

Adaptation of tree growth to elevated CO₂: quantitative trait loci for biomass in *Populus*

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Summary

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- Information on the genetic variation of plant response to elevated CO₂ (e[CO₂]) is needed to understand plant adaptation and to pinpoint likely evolutionary response to future high atmospheric CO₂ concentrations.
- Here, quantitative trait loci (QTL) for above- and below-ground tree growth were determined in a pedigree – an F₂ hybrid of poplar (*Populus trichocarpa* and *Populus deltoides*), following season-long exposure to either current day ambient CO₂ (a[CO₂]) or e[CO₂] at 600 µl l⁻¹, and genotype by environment interactions investigated.
- In the F₂ generation, both above- and below-ground growth showed a significant increase in e[CO₂]. Three areas of the genome on linkage groups I, IX and XII were identified as important in determining above-ground growth response to e[CO₂], while an additional three areas of the genome on linkage groups IV, XVI and XIX appeared important in determining root growth response to e[CO₂].
- These results quantify and identify genetic variation in response to e[CO₂] and provide an insight into genomic response to the changing environment.

Key words: elevated CO₂, genome, *Populus*, quantitative trait loci (QTL), root growth, stem growth.

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Introduction

Forest trees represent the largest terrestrial pool of biomass carbon (C) and, consequently, an understanding of their response to rising atmospheric carbon dioxide concentration, [CO₂], is of considerable importance for predicting biosphere C feedbacks and has been extensively studied over the past two decades (Norby *et al.*, 2005). Elevated [CO₂] (e[CO₂]) may act as a fertilizer, stimulating plant growth and accelerating development, but the role of CO₂ as a selective agent in microevolutionary change has been difficult to prove (Ward & Kelly, 2004). Few studies have focused on the genetic basis of response to e[CO₂], or on transgenerational effects, although there is now some evidence to suggest that high-throughput genomic and postgenomic tools may provide some insight into these effects. For example, microarrays are beginning to identify genes that

appear sensitive to e[CO₂] (Gupta *et al.*, 2005; Taylor *et al.*, 2005; Ainsworth *et al.*, 2006), and leaf growth responses to e[CO₂] have been found in *Populus* and quantitative trait loci (QTL) for this response determined (Rae *et al.*, 2006). Although trees are, in general, difficult subjects to study, the *Populus* genus offers an ideal system. Its distribution is widespread in the Northern hemisphere, in contrasting environments, and there is considerable genetic variation in biomass production (Bradshaw & Stettler, 1995; Wu, 1998a; Wullschlegel *et al.*, 2005). In addition, poplar is now recognized as the model forest tree (Taylor, 2002; Wullschlegel *et al.*, 2002), and has several advantages for genetic studies, including the availability of linkage maps, mapping pedigrees and, most importantly, the release of the complete *Populus* genome sequence, the first for a tree (Tuskan *et al.*, 2006; <http://genome.jgi-psf.org/Poptr1/Poptr1.home.html>). *Populus* species

are useful in e[CO₂] experimental systems because of their fast growth rates and rapid development of a closed canopy (Gielen *et al.*, 2001). In response to e[CO₂], biomass stimulation in *Populus* has been between 33% and 39% in a short-rotation coppice system (Gielen & Ceulemans, 2001) and 20% in a mixed stand (Kubiske *et al.*, 2006). This response is determined by enhanced stem height, branching (Calfapietra *et al.*, 2003) and leaf area (Ferris *et al.*, 2001; Taylor *et al.*, 2003), improving foliage display for light interception and driving increased photosynthesis and gross primary production (Wittig *et al.*, 2005). In a free-air CO₂ enrichment (POPFACE) experiment, poplar canopy productivity was stimulated in the first year after planting (Calfapietra *et al.*, 2003) and the first year after coppicing (Liberloo *et al.*, 2005). Seemingly, much of the increased assimilation in e[CO₂] is invested in enhanced root biomass (Pritchard & Rogers, 2000), in particular in fine root biomass (Lukac *et al.*, 2003).

Here we quantify the effect of e[CO₂] on tree growth using an interspecific inbred F₂ pedigree (Family 331) from the two contrasting species, *P. trichocarpa* T. & G. and *P. deltoides* Marsh (Bradshaw *et al.*, 1994; Bradshaw & Stettler, 1995). The F₂ generation segregates for a wide variety of traits including growth, form and phenology characters making this an ideal pedigree in which to study quantitative traits such as above- and below-ground growth (Rae *et al.*, 2004). Quantitative trait locus mapping has yielded valuable insights into plant response to a range of environmental changes including salinity (Koyama *et al.*, 2001), drought (Lanaceras *et al.*, 2004), nitrogen supply (Loudet *et al.*, 2003) and soil aluminium concentration (Hoekenga *et al.*, 2003). Quantitative trait loci and putative underlying genes have recently been proposed to explain increased leaf growth and delayed leaf senescence in response to e[CO₂] (Rae *et al.*, 2006), but no information is currently available on any tree biomass traits or below-ground growth response to e[CO₂] and associated QTL in any study to date. Here we determine QTL for above- and below-ground growth response to rising CO₂ and in particular, using an additive main effects and multiplicative interaction (AMMI) model (Gauch, 1988), determine genotype by environment (G × E) interactions and identify QTL that occur in response to altered atmospheric CO₂. This approach provides some of the first data on the genomic regions determining tree growth response to e[CO₂].

