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Leaf stomatal and epidermal cell development: identification of putative quantitative trait loci in relation to elevated carbon dioxide concentration in poplar

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Summary Genetic variation in stomatal initiation and density, and epidermal cell size and number were examined in a hybrid pedigree of *Populus trichocarpa* T. & G. and *P. deltoides* Marsh in both ambient ([aCO_2]) and elevated ([eCO_2]) concentrations of CO_2. We aimed to link anatomical traits with the underlying genetic map of F_2 Family 331, composed of 350 markers across 19 linkage groups. Leaf stomatal and epidermal cell traits showed pronounced differences between the original parents. We considered the following traits in the F_2 population: stomatal density (SD), stomatal index (SI), epidermal cell area (ECA) and the number of epidermal cells per leaf (ECN). In [eCO_2], adaxial SD and SI were reduced in the F_2 population, whereas ECA increased and ECN remained unchanged. In [aCO_2], four putative quantitative trait loci (QTL) with logarithm of the odds ratio (LOD) scores greater than 2.9 were found for stomatal traits on linkage group B: adaxial SI (LOD scores of 5.4 and 5.2); abaxial SI (LOD score of 3.3); and SD (LOD score of 3.2). These results imply that QTL for SI and SD share linkage group B and are under genetic control. More moderate LOD scores (LOD scores ≥ 2.5) suggest QTL for SI on linkage groups A and B and for SD on linkage groups B, D and X with a probable co-locating quantitative trait locus for SI and SD on linkage group D (position 46.3 cM). The QTL in both [aCO_2] and [eCO_2] for adaxial SD were co-located on linkage group X (LOD scores of 3.5 and 2.6, respectively) indicating a similar response across both treatments. Putative QTL were located on linkage group A (position 89.2 cM) for both leaf size and ECN in [aCO_2] and for ECA at almost the same position. The data provide preliminary evidence that leaf stomatal and cell traits are amenable to QTL analysis.

Key words: cell number, cell size, CO_2, leaf growth, Populus, stomatal development.

Introduction

Tree and plant growth and development may be greatly affected by exposure to elevated CO_2 concentration ([eCO_2]). Large increases in leaf area, changes in shape and initiation, as well as reductions in stomatal numbers and changes in cell production and expansion, have been recorded in tree species in response to [eCO_2] (Ceulemans et al. 1995, Norby et al. 1999, Pritchard et al. 1999, Ferris et al. 2001, Taylor et al. 2001). Although it is unknown how these changes are effected, there is evidence that altered cell wall biochemical processes and cell cycle events are both sensitive to CO_2 supply (Taylor et al. 1994, Ranasinghe and Taylor 1996).

Leaf stomatal density affects gas exchange and it has been suggested that declining stomatal numbers are correlated with rising [CO_2] over the last century (Woodward 1987, Jarvis et al. 1999, Croxdale 2000). Differences in photosynthetic pathway, surface features, light penetration and the internal architecture of leaves may impact on stomatal initiation, distribution and function, and may explain the heterogeneity of stomatal responses in leaves (Beerling and Woodward 1995, Croxdale 2000). Changes in stomatal density are often paralleled by changes in the ratio of stomata to epidermal cells, indicating that [eCO_2] affects stomatal initiation directly, rather than indirectly through the rate of leaf cell expansion (Woodward 1987, Ferris and Taylor 1994, Ceulemans et al. 1995, Ferris et al. 1996).

It is unknown how stomatal density is controlled during leaf growth. However, the establishment of cellular identity in plants is likely to be genetically regulated (Dolan and Okada 1999). Gray et al. (2000) identified a gene (*hic*) that may be involved in the negative regulation of stomatal initiation in plants exposed to [eCO_2]. Recent research on the molecular mechanisms controlling stomatal density in *Arabidopsis thaliana* (L.) Heynh. mutants suggests that an *SDD1* gene controls the development of cell lineages that lead to guard cell formation (Berger and Altmann 2000). This is consistent with research showing that the patterning of trichomes is controlled by two or more genes (Marks 1997), and that the patterning of stomata in angiosperms is likely to be coupled to the cell cycle (Croxdale 2000).

Physiological traits important for plant responses to stress are often quantitative rather than qualitative, suggesting that they are determined by several gene products, rather than a single product. Many complex traits, such as large leaf areas and stomatal index, are unlikely to be entirely described by the inheritance of a single gene (Bradshaw and Stettler 1995, Wu et al. 1997). Quantitative genetics at the molecular level is being widely investigated in both plant and animal systems (Prioul et al. 1997) and holds promise as a method for analyzing complex leaf traits (Bradshaw and Stettler 1995). For an investigation of quantitative trait loci (QTL), a pedigree must be found or produced in which the trait(s) of interest are segregating. The pedigree must have segregating variation for a collection of genetic markers, and enough of these markers to construct a genetic map of all the chromosomes. It must be possible to score the trait of interest on every individual for which there are genotype data.

