Functional Ecology 2002
16, 510–521

Hydraulic differentiation of Ponderosa pine populations along a climate gradient is not associated with ecotypic divergence

H. MAHERALI,*†‡ B. L. WILLIAMS,§** K. N. PAIGE,§‡¶ and E. H. DELUCIA*¶

**Department of Plant Biology, §Department of Animal Biology and ¶Program in Ecology and Evolutionary Biology, University of Illinois, Urbana, Illinois 61801, USA, and **Laboratory of Molecular Biology and Howard Hughes Medical Institute, University of Wisconsin, Madison, WI 53706, USA*

Summary

1. *Pinus ponderosa* occurs in a range of contrasting environments in the western USA. Xeric populations typically have lower leaf : sapwood area ratio (A_L/A_S) and higher whole-tree leaf specific hydraulic conductance (K_L) than mesic populations. These climate-driven shifts in hydraulic architecture are considered adaptive because they maintain minimum leaf water potential above levels that cause xylem cavitation.

2. Using a common garden study, we examined whether differences in biomass allocation and hydraulic architecture between *P. ponderosa* populations originating from isolated outcrops in the Great Basin desert and Sierran montane environments were caused by ecotypic differentiation or phenotypic plasticity. To determine if populations were genetically differentiated and if phenotypic and genetic differentiation coincided, we also characterized the genetic structure of these populations using DNA microsatellites.

3. Phenotypic differentiation in growth, biomass allocation and hydraulic architecture was variable among populations in the common garden. There were no systematic differences between desert and montane climate groups that were consistent with adaptive expectations. Drought had no effect on the root : shoot and needle : stem ratio, but reduced seedling biomass accumulation, leaf area ratio, A_L/A_S and K_L. Stem hydraulic conductance (K_H) was strongly size-dependent, and was lower in droughted plants, primarily because of lower growth.

4. Although microsatellites were able to detect significant non-zero ($P < 0{\cdot}001$) levels of differentiation between populations, these differences were small and were not correlated with geographic separation or climate group. Estimates of genetic differentiation among populations were low (<5%), and almost all the genetic variation (>95%) resided within populations, suggesting that gene flow was the dominant factor shaping genetic structure.

5. These results indicate that biomass allocation and hydraulic differences between desert and montane populations are not the result of ecotypic differentiation. Significant drought effects on leaf : sapwood allocation and K_L suggest that phenotypic differentiation between desert and montane climates could be the result of phenotypic plasticity.

Key-words: Ecotypes, genetic variation, hydraulic architecture, microsatellites, phenotypic plasticity

Functional Ecology (2002) **16**, 510–521

Introduction

Climatic variation can exert strong selective pressures on plant populations. Because physiological traits are linked to plant fitness, selection by climate can drive genetic differentiation in the physiology of populations occurring in contrasting environments. Ecotypic differentiation has been observed for traits such as photosynthesis (Balaguer *et al.* 2001; Sandquist & Ehleringer 1997); leaf conductance (Abrams 1994); and resistance to xylem cavitation (Kavanagh *et al.* 1999; Sparks & Black 1999). Plants are also capable of acclimating to a variety of environments through phenotypic plasticity (Bradshaw 1965). Phenotypic plasticity for physiological traits may be adaptive in perennial

†Author to whom correspondence should be addressed. E-mail: maherali@duke.edu
‡Present address: Department of Biology, Duke University, Box 90340, Durham, North Carolina 27708-0340, USA.

© 2002 British Ecological Society

plants facing temporal variation in the environment (Cordell *et al*. 1998; Schlichting & Pigliucci 1998).

Trees make several physiological adjustments to cope with atmospheric and soil water deficits. Among the most common are changes in the leaf : sapwood area ratio (Callaway, DeLucia & Schlesinger 1994; Mencuccini & Grace 1995; Whitehead, Edwards & Jarvis 1984); sapwood permeability (Maherali & DeLucia 2000a); and vulnerability to xylem cavitation (Alder, Sperry & Pockman 1996; Franks, Gibson & Bachelard 1995). Because the induction of xylem cavitation limits water transport capacity, mechanisms that enable either the resistance or avoidance of cavitation may be adaptive in arid environments. A decline in the leaf : sapwood area ratio (A_L/A_S) in response to warmer and drier climates may be the most significant response to increasing aridity (Mencuccini & Grace 1995). This shift in biomass allocation increases the physical capacity of roots and stems to supply leaves with water (leaf specific hydraulic conductance; K_L) and maintains minimum leaf water potential above levels that cause xylem cavitation (Maherali & DeLucia 2001; Mencuccini & Grace 1995; Sperry & Pockman 1993; Tyree & Ewers 1991). A reduction in A_L/A_S in response to arid climates has been documented in several tree species (Margolis *et al*. 1995; Waring & Schlesinger 1985), but is especially strong in pines (DeLucia, Maherali & Carey 2000; Mencuccini & Bonosi 2001). It is not known if this variation is a plastic response to the environment, or is the result of ecotypic differentiation. Phenotypic plasticity in pines may be of particular adaptive significance in the future because it would allow trees to acclimate in response to rapid changes in climate caused by human activities.

In western North America, Ponderosa pine (*Pinus ponderosa* L.) occurs in a range of contrasting environments and phenotypes. In the Sierra Nevada and adjacent Great Basin, a conspicuous feature of these forests is that some populations occur on isolated pockets of soil derived from hydrothermally altered andesite (Fig. 1; Billings 1950; DeLucia, Schlesinger & Billings 1988). These islands of Ponderosa pine occur in the Great Basin as far as 60 km from the eastern edge of montane forests, and are probably relicts of widespread coniferous forests present during the late Pleistocene (Billings 1950; DeLucia & Schlesinger 1990). At higher elevation, Ponderosa pine stands also occur in the same altered andesite soil within the contiguous forests of the foothills and mountains of the Sierra Nevada (Callaway *et al*. 1994; Schlesinger, DeLucia & Billings 1989). Desert populations experience 3 °C higher average annual temperature, 60% greater summer vapour pressure deficits (D), and 50% less precipitation than montane populations (Callaway *et al*. 1994; Carey, Callaway & DeLucia 1998; DeLucia & Schlesinger 1990). Desert and montane trees have striking morphological and hydraulic differences that are in accordance with adaptive expectations. For example, desert trees have 44% lower A_L/A_S and 19% higher sapwood permeability (K_S) than montane trees (Table 1; Callaway *et al*. 1994; Maherali & DeLucia 2000b).

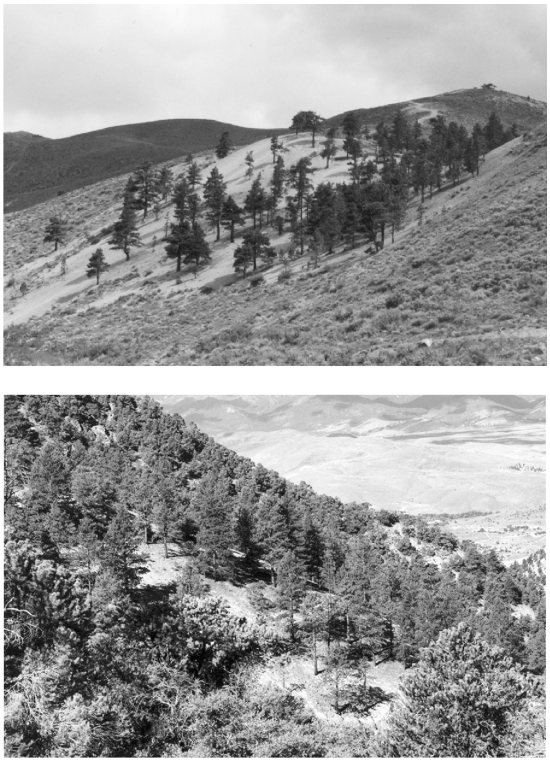

Fig. 1. Representative desert (upper panel) and montane (lower panel) populations of Ponderosa pine on soil derived from hydrothermally altered andesite, near Reno, NV. Hydrothermal alteration of andesites occurred in the late Miocene (Billings 1950) and resulted in acidic (pH ≈ 3·5) and nutrient-poor soils (Billings 1950; Schlesinger *et al*. 1989). For the desert site, the surrounding vegetation is typical of the Great Basin, dominated by *Artemisia tridentata*. *Artemisia tridentata* is excluded from altered andesite because of extreme nutrient limitation (DeLucia & Schlesinger 1990; Schlesinger *et al*. 1989), whereas Ponderosa pine is excluded from desert soils because of intense competition for water (DeLucia *et al*. 1988) and seedling predation (Callaway *et al*. 1996). For the montane site, surrounding vegetation is typical of the eastern Sierra Nevada, including *Pinus edulis*, *P. jeffreyi*, *P. lambertiana* and *Juniperus osteosperma*.

