Population divergence for heteroblasty in the Canary Island pine (*Pinus canariensis*, Pinaceae)[1]

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A heteroblastic (or vegetative phase) change is an abrupt manifestation in the general heteroblastic development during the ontogeny of plants. The Canary Island pine undergoes an especially marked and delayed heteroblastic change, including both the formation of secondary needles on dwarf shoots and the onset of preformed growth. To assess genetic and environmental effects on the heteroblastic change in this species, we followed plants from 19 populations at a dry site and a wetter site. Comparing juvenile and adult needles from the same individuals, the adult had a significantly lower rate of water loss and higher leaf mass per area. Pooling data from all seed sources, the heteroblastic change took place when plants reached a critical height, on average, at 4 years of age at the dry site and 1 year earlier at the wet site. Within a subsample of individuals of equal size, mortality was significantly higher in juvenile plants than in mature plants. However, the juvenile phase was longer in plants from dry regions when compared to plants from highly productive, wet regions. This apparent contradiction might be explained through differential resource allocation and the cost of sclerophylly and resprouting ability. Considering the life strategy of the Canary Island pine, we interpret the prolonged juvenile phase as an unavoidable trade-off for the high tolerance of adults to harsh environments.

Key words: Canary Islands; ontogeny; phase change; Pinaceae; provenance trial; survival.

Ontogeny is a key aspect in understanding the evolutionary role of phenotypic differences between organisms (McKinney and McNamara, 1991; Pigliucci, 1997). In plants, the ontogenetic sequence is often associated with morphological differences in leaf characteristics, internode dimensions, and the fate of axillary buds. While the ontogenetic transition is usually gradual, in some plant species the transition is abrupt and distinct, frequently referred to as heteroblastic change or "vegetative phase change," to distinguish it from a "reproductive phase change" (Greenwood, 1995; Jones, 1999). Heteroblasty, that is, the sequence of changes in vegetative metamers along the shoot during ontogeny, has been extensively studied in a few model plants, such as maize and *Arabidopsis* (Pigliucci, 1997; Winn, 1999; Poethig, 2002a, b), but information on the adaptive and evolutionary role of heteroblasty in woody plants is still scarce. Moreover, while ontogeny has been recognized as an important source of variation for functional studies of plants (Thomas and Winner, 2002; Wright and Mcconnaughay, 2002), few works have dealt with ontogenetic differences linked to distinct, heteroblastic morphotypes (Miller et al., 1995; King, 1999; Schreuder et al., 2001). Studies on different *Eucalyptus* species have highlighted the complexity of interpreting the adaptive role of heteroblasty (Jordan et al., 2000) and have shown high heritability for the timing of heteroblastic change and its independence from the timing of the reproductive phase change in this genus (Wiltshire et al., 1998; Jordan et al., 1999).

Although little studied in this respect, *Pinus* provides a good model to broaden our knowledge on the adaptive role of heteroblasty. After the cotyledonary stage, seedlings have only euphylls (solitary needles such as those in the adult shoots of *Pseudotsuga*, *Picea*, or *Abies*). Later, axillary shoots start developing; first, fascicles of juvenile needles (resembling those of *Cedrus* or *Larix*) appear in many species, followed by the distinctive dwarf shoots with clustered secondary needles (Doak, 1935; Lester, 1968). In most pine species, secondary needles are produced from the second vegetative period onward or even during the first growing season. Juvenile needles are bluish, short, and elliptical in shape, while adult secondary needles are dark green and significantly longer and thicker. In addition to the heterophylly (i.e., differences between leaf structures), the heteroblastic change in pines includes an abrupt change from free growth to fixed growth (Lanner, 1976). In the juvenile phase, stem units elongate shortly after their formation without forming terminal buds. By contrast, the adult, fixed growth pattern implies increasing the lapse between the formation of needle primordia and the elongation of stem units (Cannell et al., 1976; Lanner, 1976). This expansion of the period for production of initials in a bud protected with scales and resinous materials has been interpreted as an adaptation to temperate or cold climates occurring in the Tertiary (Klaus, 1989) and as a way to concentrate the entire shoot elongation in a single flush in spring for competing more favorably with herbaceous plants (Cannell et al., 1976). Growth-accelerating conditions, including watering, fertilizing, high temperatures, and long photoperiods have been found to hasten the transition from juvenile to adult foliage and reduce the time to formation of the first terminal bud in pines (Lester, 1968; Lascoux et al., 1993), putatively, when the plant achieves a critical size for undergoing heteroblastic change (Greenwood, 1995).