In poplar, a linkage map consisting of 343 restricted fragment length polymorphisms (RFLP), sequence tagged sites (STS) and random amplified polymorphic DNA (RAPD) markers has been constructed based on *Populus trichocarpa* T. & G. and *P. deltoides* Marsh. *Populus trichocarpa* has fairly thick ovate leaves supported by a short and stout petiole and is distributed west of the Rocky Mountains in North America. *Populus deltoides* is distributed east of the Rocky Mountains and has thin deltoid leaves attached to a long flat petiole (Wu et al. 1997). The differences in leaf size and morphology between these poplars are reflected in differences between them in leaf anatomy (Hinckley et al. 1989). In *P. trichocarpa*, the process of cell division is completed early in leaf development and final leaf area is achieved during the subsequent cell expansion phase, whereas the final leaf size of *P. deltoides* is determined by cell division occurring throughout development (Ridge et al. 1986). *Populus trichocarpa* leaves have a thick, loosely arranged spongy mesophyll, giving the abaxial surface a whitish color. Little is known about the genetic basis of leaf morphological variation in forest trees and there is no information on how leaf stomatal and cell traits are genetically and developmentally correlated. Here we describe preliminary results on stomatal and epidermal cell traits of leaves of the F_2 poplar population grown in an ambient or elevated concentration of CO_2.

Materials and methods

Plant pedigree, field site and exposure to CO_2

The pedigree used for QTL mapping was generated by hybridization of the maternal *P. trichocarpa* Clone 93-968 from western Washington and the paternal *P. deltoides* Clone ILL-129 from central Illinois in 1981. Two siblings, 53-246 and 53-242, from the resulting F_1 family (Family 53) were crossed to form an F_2 family (Family 331; Bradshaw and Stettler 1993, Bradshaw et al. 1994). This experiment was conducted in 16 open-top chambers (OTC) at the Forestry Commission field site, Headley, U.K. (Grid Ref. SU813382; Taylor and Dobson 1989). In May 1999, 285 labeled F_2 genotypes of Family 331 pedigree, the *P. trichocarpa* and *P. deltoides* parents and the F_1 genotypes were established from unrooted hardwood cuttings derived from a stool bed at the University of Washington, Seattle. Cuttings were grown in John Innes No. 2 compost (lime free) in plastic tubes (91 cm in height, 15 cm in diameter) in a randomized complete block design. Before planting, each cutting was dipped in Tile 250 EC fungicide solution (Ciba-Geigy Agrochemicals, Cambridge, U.K.). For each treatment, 285 genotypes were placed in eight chambers (about 36 genotypes per chamber). The plastic tubes were placed 25 cm apart in a circular pattern. The pots were buried to a depth of 10 cm for stability. Eight chambers received the ambient concentration of CO_2 ($[aCO_2]$) and the other eight chambers received an elevated concentration of CO_2 ($[eCO_2]$) at a target concentration of 600 μmol mol^{-1} CO_2. Between May and September, mean $[CO_2]$ in the $[eCO_2]$ chambers ranged between 588.29 ± 63.35 and 595.30 ± 83.52 μmol mol^{-1}, whereas in the $[aCO_2]$ chambers, mean $[CO_2]$ ranged between 400.81 ± 39.00 and 408.08 ± 38.84 μmol mol^{-1}. Each chamber was ventilated by means of a 1-hp centrifugal pump mounted in an adjacent cabinet constructed of galvanized steel. Each pump drew in the surrounding ambient air. The air was then evenly distributed throughout the chamber by means of a circular perforated polythene tube held against the walls of each chamber (Gardner et al. 1995). Across chambers, mean monthly air temperatures (± SD) outside and inside the chambers were, respectively: May: 13.2 (4.0), 16.4 (5.7) °C; June: 14.3 (4.4), 17.6 (6.1) °C; July: 18.1 (5.1), 21.3 (6.9) °C; August: 16.3 (4.5), 19.0 (6.0) °C; and September: 15.3 (4.3), 17.4 (5.6) °C. Initially, all chamber floors were treated with Stomp 400 SC, a pre-emergent herbicide, MAFF No. 04183 (BASF Plc, Cheadle Hulme, U.K.), applied with a Gloria sprayer (Gloria-Werke, Wadersloh, Germany) at a dilution of 30 ml l^{-1}. A slow-release nitrogen fertilizer (5 g Osmocote; Grace-Sierra, Nottingham, U.K.) was added to each pot after 67 days of growth. The trees were staked 68 days after planting (DAP). Water was supplied to each pot by a drip irrigation system as required. Measurements of tree development and the physiological traiting of these genotypes were made throughout the growing season. The leaf traits presented here are adaxial and abaxial stomatal density (SD) and stomatal index (SI), and adaxial epidermal cell area (ECA) and epidermal cell numbers (ECN) per leaf.