Materials and Methods

Plant material and exposure conditions

A three-generation *Populus* mapping pedigree was generated by the 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 full-siblings, 53-246 and 53-242, from the resulting F₁ family (Family 53) were crossed to form an F₂ family (Family 331; Bradshaw &

Stettler, 1993; Bradshaw *et al.*, 1994). A total of 285 members of this family were used in this study.

This experiment was conducted in 16 open-top chambers (OTC) at the Forestry Commission field site, Headley, UK (51°07' N, 0°50' W). On 13 and 14 May 1999, the *P. trichocarpa* and *P. deltoides* parents, the F₁s and F₂ genotypes were established from unrooted hardwood cuttings derived from a stool bed at the University of Washington, Seattle, WA, USA. Cuttings were grown in John Innes no. 2 compost (lime free) in plastic tubes (91 cm in high, 15 cm diameter), in a randomized complete block design. For each treatment, 285 genotypes were randomly placed into one of eight chambers (*c.* 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 of these chambers received the ambient concentration of CO₂ (a[CO₂]) while eight chambers received an elevated concentration of CO₂ (e[CO₂]) at a target concentration of 600 μmol mol⁻¹ CO₂. Details of chamber design and monitoring of CO₂ have been reported previously (Ferris *et al.*, 2002; Rae *et al.*, 2006). Measurements were conducted throughout the growing season.

Stem growth

The number of sylleptic branches produced was counted at 125 d after planting (DAP). At the end of the experiment, tree height was measured with a metre rule and stem diameter was measured using precision manual callipers. Stem volume was estimated at 152 DAP using Huber's formula:

$$V = \frac{\pi d^2 \times l}{400000}$$

(*l* = length/height (m); *d* = the mid diameter (cm); and $\pi = 3.142$).

Root density measurements

Acetate sheets (A4 size) were attached to the clear plastic sides of each rhizotron using PVC tape. On 7 June (25 DAP), a black permanent marker was used to mark the position of any primary root tips apparent in the clear rhizotron window. This procedure was repeated 3 d later using a permanent blue marker, marking the new position of the root tips and tracing the path of the growing roots. This was repeated at regular intervals using coloured pens until 15 July. By this time the roots had grown the full length of the rhizotron. The acetate sheets were removed and the root traces measured using a home-made root-measuring wheel. Mean daily growth rate was calculated for each root. A transparent acetate grid made up of nine squares each of 1.27 cm² was taped onto the transparent rhizotron window. Primary and secondary root intersections with the grid were counted to give root density. Owing to the dimensions of the grid, the number of root/grid intersections

were equivalent to the root length in cm. (i.e. 17 intersections = 17 cm) (Marsh, 1991; Murray & Bristow, 1997). A grid of total area = 19.35 cm² was used to measure root length at three locations on the rhizotron window. The results of each count were combined to give the number of roots per 58.06 cm² (19.35 × 3 = 58.06).

Data analyses

Differences between plants grown in a[CO₂] and e[CO₂] were tested using a paired *t*-test, or *z*-test for non-normally distributed data in the Minitab statistical package (version 13). The G × E interactions were explored by using Bartlett's test to identify unequal variances of traits measured in a[CO₂] and e[CO₂]. Two-way ANOVA were carried out for each molecular marker, so that marker genotype by CO₂ interactions could be identified. The AMMI model was carried out using R-script (provided by Catherine Bastien, INRA, Orleans, France) and the principal component for each trait was used as a measure of plasticity between CO₂ concentrations for each genotype.

QTL mapping

The genetic linkage map for this pedigree was produced by G. A. Tuskan *et al.* (pers. comm.; http://www.ornl.gov/sci/ipgc/ssr_resource.htm) consisting of 91 simple sequence repeats (SSRs) genotyped on 350 individuals and 92 fully informative amplified fragment length polymorphisms (AFLPs) genotyped on 165 individuals. The primer sequences of SSR markers were blasted against the poplar genome sequence and linkage groups orientated based on the physical sequence (i.e. 3' to 5').

Trait data and plasticity measures were tested for normal distribution using the Andersson-Darling test. In cases where data were non-normally distributed, Box-Cox transformations were carried out. The data were analysed for QTL using the linear regression approach put forward by Haley & Knott (1992). The analysis was carried out using the option for outbred large single full-sib families with the software QTLEXPRESS (Seaton *et al.*, 2002). Chamber effect was included as a fixed effect in the QTL analysis. Chromosome-wide permutation tests with 1000 iterations were carried out to determine *P*-values and a significance threshold of 0.05 was taken as evidence for presence of a quantitative trait locus (Churchill & Doerge, 1994). Confidence intervals for the position of a quantitative trait locus were defined as the interval in which the *F*-statistic of the presence of a quantitative trait locus was at least twofold of its maximum value. Paternal, maternal and the interaction effects at the position where the quantitative trait locus mapped were noted. In cases where non-normally distributed data were transformed, the analysis was run again using the untransformed data to calculate the true QTL effects. The positions of molecular markers that showed G × E interactions,

