Leaf heteroblasty is not an adaptation to shade: seedling anatomical and physiological responses to light

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Abstract: Heteroblastic plants produce markedly different leaf morphologies between juvenile and adult stages, while homoblastic plants exhibit little or gradual changes. We tested the hypothesis that the leaf morphology of the seedling stage of New Zealand heteroblastic species is advantageous in dealing with low light levels found in forest understorey. We used four independent contrasts of heteroblastic and homoblastic seedlings from the genera *Aristotelia*, *Hoheria*, *Pseudopanax*, and *Melicope* grown in full-sun (100% sunlight) and shade (5% sunlight) light environments in a glasshouse. The four heteroblastic species had consistently smaller leaves and lower specific leaf area than their paired homoblastic species both in sun and shade. In the shade, there were no consistent differences in leaf anatomy (thickness of leaf blade, cuticle, epidermis, and palisade mesophyll, and stomatal density × stomatal aperture length) or physiology (maximum photosynthetic rate, dark respiration, and light compensation point) between homoblastic and heteroblastic species. However, in the sun, heteroblastic *A. fruticosa*, *P. crassifolius*, and *M. simplex* had appreciably thicker leaf blades as well as higher maximum photosynthetic rates than their homoblastic congeners. These traits suggest heteroblastic seedlings possess leaf traits associated with an advantage in high-light environments. We conclude that the heteroblastic seedling leaf morphology is unlikely to be an adaptation to very low light. Alternative explanations for the functional significance of changing leaf morphology in association with life-stage should be sought.

Keywords: full sun, homoblasty, leaf anatomy, morphology

Introduction

Heteroblastic plants exhibit dramatic differences in leaf morphology between juvenile and adult stages, whereas homoblastic plants have only gradual or slight ontogenetic changes in leaf size and shape (Goebel 1900). Despite the many studies that have examined potential hypotheses for the evolution of this syndrome, no clear evidence has emerged. In New Zealand, at least 40 species in 17 families have dramatic differences in leaf shape or morphology between seedling and adult stages (Gamage 2004). This high incidence has led to suggestions that heteroblasty may have been advantageous during particular climatic regimes (in particular, the dry soil conditions that occurred during the Pleistocene period; Cockayne 1912; McGlone & Webb 1981), or in reducing herbivory by ratites and other flightless birds (Greenwood & Atkinson 1977; Atkinson & Greenwood 1989). However, changing phenotype is also a strategy used by plants to respond to predictable environmental variation (Strauss-Debenedetti & Bazzaz 1991; Strauss-Debenedetti & Berlyn 1994; Valladares 2000, 2002; Jones 2001) and here we examine the hypothesis that heteroblasty could be advantageous in dealing with the vertical light gradients found in forests.

Many studies have demonstrated that the light environment can influence the leaf form of heteroblastic plants (Ashby 1948; Njoku 1956; Cameron 1970; Jones 1995; Day 1998; James & Bell 2000). For example, Lee and Richards (1991) found that juvenile vines with entire leaves occur in humid shady conditions, while adults with lobed leaves occur in sunny and drier environments. In New Zealand, an ontogenetic study of leaf anatomy and morphology of heteroblastic *Pseudopanax crassifolius* showed that seedling leaves are anatomically comparable to the leaves of many shade plants relative to their adult or juvenile leaves (Gould 1993). The architectural self-shading in divaricate heteroblastic plants (e.g. *Aristotelia fruticosa*) may maximise carbon fixation of inner leaves by protecting against photoinhibition (Howell et al. 2002). These findings suggest that the light environment can influence the leaf form, anatomy, and physiology

of some heteroblastic plants, but it is not known how general these findings are throughout the New Zealand heteroblastic flora.

Here, we tested the prediction that at the seedling stage, heteroblastic seedlings would be more shade tolerant than congeneric homoblastic species. Four pairs of common native New Zealand woody plants (homoblastic: *Hoheria lyallii, Aristotelia serrata, Pseudopanax arboreus,* and *Melicope ternata*, heteroblastic: *H. sexstylosa*, *A. fruticosa, P. crassifolius*, and *M. simplex*) that can be found in lowland to subalpine forests were used in this experiment. We used independent contrasts of homoblastic and heteroblastic species to test for consistent differences in morphological, anatomical, and physiological leaf traits in seedlings grown in sun and shade environments.

