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GENETIC DIFFERENTIATION OF INTRINSIC WATER-USE EFFICIENCY IN THE HAWAIIAN NATIVE *ACACIA KOA*

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We examined genetic differentiation of intrinsic water-use efficiency (WUE) and the relationship between WUE and phyllode morphological and nutritional traits in the native Hawaiian tree koa (*Acacia koa* Gray, Fabaceae). We obtained both integrated (stable isotope ^{13}C composition of phyllode tissues; $\delta^{13}C$) and instantaneous (assimilation/stomatal conductance to water vapor; A/g) estimates of WUE on koa accessions grown in a progeny trial at Maunawili on the island of O'ahu. The accessions were from sites along an elevation (900–1300 m) and rainfall (ca. 2200–1300 mm) gradient on the southwestern slopes of Mauna Loa on the island of Hawai'i. The $\delta^{13}C$ values of koa phyllodes at Maunawili ranged between −28‰ and −31‰ and were positively related to elevation of the seed source. Among seed sources grown at the common site, phyllode $\delta^{13}C$ varied with similar magnitude and direction to that associated with the field populations, indicating genetic differentiation of WUE in koa. Instantaneous measurements of WUE showed similar trends of increasing WUE with elevation of the seed source. Phyllode $\delta^{13}C$ was not correlated with foliar concentrations of nitrogen (N), indicating that differences in $\delta^{13}C$ were not caused by nutrient-related changes in photosynthetic capacity but rather by stomatal limitation. Phyllode morphological traits such as specific leaf mass (SLM), length, and area were unrelated to $\delta^{13}C$. The similarity between the slopes of SLM and phyllode N content at Maunawili and Honaunau supported the contention that there is global interdependence between these two traits. The finding of genetic differentiation of WUE in koa is important for native forest restoration and seed selection.

Keywords: water limitation, $\delta^{13}C$, gas exchange, Hawaiian forests.

Introduction

The ratio between carbon gained through photosynthesis and water lost (i.e., water-use efficiency, WUE) is often positively related to drought tolerance and is a major determinant of plant fitness in a given environment (Ehleringer and Cooper 1988; Dudley 1996*b*). Intrinsic WUE expresses the ratio between CO_2 assimilation (A) and stomatal conductance to water vapor (g) and can be estimated either by instantaneous measurements of gas exchange or by time-integrated measures. Among the latter, discrimination against the stable isotope ^{13}C naturally occurs during the photosynthetic process and is functionally related to the ratio of CO_2 partial pressure in intercellular spaces (pi) to that in the ambient (pa). The pi : pa ratio in turn reflects average intrinsic WUE at the leaf level (Farquhar et al. 1982). This implies that disproportionate changes in A and g modify the pi : pa ratio and, therefore, both WUE and the carbon isotope composition of plant tissues ($\delta^{13}C$).

Previous research has shown that WUE can relate to both the environment (Garten and Taylor 1992) and the genetic makeup of the species (Zhang et al. 1993; Lauteri et al. 1997) as well as to leaf phenological characteristics. Genotypes with

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high WUE would have an adaptive advantage in situations in which water availability is a limitation for plant growth. In this article, we examined possible genetic differentiation of WUE in *Acacia koa* (koa), a nitrogen (N)-fixer tree native to Hawai'i, and the relationship between WUE and phenological traits of koa phyllodes (i.e., modified petioles functioning as leaves). Toward this end, we measured WUE and morphological traits on several accessions of koa from different altitudes; the accessions were growing in a common garden. We asked the following questions: (1) Does WUE differ between altitudes of the seed source, so that higher WUE is found in koa accessions from drier sites? (2) Does WUE correlate to morphological traits?