In a few species within the subgenus *Pinus*, particularly in the Mediterranean *P. pinea*, *P. canariensis*, *P. halepensis*, *P. brutia*, and, to a lesser extent, also in *P. pinaster*, the juvenile growth habit can persist for several years (Klaus, 1989). Particularly, the stone pine (*Pinus pinea*) and the Canary Island
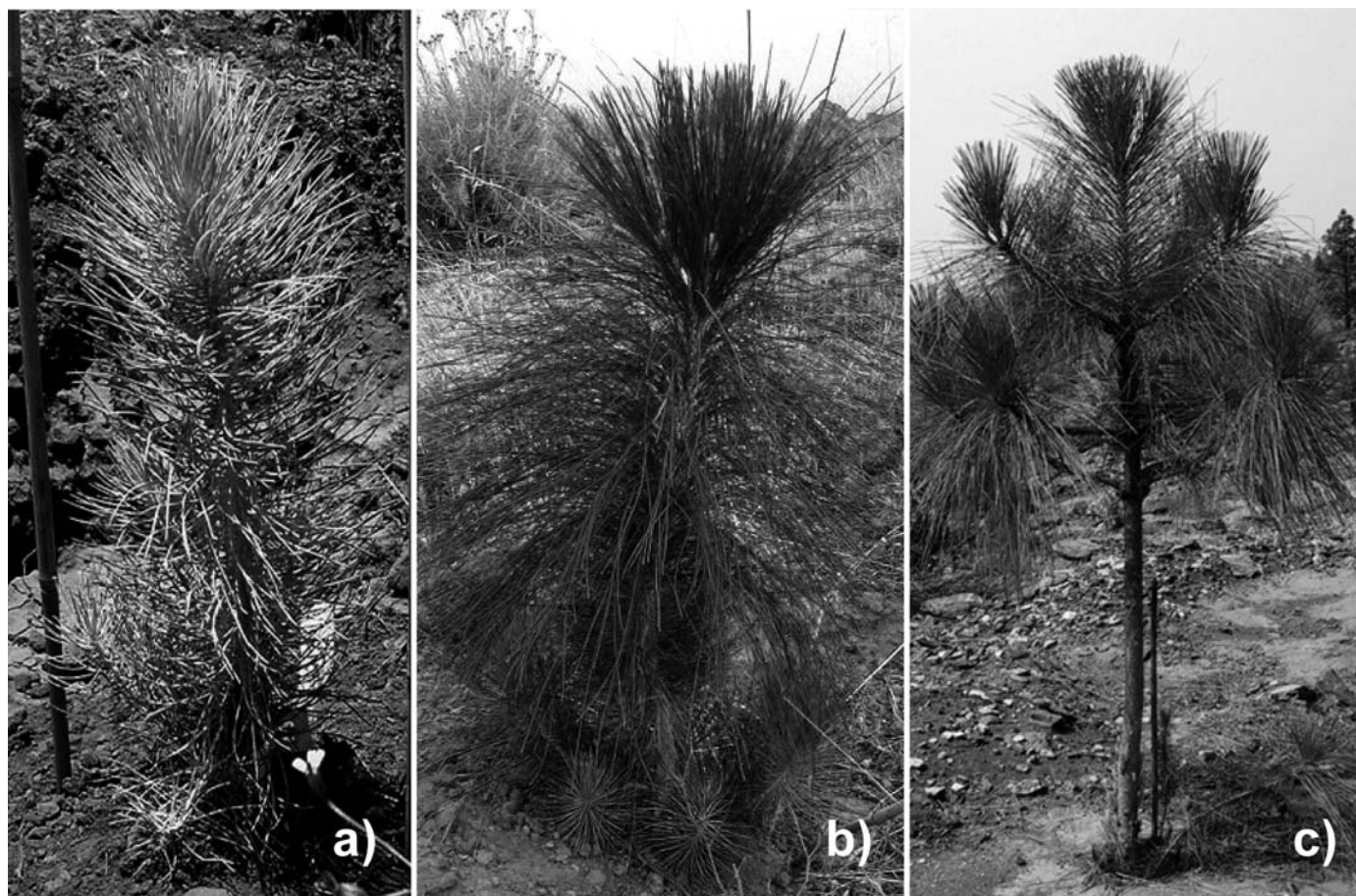
Fig. 1. Juvenile and adult vegetative phases of the Canary Island pine; (a) 3-yr-old juvenile plant (height 35 cm) with characteristic glaucous primary needles ~5 cm long; (b) 3-yr-old plant with similar height but preformed growth and secondary needles ~25 cm long; (c) 5-yr-old plant, 140 cm tall.

pine (*Pinus canariensis* Chr. Sm.) have long-lasting juvenile stages (Lester, 1968) and a marked heterophylly between primary and secondary needles.

The Canary Island pine is a drought-tolerant, fire-resistant endemism of the Canary Archipelago. This pine displays a conspicuous and delayed heteroblastic change (Fig. 1) combined with high environmental variation between natural populations, which may have contributed to ecotypic or clinal intraspecific genetic variation (Climent et al., 2004). Secondary needles have an extensive lignified hypodermis and deeply sunken stomata with a complex spatial structure, which makes them extremely well adapted to avoid water loss when compared to juvenile needles (Jimenez et al., 2000; Zellnig et al., 2001). Therefore, we expected plants before the heteroblastic change to perform differently than plants after heteroblastic change in the field. To test this expectation and to describe intraspecific variation for this characteristic, we conducted a common garden experiment with several natural populations covering the whole geographical and ecological range of the species, planted at two contrasting sites.

Here we describe the morphology of the heteroblastic change in *Pinus canariensis* and address three main questions: (1) Does heteroblastic transition in the Canary Island pine occur in plants of different sizes, or is it solely accelerated or delayed according to the rate of development? (2) Are there genetic differences among populations of *Pinus canariensis* in the age or size of plants for heteroblastic change? (3) Is there

a relationship between environment and the onset of heteroblastic change?

MATERIALS AND METHODS

Plant material—All 19 *P. canariensis* sampled populations (Table 1 and Fig. 2) are well documented to be of natural origin and naturally regenerated through historical records dating from the 16th century. In each population, cones were collected from 25 trees spaced at least 100 m apart. Cones were oven-dried to extract seeds, and within each population, seeds were pooled across parent trees. Sampled populations ranged from semi-arid pine stands, receiving 300 mm of rain per year to wet mixed pine forests with evergreen broadleaves, receiving >1500 mm per year.

Climatic characterization of these is subject to wide errors due to various factors. The network of meteorological stations covers mainly low-altitude areas, and sharp differences in rainfall and temperature occur within short distances due to the steep topography of the islands, resulting in imprecise extrapolations. A third crucial factor is the variable amount of water captured by vegetation from the mist carried by trade winds. This throughfall can account for as much as 2.2 times the incident rainfall in wet pine forests (Aboal et al., 2000). To solve this information gap, we used the average pine sapwood area per hectare (G_s, obtained from a network of 70 plots in mature pine forests) as an indirect site index for each of the eight ecological regions defined for the species (Table 1; Climent et al., 2004). This index is based on the relationship of the water-conducting area of the stem to the general water balance of the tree (Zimmermann, 1964; Waring et al., 1977) and has proven to be highly correlated with other phenotypic features of this species (Climent et al., 2004). Values of G_s ranged from 6.2 m²/ha in the driest region (region 8,

TABLE 1. Location of sampled populations of *Pinus canariensis*, grouped into eight ecological regions, site index values per region (G_s), and mean seed size per region.