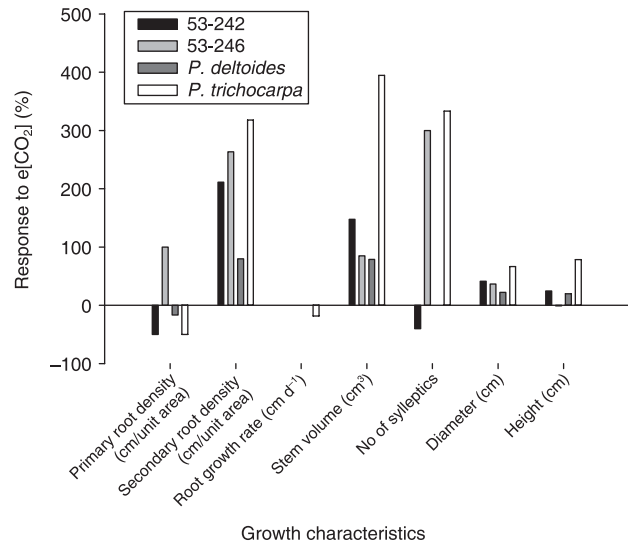


Fig. 1 The response of the grandparental species and parental clones of *Populus* to elevated CO₂ (e[CO₂]) for *P. trichocarpa*, *P. deltoides* grandparents and 53-246, 53-242 F₁ parents.

based on two-way ANOVA, were compared to the positions of QTL for plasticity measures.

The genotype data for SSRs were converted into the format for use in MAPMAKER-EXP (Lander *et al.*, 1987) and links between these SSRs and RFLPs previously mapped by Bradshaw *et al.* (1994) for this population were found so that comparisons with QTL cited in literature could be compared with those mapped in this study.

Results

Grandparental and parental responses to elevated CO₂

Figure 1 shows that *P. deltoides* had a greater stem volume than *P. trichocarpa* in a[CO₂] although both parents showed an increase in stem volume in e[CO₂], with a larger response observed in *P. trichocarpa*. In fact, *P. trichocarpa* showed nearly a fourfold increase in stem volume whereas *P. deltoides* showed less than a one fold increase, confirming previous findings that suggest the response to e[CO₂] in *P. trichocarpa* is greater than that of *P. deltoides* (Rae *et al.*, 2006). The number of sylleptic branches (branches formed without a period of dormancy) in *P. trichocarpa* was also increased by > 300% whereas in *P. deltoides* no increase in syllepsis was observed in response to e[CO₂]. Both grandparents showed a decrease in primary root density in e[CO₂], but secondary root density was increased in both *P. deltoides* and *P. trichocarpa* in e[CO₂] by one- and three-fold, respectively. In general, we can say that there were clear species differences in response to e[CO₂], leading us to speculate that this population would be a useful tool to determine the genetic basis of growth enhancement in future CO₂ atmospheres.

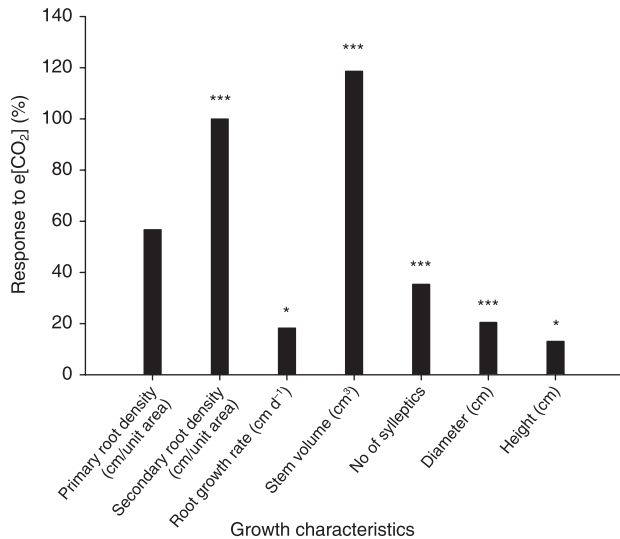


Fig. 2 Response of the *Populus* F₂ pedigree to elevated CO₂ from a cross between *P. trichocarpa* and *P. deltoides* compared with ambient CO₂. Statistical significance of results from a *t*-test: *, 0.05 > *P* > 0.01; **, 0.01 > *P* > 0.001; ***, *P* < 0.001.

Population response to elevated CO₂

Figure 2 confirms the observations from many large-scale field experiments, showing that e[CO₂] may stimulate plant growth, development and productivity, providing strong evidence that the QTL mapped from these data will be relevant and of value for further detailed molecular analysis since they ‘mimic’ responses to CO₂ in *Populus* observed in large field-scale ecosystem experiments (Liberloo *et al.*, 2005); stem volume index increased by 118% in e[CO₂] in this population. Clear effects of e[CO₂] were found for root growth, particularly apparent for the secondary root density, where a 100% increase was seen in e[CO₂] (Fig. 2), suggesting more branched rooting systems and relatively finer roots. A smaller (18%) increase was measured in primary root growth. The frequency distributions are shown in Fig. 3, with the grandparental and parental values in a[CO₂] and e[CO₂] all marked.

