Genetic Analysis of Natural Variations in the Architecture of *Arabidopsis thaliana* Vegetative Leaves

José Manuel Pérez-Pérez, José Serrano-Cartagena and José Luis Micol[1]

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, 03202 Elche, Alicante, Spain

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ABSTRACT

To ascertain whether intraspecific variability might be a source of information as regards the genetic controls underlying plant leaf morphogenesis, we analyzed variations in the architecture of vegetative leaves in a large sample of *Arabidopsis thaliana* natural races. A total of 188 accessions from the *Arabidopsis* Information Service collection were grown and qualitatively classified into 14 phenotypic classes, which were defined according to petiole length, marginal configuration, and overall lamina shape. Accessions displaying extreme and opposite variations in the above-mentioned leaf architectural traits were crossed and their F_2 progeny was found to be not classifiable into discrete phenotypic classes. Furthermore, the leaf trait-based classification was not correlated with estimates on the genetic distances between the accessions being crossed, calculated after determining variations in repeat number at 22 microsatellite loci. Since these results suggested that intraspecific variability in *A. thaliana* leaf morphology arises from an accumulation of mutations at quantitative trait loci (QTL), we studied a mapping population of recombinant inbred lines (RILs) derived from a Landsberg *erecta*-0 × Columbia-4 cross. A total of 100 RILs were grown and the third and seventh leaves of 15 individuals from each RIL were collected and morphometrically analyzed. We identified a total of 16 and 13 QTL harboring naturally occurring alleles that contribute to natural variations in the architecture of juvenile and adult leaves, respectively. Our QTL mapping results confirmed the multifactorial nature of the observed natural variations in leaf architecture.

MANY questions remain unanswered in the study of how the overall pattern of plant leaves is built, both at the level of leaf initiation and morphogenesis. Over the past decade, however, significant insights into several of the mechanisms operating in leaf ontogeny have been gained by studies of different plant species (reviewed in Hake and Sinha 1991; Smith and Hake 1992; Sinha *et al.* 1993; Telfer and Poethig 1994; Tsukaya 1995; Hall and Langdale 1996; Sylvester *et al.* 1996; Poethig 1997; Brutnell and Langdale 1998; Tsiantis and Langdale 1998; Van Lijsebettens and Clarke 1998; Scanlon 2000; Byrne *et al.* 2001). Most of the published studies illuminating this issue have approached the causal analysis of leaf development by means of isolating mutants, whose characterization has allowed the identification of genes involved in leaf ontogeny. Nevertheless, variants in the architecture of leaves can be found not only by experimentally inducing mutations but also in the diversification of the species of interest in natural races. Both of these approaches might provide a panoramic view of the range of variation of the system under study and also specific variants to be studied to identify the genes controlling plant organogenesis.

The study of natural variation has proved useful for analyzing the genetic basis of some developmental processes in the model system *Arabidopsis thaliana*. Important contributions to their genetic dissection have been made by analyzing the progeny of intercrosses involving accessions (also named ecotypes) that differ in specific traits. Such an approach has allowed the identification of single genes controlling flowering time such as *FLOWERING ALTERED* (*FLA*; Lee *et al.* 1993), also named *FRIGIDA* (*FRI*; Clarke and Dean 1994; Sanda *et al.* 1997; Johanson *et al.* 2000); *AERIAL ROSETTE* (*ART*); and *ENHANCER ROSETTE* (*EAR*; Grbic and Bleecker 1996). In addition, quantitative trait loci (QTL) analysis has been shown to be useful in the identification of novel genes involved in some developmental processes such as those of *EARLY DAY-LENGTH INSENSITIVE* (*EDI*), *FLOWERING F* (*FLF*), *FLG*, and *FLH* (Alonso-Blanco *et al.* 1998), which affect flowering time; the *ROSETTE LEAF NUMBER* (*RLN1–RLN5*) loci (Clarke *et al.* 1995), which affect vernalization responsiveness; the *REDUCED TRICHOME NUMBER* gene (*RTN*; Larkin *et al.* 1996), affecting the number of trichomes on vegetative leaves; four QTL affecting seed oligosaccharide storability [Bentsink *et al.* 2000; those of *ESPRESSO* (*ESP*), *ANDANTE* (*AND*), *NON TROPO* (*NOT*), and *RALENTANDO* (*RAN*)]; QTL affecting circadian rhythm (Swarup *et al.* 1999); at least 11 loci affecting seed size

[1]*Corresponding author:* Universidad Miguel Hernández, Campus de Elche, Edificio Vinalopó, Avenida del Ferrocarril s/n, 03202 Elche, Alicante, Spain. E-mail: jlmicol@umh.es

(Alonso-Blanco *et al.* 1999); 11 QTL associated with several floral traits (Juenger *et al.* 2000); 110 QTL for inflorescence developmental traits (Ungerer *et al.* 2002); and 12 QTL affecting light and hormone responses (Borevitz *et al.* 2002) among others.

Advances in molecular technologies, together with the information provided by genome projects, have made possible the Mendelization of QTL intervals, which are instrumental in positionally cloning the underlying genes. Recent examples are the *fw2.2* gene of tomato (Frary *et al.* 2000) and the *EDI* locus of *A. thaliana*, a QTL that turned out to be a novel allele of the *CRYPTOCHROME2* (*CRY2*) gene (El-Din El-Assal *et al.* 2001).

With a view to identifying genes involved in leaf morphogenesis, we have screened for new mutants with abnormal leaves (Berná *et al.* 1999; Robles and Micol 2001; Pérez-Pérez *et al.* 2002) and conducted genetic analyses of already obtained mutants (Serrano-Cartagena *et al.* 1999, 2000). The work presented here consists of a study of variations in the architecture of vegetative leaves in a large sample of *A. thaliana* accessions, with the purpose of ascertaining whether natural variability can act as a source of information for studying the genetic controls underlying leaf morphogenesis. We also performed a detailed morphometric analysis of leaf growth in one such accession, Enkheim-2 (En-2), which represents the genetic background for most of the mutant lines belonging to the large *Arabidopsis* Information Service (AIS) form mutants collection. Variations in the repeat number at 22 microsatellite loci were determined to measure genetic distances between the accessions under study, with the purpose of assessing correlation between their phylogeny and leaf morphology. We finally identified QTL contributing to natural variations in the architecture of the vegetative leaves of *A. thaliana* by analyzing a mapping population of recombinant inbred lines (RILs) obtained from a Landsberg *erecta*-0 (L*er*-0) × Columbia-4 (Col-4) cross.

MATERIALS AND METHODS

Plant materials, growth conditions, and crosses: Seeds of *A. thaliana* (L.) Heyhn. accessions and RILs (Lister and Dean 1993) derived from a cross between Landsberg *erecta*-0 (L*er*; NW20) and Columbia-4 (Col-4; N933) were supplied by the Nottingham *Arabidopsis* Stock Centre (NASC). The accessions used in this work are listed in Table 1, where they are noted by using the *Arabidopsis* Information Service and NASC (in parentheses) nomenclatures (Röbbelen 1965; Kranz 1978; Kirchheim and Kranz 1981; Kranz and Kirchheim 1987). The list of studied RILs included the following: N1901, N1903–N1971, N1973–N1999, N4664, and N4686.

Sterile (in 150-mm petri dishes containing 100 ml solid culture medium) and nonsterile (in pots containing a 2:2:1 mixture of perlite, vermiculite, and sphagnum moss) cultures were performed at 20° ± 1°, 60–70% relative humidity, and continuous illumination of 7000 lux as described in Ponce *et al.* (1998). Crosses were performed with forceps under a binocular microscope, by transferring pollen from mature anthers to the stigmata of previously emasculated flowers (Kranz and Kirchheim 1987).

Photography and morphometric analysis: Photographs of accession rosettes were taken with a Nikon F-601 AF camera equipped with an AF Micro Nikkor 105-mm f/2.8 macro lens 30 days after sowing. For the morphometric analysis of En-2 leaves, all the leaves from 10 plants were excised with forceps every 2 days, from the 10th to the 32nd day after sowing, immediately submerged in water to prevent dehydration, and mounted on slides before being photographed in a Leica MZ6 microscope. Morphometric analysis of these photographs was performed with the Sigmascan 2.0 program (Statistical Products & Service Solutions, Chicago).

For the morphometric analysis of RILs, seeds were sown on petri dishes. A total of 40 plants, corresponding to two RILs, were grown per petri dish. Two sowings were made per RIL. Leaves from the third and the seventh node from 15 plants of each RIL, chosen at random, were excised with forceps 25 days after sowing, immediately placed on the surface of agar medium to prevent dehydration, and covered by a transparent film. Photographs were taken with a Sony Cybershot FV-505 digital camera using a resolution of 1024 × 768 pixels. Images were digitally processed with the Adobe Photoshop 5.0 program (Adobe Systems, San Jose, CA) and morphometrically analyzed with the *NIH Image* program (developed at the U.S. National Institutes of Health and available at http://rsb.info.nih.gov/nih-image/).