Water stress is a major limitation to productivity of koa, one of the two dominant tree species in the Hawaiian forest and the most economically valuable. Accordingly, the stand basal area of mature koa stands decreased along rainfall gradients in the islands of Hawai'i (Ares and Fownes 1999) and Kaua'i (Harrington et al. 1995). On the island of Hawai'i, aboveground net primary productivity of koa decreased with rainfall and was not related to foliar nutrients. The $\delta^{13}C$ values were positively correlated to instantaneous measures of WUE (A/g) of koa seedlings in the greenhouse, and these values increased with decreased water supplies both in the field and in the greenhouse, thereby implying stomatal limitation for koa (Ares and Fownes 1999). On the island of Kaua'i, $\delta^{13}C$

of koa did not obviously change with rainfall but rather was related to stand characteristics such as leaf area index, aboveground woody biomass increment, and production per unit leaf area (Harrington et al. 1995). Because koa phyllodes had higher WUE than did juvenile leaves, it has been postulated that koa phyllodes are adaptations to drought (Hansen and Steig 1993).

Limited soil water (Jaindl et al. 1995) and increasing atmospheric vapor pressure deficit (Sharifi and Rundel 1993) depressed carbon isotope discrimination and therefore increased $\delta^{13}C$. In addition, $\delta^{13}C$ often increased with elevation (Körner et al. 1988; Sparks and Ehleringer 1997), probably as a result of reduced atmospheric CO_2 and O_2 partial pressures (Körner et al. 1991). Nevertheless, water availability seemed to be a more important factor in controlling $\delta^{13}C$ than was elevation (Friend et al. 1989; Meinzer et al. 1992; Jaindl et al. 1995).

Genotypic variation in $\delta^{13}C$ has been indicated both for agricultural crops (Johnson et al. 1995) and for forest species such as *Picea glauca* (Sun et al. 1996*b*). In general, no interactions between genotype and environmental effects on $\delta^{13}C$ were observed (Zhang and Marshall 1994; Sun et al. 1996*b*), indicating constancy of this trait across environments. Responses to selection for WUE appeared to be difficult to predict because heritabilities for isotope discrimination have been found to vary widely (Schuster et al. 1992; Donovan and Ehleringer 1994; Ehdaie and Waines 1994).

Given the fact that water supply limits koa growth in its native range, it is possible that natural selection has favored greater WUE to maximize carbon gains per unit of water loss. Genetic differentiation for a variety of plant traits (including physiological ones) has been noticed even across very small distances (Linhart and Grant 1996). Previous research has shown wide genetic variation in koa morphology (St. John 1979; Daehler et al. 1999), but genetic differentiation in terms of koa physiological traits is unknown. In this study, therefore, we hypothesized that there is genetic differentiation of WUE in koa, implying that there are both genetic variations for WUE and differences in natural selection among elevations that vary in their water regimes.

Carbon isotope discrimination has been found to relate to leaf phenotypical characteristics such as mass or thickness (expressed either as specific leaf mass, SLM, or as specific leaf area, SLA [i.e., the inverse of SLM]; Nageswara Rao and Wright 1994; Sparks and Ehleringer 1997) as well as leaf size (Meinzer et al. 1992; Kohorn et al. 1994). In peanut, SLA was proposed as a surrogate for isotope discrimination because a positive relation between SLA and discrimination was maintained over lòcations and watering regimes (Nageswara Rao and Wright 1994). It has been hypothesized that the relationship between leaf thickness and isotope discrimination arises because of increased diffusional pathway length for CO_2 from the atmosphere to the substomatal cavities (Vitousek et al. 1990). Nonetheless, the relationship between SLM or SLA and $\delta^{13}C$ does not always hold (Kohorn et al. 1994; Zhang and Cregg 1996), and leaf gas-exchange characteristics can vary independently of SLM (Meinzer et al. 1992). For koa, we hypothesized that genotypic changes in $\delta^{13}C$ run in parallel with changes in SLM and that SLM can be used as a surrogate for $\delta^{13}C$ and intrinsic WUE. Also, SLM could be used to predict

phyllode N content, as these traits would be negatively correlated as predicted by the theory of interdependence of functionally important traits in plants (Reich et al. 1997). Finally, we predicted that leaf size, a trait that can be adaptive in dry environments (Dudley 1996*b*), correlated negatively with elevation of the seed source and $\delta^{13}C$ because the higher elevation sites in which koa seeds were collected corresponded to drier sites along a gradient.