QTL mapping

Tables 1–3 show the QTL mapped in a[CO₂], e[CO₂] and plasticity to CO₂, respectively. The ‘plasticity QTL’ represent areas of the genome that appear to be sensitive to e[CO₂] and they may provide insight into future targeted work using a

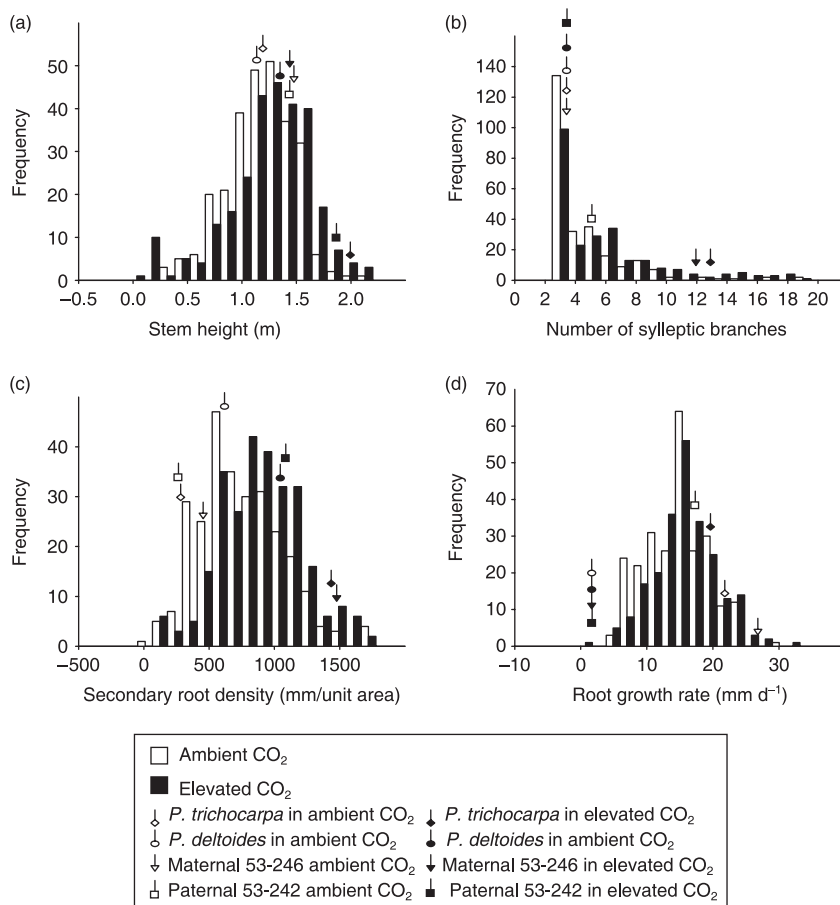


Fig. 3 Distribution of the phenotypes for (a) stem height (September), (b) number of sylleptic branches (September), (c) secondary root density and (d) root growth rate for Family 331 pedigree of poplar grown in open-top chambers at Headley, UK. Treatments: open bars, ambient CO₂; closed bars, elevated CO₂. Mean values for the grandparental species and parental clones in ambient and in elevated CO₂ are marked as shown in the key.

Table 1 Quantitative trait loci (QTL) mapped for plants grown in ambient CO₂ concentration

Trait	LG	Position (cM)	% Var	CI	<i>P</i>	Paternal	Maternal	Interaction
Stem height	IV	62	5.7	42–75	0.001	0.9987	6.9486	4.4074
Stem height	XVII	59	3.6	42–78	0.007	-7.677	-5.9244	0.6112
Stem diameter	IV	70	4.9	52–83	0.002	-0.003	0.0523	0.0102
Stem diameter	VIII	18	2.6	0–25	0.017	-0.0021	0.0117	-0.0211
Stem diameter	XII	0	2.6	0–12	0.049	0.0299	-0.0094	-0.0033
Stem diameter	XVI	63	2.3	33–87	0.034	0.0438	0.0225	0.0102
Stem diameter	XIX	23	2.9	16–32	0.018	0.0226	0.0263	0.0148
Number of sylleptic branches	VIII	19	3.4	0–27	0.021	-0.1861	-0.5288	0.1164
Number of sylleptic branches	X	5	2.9	0–18	0.041	0.5067	-0.182	-0.4709
Stem volume	IV	63	5.5	49–78	0.005	1.493	9.2929	2.1632
Stem volume	XVII	57	2.6	58–76	0.009	-9.0251	-6.8842	1.9636
Primary root density	XIV	0	2.7	0–12	0.021	0.0051	1.4813	0.3558
Secondary root density	X	43	4	28–57	0.01	2.3247	8.1443	-1.5084
Secondary root density	XII	21	2.8	6–24	0.025	5.6293	-1.1989	-2.7698
Secondary root density	XIV	18	6.1	9–28	< 0.001	-1.9107	8.846	-3.8626
Root growth rate	I	12	5.8	8–27	0.023	0.0626	0.1132	0.03
Root growth rate	XIX	22	4.5	12–32	0.005	0.0948	0.0485	0.0405

LG, linkage group; Position (cM), position to which the QTL mapped in cM; %Var, percentage variation explained by the QTL; CI, confidence interval; *P*-value, significance of a quantitative trait locus being present; Paternal, effect from the paternal parent; Maternal, effect from the maternal parent; Interaction, effect of the interaction between parental effects.