Island[a]	Region	G_s (m²/ha)	Seed size (cm²)	Population	Latitude (N)	Longitude (W)	Altitude (m a.s.l.)
Tf	1	26.6	0.56	1	28° 22′	16° 29′	1400
				2	28° 22′	16° 40′	1400
				3	28° 18′	16° 43′	1350
	2	15.9	0.61	4	28° 11′	16° 38′	2100
				6	28° 13′	16° 32′	1450
	3	36.6	0.57	7	28° 23′	16° 25′	1350
				8	28° 25′	16° 23′	1200
LP	4	31.1	0.68	9	28° 44′	17° 50′	1850
				11	28° 47′	17° 55′	1450
	5	14.0	0.63	12	28° 47′	17° 58′	800
				13	28° 40′	17° 57′	950
				15	28° 31′	17° 50′	1200
EH	6	15.4	0.53	16	27° 42′	18° 00′	900
				17	27° 44′	17° 58′	900
GC	7	9.8	0.54	18	28° 03′	15° 41′	1100
				19	28° 01′	15° 42′	950
	8	6.2	0.59	20	27° 56′	15° 44′	950
				21	27° 55′	15° 41′	1000
				22	27° 53′	15° 36′	1000

[a] Tf, Tenerife; LP, La Palma; EH, El Hierro; GC, Gran Canaria.

corresponding to Southern Gran Canaria) to >25 m²/ha in the windward areas of Tenerife and La Palma (regions 1, 3, and 4).

Experimental design and field measurement—To check for maternal effects related to seed size, 20 sound seeds per mother tree were scanned to obtain the mean projected area per population. The seedlings used in the common garden experiment were raised over 12 mo in a nursery in plastic containers with a peat-based fertilized mixture. One-year-old seedlings were planted in December 1999 in hand-opened holes following removal of scrubby vegetation. The two trial sites were located in Tenerife (Canary Islands, Spain) within the range of potential pine forests (Fig. 2). These sites were selected for this study because their soils (andosols) and annual mean temperatures (~13°C) are very similar, but they differed markedly in water availability. The north site (NS, 28°21′ N, 16°36′ W) is located at 1575 m on the windward slope of Teide Mountain (3718 m) and benefits from the mist from October to June. By contrast, the south site (SS, 28°16′ N, 16°29′ W) is located on the leeward slope of Teide Mountain, lacking the mist effect. The regional site index G_s is 26.6 m²/ha for NS and 15.9 m²/ha for SS. At each site, plants were arranged in seven randomized complete blocks in four-plant-squared experimental plots, totalling 28 plants per population, plus a borderline. The effect of differential competition within and between experimental plots was checked using standard indices (Tomé and Burkhart, 1989), and judged as inconsequential; competition throughout the experiment was negligible even at the best site (NS) due to the wide spacing (2 × 3 m) in relation to plant size.

Measurements were taken after planting in the winter of 1999/2000 (age 1 yr) and every autumn until 2004 (age 6 yr). This period covered the entire juvenile period for virtually all plants in the trials. Each year, the height, survival, and ontogenetic score (OS) of every plant were recorded. The ontogenetic score followed a categorical three-level scale (OS0, juvenile; OS1, intermediate; and OS2, adult; Fig. 3). Further analyses were based on height at age 1 (H_1) and height at age 6 (H_6).

To check for differences in sclerophylly between juvenile and adult foliage, residual transpiration (Clarke and Richards, 1988) and needle mass per area unit were assessed at age 6. A first sample of 20 juvenile needles and six dwarf shoots (with three secondary needles each) was collected at neighboring stem sections from each of 6–10 individuals of 13 populations covering all eight ecological regions at both sites, NS and SS. Needle projected area was obtained with a scanner. The images were analyzed using Scion image for Windows (Scion corporation, Frederick, Maryland, USA). Needle samples were then dried at 50°C for 5 d to determine leaf dry mass and leaf mass per unit area was calculated (in grams per square centimeter). A second sample of excised needles was used to estimate residual transpiration through the rate of water loss (RWL) following a protocol modified from Clarke and McCaig (1982). Adult and juvenile needles of 20 individuals from 10 populations were water-saturated over 1 day and weighed for saturated mass. Needles were placed in an illuminated room at 28°C and 30% relative humidity and weighed at 15-min intervals during the first 135 min and at 30-min intervals during the next 300 min to obtain a curve of water loss. To avoid the curvilinear response at the beginning (due to stomata closure) and the slope change at the end of the experiment, only data between 45 and 200 min were included in the analysis (McCaig and Romagosa, 1991). The rate of water loss (RWL) was estimated as the slope of the linear regression of cumulative water loss over time.