Detection of microsatellite variation: DNA isolation and PCR amplifications were performed following the high-throughput method described in Ponce *et al.* (1999), which is based on multiplex PCR amplification of microsatellites, followed by fluorescent semiautomated detection of the amplification products in a Perkin-Elmer (Norwalk, CT) ABI PRISM 377 DNA sequencer. Genomic DNA samples of each accession were used as templates in five parallel multiplex PCR mixtures, each of which included three to six primer pairs. Each primer pair incorporates one oligonucleotide labeled with a fluorochrome [4, 7, 2′, 4′, 5′, 7′-hexachloro-6-carboxyfluorescein (HEX), 6-carboxyfluorescein (6-FAM), or 4, 7, 2′, 7′-tetrachloro-6-carboxyfluorescein (TET) phosphoramidites]. The microsatellites coamplified in each reaction mixture and the fluorochrome used to identify the corresponding amplification product were as follows: nga1126 (TET), nga162 (6-FAM), AthGAPAab (6-FAM), nga1111 (HEX), and MBK5 (HEX) in mixture 1; nga168 (6-FAM), nga12 (HEX), and AthCTR1 (TET) in mixture 2; AthZFPG (6-FAM), T27k12-Sp6 (6-FAM), AthGENEA (TET), nga1145 (HEX), AM4 (TET), and nga1139 (HEX) in mixture 3; AthACS (TET), nga111 (6-FAM), AthCHIB (HEX), nga6 (HEX), and nga139 (TET) in mixture 4; nga361 (TET), nga1107 (TET), and AthPHYC (6-FAM) in mixture 5. The sequences of the oligonucleotides used were as described in Ponce *et al.* (1999).

Microsatellite lengths were determined using the GENESCAN 2.1 fragment analysis software (Applied Biosystems, Foster City, CA). The number of repeats of each allele was estimated by comparing the size of its amplification product with that of the Col-0 accession, which was determined by Bell and Ecker (1994).

Gene diversity (or expected heterozygosity in a random mating population, H) was estimated following Nei (1973) and Innan *et al.* (1997): $n(1 - \Sigma p^2)/(n - 1)$, where n is the number of samples and p is the frequency of an allele. The MICROSAT 1.5d program (E. Minch, unpublished data; available at http://hpgl.stanford.edu/projects/microsat/) was used to obtain genetic distance measurements. This program allows the following parameters to be calculated for microsatellite data: the $(\delta\mu)^2$ genetic distance (Goldstein *et al.* 1995a), the average square distance (D_1; Goldstein *et al.* 1995b), the

kinship coefficient (K_F; Cavalli-Sforza and Bodmer 1971), the proportion of shared alleles (P_S; Bowcock *et al.* 1994), the fuzzy set similarity measure (F_S; Dubois and Prade 1980), and the absolute difference parameter (D_{AD}; Goldstein *et al.* 1996). Distance matrices were obtained with the MICROSAT program and bootstrap resampling ($n = 1000$) was used to construct multiple phylogenetic trees with the NEIGHBOR program, which were derived into consensus trees with the CONSENSE program, both included in the PHYLIP 3.5c package (Felsenstein 1993). Trees were plotted with the TreeView program (Page 1996).

Measurement of quantitative variation and statistical analysis: Leaf morphology was studied in the third node (N3) and seventh node (N7) vegetative leaves 25 days after sowing. Measurements were taken for lamina area (LA), lamina perimeter (LP), lamina major chord or length (LL), lamina minor chord or width (LW), petiole length (PL), and petiole width (PW). In addition, the number of marginal serrations (NMS) was scored only in seventh leaves. Other measurements made 25 days after sowing were the total leaf number (TLN) and major (MRD) and minor (mrd) rosette diameters.

Data statistical analyses were performed by the SPSS version 7.5 software package (Statistical Products & Service Solutions) as described below. The normality of the studied traits was assayed by both chi-square (Snedecor and Cochran 1989) and Kolmogorov-Smirnov analyses (Chakravarti *et al.* 1967). For each trait, total phenotypic variance (V_P) was partitioned into sources attributable to genotype and error, using a random effects analysis of variance (ANOVA) as previously described (Ungerer *et al.* 2002). Components of variance were used to estimate the broad-sense heritability (H^2) of the studied traits as $V_G/(V_G + V_E)$, where V_G is the among-RIL variance and V_E is the residual (error) variance. The coefficient of genetic_variation (CV_G) was calculated as $(100 \times \sqrt{V_G})/\overline{X}$, where $\overline{X}$ is the mean value for the studied trait in the RIL population. Genetic correlations (r_G) among the studied traits in the RIL population were estimated as $\mathrm{cov}(x, y)/\sigma_x\sigma_y$ (Robertson 1959), and proximity matrices were constructed by using the SPSS version 7.5 software package.

QTL mapping: We used a mapping population of 100 RILs, derived from a L*er*-0 × Col-4 cross, whose residual heterozygosity is 0.42% (Lister and Dean 1993). Quantitative genetic analyses were conducted on the morphometric data obtained as described above from the pictures taken from excised leaves.

We constructed a linkage map using 173 molecular markers (42, 24, 29, 38, and 40 markers, respectively, for chromosomes 1, 2, 3, 4, and 5), all of which had already been genotyped by previous authors in at least 90 of the RILs studied here (information available at http://nasc.nott.ac.uk/RI_data/full_markers.text). These markers covered 519.5 cM, >85% of the Arabidopsis genome, and were spaced at intervals ranging from 0.5 to 8 cM, their average distance being 3 cM.

QTL analyses were performed by using the MAPQTL version 4.0 program (Van Ooijen and Maliepaard 1996). Marker interval candidates to possibly contain a QTL were identified by means of the consecutive use of interval mapping (IM; Lander and Botstein 1989) and multiple-QTL model mapping (MQM; Jansen 1993; Jansen and Stam 1994) methods. The IM method was used first, and different combinations of the markers linked to the identified QTL were then tested as cofactors for MQM mapping. QTL intervals were gradually refined in this way, to finally select as cofactors those markers that maximized the variance explained by each QTL. Logarithm-of-odds (LOD) scores obtained in this way were used to construct QTL likelihood plots (Lander and Botstein 1989), using the SigmaPlot 2000 version 6.0 program (Statistical Products & Service Solutions). A significance threshold of 2.7 was chosen for QTL identification after determination, separately performed for each trait, of experiment-wide significance thresholds by permutation analysis (Churchill and Doerge 1994; Doerge and Churchill 1996; Ungerer *et al.* 2002). One thousand permutations were performed for each trait, and the LOD score thresholds obtained ranged from 2.7 to 2.8 ($P < 0.05$) and from 3.3 to 3.5 ($P < 0.01$). For each identified QTL, 2-LOD support intervals were established as a 95% confidence level (Van Ooijen 1992) after fixing as cofactors the closest marker to all the detected QTL, with the exception of that cofactor linked to the QTL under study. Finally, the additive effect and the percentage of variance explained by each QTL, as well as the total variance explained by all of the QTL contributing to a single trait, were also obtained from the MapQTL 4.0 program using MQM mapping.

RESULTS

Phenotypic classification of accessions of the AIS collection: To study leaf natural variations in a wide and representative sample of *A. thaliana* plants, wild-type lines were chosen from the large AIS collection. Such natural races of different geographical origins are named ecotypes or accessions (according to Alonso-Blanco and Koornneef 2000). Seeds from 193 AIS accessions were obtained from the NASC (Table 1) and grown in a controlled environment under sterile culture conditions (see materials and methods). A total of 24 seeds, corresponding to four accessions, were sown per 150-mm petri dish. The plants were photographed every 3 days, from the sixth day after sowing. Five accessions (Dijon-M, Enkheim-T, Hojda-Obi-Garm, Cen-0, and Chi-0) had to be discarded because of their inability to germinate, despite repeated attempts, in our working conditions. The remaining 188 accessions were qualitatively classified into 14 phenotypic groups, attending to the morphology of their rosette leaves. We took into account the overall shape of the lamina (spatulate, rounded, or lanceolate), its deviations from flatness or planarity (undulate, revolute, or involute leaves), and its marginal configuration (smooth or serrated margin), as well as the compactness of the basal rosette (either bushy or loose, which is mostly dependent upon the length of the petiole) as observed 30 days after sowing (Table 1).

Leaf initiation rate in accessions of the AIS collection: In *A. thaliana*, as in many other plant species, leaves produced at different stages of development show morphological differences, which are known as heteroblasty (reviewed in Lawson and Poethig 1995; Tsukaya *et al.* 2000). This is illustrated in Figure 1, which represents variations with time in the shape and size of a given leaf during its expansion, as well as differences between different leaves within a plant (taking as examples the second and the fifth rosette leaves of the En-2 accession). Early leaves are small with rounded laminae, whereas later leaves are larger, have lanceolate laminae, and expand more rapidly than the earlier ones.