Materials and methods

Study species

To enable independent contrasts, four pairs of congeneric homoblastic and heteroblastic species were selected across separate families: *Hoheria* (Malvaceae), *Aristotelia* (Elaeocarpaceae), *Pseudopanax* (Araliaceae), and *Melicope* (Rutaceae). The homoblastic and heteroblastic species used for the experiment were all common native New Zealand woody shrubs or trees and were chosen for their seed and seedling availability during the study period. See Table 1 for a comparison of their distribution, habit, and leaf morphology.

Controlled environmental shelters

Four enclosures were constructed to simulate forest understorey (i.e. 5% of full sunlight, such as might be experienced by seedlings under a *Kunzea* canopy) and full sunlight. Each enclosure had a wooden frame 80 × 215 × 120 cm (w × l × h). Light quality in the two shade enclosures was altered to Red:Far-Red (R:FR) = 0.25 by covering the wooden frames with dye-impregnated films (PANTHER 20). In addition, three layers of shade cloth were used to cover the enclosures. The other two enclosures were covered with clear polythene so that light quality was unaltered (R:FR = 1.25). The enclosures were provided with adequate ventilation by means of electrical fans. On sunny days during summer (November–January), the averaged (± SE) maximum amount of photosynthetic photon flux density (PPFD) recorded in shade and full-sun enclosures was 51 ± 6 and 1636 ± 102 μmol m^{-2} s^{-1}, respectively. The averaged maximum air temperature was 18.2 ± 1.6 ºC and 26.4 ± 2.1 ºC, respectively. Light intensity and air temperature measures taken in an open area outside the glasshouse were 2027 ± 112 μmol m^{-2} s^{-1} and 25.5 ± 3.3 ºC.

Experimental design

One-year-old seedlings of *Hoheria* and *Aristotelia* (both homoblastic and heteroblastic species), approximately 20–25 cm in height, were planted in 2-L circular polybags filled with potting mix (a blend of compost, bark, pumice, trace elements, and Osmocote® slow-release fertiliser) in 2001. For *Pseudopanax* and *Melicope*, seeds were collected

Table 1. Habit and leaf characteristics of species used to examine light response.

Species name	Syndrome	Habit	Distribution	Seedling leaf morphology	Adult leaf morphology
Hoheria lyallii	Homoblastic	Tree	Montane and subalpine forests	Simple, 1–3 cm long × 1–3 cm wide, deeply crenate margins	Simple, 5–14 cm long × 2–10 cm wide, deeply crenate margins
Hoheria sexstylosa	Heteroblastic	Tree	Lowland forests	Small (1–1.5 cm long × 1–1.5 cm wide), lobed	Large (5–15 cm long × 2–5 cm wide), serrate margin
Aristotelia serrata	Homoblastic	Tree	Lowland–montane forests	Simple, with serrate margin	Simple, with serrate margin
Aristotelia fruticosa	Heteroblastic	Shrub	Lowland–alpine forests	Deeply toothed or lobed	Simple, 5–10 mm long × 4–5 mm wide, serrate margin
Pseudopanax arboreus	Homoblastic	Tree	Forests, open shrubland	Compound leaves	Compound leaves
Pseudopanax crassifolius	Heteroblastic	Tree	Lowland, montane or regenerating forest wide),	Long and narrow (1 m long × 1 cm sharply pointed marginal teeth	Shorter and broader (10–20 cm long × 2–3 cm wide), toothed or smooth margin
Melicope ternata	Homoblastic	Small tree	Lowland forests and margins	Trifoliate	Trifoliate
Melicope simplex	Heteroblastic	Shrub	Lowland forests and shrubland	Trifoliate	Simple

from five different parent trees and were germinated in a partially shaded glasshouse. In 2002, 3-month-old seedlings (5–10 cm in height) were planted in polybags and transferred to the environmental shelters. Due to lack of space in the glasshouses, *Hoheria* and *Aristotelia* were grown in 2001, while *Pseudopanax* and *Melicope* were grown in 2002. There were 12 seedlings per species in each light treatment with the exception of *Melicope*; due to the poor germination of *M. simplex*, only six pairs were planted in each light treatment. In each enclosure, seedlings were arranged in two blocks (six replicates of seedlings per species within a block) in regular lines at 20×20-cm spacing between bag centres. Seedlings were well watered during the experiment and the polybags had adequate drainage. After 9 months, measurements were taken of the leaf physiology, anatomy, and morphology of the seedlings.

Leaf physiology

Photosynthetic carbon gain was assessed from measurements of light response curves taken using a LI-6400 infrared-gas analyser (Licor Inc., Lincoln, Nebraska, USA) on six seedlings for each species. From each seedling a fully expanded, undamaged, newly formed mature leaf was selected for the measurements. Leaf temperature was maintained 25 ± 1 ºC and water vapour pressure deficit was 1.0–1.2 kPa during the photosynthesis measurements. Reference CO_2 was maintained at 370 ± 1 μmol mol^{-1} and relative humidity at 55 ± 5 %. The leaf was darkened for 15–20 min initially to obtain a reading of dark respiration.