Data analysis—Differences between juvenile and adult foliage were assessed by comparing the linear regression models of relative needle mass vs.
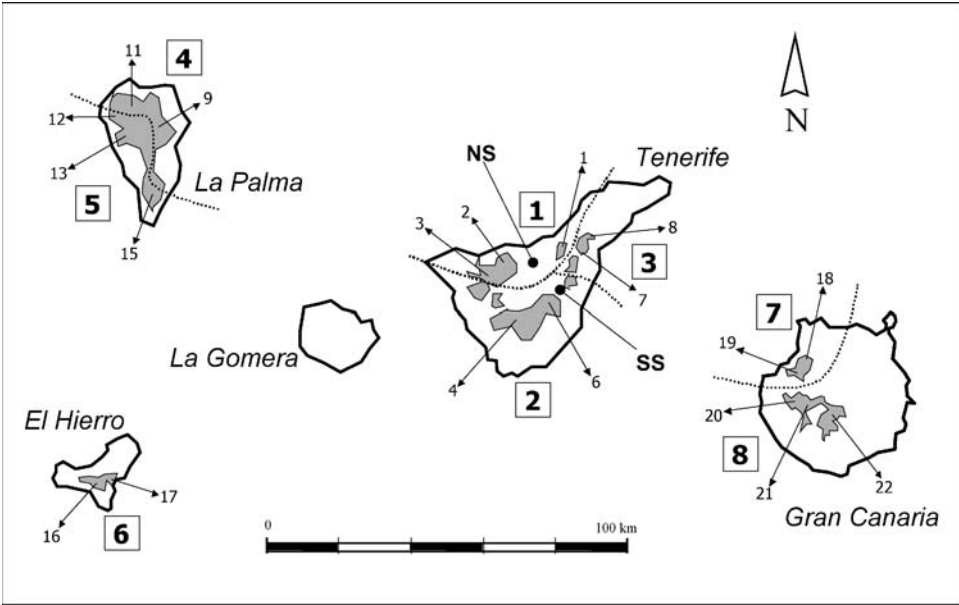

Fig. 2. Range map of the Canary Island pine at the Canary Archipelago (gray areas) with the location of sampled populations (small numbers) and trial sites (northern, wet site [NS] and southern, dry site [SS]) . Boxed numbers and dotted lines represent the ecological regions and their artificial boundaries, respectively.
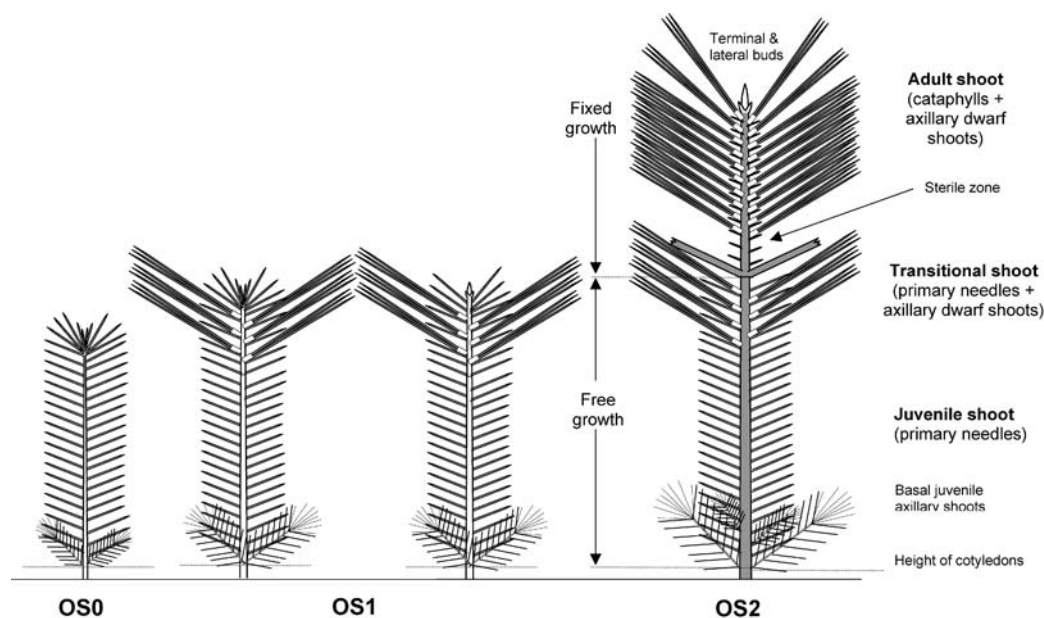
Fig. 3. Ontogenetic score to describe heteroblastic change in *Pinus canariensis*. The juvenile stage (OS0) corresponds to the free shoot growth, crowned by a rosette of primary needles. The intermediate stage (OS1) is characterized by the formation of axillary dwarf shoots with secondary needles at the upper nodes (transitional shoot) until the first formation of a cataphyllary terminal bud. The adult stage (OS2) is characterized by the subsequent growth of the first cycle with exclusively secondary needles (dwarf shoots at the axils of cataphylls). The presence of lateral branches (first whorl) was not necessarily coincident with the first cycle of fixed growth. Basal juvenile shoots were present in all plants.

time and those of needle dry mass vs. needle area. The influence of plant age and height on heteroblastic change (HC) was analyzed at the site level (pooling for populations and replicates) using logistic probability models. For this analysis, every observation for a given plant (i.e., every combination of age, height, and ontogenetic score at each measurement) was used as an independent data point, thus considerably enlarging the sample size. We used the following logistic function:

$$OS = 2e^{(a+bx)}\left(1 + e^{(a+bx)}\right)^{-1}, \qquad (1)$$

where OS had the values 0, 1, or 2 and x was alternatively age or height at the previous year. The adjusted inflexion point at the percentile 50 ($x = -a/b$, representing the age or size of maximum change) and the percentile 95 (a proxi to HC completion) were compared between sites through 95% confidence intervals. In addition, the slope of the curve at the inflexion point (b) was also determined to assess differences in the rate of change of the heterochronous process (Alberch et al., 1979).

The relationship between heteroblasty and survival was analyzed through a demographic analysis. For this purpose, each plant at each measuring date was classified into one of three categories: juvenile (J, corresponding to OS0), adult (A, pooling OS1 and OS2 because of their identical behavior facing survival), or dead (D). Between consecutive observations, the possible transitions between categories were either heteroblastic change (abbreviated JA) or death either at the juvenile (JD) or adult stage (AD). Plants conserving their juvenile or adult status were identified as JJ and AA, respectively. Rejuvenation (AJ) and the recovery of apparently dead plants (DJ) occurred at a negligible frequency; hence we preferred to consider those cases as observation errors and excluded them from the analyses. The frequencies obtained were cross-tabulated to build a transition matrix, showing the probabilities of a plant at a given year remaining in the same state, changing it, or dying over the following year (Markov chain). Plant height was compared between demographic groups to determine the relationship among plant size, heteroblasty, and survival each measuring year.