Table 2 Quantitative trait loci (QTL) mapped for plants grown in elevated CO₂ concentration

Trait	LG	Pos (cM)	% Var	CI	<i>P</i>	Paternal	Maternal	Interaction
Stem height	I	126	5.1	117–164	0.004	-3.721	-9.1701	-1.3851
Stem height	VI	137	7.1	131–139	0.001	-1.7445	-10.4205	-4.1466
Stem diameter	I	174	3.7	153–174	0.026	-0.036	-0.029	-0.0184
Stem diameter	VI	136	6.5	119–139	0.001	-0.0295	-0.0575	-0.0139
Stem diameter	X	40	4.2	28–53	0.006	-0.0011	-0.0548	-0.0024
Stem diameter	XVI	76	3.7	54–87	0.012	0.0524	0.031	-0.0085
Number of sylleptic branches	VIII	22	6.2	11–27	0.001	-0.6866	-0.7453	-0.0523
Number of sylleptic branches	XIV	45	4.1	30–58	0.005	0.7728	0.4578	0.1132
Stem volume	VI	137	6.7	129–139	0.001	-5.3457	-16.4526	-6.2626
Stem volume	X	33	3.3	22–50	0.016	1.4602	-10.6988	-4.0675
Primary root density	I	25	3.7	7–41	0.037	-1.7751	1.3883	-0.5346
Primary root density	VIII	18	3.2	15–25	0.015	-1.4587	-0.8552	0.5677
Primary root density	X	14	3.6	2–27	0.014	0.3381	2.0467	-0.1258
Primary root density	XI	25	3.7	0–48	0.016	-1.7997	0.6601	-0.7402
Secondary root density	IV	72	3	43–84	0.03	4.6116	-4.5461	1.8062
Secondary root density	V	0	3.1	0–19	0.038	5.5719	-3.127	-1.4956
Secondary root density	X	44	2.9	25–62	0.033	-1.8849	6.864	-5.6324
Root growth rate	XVI	48	3.4	25–83	0.022	0.0531	0.1411	-0.0991

LG, linkage group; Pos, position to which the QTL mapped in cM; %Var, percentage variation explained by the QTL; CI, confidence interval; *P*-value, significance of a quantitative trait locus being present; Paternal, effect from the paternal parent; Maternal, effect from the maternal parent; Interaction, effect of the interaction between parental effects.

reverse genetics approach. Using a whole-genome scan option in QTLEXPRESS, the position to which the QTL map, the confidence interval, percentage variation explained by each QTL, *P*-value, paternal, maternal (the difference in effects of the alleles inherited from each grandparent) and interaction effects (the deviation from additivity, where zero indicates complete additivity) are shown for each quantitative trait locus.

A total of 17 QTL in a[CO₂], 18 QTL in e[CO₂] and nine plasticity QTL were mapped in the pedigree for the seven traits. The QTL were mapped for all traits grown in both CO₂ treatments. The QTL for response to CO₂ were mapped for all traits with the exception of number of sylleptic branches. The variance explained by QTL ranged from 2.6 to 6.1% for a[CO₂] and 2.9–7.1% for e[CO₂]. The confidence intervals

Table 3 Quantitative trait loci (QTL) for response to CO₂ (plasticity QTL) using the component of the AMMI model as a measure of plasticity

Trait	LG	Pos (cM)	%Var	CI	P	Paternal	Maternal	Interaction
Stem height	I	118	2.9	98–128	0.020	−0.0066	−0.012	−0.0002
Stem height	IV	13	2.9	0–30	0.023	0.0082	−0.0116	−0.0059
Stem height	IX	3	1.2	0–9	0.017	0.0094	−0.0058	0.007
Stem diameter	XII	3	1.1	0–19	0.047	−0.0046	0.0095	−0.003
Stem volume	IX	3	3.0	0–9	0.038	0.0071	−0.0073	0.0094
Primary root density	XIX	32	2.6	16–32	0.037	−0.0123	0.0085	0.0068
Root growth rate	IV	82	2.2	48–84	0.038	−0.0163	0.0101	−0.0086
Root growth rate	XVI	59	2.2	27–87	0.030	−0.0021	0.0229	0.0016
Root growth rate	XIX	20	2.2	0–32	0.040	−0.0136	−0.0064	0.0011

LG, linkage group; Pos, position to which the QTL mapped in cM; %Var, percentage variation explained by the QTL; CI, confidence interval; P-value, significance of a quantitative trait locus being present; Paternal, effect from the paternal parent; Maternal, effect from the maternal parent; Interaction, effect of the interaction between parental effects.

for the position of a QTL were defined as the interval in which the *F*-statistic of the presence of a QTL was at least twofold of its maximum value. Intervals varied between 12 cM and 58 cM, with an average of 23 cM.

Figure 4 shows positions to which QTL mapped for stem and root traits in important linkage groups. The suggested presence of G × E interactions based on nonhomogeneous variances for traits scored in a[CO₂] and e[CO₂] and the number of molecular markers which showed significant genotype × CO₂ interaction from a two-way ANOVA are given in Table 4. Interestingly the above-ground stem traits showed significant differences of trait variances between a[CO₂] and e[CO₂], but the below-ground traits show a higher number of G × E interactions at molecular markers. Of the 26 marker genotype by CO₂ effects, only two share a similar position to plasticity QTL. These are for stem height on LGIX, and root growth on LGXIX.