Light response curves for full-sun leaves were measured at 14 PPFD levels starting from 1600 μmol m^{-2} s^{-1}, while shade leaves were measured starting from 1200 μmol m^{-2} s^{-1}, because a decrease in photosynthetic rate was observed above 1200 μmol m^{-2} s^{-1} light intensity during test measurements (i.e. apparent photoinhibition). Leaves were kept for 10–15 min in each light level until the photosynthetic rate was stable. All measurements were made during summer, and commenced at 0800 hours and lasted till 1400 hours. For plants with leaves smaller than the leaf cuvette, the leaf was marked, and its leaf area was measured using the leaf area meter (LI-320, Licor Inc., Lincoln, Nebraska, USA), when plants were destructively sampled. Photosynthesis was recalculated for those seedlings.

Maximum light-saturated photosynthesis was calculated as the mean of all points beyond which photosynthesis was saturated. Dark respiration was the mean of respiration rates obtained when the leaves were maintained in the dark. Apparent quantum yield was estimated from photosynthetic light response data using linear regression across three points where net CO_2 assimilation was linearly related to PPFD. The light compensation point (LCP) at which the net photosynthesis was zero was obtained from the light response curve.

Leaf anatomy

Seedling leaves that were used for physiological measurements were subsequently fixed for leaf anatomy analysis. To determine cell dimensions, 1×0.5-cm cross sections were taken across the midrib, and immediately fixed in 70% FAA (formalin:acetic acid:alcohol = 5:5:90). The strips were dehydrated in an ethyl alcohol series and then embedded in separate wax blocks. Cross sections were cut from each strip at 10-μm thickness with a rotary microtome and mounted on a slide. Three slides from each strip were prepared. The tissue was then stained with safranin and fast green (Ruzin 2000). From each of the three slides, using a light microscope, four measurements were made of leaf thickness, cuticle thickness of the upper leaf surface, upper and lower epidermal cell thickness, and the palisade cell layer thickness. Each slide was measured in different positions that avoided the midrib region.

To determine stomatal density and stomatal aperture length, 1×1-cm leaf sections were taken from the sample leaf adjacent to the section used for cross-section measurements. Each section was incubated in an oven at 50ºC in 5% sodium hydroxide to clear leaf pigments. Sections were then stained with 1–2 drops of 0.5% aqueous toluidine blue solutions and mounted on slides (Ashton et al. 1999). For each section, stomata were counted in four different fields of view using an eye-piece grid. Three closed-stoma aperture lengths were measured in four different fields of view on the abaxial side of the leaf. No stomata were found on the adaxial leaf surfaces. To obtain a relative comparison among the species of the amount of stomatal pore area (SPA) of leaf, mean stomatal aperture length was multiplied by mean stomatal density (Ashton & Berlyn 1994; Ashton et al. 1999).

Leaf morphology

At the end of the experiment, the area of the leaves (produced in sun and shade light environments) per seedling was measured using a leaf area meter (LI-3100, Licor Inc., Lincoln, Nebraska, USA). For leaf area measurements of *Pseudopanax crasssifolius*, the massive non-photosynthetic midrib was removed (Gould 1993) by cutting the leaf along the midrib. *Pseudopanax arboreus* was treated in the same manner. Specific leaf area was calculated by dividing the total leaf area of each seedling by total leaf dry mass.

Statistical analysis

The differences between congeneric pairs of homoblastic and heteroblastic species were estimated using generalised linear models in MINITAB Version 12 for each of the physiological, anatomical, and morphological measures. All data were log-transformed before analysis to meet the assumptions of normality. We tested for differences among light, block, genera, species (homoblastic or heteroblastic) nested within genera, and all two-way interactions. Block

was considered as a random factor. The traits that showed significant interactions between light and species (nested within genera) were further compared using a *t*-test between each homoblastic and heteroblastic pair within each treatment (Quinn & Keough 2002).

To assess whether homoblastic and heteroblastic species differed in the degree of their response to sun and shade, for each trait an index of plasticity was calculated as the ratio of the mean in sun and the mean in shade (see Ashton & Berlyn 1994). This index indicates both the magnitude and direction of plasticity for homoblastic and heteroblastic species for each measured variable. The index of plasticity between homoblastic and heteroblastic species was analysed using paired *t*-tests.