Material and Methods

We sampled foliage and performed gas-exchange measurements on koa accessions from the Honaunau Forest in the island of Hawai'i; the accessions were growing in a progeny trial on the island of O'ahu. The Honaunau Forest extends from ca. 600 m to 1500 m elevation on the southwestern slopes of Mauna Loa; mean annual rainfall ranges from 2500 mm at low elevation to 1200 mm at the top of the forest (Kamehameha Schools Bishop Estate, unpublished records for the period 1958–1971). The driest period lasts from October to December, and zero water storage during some months likely occurs in high-elevation, stony soils (Ares and Fownes 1999). Mean annual temperature along the gradient varies between 19° and 14°C. The koa seeds were collected along five elevational bands from ca. 900 m to 1300 m in elevation (zone 5 = 900–1000 m, zone 4 ≥ 1000–1100 m, zone 3 ≥ 1100–1200 m, zone 2 ≥ 1200–1250 m, zone 1 ≥ 1250–1300 m), but the precise elevation for every individual was not recorded. Along the 900–1300-m gradient, annual rainfall ranged between ca. 2200 mm and 1300 mm.

The progeny trial was set up on June 1996 at the Maunawili Station of the Hawai'i Agriculture Research Center (105 m above sea level). Mean annual rainfall at Maunawili is 2125 mm, and mean annual temperature is 25°C. The soil under the trial was originally classified as fine, mixed, isohyperthermic Typic Palehumult within the Kaneohe series (Dudley 1996*a*). However, newly available soil descriptions for the site indicated the presence of soils from volcanic ash and cinders without argillic horizon and with a net positive charge in the subsoil. Therefore, the soil under the koa trial is probably a clayey, sesquic, isohyperthermic Humic Xanthic Acrudox lying on top of the Kaneohe series (Gavenda et al. 1996).

Koa seedlings were raised in a greenhouse on the island of O'ahu, and 3.5-mo-old individuals were planted in the field (Sun et al. 1996*a*). Both trials were set up following an augmented randomized complete block design, with 10 plants per plot and two replicates. Spacing was 1 m × 1.5 m.

Sampling of koa foliage and physiological measurements were performed in late 1997 and early 1998. During 1997, rainfall at Maunawili amounted to 2072 mm, a figure close to the historical average, with showers occurring almost daily (fig. 1*A*). Evapotranspiration was 892 mm, and monthly records exceeded rainfall only during February (92 mm vs. 59 mm). Daily air temperature amplitudes were low, as is typical in many tropical and subtropical areas (fig. 1*B*). In 1997, mean annual temperature was 24°C, mean total solar radiation was 8.3 MJ m^{-2} d^{-1}, mean annual relative humidity was 73%, and mean daily maximum wind speed was 1 m s^{-1}.

We sampled koa foliage of 18 accessions on December 23, 1997, at Maunawili. At the time of the measurements, the
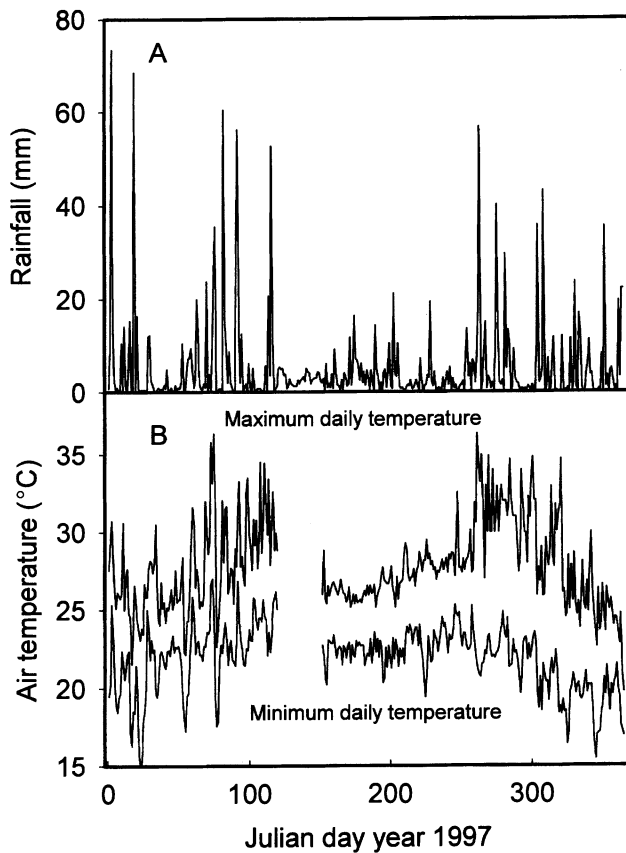
Fig. 1 Rainfall (A) and air temperature (B) during 1997 at Maunawili, island of Oʻahu.