Stomatal imprints, epidermal cell size and number

At 80–82 DAP, a single fully mature leaf was excised from each tree. Leaves were photocopied and leaf areas (mm^2) were measured with an Image Analyser (Delta-T Devices, Cambridge, U.K.). A template of 1.5 cm^2 was placed on the base of the abaxial and adaxial surface of the leaf and the area sprayed with clear lacquer (Halfords, Reddith, U.K.) and left to dry for 20–25 min. A leaf imprint was obtained with tape (Ferris and Taylor 1994). For measurements of stomatal density and index, replicas were examined with the aid of a light microscope. The number of stomata per half field of view was converted to number of stomata per mm^2. These data were used to

calculate the SI (%), which relates SD to the number of epidermal cells per unit area (ECD), where SI = (SD/(SD + ECD))100 (Ferris and Taylor 1994). The ECD measurements excluded guard cells. For measurements of cell size and number, digital images of the epidermal impressions were captured with a light microscope (Axiophot 2 Universal Microscope, Carl Zeiss, Jena, Germany) and digital imaging software (Metamorph Imaging System, West Chester, PA), which obtained one digital image per slide of mature adaxial epidermal cells from between the midrib and the major veins. The areas of 10 adaxial epidermal cells per slide were obtained randomly from the image. An estimation of ECN was calculated for each poplar genotype from the mean ECA and the leaf area. Photographs of the leaf surface of the parents were taken with a light microscope.

Data analyses and QTL mapping

The data were analyzed for QTL with MAPMAKER/QTL 1.1 software (Lander and Botstein 1989). The RFLP, STS and RAPD-based genetic map made from a total of 90 F_2 individuals of the pedigree has been described (Bradshaw et al. 1994, Bradshaw and Stettler 1995). Segregation distortion was reported for the RFLP (6%) and RAPD (14%) markers (Bradshaw et al. 1994). This has been found to be a common problem in this pedigree (Bradshaw and Stettler 1994), which may affect the mapping of QTL. The data were tested for normality using an Anderson-Darlington test. Analyzed data were for leaves sampled at 80–82 DAP unless otherwise stated. A threshold logarithm of the odds ratio (LOD) score of 2.9 was used for declaring the existence of a quantitative trait locus, corresponding to an approximate nominal significance of $P = 0.05$ for the entire genome (Lander and Botstein 1989, Bradshaw and Stettler 1995, Bradshaw 1996). However, because the F_2 sample used for mapping in this study was small, an LOD threshold of 2.5 (Stuber et al. 1992, Wu et al. 1997) was also considered to be suggestive of QTL.

Results and discussion

Leaf cell and stomatal traits in ambient and elevated [CO_2]

Table 1 summarizes the average stomatal and epidermal cell traits of the parents. Irrespective of CO_2 treatment, there were qualitative phenotypic and cellular differences between the parents: adaxial and abaxial SD and SI were higher in *P. deltoides* than in *P. trichocarpa* (Table 1, Figure 1). Compared with *P. trichocarpa*, leaf areas of the *P. deltoides* parent were larger as a result of increased cell production per leaf (Table 1, Figure 1). This suggests that cell number is more important than cell area for the production of large leaves. Donnelly et al. (1999) showed that cell cycling occurs at different rates in different tissues of the developing *Arabidopsis* leaf and that each cell layer is characterized by a distinct developmental sequence, with cycling occurring only after cells have reached a certain size. Irrespective of CO_2 treatment, *P. deltoides* had more stomata per mm^2 on both leaf surfaces than *P. trichocarpa*. Elevated [CO_2] tended to reduce SD and SI in *P. deltoides*, whereas both SD and SI remained unchanged on the adaxial leaf surface but increased on the abaxial leaf surface of *P. trichocarpa* (Table 1).