Results

Leaf physiology

There were significant interactions between light and species within each genus for maximum light-saturated photosynthetic rate, dark respiration, and light compensation point, but not for the apparent quantum yield (Table 2). In the shade, *t*-tests revealed no significant differences between each homoblastic and heteroblastic pair for any of the light-response-curve parameters, with the exception of light-saturated photosynthetic rate, which was significantly greater in the homoblastic *Aristotelia* species (Table 3). In contrast, in the full-sun treatment, three of the four heteroblastic species had significantly

Table 2. F-statistics for nested analysis of variance for morphological (mean leaf size = LS, total leaf area = TLA, specific leaf area = SLA) and physiological attributes (maximum photosynthetic rate = A_{max}, apparent quantum yield = ϕ, dark respiration = R_d, light compensation point = LCP). Light was a fixed factor while species (either homoblastic or heteroblastic) were nested within genera. Block was considered as a random factor. df = degrees of freedom. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	df	LS	TLA	SLA	A_{max}	ϕ	R_d	LCP
Light	1	125.3***	232.9***	15.9*	4645.3***	101.8***	703.3***	135.6***
Block	3	ns	ns	ns	ns	ns	ns	ns
Genera	3	265.1***	219.1***	59.4***	137.5***	11.3***	59.1***	23.7***
Species (Genera)	4	86.5***	65.3***	61.3***	38.3***	ns	7.4***	ns
Light × Genera	3	11.4***	49.8***	32.3***	56.1***	ns	15.2***	13.2**
Light × Species (Genera)	4	9.3***	21.8***	4.8**	109.4***	ns	3.2*	3.8*

Table 3. Parameters of the light response curves. Light-saturated photosynthesic rate (A_{max}), dark respiration (R_d), apparent quantum yield (ϕ), and light compensation point (LCP) for *homoblastic* and ***heteroblastic*** *Hoheria, Aristotelia, Pseudopanax,* and *Melicope* seedlings in full-sun and shade light treatments. Data are mean values from six seedlings with standard errors in parentheses. Letters represent paired *t*-test results between each homoblastic and heteroblastic pair within a light treatment. Species followed by different letters were significantly different ($P < 0.05$) within a genus.

	A_{max} (μmol CO_2 m^{-2} s^{-1})	R_d (μmol CO_2 m^{-2} s^{-1})	ϕ (mol CO_2 mol $quanta^{-1}$)	LCP (μmol m^{-2} s^{-1})
Shade				
H. lyallii	3.9(0.14)	−0.49(0.05)	0.034(0.001)	13.4(1.2)
H. sexstylosa	3.8(0.15)	−0.55(0.02)	0.035(0.003)	15.6(1.7)
A. serrata	4.0(0.06)a	−0.73(0.06)	0.036(0.004)	22.4(2.0)
A. fruticosa	2.4(0.11)b	−0.76(0.07)	0.033(0.001)	21.6(1.6)
P. arboreus	3.2(0.13)	−0.34(0.03)b	0.038(0.004)	9.3(1.6)b
P. crassifolius	3.0(0.11)	−0.56(0.02)a	0.044(0.002)	13.9(1.1)a
M. ternata	2.9(0.06)	−0.29(0.01)	0.034(0.002)	9.5(1.8)
M. simplex	2.5(0.03)	−0.33(0.03)	0.033(0.002)	10.6(1.5)
Full sun				
H. lyallii	11.5(0.29)	−1.37(0.08)	0.052(0.004)	26.9(3.3)
H. sexstylosa	11.7(0.32)	−1.48(0.05)	0.053(0.003)	28.6(1.9)
A. serrata	7.2(0.13)b	−1.26(0.03)b	0.047(0.003)	24.6(2.6)b
A. fruticosa	11.8(0.17)a	−1.44(0.03)a	0.045(0.002)	33.3(1.7)a
P. arboreus	8.9(0.41)b	−1.29(0.09)b	0.052(0.002)	25.8(2.9)
P. crassifolius	12.1(0.14)a	−1.52(0.07)a	0.056(0.002)	28.7(1.5)
M. ternata	5.8(0.13)b	−0.64(0.08)b	0.044(0.002)	16.7(1.3)b
M. simplex	8.9(0.25)a	−1.01(0.11)a	0.042(0.002)	24.3(3.5)a

higher light-saturated photosynthetic rate and dark respiration than their paired homoblastic species, while light compensation point (LCP) was significantly higher only for heteroblastic *A. fruticosa* ($t = 4.27$, $P = 0.014$) and *M. simplex* ($t = 3.61$, $P = 0.021$) than their paired homoblastic species (Table 3).