By means of a common garden experiment, we determined whether climate-driven shifts in biomass allocation and hydraulic architecture between desert and montane Ponderosa pine populations are caused by phenotypic plasticity or represent ecotypic differences. To examine the potential for phenotypic plasticity in each population, we grew seedlings in contrasting irrigated and drought treatments. To test for ecotypic differentiation in the common garden, we assessed whether populations from desert and montane climate groups diverged systematically from each other in a manner consistent with differences in the field. Our analysis was confined to those parameters that differed between desert and montane trees in the field: growth, biomass allocation and hydraulic conductance (Table 1). Because desert Ponderosa pine populations are geographically isolated (Fig. 1), they may be genetically

© 2002 British Ecological Society,

Table 1. Mean (±1 SE) biomass allocation and xylem hydraulic parameters per tree for desert and montane Ponderosa pine populations used in this study. Biomass allocation parameters were calculated from the diameter at the base of the live crown, based on relationships described by Callaway *et al*. (1994) for desert (Peavine and Desert Research Institute) and montane (Alpine co. and Virginia range) populations. Trees were from 30 to 180 years old. Hydraulic parameters are from Maherali & DeLucia (2000b) for the same populations

Parameter	Desert	Montane	*P*
d.b.h. (cm)	38·0 ± 5·3	41·2 ± 4·9	NS
Sapwood volume (dm^3)	762 ± 317	667 ± 169	NS
Height (m)	8·9 ± 1·4	14·3 ± 1·9	<0·001
Sapwood mass (kg)	315 ± 131	280 ± 71	NS
Leaf mass (kg)	30·4 ± 7·5	51·4 ± 9·4	<0·001
Leaf : sapwood area ratio ($m^2\ cm^{-2}$)	0·116 ± 0·004	0·207 ± 0·002	<0·001
Sapwood : leaf mass ratio ($kg\ kg^{-1}$)	8·21 ± 1·29	5·93 ± 0·63	<0·001
Sapwood volume : leaf area ratio ($dm^3\ m^{-2}$)	6·07 ± 0·95	3·56 ± 0·49	<0·001
Specific conductivity (K_S; $kg\ m^{-1}\ MPa^{-1}\ s^{-1}$)	0·64 ± 0·04	0·54 ± 0·03	<0·05
Xylem tension at 50% cavitation (MPa)	–2·65 ± 0·20	–2·61 ± 0·19	NS

Table 2. Climate and stand characteristics of desert and montane Ponderosa pine populations used in common garden and molecular marker studies. Mean temperature and precipitation are based on 30-year (1961–90) summaries from weather stations located near each study site (Western Regional Climate Center, Reno, NV). Mean maximum daily temperatures are the average of monthly maxima. Vapour pressure deficit (*D*) was calculated from relative humidity data obtained from the Vegetation/ Ecosystem Modeling and Analysis Project (VEMAP; Kittel *et al*. 1995). The designation of a population as either montane or desert is based on the phenotype of mature trees at each site (see Table 1)

Site	Elevation (m)	Latitude, longitude	Population size	Precipitation ($mm\ year^{-1}$)	Mean annual temperature (°C)	Maximum monthly *D* (kPa)
Montane						
Alpine co.*†	2100	38°41′ N, 119°44′ W	>1000	550	8·2	2·25
Virginia range*†	2030	39°22′ N, 119°41′ W	~500	473	8·4	2·55
Alum creek†	1800	39°27′ N, 119°50′ W	>1000	468	8·1	2·24
Desert						
Peavine*	1500	39°34′ N, 119°50′ W	≈300	245	10·5	3·58
DRI*†	1500	39°35′ N, 119°47′ W	≈300	225	11·4	4·02
Lockwood†	1500	39°30′ N, 119°36′ W	≈300	173	11·5	4·22
Pyramid lake†	1850	39°49′ N, 119°29′ W	≈500	180	10·8	4·24

*Populations used in common garden experiment.
†Populations analysed for genetic variation using microsatellite DNA.

distinct from each other and from populations in the contiguous range of the species. We therefore characterized the genetic structure of these populations using DNA microsatellites. We used this approach to determine if populations were genetically differentiated and if patterns of genetic differentiation coincided with geographic separation and phenotypic differences between environments.

Materials and methods

COMMON GARDEN STUDY

Experimental design and seed collection

We collected cones from 15 trees at two desert (Desert Research Institute, DRI; Peavine Mountain) and two montane (Alpine County; Virginia Range) populations in September 1997. Climate characteristics, approximate population size, location and elevation for each population are listed in Table 2. Soil chemistry is described by Billings (1950) and Schlesinger *et al*. (1989). Trees separated by a minimum of 20 m were selected along two 250-m-long transects that spanned each site. Fresh cones were placed in a forced convection oven for 3–4 days at 40 °C to induce opening. Within a population, seeds were pooled across trees, soaked for 48 h, stratified at 4 °C for 45 days, then germinated in 'rootainer' pots (Hummert International, Earth City, MO) filled with a mixture of quartz sand and loam (10 : 3 v/v). After 4 weeks in a greenhouse under natural light, 32 seedlings from each population were randomly selected and transplanted into 40 cm deep, 3·14 l PVC tubes. Therefore each experimental greenhouse population constituted a mixture of genotypes of the corresponding field population.

Greenhouse temperatures varied from 15 °C (night) to 25 °C (day). Natural light was supplemented with high-intensity discharge lamps suspended above the seedlings to provide a 15 h photoperiod and maintain

© 2002 British Ecological Society,

minimum incident irradiance (photosynthetically active radiation) at the tops of the pine seedlings above 700 μmol m^{-2} s^{-1}, sufficient to saturate photosynthesis (H.M., personal observation). To minimize the effects of environmental heterogeneity within the greenhouse room, we used a randomized complete block experimental design (e.g. Potvin 1993) with population and irrigation as treatments. Pots were arranged in four blocks with eight seedlings from each population randomly placed in each block. Half the seedlings in each block were randomly assigned to the drought treatment. At the start of the experiment, each pot was fertilized with 10 g slow-release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA). For the first 8 months plants were watered to field capacity every other day. At the beginning of the ninth month, a 54 day drought treatment was imposed on half the seedlings. Droughted plants received 100 ml water once a week, whereas well watered plants continued to be watered to field capacity every other day. To confirm that the drought treatment affected the water status of seedlings, we measured predawn and midday needle water potentials 2 weeks prior to final harvest using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA) on two seedlings in each population × drought treatment combination.

Hydraulic conductivity

At harvest, four seedlings from each population and irrigation treatment group were brought to an air-conditioned laboratory and placed under water, and a 3–5 cm long stem segment between the root collar and first leaf was excised and de-barked. Stem segments were placed in a water bath for 1 h to reduce resin exudation. Hydraulic conductivity (K_H) was measured as described by Sperry *et al.* (1988). Segments were cleared of air embolism by flushing them with a filtered (0·2 μm) weak HCl solution (pH = 2) under high pressure. Preliminary experiments showed that Ponderosa pine reached maximum volume flow rate (Q, kg s^{-1}) after 30 min at 100 kPa, so all segments were flushed for 1 h prior to measuring hydraulic conductivity. Measurements of K_H were made at a regulated pressure of 45 kPa. Stem efflux was collected and weighed to calculate volume flow rate.