Many stomatal and cell traits of the F_2 population were sensitive to [eCO_2]. However, the responses of SD and SI in plants exposed to [eCO_2] can be variable (Ferris and Taylor 1994, Woodward and Kelly 1995). Studies of various species have shown no effect of [eCO_2] on SD or SI or both (Radoglou and Jarvis 1992), whereas other studies have shown reduced SD or SI or both (Ferris and Taylor 1994, Ceulemans et al. 1995, Ferris et al. 1996). Figures 2 and 3 show the frequency distributions of the abaxial stomatal and adaxial cell measurements, respectively, for the F_2 genotypes, and the location of the parental and F_1 genotypes. For abaxial SD, there was a quantitative difference in estimated modal frequency of 121–150 mm^2 and 151–180 mm^2 in [aCO_2] and [eCO_2], respectively (Figure 2a), whereas the modal frequency of the abaxial SI was between 18 and 20% in both treatments (Figure 2b). Irrespective of CO_2 treatment, SD of the F_2 population for the abaxial surface ranged from < 30 to 360 mm^2, whereas the adaxial surface ranged from < 30 to 240 mm^2 (L. Long, personal communication). The responses to [CO_2] of adaxial and abaxial SD may also be influenced by light environment. In addition, [eCO_2] may affect stomatal production on the two leaf surfaces differently because intrinsic cellular development in the epidermis is variable (Sachs et al. 1993, Dunlap

Table 1. Phenotypic parental characteristics (female parent, *P. trichocarpa*, Clone 93-968 and male parent, *P. deltoides*, Clone 14-129). The parents were grown in ambient and elevated [CO_2]. Means (± SE) are shown.

Parameters	Parental characteristics			
	Male (*P. deltoides*)		Female (*P. trichocarpa*)	
	Ambient [CO_2]	Elevated [CO_2]	Ambient [CO_2]	Elevated [CO_2]
Leaf area (mm^2)	22788 (6553)	25906 (10463)	4506 (2303)	12104 (4022)
Adaxial stomatal density (mm^2)	136 (15)	121 (–)	30 (0.2)	30 (0.3)
Abaxial stomatal density (mm^2)	258 (45)	197 (45)	136 (46)	166 (75)
Adaxial stomatal index (%)	14 (3)	12 (0.7)	3 (0.2)	3 (0.6)
Abaxial stomatal index (%)	24 (0.9)	19 (4)	27 (7)	30 (10)
Adaxial epidermal cell size (μm^2)	414 (13)	455 (120)	645 (3)	730.7 (19)
Adaxial epidermal cell numbers × 10^6	5.6 (1.8)	5.5 (0.9)	0.7 (0.3)	1.7 (0.6)

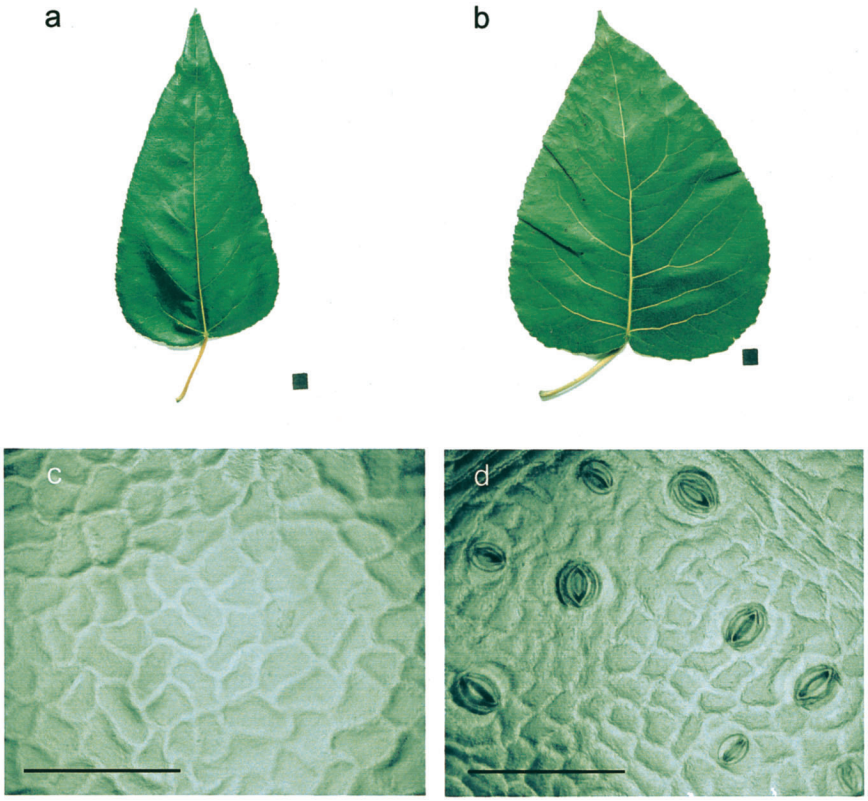

Figure 1. Top: Leaves of the parent species (a) *P. trichocarpa* (female parent) and (b) *P. deltoides* (male parent) of the F_2 Family 331. The black square = 1 cm^2. Bottom: Light microscope photographs of adaxial epidermal cell imprints showing differences in epidermal cell size and stomatal density of (c) *P. trichocarpa* and (d) *P. deltoides* grown in ambient [CO_2]. The scale bar = 100 µm.

and Stettler 2001). Figure 3a shows an estimated modal frequency of 551–600 μm^2 and 651– 700 μm^2 for ECA in [aCO_2] and [eCO_2], respectively. Figure 3b shows a modal frequency of $0.81–1.00 \times 10^6$ ECN in [aCO_2] and a bimodal frequency of $0.61–0.80 \times 10^6$ and $1.21–1.40 \times 10^6$ ECN in [eCO_2]. Clearly, ECA responded positively to [eCO_2], whereas ECN showed a variable response.