Leaf anatomy

Homoblastic and heteroblastic species showed significant differences for many of their leaf anatomical parameters (thickness of leaf blade, cuticle, palisade and spongy mesophyll, and epidermis, and stomatal pore area) in both sun and shade light environments (Table 4). Generalised linear models indicated significant effects of light environment, genera, and species within genera for all characters measured. There was also a significant interaction between light environment and species nested within genera (Table 4). However, there were no consistent differences between the paired homoblastic and heteroblastic species in the shade. For example, *t*-tests showed that two out of four heteroblastic species (*H. sexstylosa*, *P. crassifolius*) had significantly thinner leaf blades and palisade mesophyll relative to their homoblastic congeners, while homoblastic and heteroblastic species in the other two genera either did not differ, or had significant differences in the opposite direction (Table 5).

In contrast to the shade environments, there were consistent differences between homoblastic and

Table 4. *F*-statistics for nested analysis of variance for various anatomical measures: leaf blade (LB), cuticle thickness (CT), upper epidermis (UE), palisade mesophyll (PM), lower epidermis (LE), stomatal pore area (stomatal density × stomatal aperture length) (SPA). Light was a fixed factor. Species (either homoblastic or heteroblastic) were nested within genera. Block was considered as a random factor. df = degrees of freedom. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	df	LB	CT	UE	PM	SM	LE	SPA
Light	1	5694.8***	1333.2***	967.9***	8073.2***	860.4***	11.5***	1444.3***
Block	3	ns	6.4**	ns	30.6***	8.7**	77.4***	Ns
Genera	3	2585.6***	1404.7***	534.4***	1204.3***	635.3***	869.2***	193.4***
Species (Genera)	4	74.4***	77.4***	314.4***	66.5***	35.4***	21.4***	298.1***
Light × Genera	3	21.4***	187.8***	98.6***	120.3***	6.6***	10.6***	113.4***
Light × species (Genera)	4	116.2***	12.4***	222.4***	83.4***	22.4***	11.5***	31.4***

Table 5. Summary of leaf anatomical attributes (thickness of leaf blade, cuticle, upper epidermis, palisade mesophyll, and lower epidermis, and stomatal pore area (stomatal density × stomatal aperture length). Data are means from 12 seedlings of homoblastic and heteroblastic *Hoheria, Aristotelia, Pseudopanax,* and six seedlings of *Melicope* species, with standard errors in parentheses. Letters represent *t*-test results between each *homoblastic* and ***heteroblastic*** pair within a light treatment. Species followed by different letters were significantly different ($P < 0.05$) within a genus in sun or shade.

	Leaf blade (μm)	Cuticle (μm)	Upper epidermis (μm)	Palisade mesophyll (μm)	Lower epidermis (μm)	Stomatal pore area (μm mm^{-2})
Shade						
H. lyallii	83.5(2.12)a	0.85(0.03)a	15.3(0.26)a	22.2(0.47)a	12.4(0.19)a	2945(68)b
H. sexstylosa	65.7(0.98)b	0.68(0.02)b	14.3(0.23)b	17.7(0.26)b	11.5(0.17)b	3603(73)a
A. serrata	50.5(1.01)b	0.52(0.01)b	17.8(0.27)a	15.5(0.25)b	9.2(0.17)b	2834(53)a
A. fruticosa	71.0(1.56)a	1.08(0.04)a	14.0(0.55)b	18.2(0.47)a	10.8(0.48)a	2021(82)b
P. arboreus	176.1(2.15)a	1.45(0.09)	19.2(0.32)	39.6(0.33)a	18.0(0.28)b	2038(59)b
P. crassifolius	129.3(1.05)b	1.41(0.04)	20.3(0.25)	34.9(0.36)b	18.3(0.23)a	3691(77)a
M. ternata	118.9(3.67)b	0.52(0.01)b	20.4(0.42)a	34.3(0.92)	16.1(0.41)	2316(109)b
M. simplex	121.3(2.56)a	0.64(0.03)a	16.8(0.32)b	33.3(0.85)	13.3(0.29)	3273(126)a
Full sun						
H. lyallii	154.1(3.2)	1.11(0.03)	18.5(0.27)a	65.5(1.72)	12.9(0.15)a	6467(95)b
H. sexstylosa	146.5(2.5)	1.20(0.03)	16.7(0.37)b	62.0(1.52)	11.6(0.18)b	6822(121)a
A. serrata	113.9(1.9)b	0.98(0.02)b	24.8(0.35)a	43.0(0.57)b	10.8(0.17)	5550(103)b
A. fruticosa	154.7(1.9)a	1.54(0.04)a	20.8(0.38)b	66.2(0.83)a	11.2(0.14)	6144(117)a
P. arboreus	286.0(2.7)b	4.41(0.09)b	22.0(0.34)a	88.7(1.12)b	17.5(0.22)b	3240 (72)b
P. crassifolius	376.7(4.6)a	4.92(0.10)a	18.5(1.66)b	124.3(2.68)a	19.1(0.21)a	5402(88)a
M. ternata	217.5(1.9)b	0.77(0.05)b	39.1(0.40)a	46.9(0.88)b	14.2(0.33)	2090 (90)b
M. simplex	227.2(3.8)a	1.16(0.06)a	17.4(0.39)b	74.8(1.27)a	15.4(0.33)	5867(200)a