To separate the effects of plant size and heteroblasty on survival, we searched for series of plants with the same height (disregarding plant age) but different ontogenetic stage within each population. A series was considered only if any mortality was observed over the following year. This subsample consisted of 26 series totalling 188 plants and including all ecological regions. Survival between juveniles and adults was compared using Student's *t* test of angular-transformed frequencies per series. All analyses were performed with

statistical package Statgraphics Plus, version 5.1 (StatPoint, Inc., Herndon, Virginia, USA).

To separate environmental and genetic influences on heteroblasty, two variables, height (Hh) and time (Th) until heteroblastic change, were defined, respectively, as the height and age the year before the heteroblastic change for each plant. A GLM approach to ANOVA with type III sum of squares was conducted for Hh, Th, and the height at age 6, H_6, using the following model:

$$Y = \mu + S + B(S) + R + P(R) + R \times S + \xi \cdot H1 + \varepsilon, \qquad (2)$$

with Y being the response variable, μ the general mean, S the deviation due to the site, $B(S)$ the effect of the block nested within site, R the effect of the region, $P(R)$ the effect of the population within each region, $R \times S$ the interaction between region and site, and ε the residual term, thus comprising individual variation and experimental error. Initial height (H_1) was included as a covariate in the analysis of Th and H_6 to avoid biases caused by nursery effects. Site, region and population were considered fixed effects while block was considered a random factor. Comparisons among regions were done through multiple range tests with Tukey honestly significant difference (HSD) intervals. The differential site effect on heteroblasty for each region was checked graphically through environment–environment biplots (Pigliucci and Schlichting, 1996) using the confidence intervals obtained from generalized linear modeling (GLM) analysis.

RESULTS

Comparison between juvenile and adult needles—The rate of water loss was significantly lower for secondary needles than for primary needles (Fig. 4a). Furthermore, leaf mass per area was significantly higher in secondary needles than in primary needles (0.13 and 0.07 g/cm^2, respectively, Fig. 4b), and this difference was consistent for all eight ecological regions analyzed. The observed needle life span was similar between juvenile and adult foliage (2–3 yr, data not shown).

Relationship between heteroblasty and plant age, size, and survival (data from all seed sources combined)—Survival and growth in height differed sharply between the wet northern site
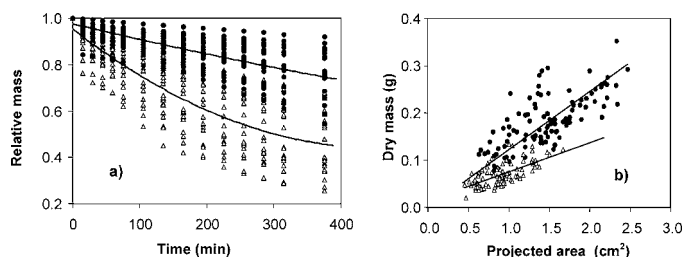
Fig. 4. Comparison of primary needles (open triangles) and secondary needles (closed circles). (a) Water loss curves. (b) Needle mass per area unit. Comparisons for rate of water loss were based on the slopes of linear regressions between 45 and 200 min.
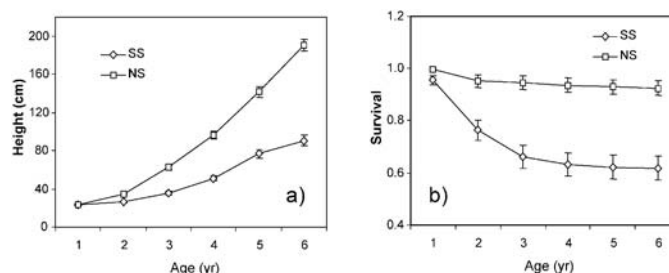

Fig. 5. Performance of *Pinus canariensis* plants at the wet, northern site (NS) and dry, southern sites (SS). (a) Height growth. (b) Survival. Bars correspond to 95% confidence intervals from mean values per four-plant experimental plot.

(NS) and the dry southern site (SS) during the study (Fig. 5a, b). At the NS, more plants survived and grew taller than at the SS. The logistic model for ontogenetic score (OS) with age as the independent variable and site as the sole categorical factor explained 76% of the observed deviance. This model showed that the onset and rate of variation of the heteroblastic change differed sharply between both sites, with earlier and faster progress at the NS (Fig. 6a). The age of maximum change (inflexion point) was ~1 yr less at NS, 2.3 yr compared to SS, 3.4 yr (significant at $P < 0.001$). Since the rate of change (slope at the inflexion point) was higher for the NS (2.2) than for the SS (1.6), a wide difference was also observed for HC completion (both significant at $P < 0.001$): 3.6 yr at the NS vs. 5.3 yr at the SS. However, when OS was regressed against plant height, logistic regressions for both sites were far more similar (Fig. 6b). This model explained 80% of the deviance and gave inflexion points of 42.8 cm at the NS and 44.2 cm at the SS, not significantly different ($P > 0.05$) while b at the NS was 0.104 vs. 0.139 at the SS ($P < 0.05$).