The above-mentioned differences between leaves in En-2 might be greater for other accessions, especially

TABLE 1

***Arabidopsis thaliana* accessions studied in this work, classified according to their leaf morphology and rosette structure**

Leaf	Rosette	Accessions
Spatulate	Bushy	An-2 (N946), Ang-0 (N948), Bs-2 (N998), Bsch-0 (N1002), Bsch-2 (N1004), Bu-4 (N1012), Bu-17 (N1036), Bu-20 (N1042), Bu-22 (N1046), Bu-25 (N1052), Bur-0 (N1028), Chi-2 (N1076), Do-0 (N1112), Dr-0 (N1114), Ei-2 (N1124), Lan-0 (N1304)
	Loose	*Ag-0 (N901)*, Ag-0 (N936), Ak-1 (N938), Bch-1 (N956), Bch-4 (N960), Bu-6 (N1016), Bu-23 (N1048), Co-2 (N1086), Co-3 (N1088), Co-4 (N1090), Di-1 (N1108), Dra-0 (N1116), Ei-6 (N1130), Es-0 (N1144), Ge-1 (N1188), Kl-3 (N1280), Ma-0 (N1356), *Sorbo (N931)*
Rounded	Bushy	Bd-0 (N962), Bla-14 (N988), La-1 (N1302)
	Loose	*Aa-0 (N900)*, *C24 (N906)*, Be-0 (N964), Bla-1 (N970), Bla-2 (N972), Bla-3 (N974), Bla-6 (N980), Bla-10 (N982), Ca-0 (N1060), Er-0 (N1142), Ge-2 (N1190), *Ler (NW20)*, Li-5-3 (N1324), Ll-0 (N1338), Ll-1 (N1340), Lz-0 (N1354)
Lanceolate	Bushy	Bla-12 (N986), Bu-15 (N1034), *Col-2 (N907)*, *Da(1)-12 (N917)*, Edi-0 (N1122), Ei-4 (N1126), Est-1 (N1150), Fr-2 (N1168), Gie-0 (N1192), *H55 (N923)*, Jl-2 (N1250), Li-3 (N1316), Li-5 (N1320), Lm-2 (N1344), Lö-1 (N1346), Lö-2 (N1348), Nc-1 (N1388)
	Loose	Chi-1 (N1074), Cvi-0 (N1096), *Cvi-0 (N902)*, Ep-0 (N1140), Gr-3 (N1202), Gü-1 (N1214), Ha-0 (N1218), Hl-0 (N1228), Hn-0 (N1234), Je-0 (N1246), Jl-5 (N1256), Mnz-0 (N1370), *Wei-0 (N3110)*
Undulate	Bushy	Bu-9 (N1022), Bu-11 (N1024), Bu-13 (N1026), Bu-19 (N1040), *Col-3 (N908)*, Gö-2 (N1196), Gr-1 (N1198), Jl-4 (N1254), Kn-0 (N1286), Lc-0 (N1306), Li-8 (N1332), Lip-0 (N1336)
	Loose	Aa-0 (N934), An-1 (N944), Bch-3 (N958), Bl1 (N968), Bu-5 (N1014), Col-0 (N1092), Ct-1 (N1094), Db-0 (N1100), Db-2 (N1104), Dra-2 (N1120), En-1 (N1136), Fr-3 (N1170), Fr-5 (N1174), Gd-1 (N1184), Gö-0 (N1194), Gr-2 (N1200), Gr-4 (N1204), In-0 (N1238), *Je54 (N924)*, Kl-4 (N1282), Kr-0 (N1296), Kro-0 (N1300), Li-7 (N1330), Ma-2 (N1358), *Mh-0 (N904)*, Mh-0 (N1366), Mrk-0 (N1374), Np-0 (N1396), *Petergof (N926)*, *RLD1 (N913)*, *Sn(5)-1 (N930)*
Revolute	Bushy	Bu-18 (N1038), Fe-1 (N1184), Fi-0 (N1156)
	Loose	*Abd-0 (N932)*, *Dijon G (N910)*, Eil-0 (N1132), El-0 (N1134), Fr-4 (N1172), Hh-0 (N1224), Jl-1 (N1248), Ma-2 (N1358), Nie-0 (N1392), *Ws (N915)*, *Ws-2 (N1601)*
Involute	Bushy	Bs-1 (N996), Bu-14 (N1032)
	Loose	Bay-0 (N954), Bu-7 (N1018), Cal-0 (N1062), Fr-7 (N1178)
Serrated margin	Bushy	Bu-3 (N1010), *Col-4 (N933)*, Dra-1 (N1118), En-2 (N1138), Est-0 (N1148), *Estland (N911)*, Fi-1 (N1158), Ga-0 (N1180), Gü-0 (N1212), Gy-0 (N1256), Hl-2 (N1230), *Kas-1 (N903)*, *Ms-0 (N905)*, Mt-0 (N1380), Mz-0 (N1382), *Rubezhnoe-2 (N928)*
	Loose	Ang-1 (N950), Be-1 (N966), Bla-4 (N976), Bla-11 (N984), Bs-5 (N1000), Bu-0 (N1006), Bu-2 (N1008), Bu-8 (N1020), Bu-24 (N1050), Cl-0 (N1082), Co-1 (N1084), Da-0 (N1098), Db-1 (N1102), Di-0 (N1106), Di-2 (N1110), *Enkheim-D (N920)*, Fr-6 (N1176), Ga-2 (N1182), Kl-1 (N1276), *Kondara (N916)*, Kä-0 (N1266), La-0 (N1298), Nd-0 (N1390), *Rubezhnoe-1 (N927)*, *S96 (N914)*, *Ws-1 (N2223)*

Underlined accessions displayed to a maximum degree the trait defining a phenotypic group and were later used in an attempt to determine the corresponding inheritance pattern. Accessions shown in italics do not belong to the AIS collection.

those displaying an increased number of leaves, which, in turn, would affect the whole aspect of the rosette. Hence, we thought it useful to determine the rates of leaf formation in the accessions under study. For that purpose, plants were photographed 6, 9, 12, 15, and 18 days after sowing, and the numbers of visible aerial

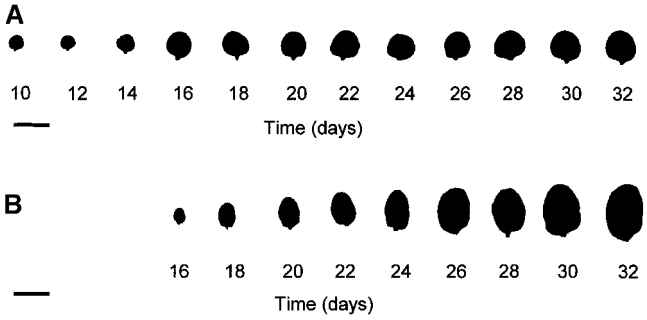

FIGURE 1.—Variation with time of the size and shape of Enkheim-2 (En-2) vegetative leaves. (A) Second rosette leaf. (B) Fifth rosette leaf. Bar, 5 mm.

organs (cotyledons, leaf primordia, and rosette leaves) were scored. The results are shown in Table 2, in which the growth rates reflect the moment when leaves and leaf primordia become visible under a dissecting microscope and not when they are produced by the shoot meristem.

As Table 2 shows, most accessions displayed the same vegetative developmental rates when cultured under the same conditions. A few exceptions were Bla-1, Bla-2, and Bla-3, which showed a delayed growth that merely seems to be a consequence of the belated appearance of their first pair of leaves. In other accessions with delayed growth, such as Condara and S96, all the leaves were produced at a lower rate. Only in 4 of the accessions studied (Do-0, Hl-0, Kä-0, and Kn-0) did vegetative leaves appear faster than in the remaining 184, with a difference with the remaining accessions of one leaf increase every 6 days. For these few accessions that showed atypical behavior, we tried to determine whether any correlation existed between their developmental profiles and the scarce information available on the environmental conditions of their habitat of origin (ANDERSON 1993); no obvious relationship was found.

Morphometric analysis of the expansion of En-2 vegetative leaves: One accession, En-2, was chosen to make quantitative the above-mentioned qualitative observations. The En-2 accession is of particular interest since it represents the genetic background of >100 mutant lines displaying altered leaf morphology, isolated by either G. Röbbelen or A. R. Kranz, which make up the so-called AIS form mutants collection (BÜRGER 1971; KRANZ 1978). A genetic analysis of these mutants has been published (SERRANO-CARTAGENA *et al.* 1999).

We morphometrically analyzed the variation with time of the shape and size of En-2 vegetative leaves, which were collected, photographed, and studied to obtain the results shown in Figure 2. In our morphometric analysis of the wild-type leaf, we chose the parameters of length, width, area, and perimeter of leaves (Table 3). We found a significant correlation between the leaf order and these parameters: At full expansion the first two leaves are smaller than the later ones. A similar correlation was observed between the length/width ratio and time, since the first two leaves are rounded, while later ones are elongated. Both the growth rate and the final length of the first pair of leaves were smaller than those of the second pair, and those of the latter, in turn, were smaller than those of the following ones. As regards the variation with time of leaf shape and size, lamina growth was seen to be much faster in the earlier stages of leaf expansion in all the studied leaves.