Hydraulic conductivity (K_H; kg m MPa^{-1} s^{-1}) was calculated as the volume flow rate divided by the pressure gradient $[Q/(dP/dx)]$. Specific hydraulic conductivity (K_S; kg m^{-1} MPa^{-1} s^{-1}) was calculated as K_H divided by the stem area of the segment, and leaf-specific hydraulic conductivity (K_L; kg m^{-1} MPa^{-1} s^{-1}) was calculated as K_H divided by the leaf area of the seedling. After measuring hydraulic conductivity, stems were perfused with filtered (0·2 μm) 0·01 basic fuschin solution under hydrostatic pressure (≈10 kPa) to determine the functional xylem area of each segment. Dye infiltration showed that the entire stem comprised functional xylem, except for a small portion of pith. Therefore stem cross-sectional area was considered equivalent to functional xylem or sapwood area.

Biomass allocation

After 10 months, seedlings were harvested, oven-dried to constant mass (70 °C for 48 h) in a forced-convection oven, and weighed. Specific leaf area (cm^2 g^{-1}) was calculated on a subsample of needles from each seedling. All-sided leaf area (A) for individual needles was calculated as $A = L(C + 2NR)$, where L is needle length; N is the number of needles per fascicle; C is the circumference of the fascicle; and R is the fascicle radius. Leaf area per seedling was calculated as the product of specific leaf area and leaf biomass.

Statistical analysis

All response variables were analysed using a two-way randomized block ANOVA with population, drought and block as fixed factors. We assumed that blocks did not interact with treatments (Neter *et al.* 1990). For size-dependent variables such as K_H and K_S, we included plant biomass as a covariate in the model. The population main effect and the population × drought interaction were partitioned using planned orthogonal 1 df contrasts (Sokal & Rohlf 1995). This analysis allowed us to examine whether significant population effects were caused by systematic differences between montane and desert climate groups. Thus for the population main effect we tested the predictions that (i) desert and montane climate groups differed; (ii) Peavine differed from DRI; and (iii) Alpine differed from Virginia. For the population × drought treatment interaction, we tested the predictions that desert and montane climate groups differed within the (i) well watered and (ii) drought-stressed treatments. Significant desert *vs* montane differences in the contrasts would be indicative of ecotypic differentiation. Although the population number in each climate group is small ($n = 2$), these 1 df contrast tests are based on N of all seedlings in the experiment, ensuring sufficient power to detect differences between climate groups (Sokal & Rohlf 1995).

We used multivariate analysis of variance (MANOVA) to determine if populations could be differentiated across jointly distributed growth and biomass allocation traits. Biomass allocation traits were highly correlated (r = 0·90–0·99), therefore we used principal components analysis (PCA) to create new, orthogonal variables for the MANOVA (e.g. Stevens 1992). We retained all principal components with an eigenvalue >1 (Kaiser's criterion; Stevens 1992). Following the MANOVA, differences between populations and climate groups were tested using planned orthogonal 1 df contrasts, as described above. All data met assumptions for MANOVA. Principal components scores were also used to visualize the dispersion between populations for growth and biomass allocation traits.

© 2002 British Ecological Society,

MOLECULAR MARKERS

Microsatellites

To identify fine-scale population structure in these stands which may not be revealed by other molecular or enzymatic markers, we used highly polymorphic microsatellite DNA markers (Hughes & Queller 1993). We initially screened seven loci, four from *Pinus radiata* (NZPR4·6, NZPR9·3, Smith & Devey 1994; NZPR1, NZPR6, Fisher *et al*. 1998); two from *P. contorta* (UAPC1, UAPC4, Hicks *et al*. 1998); and one from *P. sylvestris* (SPSY6, Soranzo *et al*. 1998). Loci UAPC1 and UAPC4 were monomorphic in our study populations. Loci SPSY6, NZPR1 and NZPR6 produced polymorphic PCR products, but diallelic patterns could not be consistently deduced when viewed on acrylamide gels. Only NZPR4·6 and NZPR9·3 (Smith & Devey 1994) were informative in *P. ponderosa*. These two loci also cross-amplified reliably in other pines (Echt & May-Marquardt 1997; Echt *et al*. 1999; Karhu *et al*. 1996).

Sample collection and DNA extraction

One-year-old needle tissue was collected from Ponderosa pine trees in May 1998 using the same sampling procedure employed for seeds, with the exception that 30 trees per site were sampled. We collected tissue from populations used in the common garden study, as well as from additional populations in each climate group (Table 1). Difficulties with primer amplification in the Peavine population prevented its use in our analysis. After collection, tissue was shipped under dry ice to the University of Illinois, then stored at –80 °C. Needles (0·2 g) were frozen with liquid nitrogen and then homogenized into a powder with a mortar and pestle. DNA was extracted from 0·1 g tissue using a 2× CTAB extraction procedure (Doyle & Doyle 1990).

PCR reactions

PCR conditions for all primers are outlined in detail elsewhere (Echt *et al*. 1999), and only conditions for NZPR4·6 and NZPR9·3 are stated here. PCR reactions were carried out using 50 ng genomic DNA, 0·2 μM of each primer, 20 mM Tris–HCl, 50 mM KCl, 0·2 mM of each dTNP, 4 mM $MgCl_2$, 1 unit of *Taq*, and water to a final volume of 20 μl. Thermocycling conditions were as follows: an initial denaturation step at 94 °C for 60 s, followed by an annealing temperature of 60 °C for 60 s and an extension step at 72 °C for 60 s. The annealing temperature was decreased by 0·5 °C each cycle until a 55 °C annealing temperature was reached, followed by an additional 20 cycles using the 55 °C annealing temperature (listed as SSRT55 by Echt *et al*. 1999). To visualize genotypes, we utilized 5′ fluorescently labelled primers in the PCR reactions and ran PCR products on an ABI PRISM 377 automated sequencer (Applied Biosystems, Foster City, CA). Genotypes were scored using Genotyper 2·0 (Applied Biosystems, Foster City, CA).

Data analysis

Allele frequencies were determined by direct count, and expected heterozygosities were calculated according to Nei (1987) as implemented in GENEPOP ver. 3·1a (Raymond & Rousset 1995). We determined total and pairwise differentiation among populations using Weir & Cockerham's (1984) estimator of genetic differentiation (θ_{ST}) as implemented in ARLEQUIN 2·000 (Schneider *et al*. 2000). The significance of these estimates was determined by permuting individuals between populations 1000 times, and critical values were adjusted for simultaneous statistical tests using the sequential Bonferroni procedure (Rice 1989). We tested for departures from random associations of allele frequencies between population pairs using a Mantel test (Raymond & Rousset 1995), with 1000 iterations of the Markov chain method (Guo & Thompson 1992) and critical values adjusted according to Rice (1989).

We partitioned genetic variance within and among populations using an ANOVA framework for molecular data (AMOVA; Excoffier *et al*. 1992; Michalakis & Excoffier 1996). Estimates of genetic variation can be influenced by assumptions concerning the model of evolution for a given molecular marker. Both an infinite allele model (IAM) and stepwise mutation model (SMM) have been applied to microsatellite data, and which model is appropriate for a given level of inquiry has been a topic of debate (Bentzen *et al*. 1996; Goldstein *et al*. 1995). To address this issue, we calculated genetic differentiation among populations (θ_{ST} and Φ_{ST}) in ARLEQUIN 2·000, where θ_{ST} is consistent with an IAM (Weir & Cockerham 1984) and Φ_{ST} is consistent with an SMM (Excoffier *et al*. 1992). The significance of the statistic was determined as described above.