Demographic analysis was done only at the dry site, SS, whilst at the wet site (NS), the probability of death for juvenile and adult plants was similarly very low (<3%, data not shown). The most frequent case at the SS (Fig. 7) was that mature plants conserved their status (AA) at all observation dates. Also, juvenile plants tended to maintain their condition (JJ) at years 1 and 2 and to undergo the heteroblastic change by age 3 or 4 yr. While both juvenile and adult mortality decreased steadily from year to year, survival was always higher for adult plants (AD transition) than for juvenile plants (JD transition). The analysis of the subsample of plants of the same height confirmed a highly significant effect ($P < 0.001$) of the ontogenetic stage on survival independently from plant size, with mean survival probabilities of 0.90 for adults vs. 0.73 for juveniles. However, in the whole data set, both JA and AA plants were significantly taller than JJ or JD plants at all five measuring dates (Fig. 8). By contrast, JD plants were not significantly smaller than JJ plants. AD plants were too scarce to attempt any inference on the relationship between their size and survival.

Intraspecific variation in heteroblastic change—The effect of site was significant for Th and H_6 but not for Hh (Table 2). Site accounted for 26% of the variation of Th and as much as 54% of the variation for total height. Both region and population within region were significant for all three variables ($P < 0.005$), with an especially high contribution of the region to the total variance for Hh. The site × region interaction was significant for Hh and H_6 ($P < 0.05$), indicating the existence

of genetic differences among ecological regions for the effect of environment on growth and heteroblastic change, especially marked for Hh (Fig. 9). Plants from the wet regions 1, 3, and 4 had significantly higher values of Hh at the dry site (SS), while the other five regions were rather similar at both sites. On the other hand, the divergence among regions was much more evident in the wet site (NS) (Fig. 9).

No geographical or altitudinal trends were found for any of the considered traits. By contrast, a strong clinal relationship was found between the indirect site index G_s and ontogeny of the plants at the NS, estimated through both Hh and Th, and their survival at the SS (Fig. 10). Regions with higher G_s had a significantly more precocious heteroblastic change ($P < 0.01$) both in age and plant size at the NS and significantly lower survival at the SS. No relationship was found between final plant height (H_6) and regional ecology using any of the mentioned variables. In addition, no relationship was observed between mean seed size per region and their survival or expressed phenotype (Hh, Th, H_6).

DISCUSSION

Heteroblasty in Pinus canariensis: relationship to plant age, size, and survival—Under the conditions of this experiment, we observed that plants of the Canary Island pine took from 2 to as much as 6 years or more (three plants still remained juvenile at the end of the experiment) before undergoing a heteroblastic change in the main shoot. We have not found previous references to such a prolonged juvenile stage in pines, apart from the "grass-stage" characteristic of some subtropical *Diploxylon* species (Kossuth, 1981; Keeley
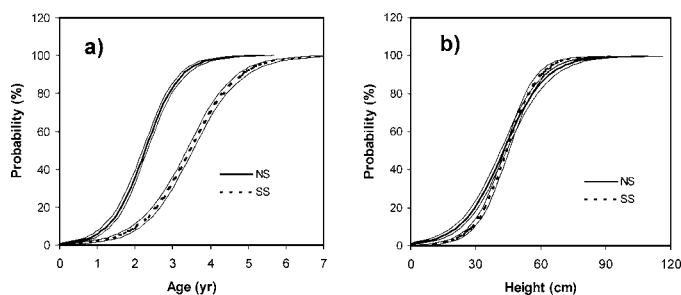

Fig. 6. Adjusted logistic functions for the probability of heteroblastic change in *Pinus canariensis* per trial site. (a) Versus plant age. (b) Versus plant height. Bars represent 95% confidence intervals.
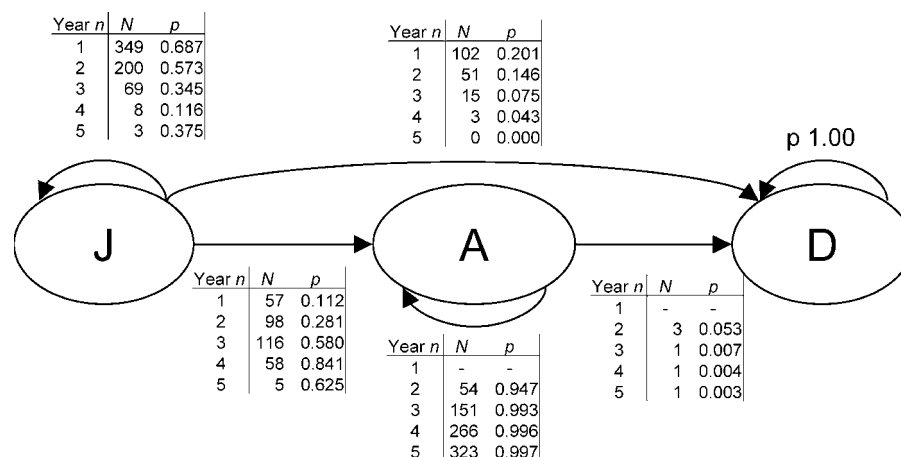
Year *n*	*N*	*p*
1	349	0.687
2	200	0.573
3	69	0.345
4	8	0.116
5	3	0.375

Year *n*	*N*	*p*
1	102	0.201
2	51	0.146
3	15	0.075
4	3	0.043
5	0	0.000

p 1.00

Year *n*	*N*	*p*
1	57	0.112
2	98	0.281
3	116	0.580
4	58	0.841
5	5	0.625

Year *n*	*N*	*p*
1	-	-
2	54	0.947
3	151	0.993
4	266	0.996
5	323	0.997

Year *n*	*N*	*p*
1	-	-
2	3	0.053
3	1	0.007
4	1	0.004
5	1	0.003

Fig. 7.   Markov chain showing transitions among states from year *n* to year *n* + 1 for *Pinus canariensis* plants at the dry site, showing absolute (*N*) and relative frequencies (*p*) per row subtotals. A, adult, OS1 and OS2; J, juvenile, OS0; D, dead.

and Zedler, 1998; Koskela, 2000). However, it is important to note that the grass stage is characterized by the lack of elongation of otherwise adult metamers, while juvenile Canary Island pine bears exclusively primary needles. In addition to the variation in the timing of the vegetative phase change, which could be interpreted as deriving merely from an accelerated or delayed development, we can respond affirmatively to the existence of genetic variability in the heteroblastic process of *P. canariensis*, in parallel with findings in *Eucalyptus globulus* (Jordan et al., 2000).