Discussion

Altered plant growth and development in e[CO₂] often includes stimulated above-ground productivity, altered root architecture and increased biomass (Curtis & Wang, 1998; Pritchard *et al.*, 1999; Norby *et al.*, 2005; Kubiske *et al.*, 2006), although, for trees, these responses may vary depending on species and tree age (Körner, 2006). These changes are of adaptive significance since they are likely to affect plant competitive ability and fitness and yet the genetic basis of plant response to elevated CO₂ has rarely been studied. By exposing a mapping population to e[CO₂], we have revealed these growth responses as well as detected the underlying QTL determining growth and development traits. By combining these results with those already reported for growth in this mapping population (Bradshaw & Stettler, 1995; Wu, 1998a) and using the results from expression studies in e[CO₂] (Taylor *et al.*, 2005), we now have an extensive dataset identifying the basis of genetic adaptation of *Populus* in e[CO₂]. Feder & Mitchell-Olds (2003)

suggest postgenomic technologies allow high-throughput discovery of candidate genes and polymorphisms that affect traits of interest in a functional ecological context and we can now begin to investigate these target genes in much more detail (Taylor *et al.*, 2006).

Quantitative trait loci mapped to 13 of the 19 linkage groups. The number of QTL detected per trait ranged from 1 to 4 with an average of 2.6 per trait in a[CO₂] and 2.4 in e[CO₂]. This is in keeping with similar studies (Kearsey & Farquhar, 1998). Individual QTL were shown to explain relatively little of the total phenotypic variance (between 2.9 and 7.1%). Previous studies reporting similar results, have suggested that stem traits were controlled by several loci with relatively large effect (Bradshaw & Stettler, 1995; Grattapaglia *et al.*, 1996). These studies, however, were carried out with considerably smaller sample sizes and so were less likely to detect QTL of small effect. More recent work has tended to report QTL that explain relatively low percentage variance. For example, work by Wullschleger *et al.* (2005) reported QTL for above- and below-ground biomass traits ranging from 7.5 to 18.3%; Rae *et al.* (pers. comm.) report QTL for stem traits that ranged from 0.3 to 24.1%; and in a study of leaf traits report QTL that explained variance ranging from 1.8 to 9.7% (Rae *et al.*, 2006). As Rae *et al.* (2006) have noted, the low variance explained is suggestive of additional, undetected QTL of small effect.

Quantitative trait loci for growth and biomass mapped in a related backcross pedigree sharing the same maternal grandparent have been reported previously (Wullschleger *et al.*, 2005). The use of the same microsatellite primers in the production of the linkage maps for the two pedigrees enabled the identification of QTL which collocate to similar regions. For example, in the backcross pedigree, a QTL for stem biomass mapped at the top of LGVI to a similar region as stem height, diameter and volume QTL in the F₂ pedigree; on LGXII, QTL for above- and below-ground traits mapped to the same region in the backcross pedigree as to QTL for stem diameter and secondary root density in the F₂; on LGXIV a QTL for