Table 2 shows the mean values of the population of the F_2 genotypes. Adaxial SD and SI decreased in the mature leaves sampled ($P \leq 0.02$) in [aCO_2], but the abaxial values did not. Mean adaxial SD and SI of plants grown in [aCO_2] was 63.1 mm^2 and 7.5%, respectively. Mean SD and SI for the adaxial leaf surface of plants grown in [eCO_2] was 55.3 mm^2 and 6.8%, respectively. The coefficient of variation was similar across treatment means for both SI and SD, although a greater measure of relative variability was seen on the adaxial leaf surface. If [eCO_2] affects cell differentiation in the meristem and thus stomatal initiation, then SD will change without ECD changing in parallel and thus SI will be affected (Ferris and Taylor 1994). We found that [eCO_2] affected stomatal initiation, particularly on the adaxial leaf surface. A similar response has been observed in several tree species including *Acer* and *Quercus* (Woodward 1987) and *Populus* (Ceulemans et al. 1995). Evidence from several plant model systems suggests that positive and negative genetic regulators determine the differentiation of particular cell types in defined locations, once positional cues are perceived (Dolan and Okada 1999). Mean ECA of the F_2 population increased in [eCO_2] ($P < 0.001$), whereas ECN remained unaltered ($P = 0.107$), suggesting that cell expansion is often sensitive to CO_2 supply (Ferris et al. 2001, Taylor et al. 2001).

Putative QTL for leaf cell and stomatal traits

Putative QTL were determined based on the linkage map described by Bradshaw and Stettler (1995). We found that the parent species differ in the stomatal and epidermal cell traits measured, indicating that there is likely to be segregation of genes for these traits in the F_2 population. Table 3 shows the position, confidence interval and linkage group associated with the putative QTL identified for stomatal and epidermal cell traits. Both putative QTL (LOD scores > 2.9) and suggested QTL (LOD scores between 2.5 and 2.9) are shown. Putative QTL are shown for SI in [aCO_2] on the adaxial leaf surface with two putative QTL on linkage group B at position 8.0 cM (LOD score of 5.4) and at position 25.5 cM (LOD score 5.2). One quantitative trait locus is shown on linkage group D at position 46.3 cM (LOD score 3.3) and one quantitative trait locus on linkage group P at position 33.6 (LOD score of 3.6). Closely linked QTL on linkage group B may cause the calculated genetic variance to be overestimated, resulting in an inflated percentage variation (Martinez and Curnow 1992). The small sample size may also affect the accuracy of the calculated genetic variance, and the inclusion of suggestive QTL with LOD thresholds of 2.5 may result in overestimation of the total genetic variance. Putative QTL are shown for SI in [aCO_2] on the abaxial leaf surface with two putative QTL on linkage group B, with an LOD score of 3.3 at position 83.9 cM and an LOD score of 2.7 at position 0 cM