Juvenile and adult leaves of *P. canariensis* diverge in numerous morphological features whose functional relevance merits further research. As formerly postulated from anatomical attributes (Jimenez et al., 2000; Zellnig et al., 2001), our results confirmed the higher leaf mass per area and resistance to water loss of secondary needles. In fact, this increase in leaf sclerophylly along the ontogenetic sequence in the Canary Island pine seems an abrupt manifestation of a well-established general trend in trees (Greenwood, 1995; Miller et al., 1995; Steinbauer, 2001; Thomas and Winner, 2002).

The results of the demographic analysis together with the analysis of a series of plants of the same size suggest a direct effect of heteroblasty on survival, independent of plant size in *P. canariensis*, with significantly higher survival in adult plants under dry conditions. Even when it is clear, both from the logistic analysis and from size comparisons, that most plants undergo a phase change when they reach a critical size (~43 cm), our results point out that in this species the ontogenetic status might be a better indicator of plant vigor than plant size per se (V. Luis, unpublished manuscript).

Environmental and genetic effects on heteroblasty in P. canariensis—The time needed to reach vegetative maturity was significantly affected by the environment such that at the dry site the juvenile stage lasted, on average, about 1 year more than at the wet site. This is congruent with the idea that limitations to carbon gain elicit the maintenance of the juvenile condition, while treatments favoring growth tend to hasten maturation (Lascoux et al., 1993; Poethig, 2002b; Williams et al., 2004). However, the environmental effect on heteroblasty was far less marked when this process was observed on a plant height basis. We found significant genetic divergence among ecological regions, such that plants from less productive regions underwent heteroblastic change later and at a taller size compared to plants from more productive, wetter areas. On the other hand, seeds from highly productive regions (1, 3 and 4) yielded significantly lower survival at the dry site compared to the intermediate and less productive regions. The existence of genetic differences for heteroblasty among seed sources of *Pinus canariensis* is coherent with the low gene flux among populations detected within the bigger Islands (Gomez et al., 2003).

The interpretation of the significant genotype × environment interaction for the height to heteroblastic change deserves special attention. One possible explanation is that different levels of phenotypic plasticity for heteroblasty have been selected in resource-rich regions and resource-poor regions (Fig. 9). This is congruent with the idea that plasticity is selected when the environment experienced by individuals during their lifetime or from one generation to the next is heterogeneous (Van Tienderen, 1991). In fact, individuals from favorable regions of the Canary Island pine must adapt to drastic environmental changes during their long life span, ranging from strong competition with understory species in early years to the dominant position of adult trees. The steep
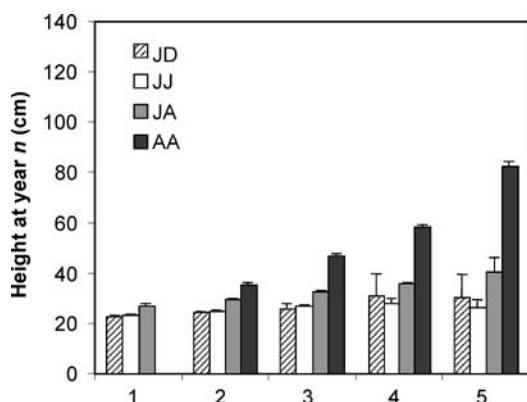

Fig. 8.   Comparison of height of *Pinus canariensis* at year *n* (means + SE) among transition classes from year *n* to *n* + 1: juveniles that died (JD), plants that stayed juvenile (JJ), juveniles that became adult (JA), and adult plants remaining alive (AA).

TABLE 2. Results of generalized linear modeling (GLM) analysis for the time and height until the heteroblastic change (Th and Hh, respectively) and height at six years (H_6), including a proxy to the percentage of the variation explained by each factor calculated with the sums of squares (SS).

Variable	Source	SS	df	MS	F	P	Explained variation (%)
Th	Site	291	1	290.5	79.34	0.0000	24.9
	Block(Site)	47	12	3.9	4.27	0.0000	4.0
	Region	60	7	8.5	9.25	0.0000	5.1
	Population(Region)	24	11	2.2	2.35	0.0076	2.0
	Site × Region	12	7	1.8	1.91	0.0649	1.1
	H_1	45	1	45.0	48.88	0.0000	3.9
	Residual	683	741	0.9			58.6
	Total	1166	780				
Hh	Site	776	1	775.5	2.34	0.1507	0.7
	Block(Site)	4250	12	354.2	3.22	0.0002	4.1
	Region	10190	7	1455.7	13.23	0.0000	9.8
	Population (Region)	3213	11	292.1	2.65	0.0024	3.1
	Site × Region	2310	7	329.9	3.00	0.0041	2.2
	Residual	81 324	739	110.0			78.6
	Total	103 474	777				
H_6	Site	1 649 720	1	1 649 720.0	148.43	0.0000	53.1
	Block(Site)	144 773	12	12 064.5	8.12	0.0000	4.7
	Region	34 065	7	4866.4	3.27	0.0020	1.1
	Population(Region)	53 489	11	4862.6	3.27	0.0002	1.7
	Site × Region	23 058	7	3293.9	2.22	0.0312	0.7
	H_1	38 717	1	38 716.7	26.05	0.0000	1.2
	Residual	1 088 110	732	1486.5			35.0
	Total	3 106 760	771				

Note: MS, mean square

slopes prevalent in northern regions may also contribute to the sharp microenvironmental differences to which closely related genotypes would be exposed from one generation to the next. In this scenario, plasticity may help individuals to benefit from enhanced local conditions. On the contrary, the lack of plasticity of the unfavorable, less productive regions is coherent with the high evolutionary costs of stress tolerance (Lortie and Aarssen, 1996). However, if, as the results at the regional level suggest, traits conferring drought resistance are genetically correlated with a delayed phase change, the high mortality of seed sources from wetter regions at the drier site might have caused a directional selection favoring drought-tolerant, less precocious genotypes from those regions. If this is the case, the observed genotype × environment interaction may simply reflect the difference in the genotypes being evaluated at both sites.