Results

GROWTH AND BIOMASS PARTITIONING

Droughted seedlings had lower predawn (–0·22 *vs* –1·09 MPa) and midday (–0·82 *vs* –1·98 MPa) water potentials than well watered seedlings, confirming the efficacy of the treatment. Drought reduced total growth and leaf area ratio (LAR, leaf area per unit dry mass) but had no effect on root : shoot ratio and needle : stem mass ratio (Fig. 2; Table 3). Populations in the desert climate group produced more biomass than those in the montane climate group, but only when droughted (Table 3). Populations differed with respect to root : shoot ratio, needle : stem mass ratio, and LAR. However, these traits did not differ systematically between montane and desert climate groups, as determined by 1 df contrasts. Instead, most of the significant effects were caused by differences among populations within montane or desert

© 2002 British Ecological Society,

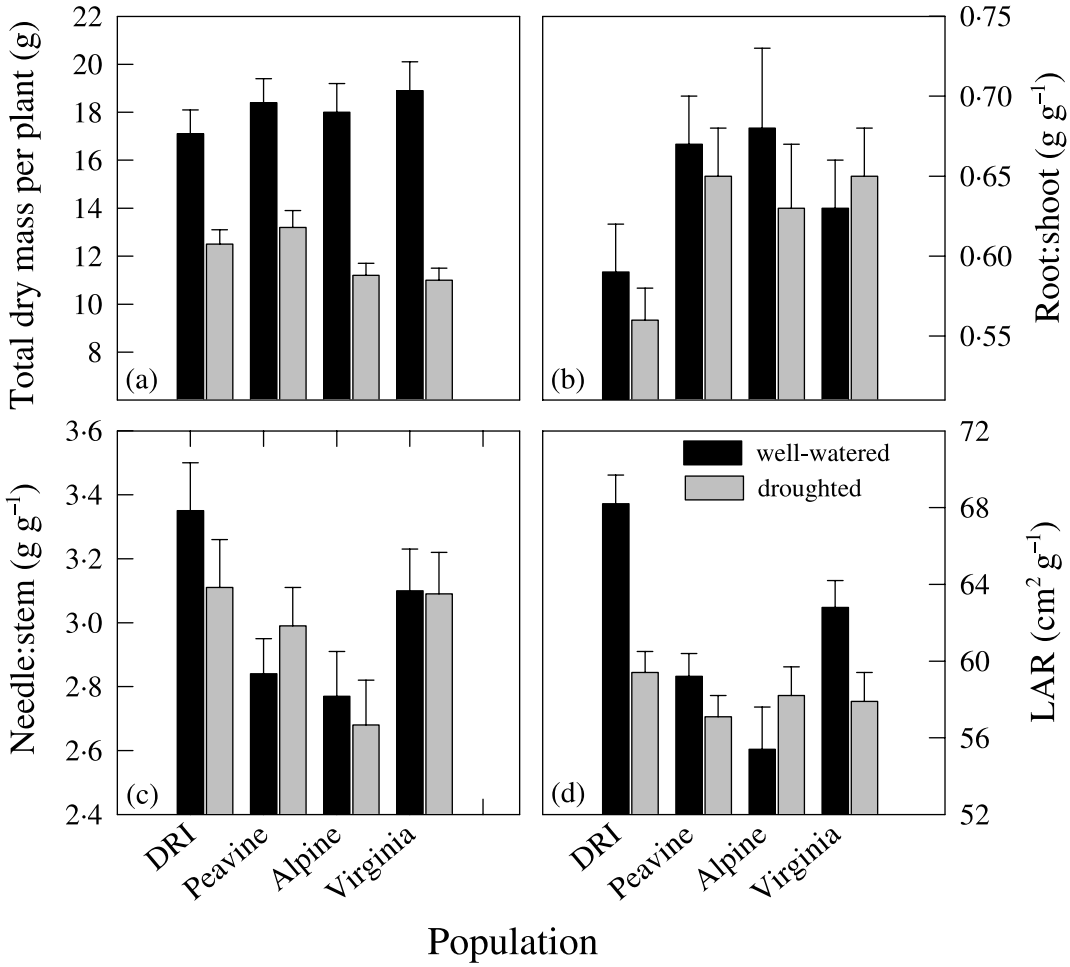

Fig. 2. Mean (±1 SE) (a) total dry mass per plant; (b) root : shoot mass ratio; (c) needle : stem mass ratio; (d) leaf area ratio (LAR) of well watered and droughted 10-month-old seedlings from desert (DRI, Peavine) and montane (Alpine, Virginia) Ponderosa pine populations grown in a common garden. Treatment and population differences are listed in Table 3.

climate groups. For example, the seedlings in the DRI population allocated more tissue to shoots and less to roots than the Peavine population, whereas the Virginia and Alpine populations differed in needle : stem mass ratio and LAR (Fig. 2; Table 3). There was no population or drought effect on specific leaf area ($P > 0{\cdot}50$, data not shown).

There was no significant effect of drought on proportional allocation to stems, needles and roots (Table 4). In contrast, there was a significant population effect

Table 3. ANOVA results for growth and biomass allocation patterns on 10-month-old Ponderosa pine seedlings. Significant ($P < 0{\cdot}05$) treatment effects are in bold. Differences between climate groups and among populations were tested with 1 df contrasts. For the population main effect the predictions tested were (i) desert and montane climate groups differed; (ii) Peavine differed from DRI; and (iii) Alpine differed from Virginia. For the population × drought treatment interaction the predictions tested were that desert and montane climate groups differed within (i) droughted; (ii) well watered treatments

		Total biomass			Root : shoot ratio			Needle : stem mass ratio			Leaf area ratio		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Population	3	8·42	0·76	0·52	**0·050**	**3·31**	**0·02**	**1·47**	**5·31**	**0·002**	**295·2**	**9·30**	**<0·001**
Desert *vs* montane		8·81	0·79	0·38	0·031	2·06	0·15	0·82	2·96	0·09	**187·7**	**5·92**	**0·02**
DRI *vs* Peavine		15·1	1·36	0·25	**0·116**	**7·66**	**0·007**	**1·54**	**5·56**	**0·02**	**512·9**	**16·2**	**0·001**
Alpine *vs* Virginia		1·20	0·11	0·74	0·049	0·32	0·57	**2·11**	**7·61**	**0·007**	**196·7**	**6·20**	**0·02**
Drought	1	**1192·9**	**107·4**	**<0·001**	0·013	0·86	0·36	0·094	0·34	0·56	**336·6**	**10·6**	**0·002**
Population × drought	3	16·8	1·52	0·21	0·007	0·43	0·73	0·21	0·77	0·51	**91·8**	**5·93**	**0·04**
Desert *vs* montane (ds)		**47·6**	**4·29**	**0·04**	0·019	1·23	0·27	0·44	1·57	0·21	0·89	0·03	0·87
Desert *vs* montane (ww)		6·87	0·62	0·43	0·013	0·85	0·36	0·39	1·40	0·24	**334·3**	**10·5**	**0·002**
Block	3	28·6	2·57	0·057	0·046	3·01	0·03	0·33	1·18	0·32	188·4	2·89	<0·001
Error	115	11·1			0·015			0·28			31·7		

Table 4. Mean (±1 SE) proportional biomass allocation to stems, needles and roots of 10-month-old seedlings from desert (DRI, Peavine) and montane (Alpine, Virginia) Ponderosa pine populations grown in a common garden. Population means across treatments are reported because there were no drought effects. Statistical differences ($P < 0{\cdot}05$) among populations are indicated by different letters

Population	Stems	Needles	Roots
Desert			
DRI	0·154 ± 0·0059a	0·483 ± 0·0094a	0·363 ± 0·0105a
Peavine	0·156 ± 0·0050a	0·448 ± 0·0089b	0·396 ± 0·0095b
Montane			
Alpine co.	0·167 ± 0·0061b	0·443 ± 0·0145b	0·391 ± 0·0152b
Virginia range	0·151 ± 0·0041a	0·461 ± 0·0110b	0·388 ± 0·0107b

on these traits, but with no consistent pattern of differentiation between montane and desert climate groups. For example, the DRI seedling population differed from all others with respect to allocation to needles and roots (Table 4), whereas the Alpine seedling population allocated more biomass to stems than the other populations.

HYDRAULIC ARCHITECTURE

Hydraulic conductivity (K_H) and specific hydraulic conductivity (K_S) were higher in well watered plants, but this difference was largely caused by significant size-dependent differences in K_H (Table 5). Well watered plants were larger than droughted plants (Fig. 2), and consequently had higher K_H (Fig. 3). Droughted plants had lower leaf-specific hydraulic conductivity (K_L) and leaf : sapwood area ratio (A_L/A_S) than well watered plants (Fig. 3c,d). Although there was a significant population effect on all hydraulic architecture variables (either as a main population effect or a 1 df contrast), there were no systematic differences between desert and montane climate groups (Table 5).