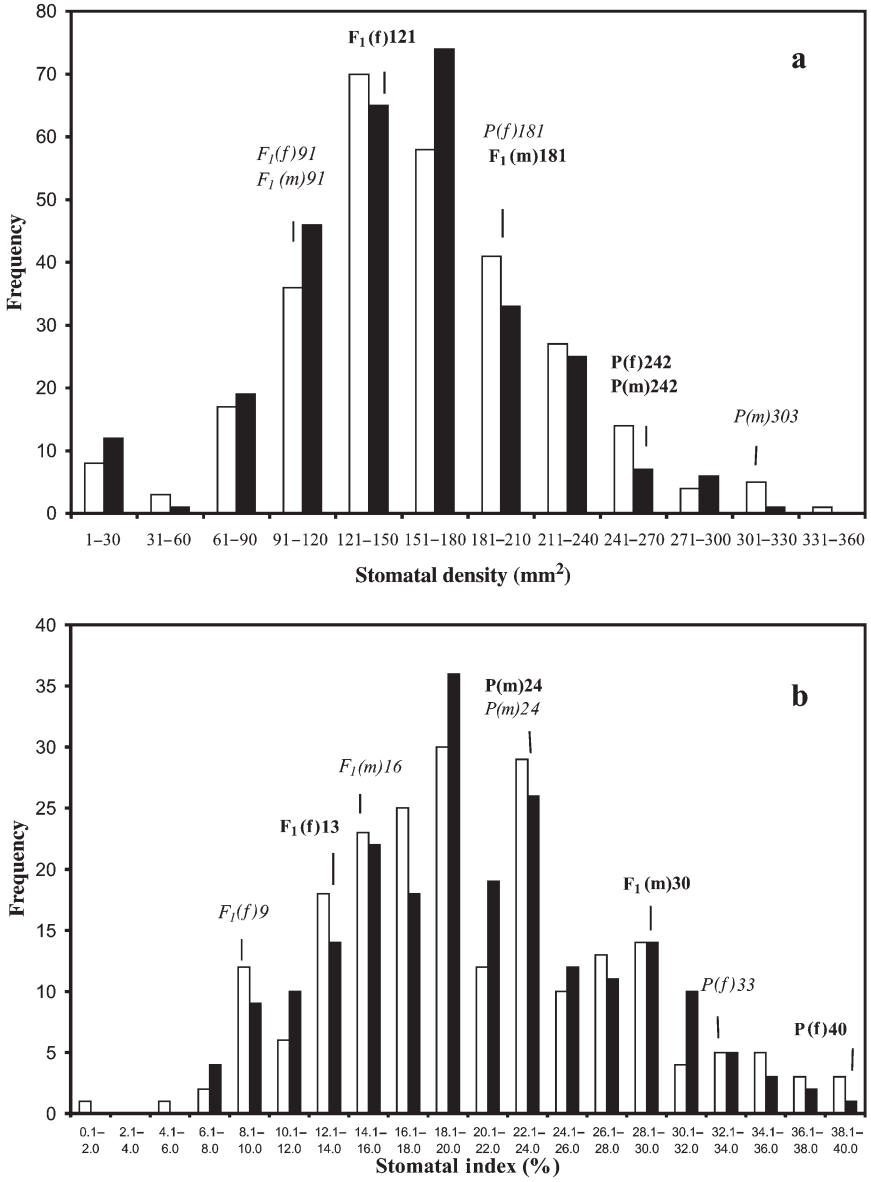

Figure 2. Distribution of the phenotypes for (a) abaxial stomatal density and (b) abaxial stomatal index of fully mature leaves of the F_2 Family 331 of poplar grown in open-top chambers at Headley in 1999. Treatments are either ambient [CO_2] (open bar, italic text) or elevated [CO_2] (solid bar, bold text). The original parents, P (*Populus trichocarpa*, female (f) and *P. deltoides*, male (m)), and the F_1 hybrids (*P. trichocarpa* × *P. deltoides*) are shown.

(Table 3). These QTL explain 41 and 58% of the phenotypic variance, respectively. The finding that QTL for adaxial and abaxial SI are situated close to each other on linkage group B suggests that this area of the genome exerts some control on the trait for stomatal initiation on both leaf surfaces.

For the abaxial SD in [aCO_2], two QTL are located on linkage group B with an LOD score of 3.2 at position 90.5 cM and an LOD score of 2.7 at position 30 cM. For adaxial SD, there are suggestions of co-locating QTL on linkage group X: in [aCO_2] there is a quantitative trait locus at position 23.9 cM with an LOD score of 3.5; and in [eCO_2] there is a quantitative trait locus at position 21.9 cM with an LOD score of 2.6. These QTL explain 50 and 34% of the phenotypic variance, respectively, which indicates that the response is the same in both CO_2 environments and that the phenotypic variance is quite high in both environments. Similarly, the quantitative trait locus for adaxial SI in [aCO_2] on linkage group D at position 46.3 cM (LOD score of 3.3) is co-located with a suggested quantitative trait locus for adaxial SD in [eCO_2] at the same position (LOD score 2.5, Table 3). Both abaxial and adaxial SI and SD appear to be under the control of two or more genes because individual QTL explain only part of the phenotypic variance (Table 3), although other variation may be associated with the environment. The consistency of QTL in populations grown under different environmental conditions implies that this quantitative trait locus has some robustness. Other results with LOD scores between 2.5 and 2.9 suggest that there are likely other QTL for SD and SI, e.g., on linkage groups A and D for SI (Table 3).

In Family 331, QTL have been identified for petiole length and flatness, leaf pigmentation, single leaf area and lamina angle (Wu et al. 1997). A few QTL with large effects have also been observed for growth, form, phenology and disease resistant traits in the same pedigree (Bradshaw and Stettler 1995).