Study of the inheritance patterns of leaf form variants in accessions of the AIS collection: Although it has tradi-

TABLE 2

Time profiles of organ appearance during the vegetative growth of *Arabidopsis thaliana* AIS ecotypes

	Predominant pattern		Other patterns	
Days after sowing	Visible organs	No. of accessions	Visible organs	Accessions deviating from the predominant pattern
6	2 C, 2 P	188		
9	2 C, 2 L	185	2 C, 2 P	Bla-1, Bla-2, Bla-3
12	2 C, 4 L	178	2 C, 2 L, 2 P	Bla-1, Bla-2, Bla-3, Condara, Cvi-0, Dijon G, Estland
			2 C, 4 L, 1 P	Do-0, Hl-0, Kn-0
15	2 C, 5 L, 1 P	172	2 C, 3-4 L, 1 P	Abd-0, Bla-1, Bla-2, Bla-3, Bla-6, Condara, Cvi-0, Dijon G, Estland, Lm-2, S96, Ws-1
			2 C, 6-7 L, 1 P	Do-0, Hl-0, Kä-0, Kn-0
18	2 C, 7 L, 1 P	165	2 C, 5-6 L, 1 P	Abd-0, Bla-1, Bla-2, Bla-3, Bla-6, Bla-10, Bla-11, Co-3, Condara, Cvi-0, Estland, Dijon G, Fi-0, Jl-4, Lm-2, RLD1, S96, Wei-0, Ws-1
			2 C, 8-9 L, 1 P	Do-0, Hl-0, Kä-0, Kn-0

C, cotyledons; L, vegetative leaves; P, leaf primordia. Distinction between leaves and leaf primordia was made according to the presence or absence, respectively, of a visible leaf petiole.

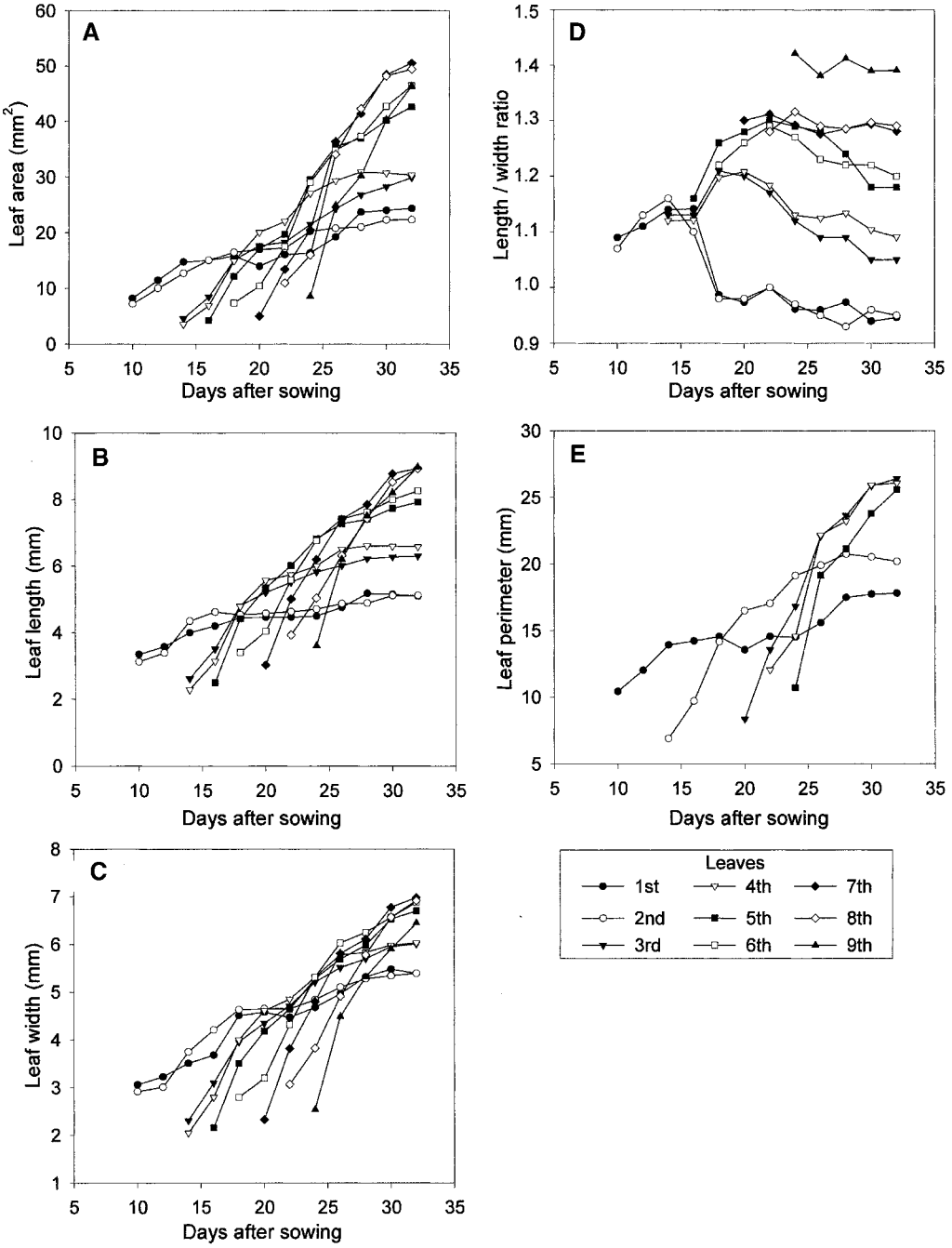

FIGURE 2.—Morphometric analysis of the expansion of En-2 vegetative leaves. Variations with time are represented for (A) area, (B) length, (C) width, (D) length/width ratio, and (E) perimeter of all rosette leaves.

tionally been assumed that traits displaying continuous variation are polygenic, several individual genes with large effects responsible for apparently quantitative variations have been identified thanks to the study of *A. thaliana* accessions (see the Introduction). To determine whether the morphological traits under study were monogenic or polygenic, crosses were performed involving pairs of accessions, each of them displaying a given trait in one of two extreme and opposite ways. Transmission of the following three traits was studied: petiole length (long or short), leaf marginal configuration (entire or serrated), and overall leaf shape (lanceolate or rounded). Figure 3 includes photographs of the studied accessions, some of which showed in an extreme manner more than one of the above-mentioned traits.

Reciprocal intercrosses were attempted between the accessions indicated in Table 4, and when their F_1 progeny was studied a low degree of phenotypic variation was found in all cases between individuals presumed to be of identical genotype, as is to be expected for outcrosses of inbred lines. The results obtained suggested inheritance patterns deviating from those expected for traits depending upon single biallelic genes. On the one hand, different results were obtained for the mode of inheritance of a given trait, when comparing all the crosses involving a given accession, the only exception

TABLE 3

Morphometric analysis of fully expanded En-2 vegetative leaves

	First	Second	Third	Fourth	Other leaves
Area	24.37 ± 8.17	22.34 ± 6.66	29.88 ± 5.97	30.34 ± 9.62	>42
Perimeter	17.80 ± 2.82	17.07 ± 2.46	19.70 ± 2.25	20.18 ± 2.90	>24
Length	5.10 ± 1.04	5.12 ± 0.85	6.30 ± 0.75	6.58 ± 0.91	>7.9
Width	5.39 ± 0.88	5.39 ± 0.72	6.01 ± 0.77	6.03 ± 1.09	>6.5
Length/width ratio	0.95	0.95	1.05	1.09	>1.18

Mean values of 10 measurements ± standard deviations are shown. Length, width, and perimeter are indicated in millimeters and area in square millimeters.

being the Li-5-3 accession (with long petiole; see Table 4), whose F_1 progeny showed long petiole. On the other hand, at least in two cases, those of Ga-0 × Gr-3 (differing in their petiole length) and Hl-0 (with lanceolate leaves) × La-1 (with rounded leaves), differences between reciprocal crosses were observed.