DIFFERENCES IN MULTIVARIATE SPACE

The first three principal components explained 57, 27 and 14% of the variance in the traits, respectively.

Table 6. Results of a multivariate ANOVA performed on principal components analysis (PCA) scores calculated from growth and biomass allocation variables measured on four populations of 10-month-old Ponderosa pine seedlings. For the population main effect the predictions tested were (i) desert and montane climate groups differed; (ii) Peavine differed from DRI; and (iii) Alpine differed from Virginia. PCA scores are plotted in Fig. 4

	MANOVA effects			
Source	df	Pillai's trace	*F*	*P*
Population	9, 366	0·188	2·73	0·004
Desert *vs* montane	3, 120	0·034	1·41	0·24
DRI *vs* Peavine	3, 120	0·093	4·08	0·009
Alpine *vs* Virginia	3, 120	0·073	3·15	0·03

Allocation to needles (+), roots (–), root : shoot ratio (–) and LAR (+) were significantly correlated with the first principal component axis. Allocation to stems (–) and needle : stem mass ratio (+) were significantly correlated with the second principal component axis, whereas only total biomass (+) was correlated with the third principal component axis. Significant population-level differences emerged when growth and biomass allocation variables were considered jointly and pooled across drought treatment in the MANOVA. However, there were no systematic differences between desert and montane climate groups. Instead, all populations differed from each other (Fig. 4; Table 6).

MOLECULAR MARKERS

Twenty-eight and seven alleles were observed at loci NZPR4·6 and NZPR9·3, respectively. Expected heterozygosities averaged 0·916 ± 0·024 ($N = 6$) for locus NZPR4·6 and 0·678 ± 0·076 ($N = 6$) for locus NZPR9·3, values similar to those found for these markers in *P. radiata* (Smith & Devey 1994) and *P. sylvestris* (Karhu *et al.* 1996). Pairwise θ_{ST} estimates (Weir & Cockerham 1984) indicated some differentiation among populations

Table 5. ANOVA results for hydraulic architecture components for 10-month-old Ponderosa pine seedlings. Significant ($P < 0{\cdot}05$) treatment effects are in bold. Statistical tests are as listed in Table 3

		Hydraulic conductivity			Specific conductivity			Leaf-specific conductivity				Leaf : sapwood area ratio			
Source	df	MS	*F*	*P*	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
		($\times 10^{-14}$)			($\times 10^{-3}$)			($\times 10^{-10}$)							
Population	3	**365·5**	**2·97**	**0·05**	9·71	1·42	0·27	3	5·95	2·63	0·08	3	**411·6**	**2·59**	**0·05**
Desert *vs* montane		332·2	2·70	0·12	0·50	0·07	0·79		3·23	1·43	0·25		36·4	0·23	0·63
DRI *vs* Peavine		**634·0**	**5·15**	**0·03**	**28·5**	**4·16**	**0·05**		4·63	2·05	0·17		492·8	3·11	0·08
Alpine *vs* Virginia		107·9	0·87	0·36	0·18	0·03	0·87		**9·98**	**4·41**	**0·05**		**709·9**	**4·48**	**0·04**
Drought	1	1·04	0·008	0·92	9·81	1·43	0·25	1	**12·8**	**5·67**	**0·03**	1	**842·3**	**5·31**	**0·03**
Population × drought	3	170·0	1·38	0·28	6·28	0·82	0·45	3	5·69	2·51	0·09	3	85·3	0·54	0·66
Desert *vs* montane (ds)		2·51	0·02	0·89	4·34	0·63	0·44		0·84	0·37	0·55		14·0	0·09	0·77
Desert *vs* montane (ww)		**771·1**	**6·26**	**0·02**	9·69	1·41	0·25		**12·0**	**5·27**	**0·03**		154·0	0·97	0·33
Total biomass	1	**4944·1**	**40·2**	**<0·01**	**60·3**	**8·80**	**<0·01**	–	–	–	–	–	–	–	–
Block	3	448·7	3·65	0·03	25·1	3·65	0·03	3	6·38	2·81	0·06	3	222·4	1·40	0·25
Error	20	123·1			6·85			21	2·27			50	158·5		

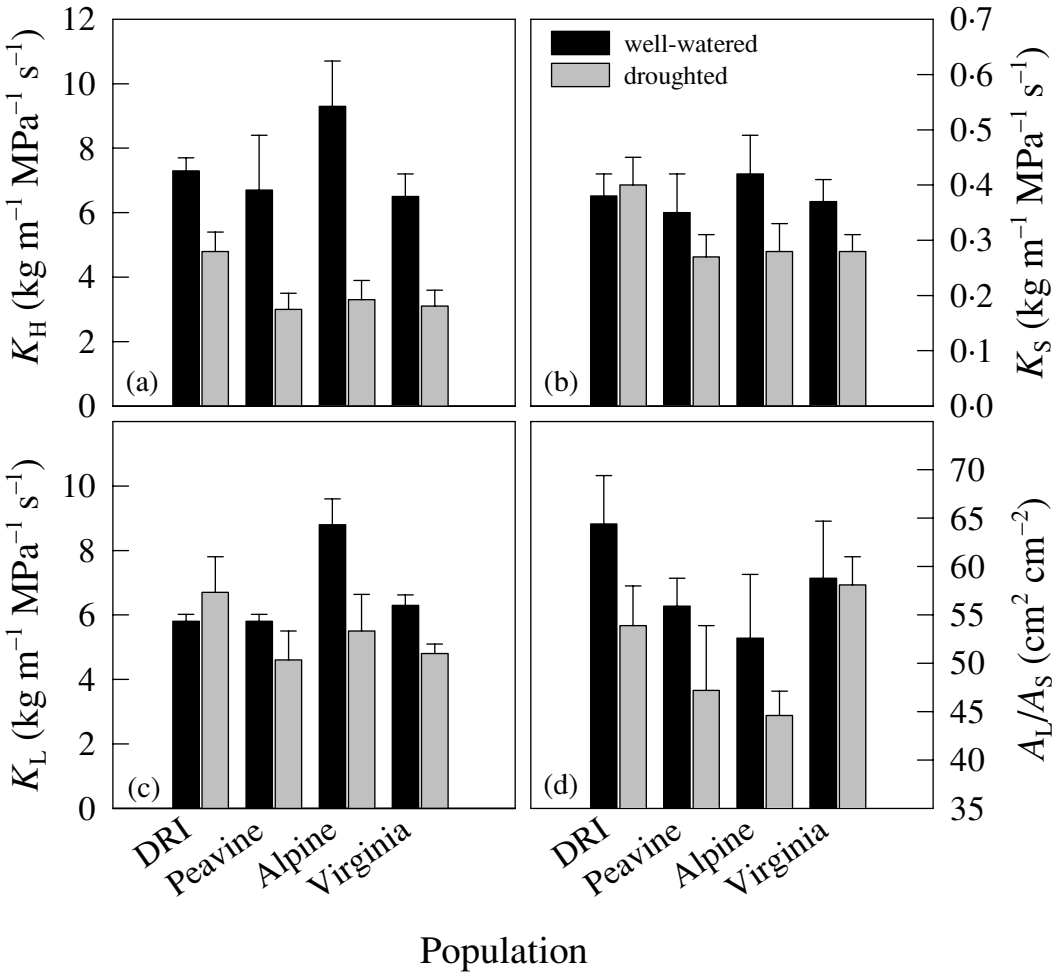

Fig. 3. Mean (±1 SE) (a) hydraulic conductivity (K_H; $\times 10^{-6}$); (b) specific hydraulic conductivity (K_S); (c) leaf-specific hydraulic conductivity (K_L; $\times 10^{-5}$); (d) leaf : sapwood area ratio (A_L/A_S) of well watered and droughted 10-month-old seedlings from desert (DRI, Peavine) and montane (Alpine co., Virginia range) Ponderosa pine populations grown in a common garden. Treatment and population differences are listed in Table 5.