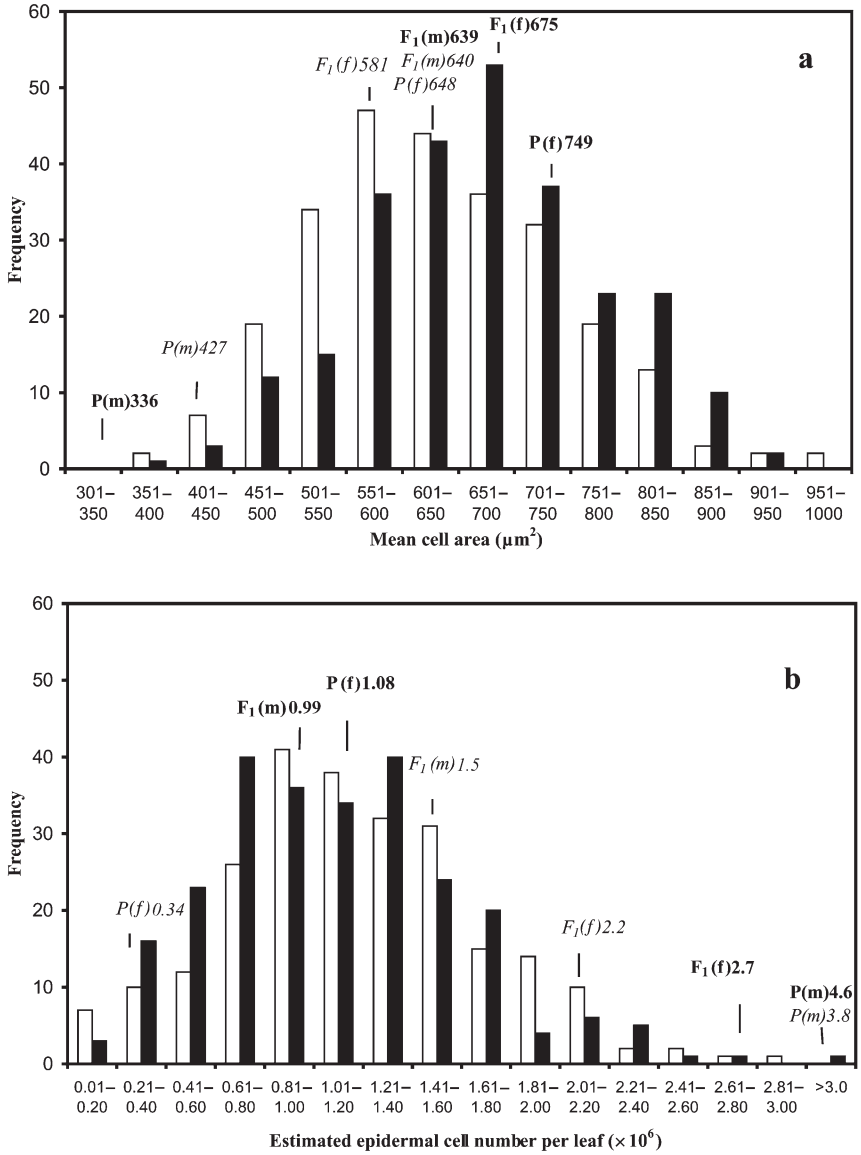

Figure 3. Distribution of the phenotypes for (a) adaxial epidermal cell area and (b) epidermal cell numbers per leaf of fully mature leaves of the F_2 Family 331 of poplar grown in open top chambers at Headley in 1999. Treatments are either ambient [CO_2] (open bar, italic text) or elevated [CO_2] (solid bar, bold text). The location of the parents and F_1 genotypes are shown as described in Figure 2.

Also, QTL have previously been mapped for specific leaf area in barley (*Hordeum*) (Yin et al. 1999), leaf length and width in *Brassica oleracea* L. (Rae 2000) and leaf area in cotton (*Gossypium*) (Jiang et al. 2000). However, there have been no studies on leaf cellular development combined with QTL analysis. Leaf area development is central to the production of stem

Table 2. Stomatal index, stomatal density, leaf cell size and leaf cell number of mature leaves of the F_2 Family 331 of poplar grown in ambient and elevated [CO_2]. Means (± SD) are shown. The coefficient of variation is shown in square brackets.

Parameters	Means of F_2 population			
	Ambient [CO_2]	Elevated [CO_2]	% Change[3]	*t*-test
Adaxial stomatal density[1] (mm^2)	63.1 (32.2) [50%]	55.3 (27.5) [50%]	–12.4	$P \le 0.001$; $t = -3.2$
Abaxial stomatal density[1] (mm^2)	151.1 (57.5) [38%]	142.3 (49.5) [35%]	–6	$P = 0.061$; $t = -1.86$
Adaxial stomatal index[2] (%)	7.5 (4.1) [54%]	6.8 (3.7) [54%]	–9	$P \le 0.02$; $t = -2.39$
Abaxial stomatal index[2] (%)	21.2 (9.1) [43%]	20.8 (0.42) [34%]	–1.8	$P = 0.49$; $t = -0.69$
Leaf cell size	633 (111) [18%]	671 (105) [15.6]	+6	$P = 0.001$; $t = 4.024$
Leaf cell number[1] × 10^6	1.1 (0.6) [55%]	1.07 (0.5) [47%]	–2.7	$P = 0.11$; $t = -1.61$

[1] Counts were SQRT transformed before analysis.
[2] Percentages were ARCSIN transformed before analysis.
[3] % Change = (([eCO_2] – [aCO_2])/[aCO_2])100; df = 563–568.

