasticity

Phenotypic responses of koa at transition to irradiance and light quality in our study suggest that light drives both processes in a complex way. Others have observed complex heteroblastic responses to light availability. Ostria-Gallardo *et al.* (2015a) noted that greater light intensity increased the complexity of compound leaves in *Gevuina avellana* but found that ontogeny had a larger effect on heteroblastic development. Ostria-Gallardo *et al.* (2015b) reported high quantities of protein transcripts related to R:FR response and shade avoidance in shorter-transitioned trees, but light quality diminished in importance as a trigger as the tree grew in height into forest gaps. We observed delays in transition rates over chronological and ontogenetic timescales. Koa were taller with a smaller biomass at transition in the lower light conditions, but the average height growth per day until transition did not vary among light treatments (Table 4). This suggests that the increased height at transition for the lower light levels was a plastic response to light availability and adaptation to gap-phased regeneration.

We did observe a partial reversion of the phenotype in response to changes in R:FR at low irradiance (e.g. 25Cloth phenotype reverting towards trait values of Full, which shared the same R:FR despite different irradiances), despite not observing changes in the rate of transition. Although we did not find significant differences in heteroblastic rates in response to R:FR, our results are in line with the hypothesis that heteroblasty is an irradiance-mediated process rather than a light quality-mediated process (Chitwood and Sinha, 2016). Nevertheless, a similar trial with *A. implexa* (Forster *et al.*, 2009a) found a delay in transition from the sixth to the eighth node in response to reduced R:FR. As in our study, they observed phenotypic changes as well at transition in response to changes in R:FR. Reduced R:FR increased slenderness, internode length, SMR, LAR and SLA and reduced RMR and the net assimilation rate of transitioned seedlings. In low-light, unreduced R:FR conditions, we observed a partial reversion of the phenotype for slenderness, LAR, SLA, LMR and DFB.

We did not find significant responses at transition to water availability for slenderness, LAR, RMR, LMR or SLA, all of which have been shown to respond to water stress in another *Acacia* species (Forster *et al.*, 2016). Our findings agree with those of Craven *et al.* (2010), in which growth and biomass traits (total, not ratios as in our study) of koa were responsive to light availability but not to water availability. Our results also contrast with Forster *et al.* (2016) in *A. implexa*, however, in which significant trait responses to reduced water availability were found for slenderness, internode length, RMR, LMR, LAR and SLA. This suggests that the studied phenotypes of koa are less affected by water availability compared with other *Acacia* species, despite the changes found in the ontogenetic trajectory in response to water availability.

Conclusions

Survival functions were used for the first time to help elucidate the roles of abiotic stressors in modifying ontogenetic trajectories. Our results demonstrate the importance of utilizing species with elongated transition times for the purpose of studying abiotic modifiers of ontogenetic trajectory. Our results elucidate the importance of water availability in regulating the timing of transition in heteroblastic *A. koa*. Koa's wide climatic range, and our finding that ontogenetic trajectories and resource allocation for contrasting ecotypes conform to the growth–tolerance trade-off hypothesis (Grime, 2006) suggests the need for field research to assess: (1) the durability of ecotype-related differences; (2) the adaptive significance of plasticity in ontogenetic trajectory; (3) differences in plasticity between ecotypes; and (4) the importance of plasticity for resilience of populations to changes in climate.

FUNDING

This work was supported by the van Eck Forest Foundation at Purdue University.

ACKNOWLEDGEMENTS

We thank the many people that helped in the greenhouse and laboratory, including Carmen Rose, Amy Miller, Safi Khurram, Jon Moore, Micah Stevens, Andrei Toca, Mercedes Uscola Fernandez and Silvia Medina Villar. We thank Mike Gosney. We thank Ben Kellner and Nathan Lichti for statistical guidance. We thank James B. Friday and Nick Dudley in Hawaii for supplying seeds and discussions regarding the relevance of the research.

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