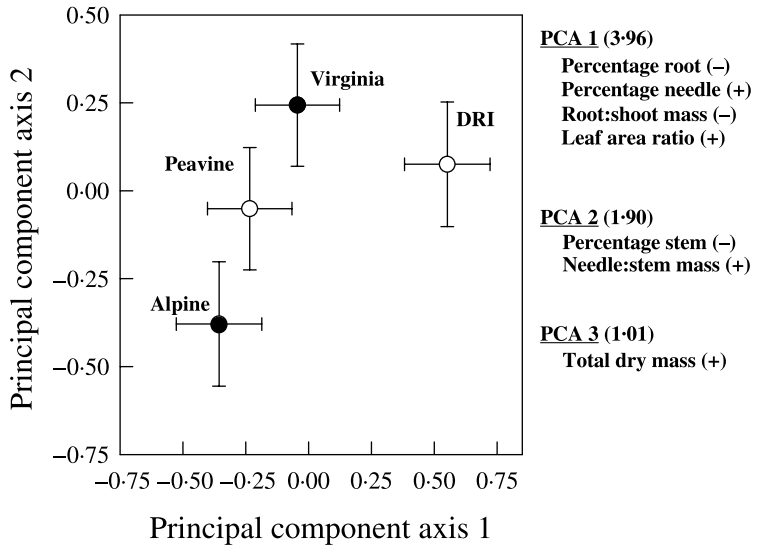

Fig. 4. A plot of mean (±1 SE) principal components analysis (PCA) scores for growth and biomass allocation variables measured on 10-month-old seedlings from desert and montane Ponderosa pine populations grown in a common garden. Dependent variable loadings for each axis are shown with eigenvalues and the nature of the correlation (positive or negative) in parentheses. Eigenvalues >1 are considered significant. ○, Desert (DRI, Peavine) populations; ●, montane (Alpine, Virginia) populations. Statistical differences among populations and climate groups are listed in Table 6.

(Table 7). Mean (±1 SE) pairwise θ_{ST}, however, was quite low (0·045 ± 0·007, $N = 15$). Pairwise θ_{ST} was also poorly correlated with pairwise straight-line geographic distance among all populations ($r^2 = 0{\cdot}03$, $F_{1,13} = 0{\cdot}425$, $P = 0{\cdot}53$). Tests of non-random associations among alleles indicated that most populations were genetically similar regardless of climate group or geographic separation. Significant differentiation based on non-random allelic associations was detected only for the Pyramid lake population when compared to DRI, Lockwood and Alum creek. Hierarchical analysis of genetic variance (AMOVA) detected significant non-zero differentiation among all populations using both θ_{ST} (infinite allele model) and Φ_{ST} (stepwise mutation model) (Table 8). Nonetheless, θ_{ST} and Φ_{ST} were low and indicated that >95% of the genetic variation was within, rather than among, populations.

Discussion

Ponderosa pine populations from desert and montane climate groups showed little evidence of ecotypic differentiation in a common garden. There were significant population-level effects on biomass allocation and

Table 7. Results of pairwise multilocus tests of differentiation between study populations of Ponderosa pine in western Nevada and eastern California. Values below the diagonal correspond to θ_{ST} (Weir & Cockerham 1984); those above the diagonal correspond to exact *P* values in a test of non-random associations among alleles (Raymond & Rousset 1995). For θ_{ST}, bold type indicates that it is significantly different from zero at $\alpha = 0{\cdot}05$. For tests of non-random associations among alleles, bold type identifies *P* values <0·05. Pairwise straight-line distances (km) listed in parentheses next to each θ_{ST} or *P* value

Sample location		DRI (Desert)		Lockwood (Desert)		Pyramid lake (Desert)		Alum creek (Montane)		Virginia range (Montane)		Alpine co. (Montane)	
DRI	(Desert)	–	–	0·151	(14)	**0·025**	(39)	0·443	(14)	0·100	(29)	0·124	(98)
Lockwood	(Desert)	0·001	(14)	–	–	**0·011**	(40)	0·055	(20)	0·229	(18)	0·114	(89)
Pyramid lake	(Desert)	**0·064**	(39)	**0·099**	(40)	–	–	**0·032**	(53)	0·155	(59)	0·205	(130)
Alum creek	(Montane)	**0·050**	(14)	**0·048**	(20)	**0·048**	(53)	–	–	0·646	(24)	0·632	(85)
Virginia range	(Montane)	0·038	(29)	**0·054**	(18)	0·017	(59)	0·026	(24)	–	–	0·759	(70)
Alpine co.	(Montane)	**0·067**	(98)	**0·066**	(89)	**0·067**	(130)	0·001	(85)	0·030	(70)	–	–

Table 8. Hierarchical analysis of genetic variance (AMOVA) among populations of Ponderosa pine. Estimates of θ_{ST} incorporate variance in allele frequencies, consistent with an infinite allele model (IAM); Φ_{ST} incorporates variance in the size of alleles, consistent with a stepwise mutation model (SMM)

Source of variation	df	SS	MS	Variation explained (%)	θ_{ST}	*P*	df	SS	MS	Variation explained (%)	Φ_{ST}	*P*
Among populations	5	9·81	0·03	4·64	0·046	<0·001	5	53·8	0·49	2·93	0·029	<0·001
Within populations	336	174·9	0·52	95·36			336	5699·7	16·29	97·07		
Total	341	184·7	0·55				341	5753·5	16·79			

some hydraulic architecture parameters, but these differences did not correspond to the climate of origin. For example, if ecotypic differentiation had occurred, we would expect desert populations to differ systematically from montane populations in a manner consistent with adaptive expectations. Yet there were generally no significant differences between climate groups for growth, biomass allocation or hydraulic variables (Figs 2 and 3; Tables 3–5). Instead, populations generally differed from each other regardless of climate group (Fig. 4; Table 6). In addition, seedling phenotypes were sometimes the opposite of ecotypic expectations. For example, A_L/A_S was lowest and K_L was highest in the Alpine county population (Fig. 3), a site that experiences the least water limitation (Table 1).

Our molecular studies suggested that genetic differentiation among populations, although statistically significant, was relatively low and was not associated with geographic distance or climate group. Pairwise θ_{ST} (Table 7) and total estimates (θ_{ST}, Φ_{ST}; Table 8) of genetic differentiation among populations were quite small, and lower than that of most pines (Jorgensen & Hamrick 1997). In the context of the considerable statistical power provided by highly polymorphic microsatellite markers, these estimates of differentiation are unlikely to be biologically significant (e.g. Hedrick 1999). Moreover, pairwise θ_{ST} was not correlated with pairwise geographic distance among populations ($r^2 = 0{\cdot}03$; Table 7). The low incidence of non-random associations among alleles in pairwise comparisons also indicates that most populations were genetically similar. Non-random allelic associations, when detected, were not consistent with geographic distance or climate group. For example, although Pyramid lake was genetically differentiated from some desert populations, it was also genetically indistinguishable from the two furthest montane populations – Virginia range and Alpine county (Table 7).

We also found that almost all the genetic variation in desert and montane Ponderosa pine was partitioned within, rather than among, populations (Table 8). This result, which was supported by both infinite allele model analysis (IAM; θ_{ST}) and stepwise mutation model analysis (SMM; Φ_{ST}) (Table 8), is also consistent with low pairwise θ_{ST} and the lack of non-random allelic associations among population pairs (Table 7). The genetic similarity among Ponderosa pine populations suggests that ongoing gene flow exerts the strongest influence on genetic structure (Hutchinson & Templeton 1999). This interpretation is consistent with evidence of significant pollen movement among Ponderosa pine populations, even in the face of considerable geographic isolation (Hamrick *et al.* 1989; Hamrick *et al.* 1994; Mitton 1995). Because these stands may be relicts of contiguous forests present during the late Pleistocene, the lack of differentiation among populations could also be attributed to their common descent.

Our results corroborate previous studies of Ponderosa pine using molecular markers, which indicate that genetic differentiation among populations is low and that most of the genetic variation resides within populations (Hamrick *et al.* 1989; Hamrick *et al.* 1994; Latta & Mitton 1999; Mitton 1995; Niebling & Conkle 1990). The small amount of genetic differentiation we observed among populations probably arose from random genetic drift, rather than natural selection. This conclusion is supported by the absence of ecotypic differentiation, the spatial isolation between some stands (Hamrick

et al. 1989), and historical evidence that populations experienced a demographic bottleneck in the late 19th century because of logging (Billings 1950).