Table 3. Chromosomal location, effects and modes of gene action of putative QTL for leaf, stomatal and cell traits measured in ambient [CO_2] ([aCO_2]) and elevated [CO_2] ([eCO_2]) in F_2 Family 331. Abbreviations: LG = linkage group; LOD = location logarithm of the odds ratio; %Va = percent variance; SI = stomatal index; SD = stomatal density; ECA = epidermal cell area; and ECN = the number of epidermal cells per leaf.

Parameters	LG[1]	Position[2]	Confidence interval	LOD[3]	%Va[4]
Adaxial SI ([aCO_2])[5]	B	8.0	0.0–18.0	5.4	86
	B	25.5	21.7–32.0	5.2	62
	D	46.3	14.1–68.5	3.3	60
	P	33.6	16.2–40.9	3.6	52
Abaxial SI ([aCO_2])	A	46.5	20.7–25.0	2.5	55
	B	0	0.0–14.0	2.7	58
	B	83.9	77.8–99.9	3.3	41
Adaxial SD ([aCO_2])	X	23.9	19.9–45.6	3.5	50
Abaxial SD ([aCO_2])	B	30	0.0–74.6	2.7	27
	B	90.5	79.8–99.9	3.2	27
Adaxial SD ([eCO_2])	D	46.3	7.4–84.3	2.5	51
	X	21.9	0.0–47.6	2.6	34
Adaxial ECA ([aCO_2])	A	116.6	54.5–138.9	2.7	38
Adaxial ECA ([eCO_2])	A	88.6	62.4–124.6	3.3	32
	C	84.3	55.6–124.3	4.1	62
Adaxial ECN ([aCO_2])	A	89.2	72.1–114.4	3.3	30
Leaf area ([aCO_2])	A	89.2	74.1–110.4	3.3	28
	F	76.2	16.3–96.1	4.1	37

[1] LG = linkage group referring to the map in Bradshaw and Stettler (1995).
[2] Position = QTL position described by the distance (cM) from the top of the linkage group.
[3] LOD scores between 2.5 and 2.9 are suggestive of QTL (Stuber et al. 1992, Wu et al. 1997).
[4] %Va = percentage of the total phenotypic variance explained by the quantitative trait locus.
[5] Measurements at 130–134 DAP.

wood in *Populus* (Ridge et al. 1986) and unlike photosynthetic traits, which are also known to be under genetic control (Isebrands et al. 1988), leaf cell traits may be robust enough to be developed as markers for accelerated tree breeding.

The most relevant finding for the leaf cell traits was the co-location of QTL for epidermal cell area (positions 116.6 and 88.6 cM), cell number and leaf area (position 89.2 cM) on linkage group A of the *Populus* genome, which map to almost the same position. Perhaps just one quantitative trait locus affects all of these traits. The co-location of QTL has recently been used to infer functional relationships, e.g., between leaf length and the concentration of tissue sucrose (Prioul et al. 1997). However, this is the first time that leaf stomatal and cell traits have been linked to QTL. The QTL may allow the identification of candidate genes; i.e., genes that can be mapped to determine their position in relation to the quantitative trait locus in question, such as that described by Frewen et al. (2000) for bud break in *Populus*.

Conclusions

We obtained evidence for putative QTL for stomatal numbers, epidermal cell characteristics and leaf area, including several QTL for stomatal traits in leaves of poplar grown in [aCO_2]. Adaxial SI and abaxial SD and SI were associated with linkage group B, whereas adaxial SD was associated with linkage group X. The indication of co-locating putative QTL on linkage group X for adaxial stomatal density and on linkage group A for leaf area, cell area and cell numbers suggests that these putative QTL are worthy of further study. The relatively high percentage variation may mean that there are a few QTL with large effects rather than many with small effects (Bradshaw et al. 1995). The use of other QTL programs for an outbreeding pedigree may increase the resolution of QTL detection (Prioul et al. 1997). The detection of QTL for leaf cellular traits of *Populus* is a first step toward determining genes underlying morphological traits. Work is ongoing to increase the number of molecular markers and number of F_2 individuals genotyped. Further studies with increased sample and replicate sizes will help to clarify these problems.

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