trees were 18 mo old and 1–3 m tall. Phyllode samples were taken from the upper crown of at least three trees for each koa accession and pooled to create a composite sample. Phyllode total length was measured with a ruler, and phyllode areas were recorded with a LI-3000 leaf area meter (LI-COR, Lin-

coln, Nebr.). Phyllode samples were then dried at 70°C, weighted for calculation of SLM (SLM : g m^{-2}), and ground to a fine powder with a mortar and pestle. Determinations of $\delta^{13}C$ and leaf N content were conducted at the Duke University Phytotron Facility in Durham, North Carolina. Data on SLW, $\delta^{13}C$, and leaf N content were compared to previous measurements in koa populations growing at the Honaunau Forest (measurements were conducted following similar sampling and analytical procedures).

Gas-exchange measurements were carried out on February 5 and 6 and on April 24, 1998. Records were taken on 13 accessions between 0900 and 1300 hours on 5-cm^2 portions of fully expanded phyllodes that were positioned as close as possible to their natural orientation; records were taken with a CIRAS-1 portable open photosynthesis system (PP Systems, Hitchin Herts, United Kingdom). For each accession, three leaves on each of two trees were measured, and averages were obtained. Measurements were taken under photosynthetic photon flux densities in excess of 600 μmol m^{-2} s^{-1}. A boundary layer conductance of 5000 mmol m^{-2} s^{-1} was used in all measurements. Instantaneous photosynthetic N use efficiency (PNUE) was determined by dividing average CO$_2$ assimilation per average leaf N content per unit leaf area.

Linear regression was used to relate $\delta^{13}C$ and instantaneous WUE to elevation of the seed source and was used as well to relate SLM to phyllode N. General linear contrasts were examined to compare the effects of the different elevations of the seed source on WUE, with the significance of the preplanned contrasts determined by the Student's t-test. Simple (Pearson) correlation analysis was used to relate $\delta^{13}C$ and elevation of the seed source to phyllode morphological and N traits.

Results

Phyllode $\delta^{13}C$ and N Content

The $\delta^{13}C$ values of the Honaunau accessions growing at Maunawili ranged between −28‰ and −31‰, with a mean value of −29.8‰ (table 1). Elevation of the koa seed source

Table 1

Phyllode $\delta^{13}C$, N Content, and Morphological and Physiological Traits of Koa Accessions at Maunawili and in Their Natural Range at Honaunau

	Maunawili (105 m a.s.l.)	Honaunau
Elevational range of koa accessions	900–1300	800–1500
Number of accessions	18	8
$\delta^{13}C$ (‰)	−29.8 ± 0.22	−29.1 ± 0.25
Phyllode N (%)	2.49 ± 0.09	2.80 ± 0.10
SLM (g m^{-2})	121.4 ± 5.4	120.4 ± 6.5
Phyllode length (cm)	15.8 ± 0.39	...
Phyllode area (cm^2)	31.5 ± 7.76	...
A (μmol m^{-2} s^{-1})	13.7 ± 3.96	...
g (mmol m^{-2} s^{-1})	0.20 ± 0.07	...
A/g (μmol m^{-2} s^{-1}/mmol m^{-2} s^{-1})	75.2 ± 29.0	...
PNUE (μmol s^{-1} mol^{-1})	0.066 ± 0.023	...