To determine if biomass allocation and hydraulic architecture traits could respond to environmental variation through phenotypic plasticity, we exposed seedlings to a drought treatment. Plastic responses to drought were observed in a few hydraulically important traits, including A_L/A_S and K_L (Table 5; Fig. 3). Some of these responses are consistent with adaptation to arid climates (DeLucia *et al*. 2000; Mencuccini & Grace 1995). For example, all populations reduced A_L/A_S in response to drought (Fig. 3). Low A_L/A_S, by increasing K_L, permits plants to maintain transpiration without inducing water potentials that cause xylem cavitation (Sperry & Pockman 1993; Tyree & Ewers 1991). Because cavitation in response to drought can lead to death (Tyree & Ewers 1991), mechanisms that facilitate its avoidance (such as low A_L/A_S) may have a selective advantage in arid climates. This response may be especially important in Ponderosa pine, because populations in desert and montane climate groups do not differ in the vulnerability of xylem to drought-induced cavitation (Maherali & DeLucia 2000b; Maherali & DeLucia 2001). Also, there were no population × environment interactions for hydraulic architecture traits, suggesting that plastic responses to drought, when they occurred, were similar in all populations regardless of climate of origin or degree of spatial isolation. By providing a buffer during natural selection, phenotypic plasticity in Ponderosa pine may preclude the existence of significant ecotypic differentiation between desert and montane populations (e.g. Rehfeldt 1979). Phenotypic plasticity could also maintain genetic variation within populations in the face of strong selection (Sultan 1987), as observed in our study (Table 8).

Although drought reduced A_L/A_S, this response did not translate directly into an increase in K_L. At the seedling or branch level, hydraulic conductivity (K_H) is often strongly correlated with size (Maherali, DeLucia & Sipe 1997). Our analysis of hydraulic architecture was therefore confounded by the negative influence of water stress on seedling biomass and, by extension, K_H (Fig. 4; Table 4). A size-dependent reduction in K_H, despite reduced A_L/A_S, was probably responsible for the modest reduction in K_L observed in droughted plants.

Despite its influence on A_L/A_S and K_L, drought had relatively weak effects on several other biomass allocation and hydraulic variables. For example, it was surprising that drought did not increase the root : shoot ratio, a particularly labile trait (Kramer & Boyer 1995). These results suggest two interpretations: either the drought treatment was not long or severe enough to cause phenotypic differences in all the response variables; or many hydraulic architecture traits are not plastic in Ponderosa pine. Phenotypic plasticity in allocation and hydraulic traits in response to moisture deficits is typically observed in experiments with seedlings for Ponderosa (Maherali & DeLucia 2000a) and other pines (*Pinus halepensis*, Tognetti *et al*. 1997). Plastic responses are also observed in genetically similar mature plantation populations grown in contrasting environments (*P. sylvestris*, Mencuccini & Grace 1995; *Pinus taeda*, Ewers *et al*. 2000; Hacke *et al*. 2000). These findings, coupled with our observations of plasticity in LAR, A_L/A_S and K_L, provide support for the former interpretation.

The potential for rapid changes in climate over the next century will challenge the ability of long-lived tree species, such as Ponderosa pine, to evolve in response to novel selection pressures (Geber & Dawson 1993). In this context, the capacity for plastic responses to the environment will be important for the persistence of a species, as well as for prediction of vegetation responses to climate in the future. For example, many studies employ observations of spatial variation in climate–vegetation relationships to predict how trees and forest ecosystems will respond to future climate change (Callaway *et al*. 1994; DeLucia *et al*. 2000; Mencuccini & Bonosi 2001). These predictions are dependent on the assumption that spatial variation in phenotypes is caused by plasticity. For Ponderosa and other pines, a negative relationship between A_L/A_S and increasing aridity suggests that trees in a warmer and drier future will have less photosynthetic tissue per unit structural mass. Such a shift would reduce growth (Callaway *et al*. 1994), and may offset the stimulation of net primary production by elevated CO_2 (Carey *et al*. 1998; DeLucia *et al*. 2000; Maherali & DeLucia 2000a). If structural and hydraulic differences in contrasting climates in pines are not driven by ecotypic differentiation, but instead by phenotypic plasticity, then the power of these predictions is strengthened.

Acknowledgements

This research was supported by grants from the International Arid Lands Consortium (96R-08) and the National Science Foundation (IBN-9800932). We thank J. S. Coleman, L. Piehl, K. L Griffin and J. T. Ball of the Desert Research Institute (Reno, NV) for providing laboratory space and logistical support. We thank C. M. Caruso for assistance in the greenhouse and advice on the experimental design and statistical analyses. We are grateful to C. S. Echt for screening microsatellite markers in Ponderosa pine and to L. Deverno for advice on DNA extraction. We also thank C. K. Augspurger, C. M. Caruso, J. O. Dawson and T. L. Phillips for helpful comments on the manuscript.

References

Abrams, M.D. (1994) Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. *Tree Physiology* **14**, 833–842.

Alder, N.N., Sperry, J.S. & Pockman, W.T. (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia* **105**, 293–301.

Balaguer, L., Martinez-Ferri, E., Vallardes, F. *et al.* (2001) Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology* **15**, 124–135.

Bentzen, P., Taggart, C.T., Ruzzante, D.E. & Cook, D. (1996) Microsatellite polymorphism and the populations structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 2706–2721.

Billings, W.D. (1950) Vegetation and plant growth as affected by chemically altered rocks in the Great Basin. *Ecology* **31**, 62–74.

Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* **13**, 115–155.

Callaway, R.M., DeLucia, E.H. & Schlesinger, W.H. (1994) Biomass allocation of montane desert ponderosa pine: an analog for response to climate change. *Ecology* **75**, 1474–1481.

Callaway, R.M., DeLucia, E.H., Moore, D., Nowak, R. & Schlesinger, W.H. (1996) Competition and facilitation: contrasting effects of *Artemisia tridentata* on desert vs. montane pines. *Ecology* **77**, 2130–2141.

Carey, E.V., Callaway, R.M. & DeLucia, E.H. (1998) Increased photosynthesis offsets costs of allocation to sapwood in an arid environment. *Ecology* **79**, 2281–2291.

Cordell, S., Goldstein, G., Mueller-Dombois, D., Webb, D. & Vitousek, P.M. (1998) Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia* **113**, 188–196.

DeLucia, E.H. & Schlesinger, W.H. (1990) Ecophysiology of Great Basin and Sierran vegetation on contrasting soils. *Plant Biology of the Basin and Range, Ecological Studies*, Series 80 (eds C. B. Osmond, L. F. Pitelka & G. M. Hidy), pp. 143–178. Springer-Verlag, Berlin.

DeLucia, E.H., Schlesinger, W.H. & Billings, W.D. (1988) Water relations and the maintenance of Sierran conifers on hydrothermally altered rock. *Ecology* **69**, 303–311.

DeLucia, E.H., Maherali, H. & Carey, E.V. (2000) Climate-driven changes in biomass allocation in pines. *Global Change Biology* **6**, 587–593.

Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *BRL Focus* **12**, 13–15.

Echt, C.S. & May-Marquardt, P. (1997) Survey of microsatellite DNA in pine. *Genome* **40**, 9–17.

Echt, C.S., Vendramin, G.G., Nelson, C.D. & Marquardt, P. (1999) Microsatellite DNA as shared genetic markers among conifer species. *Canadian Journal of Forest Research* **29**, 365–371.

Ewers, B.E., Oren, R. & Sperry, J.S. (2000) Influence of nutrient versus water supply on hydraulic architecture and water balance in *Pinus taeda*. *Plant Cell and Environment* **23**, 1055–1066.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

Fisher, P.J., Richardson, T.E. & Gardner, R.C. (1998) Characteristics of single- and multi-copy microsatellites from *Pinus radiata*. *Theoretical and Applied Genetics* **96**, 969–979.