To determine if a single gene was responsible for the variation in the morphological traits under study, we tried to analyze their F_2 phenotypic segregations. About 50 F_2 individuals derived from selfed F_1 plants were studied from all the successful crosses between accessions differing in marginal configuration, from three crosses between accessions differing in petiole length [Ga-0 × Bla-1 (F_1 progeny with long petiole), Ga-0 × Gr-3 (F_1 with short petiole), Bla-14 × Bla-1 (F_1 progeny with intermediate petiole length)], and from three crosses between accessions differing in overall leaf shape [Hl-0 × Bla-14 (F_1 with lanceolate leaves), La-1 ×

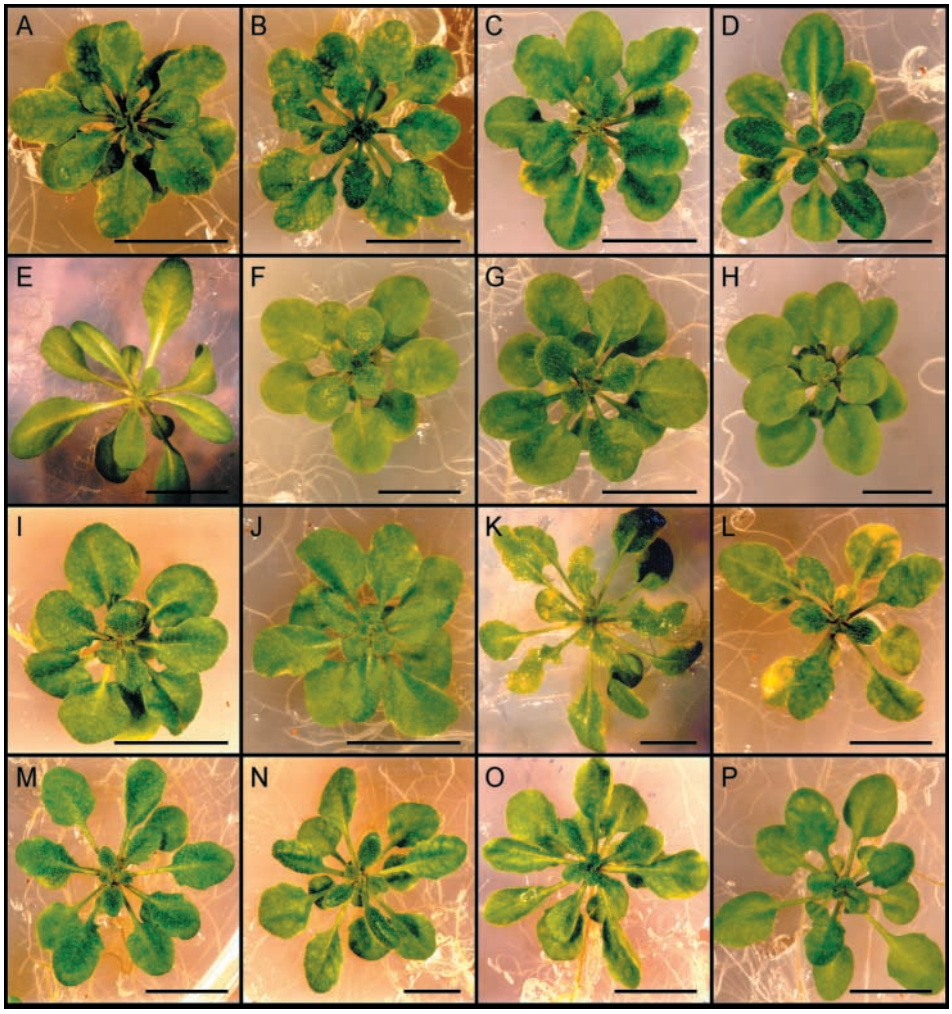

FIGURE 3.—Representative individuals of accessions belonging to the phenotypic classes chosen to study the inheritance patterns of natural variations in leaf morphology: (A) Ga-0, (B) Kä-0, and (C) Be-1, with serrated leaf margin; (D) Aa-0, (E) Cvi-0, and (F) Bd-0, with entire leaf margin; (G) La-1 and (H) Bla-14, with rounded leaves; (H) Bla-14, (I) Fi-0, and (J) Hl-2, with short petiole; (K) Bla-1, (L) Gr-3, and (M) Li-5-3, with long petiole; (N) Ep-0, (O) Hl-0, and (P) Jl-5, with lanceolate leaves. Photographs were taken 30 days after sowing. Bars, 10 mm.

TABLE 4

Phenotypes in the F_1 progeny of crosses between accessions differing in leaf morphological traits

	Long petiole				
Short petiole	Bla-1	Cvi-0	Gr-3	Jl-5	Li-5-3
Bla-14	I/I	−/I	L/L	L/L	L/L
Fi-0	S/−	−/−	−/−	−/−	−/−
Ga-0	L/−	I/I	S/I	−/−	L/L
Hl-2	−/−	−/−	I/I	−/−	L/L
	Entire margin				
Serrated margin	Aa-0	Bd-0	Bla-14		Cvi-0
Be-1	E/E	I/I	E/−		E/E
Ga-0	−/−	W/W	−/−		W/W
Kä-0	I/I	I/−	I/−		−/W
	Rounded leaf				
Lanceolate leaf	Bd-0		Bla-14		La-1
Cvi-0	−/I		I/−		D/−
Ep-0	−/−		D/−		I/I
Hl-0	D/−		D/D		I/R
Jl-5	D/−		I/I		I/I

The phenotype of F_1 individuals is indicated by two uppercase letters separated by a slash. The letter to the left of the slash indicates the phenotype of the progeny of a cross between the accessions heading the row and the column, respectively, as female and male parentals. The letter to the right of the slash indicates the phenotype of the progeny of the reciprocal cross. I, intermediate phenotype; S, short petiole; L, long petiole; W, serrated margin; E, entire margin; D, lanceolate leaf; R, rounded leaf; −, unsuccessful cross.

Hl-0 (F_1 with rounded leaves), and Jl-5 × Bla-14 (F_1 with intermediate leaves)]. Since the F_2 population constituted a phenotypic continuum in all cases, we concluded that the natural variability of the studied traits was likely to be of multigenic nature.

Microsatellite repeat number variation among accessions: To determine genetic distances between the accessions under study, PCR amplification products were obtained and sized by means of fragment analysis at 22 microsatellites in those accessions displaying the traits in an extreme manner (see Table 1), only homozygous individuals being found (Table 5). The only exception was Gr-3, for which two alleles were detected in a single locus. We estimated the level of microsatellite polymorphism on the basis of the number of alleles and gene diversity, the latter being found to vary from 0.309 (AthPHYC, 2 alleles) to 0.984 (nga6, 15 alleles) with an average of 0.827 over the 22 microsatellites.

Several distance matrices were obtained with the MICROSAT program, calculated on the basis of different genetic distance measurements (see MATERIALS AND METHODS). Consensus phylogenetic trees were constructed from resampled data using either the neighbor-joining or the UPGMA methods. According to the phylogenetic trees obtained, two examples of which are presented in Figure 4, the studied accessions group into clusters that, in general, do not correlate with those made according to petiole length, marginal configuration, and overall lamina shape of vegetative leaves. This is in agreement with our results in the bootstrap analysis, given that most of the nodes are not well supported, suggesting star-like phylogenies for the accessions, as previously proposed (SHARBEL *et al.* 2000; NORDBORG *et al.* 2002). Only in a few cases were accessions with similar leaf phenotypes clustered together (Jl-5, L*er*, and Aa-0, with long petiole, entire margin, and lanceolate leaves).

Morphometric analysis of leaf architecture in the recombinant inbred lines: We morphometrically analyzed several leaf architectural traits (see MATERIALS AND METHODS) in a mapping population of 100 RILs. We chose the third node leaf, assumed to be representative of juvenile leaves in the L*er*-0 and Col-4 accessions, and the seventh node leaf, assumed to be a typical adult leaf. Both types of leaves were collected 25 days after sowing, from plants grown in strictly controlled environmental conditions (see MATERIALS AND METHODS). Although third leaves of L*er*-0 and Col-4 were fully expanded, seventh leaves were not. With this approach we sought to identify not only the QTL involved in leaf morphology and expansion but also others responsible for the heteroblastic differences between juvenile and adult leaves.