Nongenetic differences associated with environmental conditions faced during seed development (carryover effects,
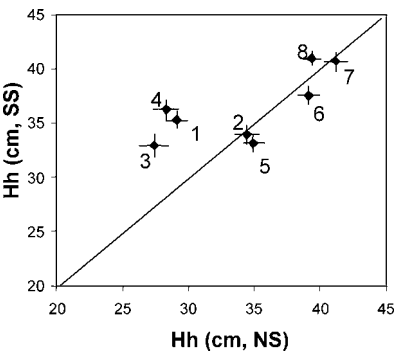

Fig. 9. Plot of mean values for the height of heteroblastic change (Hh) for plants of *Pinus canariensis* from each ecological region (1–8) at the wet (northern site, NS) and dry (southern site, SS) trial sites.

after-effects) have been found in some forest species to inflate estimates of genetic variation (Lopez et al., 2003). However, we found no relationship between seed size and heteroblasty or survival per population or ecological region, which allows us to discard at least this source of maternal effects in our experiment.

Is heteroblasty in the Canary Island pine adaptive?—In the light of our results, the adaptive interpretation of heteroblasty in the Canary Island pine is nontrivial and somewhat counterintuitive. While, at the individual level, plants with adult foliage were found to be better adapted to drought, drought-adapted populations had a longer delay until the heteroblastic change.

So, if the adult phenotype is better, why don't plants from dry environments undergo heteroblastic change earlier? Is there any advantage in maintaining the juvenile condition in stressful environments? The response to the first question may be simply because they can't. The construction of evergreen sclerophyllous foliage involves high energetic costs (Poorter and Villar, 1997; Villar and Merino, 2001); hence an early formation of adult, drought-resistant leaves may be unafford-able under environmental restrictions, as postulated for the heteroblastic, drought-tolerant conifer *Juniperus occidentalis* (Miller et al., 1995). Furthermore, the maintenance of a longer juvenile phase may be the only way to construct adult individuals with high survivability through differential resource allocation, and throughout the entire life cycle of the species, is probably adaptive. The Canary Island pine life history is characterized by a long life span, postfire resprouting ability, delayed sexual maturity, and low fecundity (Climent et al., 2004). All these features indicate a tendency to the *K* reproductive strategy and imply a need to allocate a large quantity of resources to maintenance and survival (Wilson, 1983), for example, producing a deep taproot and thick bark and accumulating great amounts of starch in the xylem
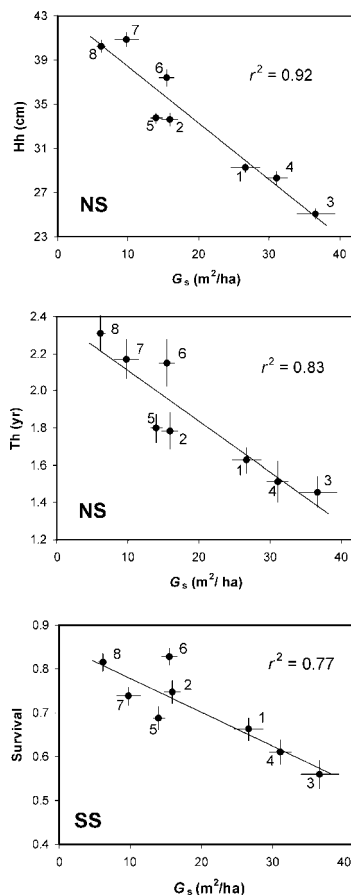
Fig. 10.   Correlations of the height and age of transition to the adult phase (Hh and Th, respectively) of *Pinus canariensis* at the wet site and survival at the dry, northern site (NS) with site quality (G_s), per ecological region. Bars correspond to standard errors among individual trees for Hh and Th, among four-tree experimental plots for survival. G_s error bars are derived from 6 to 13 values (sampling plots) per region. Numbers within the data fields correspond to ecological regions.

(Climent et al., 1998, 2004). We can see similarities between the Canary Island pine's life strategy and that of lignotuberous species, including grass-stage pines, that may indicate parallel adaptations to a combination of predictable fires with low site productivity (Keeley and Zedler, 1998). The energetic cost of storing and protecting stored materials (water and carbohydrates) leads to a loss of juvenile competitiveness, i.e., growth and survival potential, as seen when comparing resprouting and non-resprouting species in Mediterranean-type fire-prone ecosystems (Bell, 2001; Pausas et al., 2004).

In addition, shoot preformation occurring in the adult shoot implies the uncoupling of metamers formation within the winter bud and their elongation in the following spring (Cannell et al., 1976; Jones and Watson, 2001). Preformation severely limits morphological responses to environmental restrictions during the growing season, with the exception of abortion of preformed organs (Diggle, 1997). This can be too risky for young plants until they are able to develop a deep root system and to store enough water and carbohydrates.

We interpret the long juvenile phase observed in *P. canariensis* as the unavoidable trade-off of constructing adult trees highly tolerant to stress and capable to resprout and hence

attaining high reproductive success under the severe environmental constraints (drought, fire, or both) prevailing in most of its natural habitat. Further experiments will be necessary to unveil the precise role of heteroblasty in the ontogenetic shifts of biomass allocation and functional traits and the relevance of ontogenetic plasticity to the early performance of the Canary Island pine and other related taxa.

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