Note. Values are means ± standard error. CO$_2$ assimilation (A), stomatal conductance to water vapor (g), intrinsic water-use efficiency (A/g), and photosynthetic nitrogen use efficiency (PNUE) were determined on 13 accessions. m a.s.l. = meters above sea level; N = nitrogen; SLM = specific leaf mass.
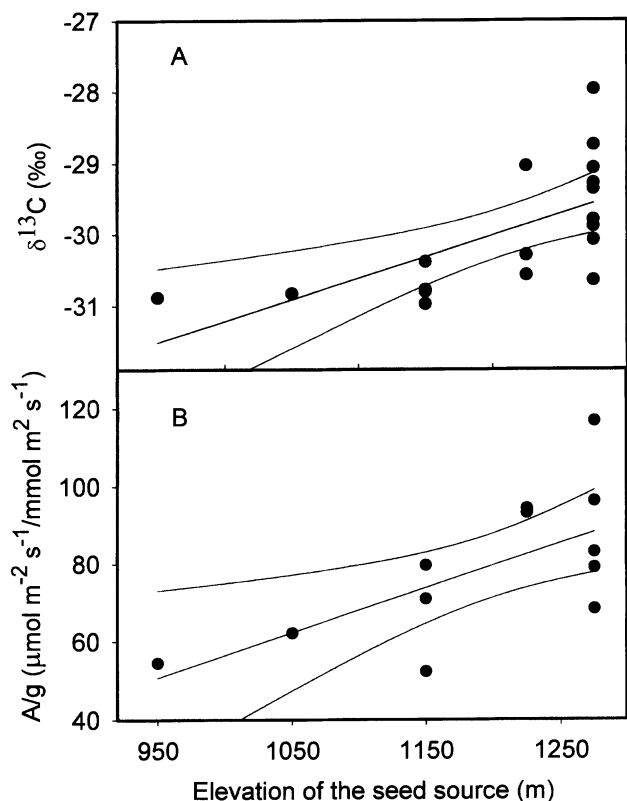
Fig. 2 Elevation of the seed source of koa accessions versus (A) phyllode carbon isotope composition (δ^{13}C; $r^2 = 0.40$, $P = 0.005$) and (B) instantaneous water-use efficiency (A/g; $r^2 = 0.43$, $P = 0.015$) at Maunawili, island of O'ahu. Elevations were the midpoints of five elevation bands in which the seeds were collected at the Honaunau Forest, island of Hawai'i. Similar results were obtained when δ^{13}C and A/g were regressed against elevation expressed as discrete categories from 5 (low elevation) to 1 (high elevation; $r^2 = 0.45$, $P = 0.002$ for δ^{13}C, and $r^2 = 0.44$, $P = 0.013$ for A/g). Ninety-five percent confidence intervals of means are shown.

was positively correlated to δ^{13}C at Maunawili (fig. 2A), indicating genetic differentiation of WUE. There was still a significant effect ($P = 0.03$) if only those elevations above 1150 m were tested. Preplanned contrasts revealed significant differences in δ^{13}C between the 1150 m and 1225 m elevations ($P = 0.04$, Student's t-test) and between the 1150 m and 1275 m elevations ($P = 0.002$), whereas the contrast between 1225 m and 1275 m elevations was not significant ($P = 0.12$). In agreement with our previous findings, phyllode N content, both on a weight and a leaf area basis, did not correlate with δ^{13}C (table 2).

Gas Exchange and Phyllode Morphology

Gas exchange data showed that instantaneous estimates of WUE (A/g) correlated with elevation of the seed source (fig. 2B). This implies that A and g did not vary in parallel along the tested elevation range. When only elevations above 1150

m were tested, the effect of elevation of the seed source on instantaneous WUE was not significant ($P = 0.12$). Photosynthetic N use efficiency was not correlated with δ^{13}C (table 2), indicating that a trade-off between WUE and N use efficiency did not occur.

Phyllode length and area and SLM correlated with neither elevation of the seed source nor with δ^{13}C (table 2). There was a trend toward increasing phyllode area with elevation of the seed source; this trend was in opposition to what may have been expected for sites with less rainfall, such as those at high elevation at Honaunau.