We found variation both among RILs and between

TABLE 5

Variation at the studied microsatellite loci

Locus	Chromosome	Repeat motif	No. of microsatellite repeats																		No. of accessions scored	No. of alleles found	Average no. of repeats	Variance	Gene diversity
			Aa-0	Bd-0	Be-1	Bla-1	Bla-14	Cvi-0	Ep-0	Fi-0	Ga-0	Gr-3	Hl-0	Hl-2	Jl-5	Kä-0	La-1	Li-5-3	*Ler*	Col-0					
AthACS	1	$(A)_n$	NS	NS	31	36	39	39	31	37	34	37	37	NS	38	37	36	37	31	36	15	6	35.73	7.00	0.838
AthZFPG	1	$(AG)_n$	14	9	8	NS	19	14	9	9	7	8	12	11	8	12	18	9	13	16	17	10	11.15	13.17	0.919
T27k12-Sp6	1	$(AT)_n$	16	11	18	10	38	15	16	35	19	35	8	20	35	8	28	NS	17	12	17	13	19.85	96.20	0.963
AthGENEA	1	$(A)_n$	37	53	52	45	35	47	51	50	38	45	39	47	39	42	44	39	32	39	18	13	43.00	35.89	0.948
nga111	1	$(GA)_n$	84	23	26	16	24	27	31	14	23	24	27	23	33	26	22	25	33	16	18	11	27.39	211.13	0.948
nga1145	2	$(GA)_n$	8	8	8	8	8	8	8	8	4	7	8	9	8	8	8	8	17	14	18	6	8.58	7.40	0.490
nga1126	2	$(AG)_n$	14	15	13	17	14	13	12	NS	13	23	17	14	23	14	18	15	24	14	17	8	15.91	12.89	0.882
nga361	2	$(AG)_n$	15	15	NS	23	19	11	22	21	19	22	11	21	13	15	12	21	21	14	17	9	17.21	16.38	0.912
nga168	2	$(GA)_n$	17	24	18	17	18	17	25	21	22	22	21	18	19	16	19	18	18	25	18	8	19.36	7.49	0.889
AthCHIB	3	$(AT)_n$	NS	23	11	12	17	7	19	12	18	26	21	0	14	17	13	18	11	14	17	12	14.62	34.87	0.963
nga162	3	$(GA)_n$	14	9	11	11	15	22	20	16	16	6	15	10	12	16	15	14	13	21	18	12	14.06	16.58	0.948
AthGAPAab	3	$(CTT)_n$	13	10	10	10	10	10	10	10	17	13	13	10	10	10	10	10	13	10	18	3	11.09	3.82	0.451
nga6	3	$(GA)_n$	32	20	37	30	26	37	40	19	17	39	36	34	33	15	17	14	20	31	18	15	27.39	76.18	0.984
nga12	4	$(GA)_n$	7	NS	7	7	7	7	14	11	11	8	13	15	8	8	8	14	10	14	17	7	9.88	8.66	0.853
nga1111	4	$(GA)_n$	20	18	19	16	10	19	16	19	17	11	19	18	18	21	17	23	19	16	18	9	17.25	9.09	0.892
AM4	4	$(AT)_n$	5	13	15	12	8	3	12	NS	11	5	7	11	6	6	NS	15	8	19	16	10	9.47	19.61	0.950
nga1139	4	$(GA)_n$	20	13	25	22	13	12	20	13	15	16	13	17	14	13	15	14	25	20	18	9	16.67	17.22	0.895
nga1107	4	$(AG)_n$	18	17	17	18	18	17	17	17	17	17	17	17	17	19	17	17	17	27	18	4	17.67	5.36	0.471
AthCTR1	5	$(AG)_n$	11	9	8	8	9	4	8	9	9	10	16	10	8	12	11	16	8	16	18	7	9.94	10.19	0.863
nga139	5	$(AG)_n$	12	30	30	30	21	33	31	31	35	32	14	32	14	14	NS	14	13	29	17	10	24.18	69.91	0.919
AthPHYC	5	$(ATT)_n\,(GAA)_n$	22	22	22	22	22	22	22	22	17	17/22	22	22	22	17	22	22	22	17	18	2	21.12	3.63	0.309
MBK5	5	$(CTT)_n$	10	7	10	9	22	17	10	13	18	17	15	13	NS	15	13	13	8	15	17	9	13.37	14.39	0.904

Repeat motif and number of repeats were obtained from Bell and Ecker (1994). Average and variance are represented by the number of microsatellite repeats. Gene diversity was calculated as indicated in materials and methods. NS, not scored.

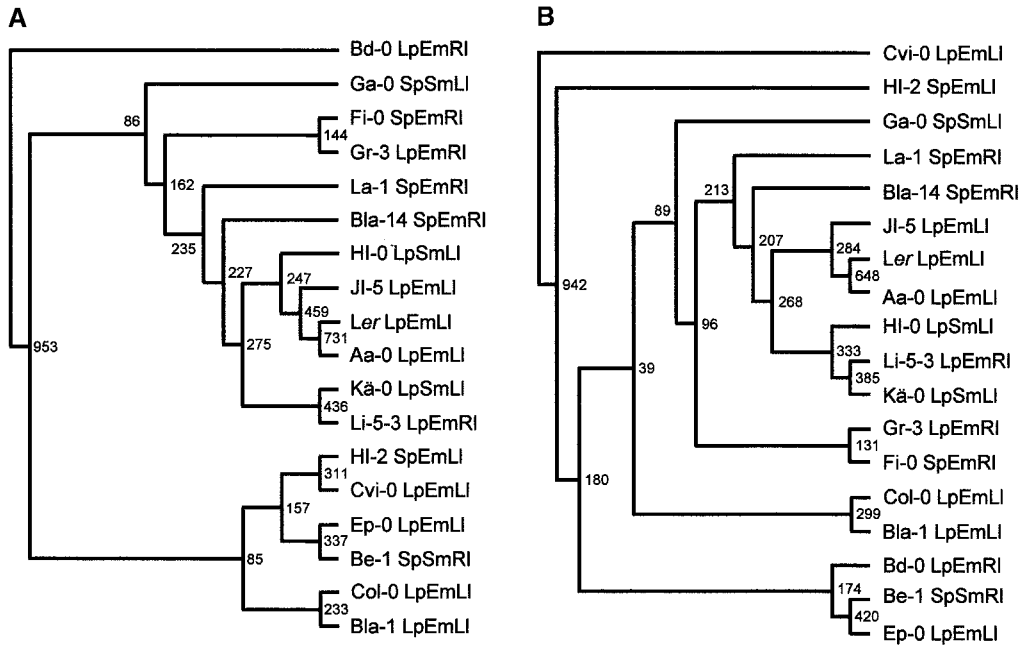

FIGURE 4.—Neighbor-joining trees of the *Arabidopsis thaliana* accessions studied in this work. Phylogram trees were constructed from a distance matrix calculated using the distance measurements D_1 (A) and D_{KF} (B). Sp and Lp, short and long petiole; Em and Sm, entire and serrated margin; Ll and Rl, lanceolate and rounded leaves. The numbers at the branch-points indicate the number of times that the accessions to the right of the branch point grouped together out of 1000 bootstrap replicates.

their parental accessions (Figure 5 and Table 6). Normal distributions of the phenotypic traits under study were obtained (Figure 6) for all the parameters analyzed, as was to be expected for quantitative traits controlled by multiple loci. In addition, in some RILs we found phenotypic values that were higher or lower than those shown by their ancestor accessions, indicating that L*er*-0 and Col-4 may contain both positive and negatively balanced alleles for leaf architectural traits. The largest of these transgressions was observed for petiole length in seventh leaves (PLN7), with the N1909 line displaying a 78.8% increase above the Col-4 parental values and a 55.9% decrease of the N1977 line compared with L*er*-0 values. The smallest transgression was shown for lamina length in third leaves (LLN3), with a 12.2% increase of N1909 with regard to L*er*-0 and a 12.7% decrease of N1930 with regard to Col-4. In addition, broad-sense heritabilities of the traits under study were calculated (see MATERIALS AND METHODS), which ranged from 84.3% for LWN7 to 98.6% for LPN3. These high values of heritability are very likely due to the strictly controlled environmental conditions used in this study.

We determined the statistical correlations between the above-mentioned variables (Table 7), as estimative

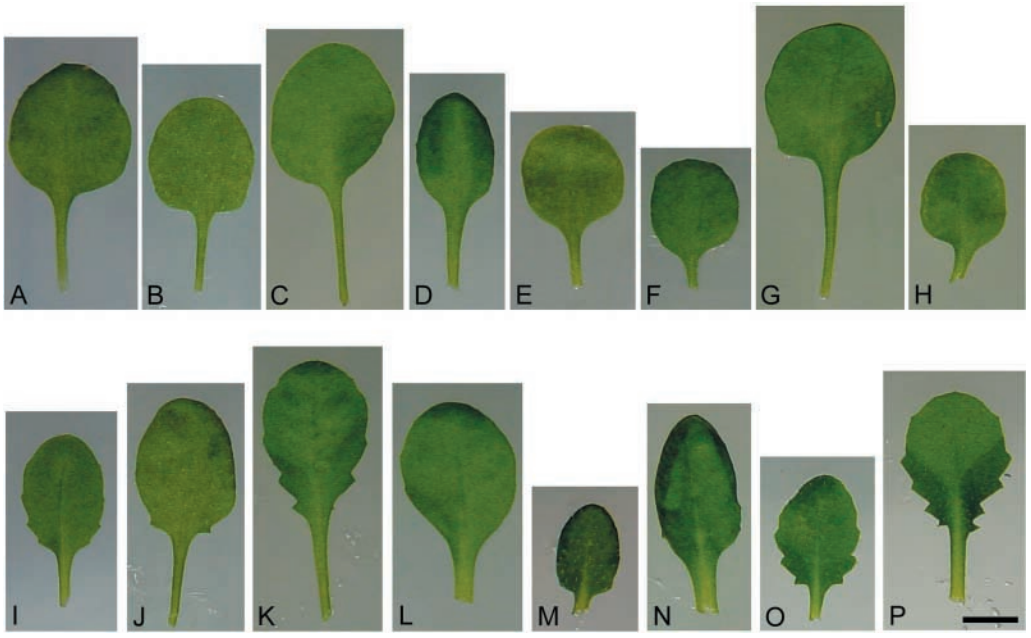

FIGURE 5.—Third (A–H) and seventh (I–P) leaves of selected RILs and their parental accessions (A and I, L*er*-0; B and J, Col-4). Notable phenotypic variations are shown for leaves of some RILs displaying long petioles and large lamina (C and K, N1909; G, N1971), lanceolate (D, N1931) or rounded lamina (E, N1940), short petiole and small lamina (F, N1950; H, N1977; M, N1957; O, N1983), rounded lamina and smooth margin (L, N1935), lanceolate lamina and thick petiole (N, N1977), and highly serrated margin (P, N1989). Bar, 5 mm.