heteroblastic pairs in full sun (Table 5). Three of the four heteroblastic species had significantly thicker leaf blades, cuticles, and palisade mesophyll while the two species of *Hoheria* did not differ, and there were no consistent differences in the thickness of the lower epidermis. The leaves of the heteroblastic pair of all genera also had significantly more stomatal pore area (stomatal density × stomatal aperture length) but thinner upper epidermis. Only *P. crassifolius* had multiple layers of collenchyma cells below the upper epidermis (hypodermis) in full sun (results not shown).

Leaf morphology

Generalised linear models of mean individual leaf size, total leaf area, and specific leaf area showed significant differences ($P < 0.05$) between light, genera, and species nested within genera. There were significant interactions between light and species nested within genera (Table 2), suggesting homoblastic and heteroblastic seedlings responded differently to light environments. All heteroblastic species had significantly smaller leaves than their paired homoblastic species in both environments, and lower total leaf area in the sun (Fig. 1). In the shade, total leaf area was lower for the heteroblastic species pairs in three *t*-tests, but the total leaf area of heteroblastic and homoblastic species of *Aristotelia* species did not differ (Fig. 1). For three of the four *t*-tests, specific leaf area was significantly lower in the heteroblastic species both in sun and shade (Fig. 1). Specific leaf area did not differ between heteroblastic *Hoheria sexstylosa* and homoblastic *H. lyallii* in either environment (Fig. 1).

Plasticity

Paired *t*-tests using an index of plasticity values showed that heteroblastic species had significantly greater change across environments for light-saturated photosynthetic rate ($t = 3.14$, $P = 0.026$) and palisade mesophyll thickness ($t = 2.89$, $P = 0.034$) than their paired homoblastic species (Table 6). There were no consistent differences in the degree of responses between paired homoblastic