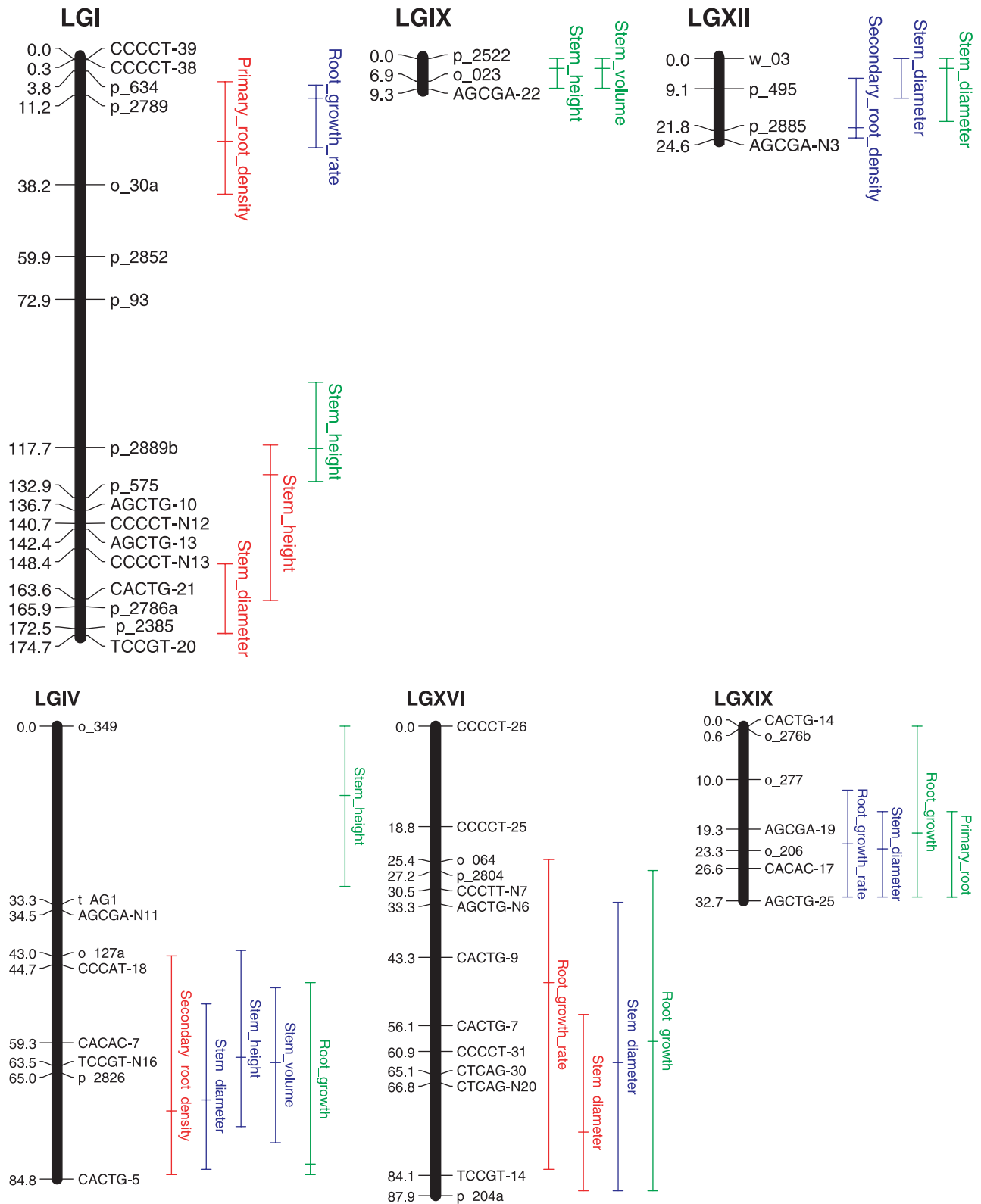


Fig. 4 Diagram of quantitative trait loci (QTL) mapped to selected, important linkage groups for above- and below-ground growth traits. The QTL for traits scored in ambient CO₂ (blue), or elevated CO₂ (red) and response (plasticity) QTL (green) are shown.

Table 4 The genotype by environment interaction shown in two ways for each trait

	Trait	G × E	Number of G × E effects at markers
Above ground	Stem diameter (mm)	***	2
	Stem height (m)	***	2
	Number of sylleptic branches	***	0
	Stem volume index	**	2
Below ground	Primary roots (mm per unit area)		9
	Secondary roots (mm per unit area)		3
	Root growth rate (mm d ⁻¹)		6

G × E, significant differences between variances for the pedigree grown in ambient (a[CO₂]) and elevated (e[CO₂]) CO₂ (**, $P < 0.01$; ***, $P < 0.001$); Number of G × E effects, number of markers that showed significant genotype by CO₂ effect.

branch biomass in the backcross pedigree mapped to a the same region as a QTL for the number of sylleptic branches in the F₂ pedigree.

The QTL from this study can also be compared with QTL for stem traits and the number of sylleptic branches mapped in the same F₂ pedigree when grown in contrasting environments in Europe (Rae *et al.*, in press). Stem trait QTL collocate to the same marker intervals on LGIV, LGIX, LGXVIII and LGXIX, while QTL for the number of sylleptic branches collocate on LGVIII and LGX.

In addition, the microsatellites used in the current study were assessed for grouping with the restriction fragment length polymorphism (RFLP) markers used previously to produce a linkage map in this F₂ pedigree (Bradshaw *et al.*, 1994). This enabled an approximate comparison of QTL previously mapped. For example, both Bradshaw & Stettler (1995) and Wu & Stettler (1994) previously mapped QTL for stem traits to linkage groups D, E, M, and O which correspond to LGXVI, LGXI, LGI, and LGXIX in the present study and a biomass QTL to linkage group J which corresponds to LGX. With the exception of LGXI, QTL for stem traits also mapped to these linkage groups in the present study. Although it is not possible to precisely align the maps used in this previous work, it is possible to use this information to confirm the presence of stem and biomass QTL across the different studies and even different mapping pedigrees.

Elevated [CO₂] resulted in the production of larger trees with increased secondary root densities (Figs 1 and 2) in all generations, with the F₂ generation segregating for all measured traits (Fig. 3). Although some QTL from the a[CO₂] and e[CO₂] treatments collocated on the genetic linkage map, there were many QTL that were identified solely in the different growing conditions. Genotype by environment (G × E) interactions were tested for each trait by testing for homogeneity of variances between CO₂ treatments. Differences in the population variances suggested that the genotypes were acting differently in the two environments. Significant G × E interactions were seen for stem height, diameter, number of sylleptic branches and volume, but not for below-ground traits. The variances of traits measured

in e[CO₂] were consistently higher than for traits in a[CO₂]. The below-ground traits showed little difference between variances but a higher number of G × E interactions at molecular markers. This implies that e[CO₂] increases variation for stem traits but affects marker genotype rank order for root traits. The highest number of marker G × E effects was seen for primary root density and root growth rate, yet this was not reflected in the number of plasticity QTL detected. The percentage variance explained for plasticity QTL was low, suggesting that there may be many QTL of small effect that are relevant in e[CO₂] that were not detected. These observations indicate that further study of the complexities of G × E interactions in relation to e[CO₂] is warranted.

The AMMI model combines analysis of variance and principal components analysis into a single model with additive and multiplicative parameters, and has been used previously to investigate the mode of genetic control in response to environmental differences (Emebiri & Moody, 2006). Emebiri & Moody (2006) report that, for a barley pedigree analysed for heading date, the allele sensitivity model, put forward by Via *et al.* (1995) appears to be prevalent. This model suggests that the constitutive gene is itself regulated indirect response to the environment, by activation of different alleles in different environments, therefore collocation is seen between stability/plasticity QTL and trait QTL showing G × E interactions. However, Rae *et al.* (in press) suggest that a gene regulation model is more prevalent for stem traits in poplar grown in contrasting environments. This model implies that one or more regulatory loci are under the direct influence of the environment and the constitutive gene is switched on and off by a regulatory gene, and therefore plasticity QTL do not collocate to trait QTL showing G × E interaction. In the present study, the lack of collocation between molecular markers showing G × E interactions with plasticity QTL suggests that the gene regulation model is also prevalent under e[CO₂]. Work on *Populus* by Wu (1998b) also concluded that the gene regulation model was the more likely mechanism controlling phenotypic plasticity for growth traits across heterogeneous environments.