TABLE 6

Phenotypic values for the studied leaf traits of the parental lines and the RIL population

	L*er*-0	Col-4	Mean of the RIL population	Highest value (RIL)	Lowest value (RIL)	V_G	V_E	H^2	CV_G
Third leaf									
LAN3	65.92 ± 16.08	51.23 ± 13.44	56.88 ± 9.11	83.21 (N1909)	37.93 (N1921)	5414.855	203.199	0.964	129.37
LPN3	30.58 ± 3.80	27.05 ± 3.65	28.39 ± 2.90	35.21 (N1909)	23.31 (N1921)	955.924	13.299	0.986	108.90
LLN3	9.86 ± 1.29	8.79 ± 1.21	9.18 ± 0.69	11.06 (N1909)	7.67 (N1930)	97.048	1.451	0.985	107.31
LWN3	8.40 ± 1.12	7.30 ± 1.12	7.73 ± 0.71	9.49 (N1909)	6.13 (N1970)	71.586	1.112	0.985	109.45
PLN3	6.05 ± 1.47	5.00 ± 1.65	5.54 ± 1.42	9.86 (N1909)	3.21 (N1977)	60.785	1.868	0.971	140.73
PWN3	1.19 ± 0.17	0.99 ± 0.14	1.14 ± 0.16	1.68 (N1951)	0.83 (N1973)	1.745	0.034	0.981	115.88
Seventh leaf									
LAN7	45.34 ± 11.57	47.82 ± 10.95	48.35 ± 9.42	67.49 (N1935)	28.19 (N1945)	1115.993	183.788	0.876	69.09
LPN7	26.40 ± 3.61	27.14 ± 2.30	27.03 ± 2.94	34.20 (N1909)	20.20 (N1945)	110.260	13.893	0.899	38.85
LLN7	9.31 ± 1.52	9.07 ± 1.06	9.27 ± 1.02	11.66 (N1909)	6.88 (N1945)	13.408	1.641	0.902	39.50
LWN7	6.13 ± 0.91	6.65 ± 0.88	6.49 ± 0.67	8.11 (N1955)	5.03 (N1957)	5.387	1.232	0.843	35.76
PLN7	3.29 ± 1.32	4.47 ± 1.67	4.50 ± 1.40	7.99 (N1954)	1.45 (N1957)	27.259	2.105	0.933	116.02
PWN7	1.19 ± 0.22	0.99 ± 0.14	1.18 ± 0.19	1.73 (N1977)	0.74 (N1904)	0.533	0.044	0.930	61.88
Rosette									
TLN	9.13 ± 0.76	11.00 ± 0.93	10.54 ± 1.11	12.73 (N1936)	8.07 (N1974)	227.243	19.501	0.927	143.02
MDR	29.75 ± 4.02	29.07 ± 3.14	33.80 ± 5.01	45.93 (N1971)	19.83 (N1976)	191.962	19.415	0.916	40.99
mdr	25.48 ± 3.58	25.25 ± 3.66	30.06 ± 4.65	40.66 (N1909)	17.29 (N1917)	18.204	0.527	0.973	14.19

Mean values ± standard deviations are shown. A total of 15 plants for each of the 100 RILs studied and at least 45 plants for each parental were studied (see MATERIALS AND METHODS). Highest and lowest values for each trait are shown together with those lines representing the extremes of the distribution. LA, lamina area; LP, lamina perimeter; LL, lamina length; LW, lamina width; PL, petiole length; PW, petiole width; TLN, total leaf number; MRD, major rosette diameter; mrd, minor rosette diameter. N3 and N7, third and seventh node leaves, respectively. LP, LL, LW, PL, PW, MRD, and mrd are indicated in millimeters and LA in square millimeters. The among-RIL variance (V_G) and residual variance (V_E) were used to calculate broad-sense heritabilities (H^2) and coefficients of genetic variation (CV_G) as described in MATERIALS AND METHODS.

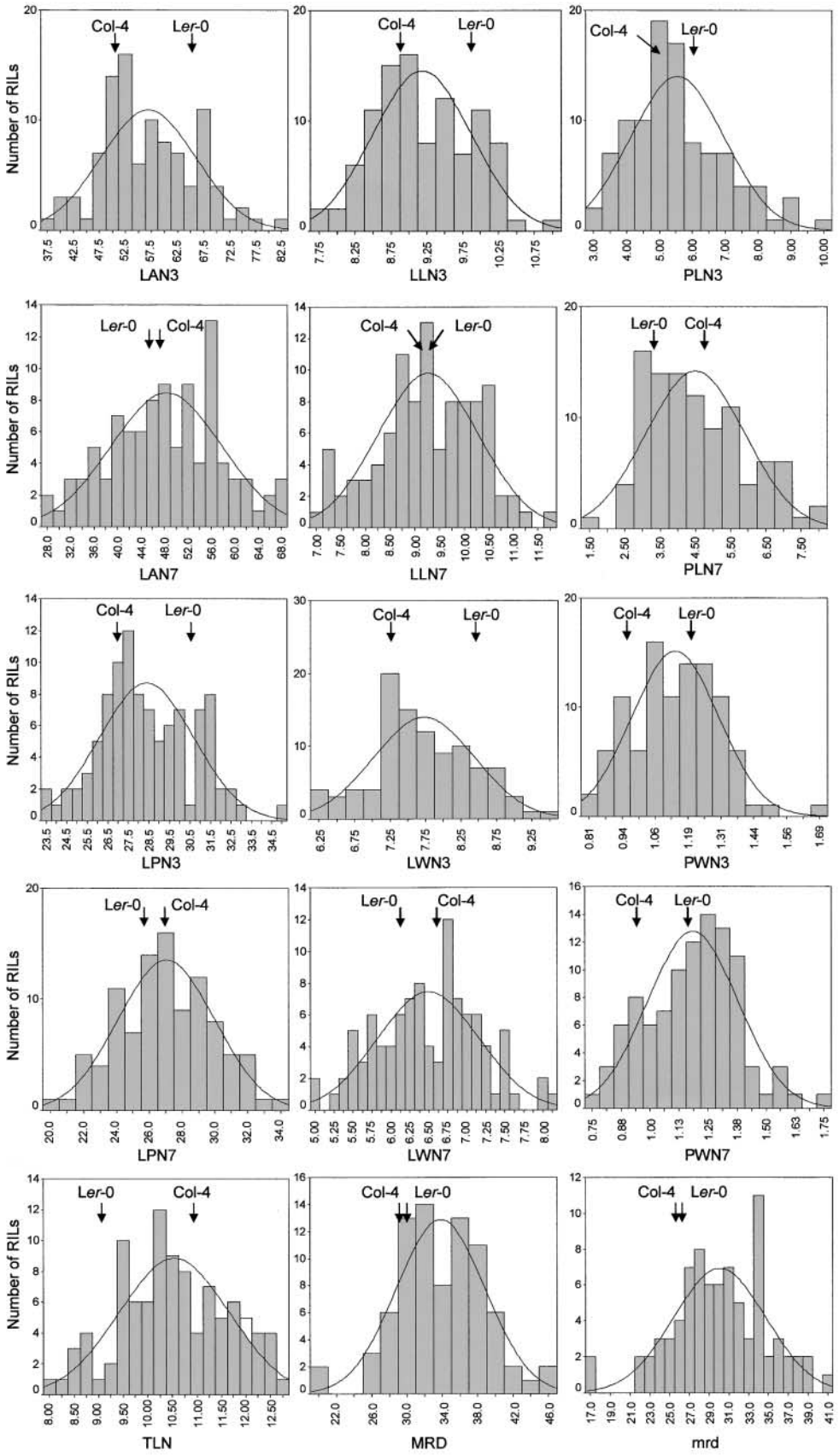

FIGURE 6.—Frequency distribution for the morphological traits studied in the RIL population and parental lines. N3, third node leaf; N7, seventh node leaf; TLN, total leaf number. Lamina perimeter (LP), lamina length (LL), petiole length (PL), lamina width (LW), petiole width (PW), major rosette diameter (MRD), and minor rosette diameter (mrd) are indicated in millimeters and lamina area (LA) is in square millimeters. Normal curves that fit the observed data are shown.