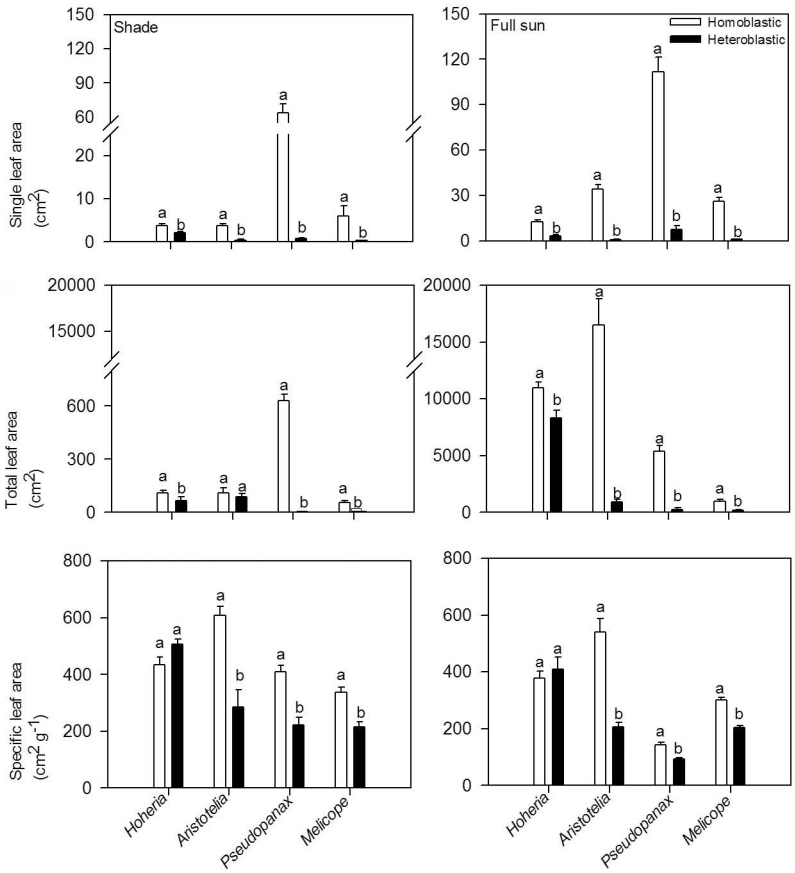

Figure 1. Leaf morphological attributes (mean leaf size, total leaf area/seedling, and specific leaf area) for homoblastic and heteroblastic, *Hoheria*, *Aristotelia*, *Pseudopanax*, and *Melicope* species grown in full sun (100% PPFD = 1600 μmol m^{-2} s^{-1}) and shade (5% PPFD = 50 μmol m^{-2} s^{-1}) light treatments. Data are mean values from 12 seedlings of each homoblastic and heteroblastic species with the exception of *Melicope* which had only six pairs of seedlings. Bars indicate ± SEM. Means followed by different letters were significantly different at $P < 0.05$ level within a genus in sun or shade.

and heteroblastic species for any of the other foliar traits (Table 6).

Discussion

Independent comparisons of heteroblastic and homoblastic species pairs showed that the four heteroblastic species had consistently smaller leaves (Fig. 1), and in the sun, had higher light-saturated photosynthetic rates, dark respiration rates (Table 3), and thicker anatomical attributes (Table 5). Plasticity of heteroblastic species was greater than that of homoblastic congeners only for maximum photosynthetic rate and stomatal pore area (Table 6). The question arises whether these differences are a feature of heteroblasty in general (with *Hoheria* differing from the usual syndrome), or due to chance sampling of the four species pairs. A comparison of a greater number of heteroblastic species would test whether these results were simply due to a low sample size. Regardless, our original hypothesis was that heteroblastic species would show more shade-tolerant traits than closely related homoblastic species. We can reject this hypothesis unequivocally.

In addition to the smaller leaf size of the heteroblastic species than homoblastic species, throughout the experiment, we also observed morphological differences in leaf lobing between sun and shade environments, but did not document these statistically. While there were not many changes in leaf shape of homoblastic species in the shade, all heteroblastic species produced entire or less dissected leaf blades, while in the sun leaves were more dissected and *Melicope simplex* produced trifoliate leaves (H. Gamage, pers. obs). These observations showed that heteroblastic species do have plastic responses to light environment, and the 'more usual' leaf morphology (i.e. that described by field guides or Floras, e.g. Allan 1961) is similar to that observed in a high-light environment. However, a study of heteroblastic species across environments in New Zealand and New Caledonia showed that while leaf lobing was characteristic of juveniles in both countries, there was no effect of habitat on the degree of lobing (Burns & Dawson 2006), and so the result of this study might be a response to very low light levels.

Irrespective of the differences in leaf morphology of heteroblastic species in the shade, there were no significant differences in leaf physiology relative to homoblastic species. Thus in shade, the homoblastic species did not have higher photosynthetic carbon gain than the heteroblastic species. However, other studies have found that apparently shade tolerant plants do not have increased photosynthetic capacity in response to reduced light quantity and quality (Red:Far-Red ratio; Reich et al. 2003). In a comparison of photosynthetic characteristics of six Australian rainforest tree species to different light quantity and quality, Turnbull (1991) found the degree of acclimatisation to light environment was not clearly related to successional status (and hence ability to survive in shade). In addition, photosynthetic light acclimatisation of the site-specific pioneer tree species *Piper auritum* and site-generalist shrub *P. hispidum* also showed no striking differences in gas exchange characteristics in response to contrasting light environments (Walters & Field 1987). Thus, it may be that photosynthetic carbon gain alone is not a consistent indicator of the ability to survive in shade.

The differences in leaf traits between homoblastic and heteroblastic species do suggest some functional explanations that warrant further testing. The combination of small leaf size, high leaf lobing, and more stomatal pores in a unit area suggests upregulating photosynthesis in high light, or due to increased transpiration rates (more stomatal pores per unit area and thinner epidermal layers), or maintaining leaf temperatures favourable to photosynthesis may be important for these heteroblastic seedlings (see Givnish & Vermeij 1976; Fetcher 1981; Gurevitch 1988).

Table 6. Plasticity values for foliar traits calculated as the ratio of mean value in full sun and shade (sun/shade) for *homoblastic* and ***heteroblastic*** species. Traits that increased from shade to sun: Plasticity > 1; traits that increased from sun to shade: Plasticity < 1. Asterisks indicate significantly higher plasticity for the heteroblastic species, compared with its homoblastic pair.