Franks, P.J., Gibson, A. & Bachelard, E.P. (1995) Xylem permeability and embolism susceptibility in seedlings of *Eucalyptus camaldulensis* Dehnh. from two different climatic zones. *Australian Journal of Plant Physiology* **22**, 15–21.

Geber, M.A. & Dawson, T.E. (1993) Evolutionary responses of plants to global change. *Biotic Interactions and Global Change* (eds P. M. Kareiva, J. G. Kingsolver & R. B. Huey), pp. 179–197. Sinauer Associates Inc., Sunderland, MA.

Goldstein, D.B., Linares, A.R., Cavalli-Sforza, L.L. & Feldman, M.W. (1995) An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463–471.

Guo, S.W. & Thompson, E.A. (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–371.

Hacke, U.G., Sperry, J.S., Ewers, B.E., Ellsworth, D.S., Schafer, K.V.R. & Oren, R. (2000) Influence of soil porosity on water use in *Pinus taeda*. *Oecologia* **124**, 495–505.

Hamrick, J.L., Blanton, H.M. & Hamrick, K.J. (1989) Genetic structure of geographically marginal populations of Ponderosa pine. *American Journal of Botany* **76**, 1559–1568.

Hamrick, J.L., Schnabel, A. & Wells, P.V. (1994) Distributions of genetic diversity within and among populations of Great Basin Conifers. *Natural History of the Colorado Plateau and Great Basin* (eds K. T. Harper, L. L. St Clair, K. H. Thorme & W. M. Hess), pp. 147–161. University Press of Colorado, Niwot, CO.

Hedrick, P.W. (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**, 313–318.

Hicks, M., Adams, D., O'Keefe, S., Macdonald, E. & Hodgetts, R. (1998) The development of RAPD and microsatellite markers in lodgepole pine (*Pinus contorta* var. *latifolia*). *Genome* **41**, 797–805.

Hughes, C.R. & Queller, D.C. (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology* **2**, 131–137.

Hutchinson, D.W. & Templeton, A.R. (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**, 1898–1914.

Jorgensen, S.M. & Hamrick, J.L. (1997) Biogeography and population genetics of whitebark pine, *Pinus albicaulis*. *Canadian Journal of Forest Research* **27**, 1574–1585.

Karhu, A., Hurme, P., Karjalainen, M. *et al.* (1996) Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? *Theoretical and Applied Genetics* **93**, 215–221.

Kavanagh, K.L., Bond, B.J., Aitken, S.N., Gartner, B.L. & Knowe, S. (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiology* **19**, 31–37.

Kittel, T.G.F., Rosenbloom, N.A., Painter, T.H., Schimel, D.S. & VEMAP, Participants (1995) The VEMAP Integrated database for modeling United States ecosystem/vegetation sensitivity to climate change. *Journal of Biogeography* **22**, 857–862.

Kramer, P.J. & Boyer, J.S. (1995) *Water Relations of Plants and Soils*. Academic Press, New York, NY.

Latta, R.G. & Mitton, J.B. (1999) Historical separation and present gene flow through a zone of secondary contact in ponderosa pine. *Evolution* **53**, 769–776.

Maherali, H. & DeLucia, E.H. (2000a) Interactive effects of elevated CO_2 and temperature on water transport in ponderosa pine. *American Journal of Botany* **87**, 243–349.

Maherali, H. & DeLucia, E.H. (2000b) Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiology* **20**, 859–867.

Maherali, H. & DeLucia, E.H. (2001) Influence of climate-driven shifts in biomass allocation on water transport and storage in ponderosa pine. *Oecologia* **129**, 481–491.

Maherali, H., DeLucia, E.H. & Sipe, T.W. (1997) Hydraulic adjustment of maple saplings to canopy gap formation. *Oecologia* **112**, 472–480.

Margolis, H., Oren, R., Whitehead, D. & Kaufmann, M.R. (1995) Leaf area dynamics of conifer forests. *Ecophysiology of Conifer Forests* (eds W. K. Smith & T. M. Hinckley), pp. 181–223. Academic Press, New York, NY.

Mencuccini, M. & Bonosi, L. (2001) Leaf/sapwood area ratios in Scots pine show acclimation across Europe. *Canadian Journal of Forest Research* **31**, 442–456.

Mencuccini, M. & Grace, J. (1995) Climate influences the leaf area/sapwood area ratio in Scots pine. *Tree Physiology* **15**, 1–10.

© 2002 British Ecological Society,

Michalakis, Y. & Excoffier, L.A. (1996) A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. *Genetics* **142**, 1061–1064.

Mitton, J.B. (1995) Genetics and the physiological ecology of conifers. *Ecophysiology of Conifer Forests* (eds W. K. Smith & T. M. Hinckley), pp. 1–36. Academic Press, New York, NY.

Nei, M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY.

Niebling, C.R. & Conkle, M.T. (1990) Diversity of Washoe pine and comparison with allozymes of ponderosa pine races. *Canadian Journal of Forest Research* **20**, 298–308.

Neter, J., Wasserman, W. & Kutner, M.H. (1990) *Applied Linear Statistical Models*. Irwin, Homewood, IL.

Potvin, C. (1993) ANOVA: experiments in controlled environments. *Design and Analysis of Ecological Experiments* (eds S. M. Scheiner & J. Gurevitch), pp. 46–68. Chapman & Hall, New York, NY.

Raymond, M. & Rousset, F. (1995) GENEPOP Version 1·2: population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Rehfeldt, G.E. (1979) Ecotypic differentiation in populations of *Pinus monticola* in north Idaho – myth or reality? *American Naturalist* **114**, 627–636.

Rice, W.R. (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Sandquist, D.R. & Ehleringer, J.R. (1997) Intraspecific variation on leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments. *New Phytologist* **135**, 635–644.

Schlesinger, W.H., DeLucia, E.H. & Billings, W.D. (1989) Nutrient-use efficiency of woody plants on contrasting soils in the western Great Basin, Nevada. *Ecology* **70**, 105–113.

Schlichting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution, a Reaction Norm Perspective*. Sinauer Associates Inc., Sunderland. MA.

Schneider, S., Roessli, D. & Excoffier, L. (2000) ARLEQUIN: A software for population genetics data analysis, Version 2·000. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Smith, D.N. & Devey, M.E. (1994) Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genome* **37**, 977–983.

Sokal, R.R. & Rohlf, J. (1995) *Biometry*, 3rd edn. W.H. Freeman, New York. NY.

Soranzo, N., Provan, J. & Powell, W. (1998) Characterization of microsatellite loci in *Pinus sylvestris*. *Molecular Ecology* **7**, 1260–1261.

Sparks, J.P. & Black, R.A. (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiology* **19**, 453–459.

Sperry, J.S. & Pockman, W.T. (1993) Limitations of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant Cell and Environment* **16**, 279–287.

Sperry, J.S., Donnelly, J.R. & Tyree, M.T. (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell and Environment* **11**, 35–40.

Stevens, J. (1992) *Applied Multivariate Statistics for the Social Sciences*. Lawrence Erlbaum, Hillsdale, NJ.

Sultan, S.E. (1987) Evolutionary implications of phenotypic plasticity in plants. *Evolutionary Biology* **21**, 127–178.

Tognetti, R., Michelozzi, M. & Giovanelli, A. (1997) Geographical variation in water relations, hydraulic architecture and turpene composition of Aleppo pine seedlings from Italian provenances. *Tree Physiology* **17**, 241–250.

Tyree, M.T. & Ewers, F.W. (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**, 345–360.

Waring, R.H. & Schlesinger, W.H. (1985) *Forest Ecosystems: Concepts and Management*. Academic Press, New York, NY.

Weir, B.S. & Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Whitehead, D., Edwards, W.R.N. & Jarvis, P.G. (1984) Relationships between conducting sapwood area, foliage area and permeability in mature *Picea stichensis* and *Pinus contorta* trees. *Canadian Journal of Forest Research* **14**, 940–947.

Received 1 October 2001; revised 1 March 2002; accepted 25 March 2002

© 2002 British Ecological Society,