There was a negative correlation between SLM and phyllode N concentration; this negative correlation also agreed with the trend found for the local population at Honaunau (fig. 3A, 3B). Accordingly, the slopes of the SLM versus phyllode N relationship among Honaunau and Maunawili did not differ ($P = 0.05$).

Discussion

Research in common-garden experiments has shown either the presence (Zhang et al. 1993; Zhang and Mashall 1994) or the absence (Morecroft and Woodward 1990; Read and Farquhar 1991; Cordell et al. 1998) of a relationship between elevation of the seed source and δ^{13}C, a time-integrated index of WUE. In part, these different results can arise because the association of environmental conditions with altitude depends on the local climate. In this study, the results support genetic differentiation of δ^{13}C in koa along an elevation range that corresponds to a gradient of decreasing rainfall. Although seed sources from low elevations were less represented, significant differences in δ^{13}C were still found when only those elevations above 1150 m were compared. The lack of significance for the A/g relationship among the high-elevation sites can be explained by the greater variation usually encountered for instantaneous measures of WUE.

Although physiological variation as a function of the environment of the seed source has long been known for other taxa (Clausen et al. 1940; Monson and Grant 1989), our finding is important to understand fitness mechanisms in koa given the crucial role that water availability plays in koa growth and natural distribution. Also, this knowledge may encourage further research on selection of koa genotypes for WUE and on the links between WUE and koa growth and wood yield across genotypes. The relationship between WUE and fitness could be explored further by comparing WUE of koa genotypes found growing in humid environments, where WUE would not be adaptive.

The range of 3‰ in δ^{13}C observed in the progeny trial was quite noticeable for koa accessions growing in the same environment, specially if we consider that elevation of the seed sources spanned a range of only ca. 400 m. Elevation, however, likely contributes to genetic isolation in the insect-pollinated koa, as koa's flowering time varies with altitude. Genetic isolation of koa, therefore, would be more dependent on asynchronous flowering than on the travel distance limitation of the insects. Greater genetic differentiation of WUE can be expected if accessions from broader gradients are compared in a trial.

The absence of a correlation between phyllode δ^{13}C and N

Table 2

Pearson Correlation Coefficient of $\delta^{13}C$ (‰) and Elevation of the Seed Source with Different Traits of Koa Accessions Growing at Maunawili

	$\delta^{13}C$ (‰)	Elevation of the seed source (m)
N_w (%)	−0.16	...
N_a (g m^{-2})	0.23	...
PNUE (μmol s^{-1} mol^{-1})	−0.18	...
SLM (g m^{-2})	0.29	−0.21
Phyllode length (cm)	0.29	−0.33
Phyllode area (cm^2)	0.39	−0.41

Note. N_w = phyllode nitrogen (N) content on a weight basis; N_a = phyllode N content on an area basis; PNUE = photosynthetic N use efficiency; SLM = specific leaf mass. All correlations were not significant at the 0.05 level of probability.

content, both on a weight and a leaf area basis, agreed with our previous findings determined under field and greenhouse conditions (Ares and Fownes 1999). These results indicated a consistent pattern, thus showing that differences in $\delta^{13}C$ were not caused by nutrient-related changes in photosynthetic capacity but rather by stomatal limitation. Also, the lack of correlation between PNUE and $\delta^{13}C$ pointed out that intrinsic WUE was not traded off against N use efficiency. Gas-exchange data confirmed the genetic differentiation of WUE in koa.

Phyllode morphological traits, such as SLM, length, and area, were not related to elevation of the seed source. This pattern opposed the one in ohʻia (*Metrosideros polymorpha*), the other dominant tree species in Hawaiʻi, in which the positive relationship between SLM and elevation under field conditions was maintained in common garden plants (Cordell et al. 1998). On the other hand, to some extent, koa seems to display greater genetic differentiation of WUE than does ohʻia.