	Single leaf area	Total leaf area	Specific leaf area	Leaf blade thickness	Palisade mesophyll thickness	Stomatal pore area	Maximum photosynthetic rate	Light compensation point
H. lyallii	3.5	120.6	0.83	1.8	2.0	2.2	2.1	2.0
H. sexstylosa	1.6	29.3	0.73	2.2*	3.5*	1.8	3.2*	1.8
A. serrata	9.3	129.9	0.89	2.3	2.8	1.9	1.8	1.2
A. fruticosa	2.0	94.3	1.04*	2.2	3.7*	3.0*	4.9*	1.5*
P. arboreus	1.8	8.5	0.32	1.6	2.2	1.6	2.8	2.8
P. crassifolius	8.3*	58.2*	0.60	2.9*	3.6*	1.5	4.0*	2.0
M. ternata	4.4	17.6	0.89	1.8	1.4	0.9	2.0	1.7
M. simplex	3.5	63.3	0.94*	1.9	2.5*	1.8*	3.6*	2.4*

Interestingly, the differences in stomatal pore area and epidermal layers found between the homoblastic and heteroblastic pairs do not suggest adaptations to a xeric environment (see McGlone & Webb 1981), although comparative studies of water-use efficiencies in similar environments would test this further. Leaf size can have important effects on leaf temperature. For example, the mangrove species *Ceriops tagal* had a six-fold decrease in leaf area and 50–15 mm decrease in leaf width with increasing exposure from shade to sunlight, and the resulting decreases in boundary layer conductance in the smaller leaves meant that temperatures were closer to ambient air temperatures (Ball et al. 1988). On the other hand, highly lobed leaves likely also appear more cryptic to herbivores, and while experimentation using moa is not possible, other indirect experimental tests of the function of small leaf size and leaf lobing are clearly warranted.

We made no attempt during this experiment to control for the developmental ontogeny of the leaves, and as a consequence, leaves measured in the full-sun environment likely developed at later nodes than the shaded leaves. Gould's (1993) experiments on *Pseudopanax* also reveal ontogeny is likely very important in determining leaf anatomical traits. Gould (1993) found significant differences between seedling and juvenile leaf anatomy, and these differences were similar to the leaf anatomical differences found between sun and shade treatments in this study; and it is likely plants grown in full sun were developmentally 'juvenile', while the plants in the shade were still developmentally 'seedlings'. However, Jones (1995) found differences in leaf morphology between sun and shade leaves at the same developmental position were the result of plastic responses occurring at the level of individual developing organs rather than at the level of whole-plant transition from juvenile to adult stages, and consequently suggested that there is no support for the historical thought that shade prolongs juvenile development of heteroblastic plants (Goebel 1900; Njoku 1956; Cameron 1970). Future studies of ecological or physiological differences between heteroblastic and homoblastic species should endeavour to take ontogenetic as well as environmental differences into account to examine this further.

We found few consistent differences between homoblastic and heteroblastic species in plasticity of leaf physiological, anatomical, and morphological traits. However, heteroblastic species showed greater plasticity for maximum photosynthetic rate and palisade mesophyll thickness (Table 6). The greater variation in light-saturated photosynthetic rate found in the heteroblastic species (Table 3) could be a direct result of greater plasticity in palisade mesophyll thickness. Another possible explanation may be that heteroblastic plants were not able to acclimatise to very low light. Thus, the apparent plasticity in photosynthesis may reflect a stress response where heteroblastic plants in shade were no longer able to photosynthesise efficiently. This possibility would also explain the finding that both heteroblastic and homoblastic species had smaller leaves in the shade than in the sun (i.e. plasticity index >1), which is inconsistent with the general tendency of sun leaves to be small while shade leaves are large (Givnish 1988; Lee & Richards 1991). Differences in leaf size are likely due to the trade-off between higher photosynthetic rates per unit leaf area and the increased evaporative demands arising in high light (Givnish 1988), and so in water-limited environments, or high-temperature environments the effect is likely to be greater (Lewis 1972; Fetcher 1981; Givnish 1988; Bongers & Popma 1990; Strauss-Debenedetti & Bazzaz 1991; Ashton 1995). In this experiment, plants were well watered, and probably not temperature stressed, and so it is likely the very low light levels in the shade treatment restricted carbon gain in this environment. Testing the responses of other homoblastic–heteroblastic species pairs to different light environments is needed to determine if this result is a characteristic of the New Zealand heteroblastic syndrome.

In summary, our results suggest few consistent responses by heteroblastic seedlings to different light environments. It seems heteroblastic seedlings are not shade tolerant, and alternative hypotheses for the evolution or maintenance of heteroblasty should be sought.

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