A number of interesting regions of the genome worth future study were uncovered (Fig. 4). For above-ground tree growth, three areas of the genome on linkage groups I, IX and XII appear important. From previous studies in *Populus*, there is evidence that above-ground biomass QTL collocate to the region of interest on LGIX, but not on LGI or LGXII. This suggests that the presence here of the plasticity QTL on LGI and LGXII represent a distinct response to changing CO₂, with these regions of the genome becoming important in determining above-ground tree growth in e[CO₂]. The presence of a QTL on LGXII for stem diameter identified in a[CO₂] but not e[CO₂] and a QTL for stem diameter plasticity imply that this region of the genome is switched off in e[CO₂].

Leaf development, longevity, petiole orientation and stomatal density act together to determine the amount of intercepted radiation, carbon gain and water loss in a forest canopy which, together with photosynthetic efficiency, determines net primary productivity. It is therefore of interest to compare QTL for leaf traits with those for growth and biomass. Of particular interest is the comparison of QTL for response to elevated CO₂ mapped previously in this pedigree for leaf traits (Rae *et al.*, 2006). The collocation of QTL for leaf traits and stem traits is to be expected and may indicate the location of genes with pleiotropic effects.

A plasticity QTL for stem height in the present study collocates to a response QTL for senescence index on LGI. This is of particular importance as elevated CO₂ was shown to reduce leaf senescence therefore extending the growing season. Stem yield differences have been shown to depend on leaf senescence and longevity in a clonal trial of a *P. tremuloides* × *P. tremula* population (Yu *et al.*, 2001). Moreover leaf longevity is increased in *Populus* species in e[CO₂] (Tricker *et al.*, 2004). Canopy duration is of interest in this mapping pedigree due to the difference between the two grandparental species. When grown in their natural habitats, *P. trichocarpa*, which comes from western Washington, has a longer growing season than *P. deltoides* which originates from central Illinois. However, when grown in southern UK, the growing season of the grandparental species differs by only 3 wk because of the earlier bud flush rather than later bud set of *P. trichocarpa* (Rae *et al.*, 2004), but in the present study senescence index was seen to be higher in *P. deltoides*, suggesting that leaf photosynthesis activity was reduced at the end of the growing season.

Several QTL for stem characters in a[CO₂] mapped to between 62 cM and 70 cM on LGIV and this is an area where QTL for leaf cell area, number of cells, leaf area and senescence index have also been mapped (Rae *et al.*, 2006). On LGVI, QTL for stem volume, height and diameter in e[CO₂] collocate with QTL for the number of leaves in both e[CO₂] and a[CO₂] and with a response QTL for (late) leaf area. Interestingly, although there is no plasticity QTL present here, these QTL also collocate with a candidate gene (PU10293) for altered leaf development in e[CO₂] (Taylor *et al.*, 2005). The QTL for stem volume and diameter in e[CO₂] only are also located on LGX between 33 cM and 40 cM, collocating with QTL for

both (late) leaf area and specific leaf area in e[CO₂] (Rae *et al.*, 2006), as well as with a QTL for leaf number found by Wu (1998a). These coinciding QTL indicate an important area of the genome for control of growth in e[CO₂] and are collocated with three candidate genes (Taylor *et al.*, 2005). Rae *et al.* (2006a) showed that LGXII, though small, is of interest for leaf growth response QTL for e[CO₂]. Here a plasticity QTL for stem diameter collocated to response QTL for leaf area in young and mature leaves, petiole length and leaf cell number, implying that the increase in cell number led to increased leaf area and therefore increased stem growth. Together these QTL may indicate the genetic basis of an important mechanism for increased stem and root growth in e[CO₂]. Candidate genes determining response to elevated CO₂ identified from microarray analysis (Taylor *et al.*, 2005) are located in this region of LGXII and suggest it as a genomic region worthy of further study.

Three areas of the genome on linkage groups IV, XVI and XIX were identified as important in determining below-ground response to e[CO₂], with plasticity QTL suggesting that these regions are switched on in e[CO₂] on LG IV and XVI and switched off in LG XIX. There is limited evidence to suggest what these QTL represent at the level of the gene, however, they may be relevant in developing improved *Populus* trees with enhanced ability to sequester carbon.

In summary, we have presented the first study of plant growth in e[CO₂], where experimental genetic variation has been used to identify the underlying genomic regions determining above- and below-ground biomass responses to future high CO₂ atmospheres. These findings represent an informative starting point for further elucidation of the genes most responsive to elevated CO₂. The combination of this mapping analysis with other high-throughput technologies now available in systems biology (Taylor *et al.*, 2005) should lead to an understanding of microevolutionary response to e[CO₂], and be of significance to future evolutionary and ecological understanding and aid future plant breeding and selection.

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