The morphological variation in SLM (i.e., increased SLM with increasing elevation) that we previously found in koa native populations at Honaunau (Ares and Fownes 1999) probably reflected acclimation rather than adaptation. The positive correlation between SLM and $\delta^{13}C$ may have initially indicated that phyllode thickness directly affected carbon discrimination processes. Rather, it was probably the case of two variables that were changing in parallel but that were not linked by a causative relationship. Carbon discrimination patterns in koa may have reflected biochemical causes, such as changes in protein structure, rather than changes in leaf thickness, size, and shape, as noticed in other studies (Kohorn et al. 1994; Sparks and Ehleringer 1997). Although changes in biochemical components seem more difficult to develop than morphological adaptation, variations in proteins among tree genotypes have previously been found to be unrelated to morphological traits (Linhart et al. 1989).

The slope of the relationship between SLM and N showed a striking similarity between the experiment site and the native range of the koa populations. This similarity agrees with the contention of global commonality among some plant traits, a commonality induced by evolutionary convergence (Reich et al. 1997). The lower N content of leaves with higher SLM is probably the consequence of less photosynthetically active tissue and, therefore, less photosynthetic enzymes in thicker

leaves. The SLM values could be used to estimate phyllode N content in koa and subsequently A, but these values are probably of little consequence in terms of estimating WUE, considering the lack of a relationship between WUE and phyllode N.

In summary, we observed a genetic component of intrinsic WUE in a Hawaiian native tree (i.e, koa) for the first time. Therefore, variations in WUE across koa genotypes reflect selective pressures within koa's native range, in which water availability is the main limitation to koa. Our results provided additional evidence that changes in WUE in koa are linked to stomatal limitation rather than to a nutritional control of photosynthesis.

The findings on environmental effects (Ares and Fownes 1999) and genetic differentiation of WUE in koa are significant for native forest restoration because they document that genetic variability among natural populations in a contiguous landscape occurs and is physiologically significant. As a consequence, subpopulations are important candidates for conservation. Furthermore, the selection of germplasm for forest planting becomes crucial either in terms of obtaining seed that is well-matched to the environment or in terms of avoiding genetic pollution of existing populations.
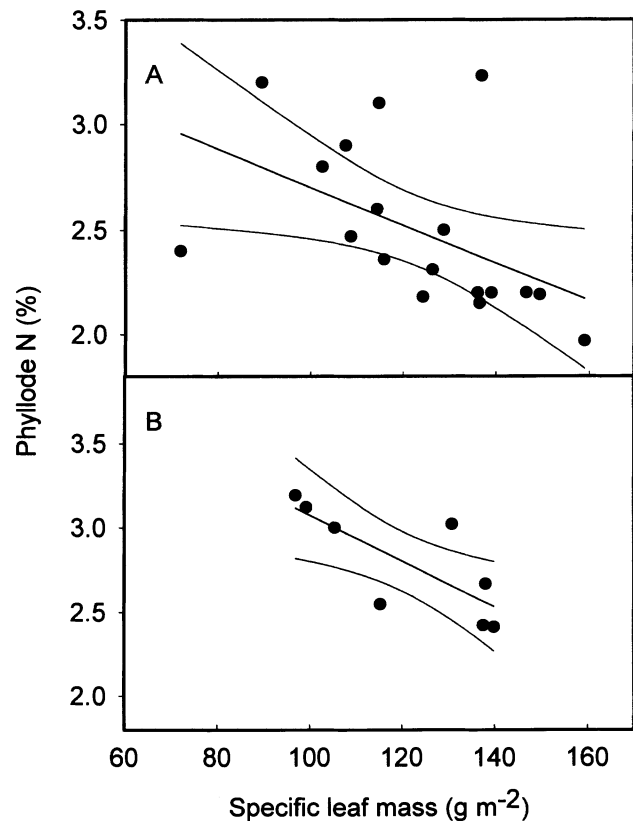

Fig. 3 Specific leaf mass versus phyllode N of koa accessions from Honaunau growing in (A) the common garden at Maunawili (r^2 = 0.26, P = 0.03) and (B) in their natural range at Honaunau (r^2 = 0.60, P = 0.02). Ninety-five percent confidence intervals of means are shown.

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