TABLE 7

Genetic correlations between third and seventh node leaf parameters

	LAN7	LPN3	LPN7	LLN3	LLN7	LWN3	LWN7	PLN3	PLN7	PWN3	PWN7
LAN3	0.527[a]	0.992[a]	0.560[a]	0.943[a]	0.554[a]	0.961[a]	0.420[a]	0.664[a]	0.636[a]	*0.025*	*−0.077*
LAN7		0.522[a]	0.980[a]	0.416[a]	0.940[a]	0.572[a]	0.938[a]	0.223[b]	0.547[a]	*−0.114*	0.209[b]
LPN3			0.559[a]	0.953[a]	0.547[a]	0.945[a]	0.417[a]	0.665[a]	0.625[a]	*0.019*	*−0.090*
LPN7				0.468[a]	0.958[a]	0.583[a]	0.882[a]	0.273[a]	0.571[a]	*−0.166*	*0.141*
LLN3					0.494[a]	0.822[a]	0.271[a]	0.641[a]	0.564[a]	*0.094*	*−0.081*
LLN7						0.551[a]	0.772[a]	0.268[a]	0.563[a]	*−0.094*	0.226[b]
LWN3							0.511[a]	0.623[a]	0.643[a]	*−0.017*	*−0.064*
LWN7								*0.118*	0.439[a]	*−0.098*	0.203[b]
PLN3									0.754[a]	*−0.080*	−0.266[a]
PLN7										−0.225[b]	*−0.147*
PWN3											0.687[a]

Genetic correlations were estimated as indicated in MATERIALS AND METHODS. Not significant correlations are indicated in italics.

[a] Highly significant correlations ($\alpha = 0.01$).

[b] Significant correlations ($\alpha = 0.05$).

of genetic correlations between the characters under study, and found that all the pairs of traits of a given type of leaf displayed positive and highly significant ($\alpha = 0.01$) correlations, the only exception being those involving petiole width. We found that the area, perimeter, length, and width of the lamina were highly correlated, suggesting the existence of a common genetic control. On the contrary, petiole and lamina lengths were slightly correlated (Table 7), suggesting that several loci differentially participate in the elongation processes of these two leaf subdomains. Similar positive correlations among morphological traits have also been found for floral organs in *A. thaliana* (JUENGER *et al.* 2000). We found lower correlations between the same traits in leaves from different nodes than between the different traits from the same leaf node, perhaps reflecting differences in leaf developmental profiles between juvenile and adult leaves. As an example, LLN3 showed strong genetic correlations with LAN3 ($r_G = 0.943$), LWN3 ($r_G = 0.822$), or PLN3 ($r_G = 0.641$) and a lower degree of genetic correlation with LLN7 ($r_G = 0.494$).

We analyzed the NMS in the studied lines as an estimate of the number of hydathodes, which is known to differ between juvenile and adult leaves (TSUKAYA and UCHIMIYA 1997; CANDELA *et al.* 1999). Only leaves from the seventh node showed any variation, which was not sufficient to carry out a quantitative analysis, the mean values obtained ranging from RILs with none up to 10 marginal serrations (data not shown).

In addition, we analyzed the TLN in the RIL population 25 days after sowing, where differences could be due to differences in both the flowering time and the time profile of leaf production. Significant variation was found with differences of up to four leaves between extreme values. Major and minor rosette diameters were also measured but not used for QTL mapping because differences between these parameters are dependent not only on leaf length but also on the angle of the petiole to the stem (not measured).

QTL mapping: The above-mentioned results on the morphometric analysis of two representative leaves, one juvenile and one adult, from 15 plants from each of 100 RILs were used for QTL mapping. We first determined the number and location of the QTL contributing to the traits under study in juvenile and adult leaves: lamina area and perimeter, lamina width and length, petiole length and width, number of leaf marginal serrations, total leaf number, and rosette diameters (Table 8). A LOD score significance threshold of 2.7 was calculated for QTL identification (see MATERIALS AND METHODS). We have taken into account, however, four putative QTL (see ju-*PLE2* and ju-*LaWI1* in Table 9 and ad-*LaSI2* and ad-*PLE1* in Table 10), whose LOD scores are below the 2.7 threshold but still make a significant contribution to the explained variance. The QTL identified (from 2 to 11 QTL for PWN7 and LPN3 or LWN3, respectively) explained a higher percentage of the phenotypic variance found for juvenile leaves (average of 69.3%) than for the adult ones (56.9%). In all cases, a large percentage of this variance was explained by one or two QTL with a strong effect, and the remaining variation was apparently due to a large number of weaker (with smaller effects) QTL, as previously proposed (ROBERTSON 1985). The proportion of the total phenotypic variance explained for each leaf trait by these QTL ranged from 48.0% in LWN7 to 72.3% in PLN3.

Since the genetic correlations between the traits under study were highly significant and similar heritabilities were found, we assumed it would be possible to detect the same QTL in different analyses, for which reason we grouped all the QTL that displayed neighboring map positions identified from the analyses of different leaf parameters (Tables 9 and 10). As a result, we were able to distinguish between the QTL affecting all the studied parameters of the whole organ (leaf size

TABLE 8

Summary of QTL results of leaf architecture analysis in *Arabidopsis thaliana* vegetative leaves

Trait	No. of QTL found[a]	Variance explained (%)	Main QTL[b]	Candidate loci[c]
LAN3	10	69.8	18.2 (5, 86.2)	*EXI2, ELO3*
			15.3 (2, 50.6)	*ER*
LAN7	8	52.4	14.6 (5, 86.2)	*EXI2, ELO3*
			12.3 (2, 69.7)	—
LPN3	11	70.3	18.6 (5, 86.2)	*EXI2, ELO3*
			15.9 (2, 50.6)	*ER*
LPN7	8	54.4	16.0 (2, 69.7)	—
			15.2 (5, 86.3)	*EXI2, ELO3*
LLN3	10	71.2	21.5 (5, 86.2)	*EXI2, ELO3*
			17.1 (2, 50.6)	*ER*
LLN7	6	50.0	15.5 (5, 86.3)	*EXI2, ELO3*
			9.2 (2, 69.7)	—
LWN3	11	66.4	15.6 (5, 86.2)	*EXI2, ELO3*
			13.7 (2, 50.6)	*ER*
LWN7	4	48.0	11.5 (5, 39.6)	—
			9.0 (5, 90.6)	—
PLN3	9	72.3	26.7 (2, 50.6)	*ER*
			9.6 (1, 117.2)	—
PLN7	6	69.6	21.3 (2, 50.6)	*ER*
			16.4 (4, 69.4)	—
PWN3	5	65.6	19.1 (2, 50.6)	*ER*
			7.8 (2, 73.8)	—
PWN7	2	67.2	44.1 (2, 50.6)	*ER*
			5.4 (5, 86.2)	—
RMD25	7	70.4	18.1 (2, 50.6)	*ER*
			9.3 (4, 69.4)	—
rmd25	8	72.7	18.0 (2, 50.6)	*ER*
			9.6 (2, 44.2)	—
TLN25	4	44.7	12.6 (4, 65.7)	*FWA*
			11.3 (2, 69.7)	*TOC2*

[a] A significance threshold of 2.7 was chosen for QTL identification, as described in MATERIALS AND METHODS.

[b] QTL with the highest percentage of variance for the studied traits are shown. Numbers indicate the percentage of variance explained. Those in parentheses correspond to the chromosome number and map position, respectively.

[c] Candidate loci were proposed from those mutations already described as perturbing leaf morphology and that mapped close to the QTL.

QTL or *LSI*), the lamina (lamina size QTL or *LaSI*), or the petiole (petiole size QTL or *PSI*) from those affecting either the organ's length (lamina length QTL or *LaLE* and petiole length QTL or *PLE*) or its width (lamina width QTL or *LaWI* and petiole width QTL or *PWI*). For juvenile leaves, a total of 16 QTL were found in this way (Table 9 and Figure 7). Five QTL were shown to affect whole leaf size (ju-*LSI1* to ju-*LSI5*), 2 of which, those linked to *er* (ju-*LSI2* in chromosome 2) and to *g4028* (ju-*LSI5* in chromosome 5), would explain >40% of the observed variance. One QTL was specific for petiole size (ju-*PSI1*), and 3 were responsible for ~25% of the variance in lamina size (ju-*LaSI1* to ju-*LaSI3*). Several minor-effect QTL for length (1 QTL for leaf length and 3 for petiole length) or width (3 QTL for lamina width) were also identified. For these juvenile leaf QTL, LOD values ranged from 2.47 (ju-*LaWI2*, identified in the LWN3 analysis) to 14.45 (ju-*LSI2*, identified in the PLN3 analysis) and the 2-LOD intervals ranged from 44.4 cM (ju-*PLE2*, identified in the PLN3 analysis) to 4.4 cM (ju-*LSI5*, identified in the LAN3 analysis) with an average of 13.6 cM.

In the case of adult leaves we found 13 QTL (Table 10 and Figure 8), 4 of which affected leaf size (the one linked to the *g4028* marker was also found in juvenile leaves), 5 lamina size QTL (that of ad-*LaSI1* on chromosome 2 was responsible for 10% of the variance), and 4 QTL affecting only petiole size (2 of which were responsible for nearly 50% of the variance). Surprisingly, 1 of these 2 QTL was that closest to the *er* marker, which also affected juvenile leaf size but was not detected in the adult leaf lamina analyses. For the adult leaves, LOD values ranged from 1.47 (ad-*LSI2* detected with the PWN7 analysis) to 18.30 (ad-*PSI1* detected with the

TABLE 9

QTL involved in juvenile leaf morphology

QTL	Trait	Cofactor marker	LOD	Map interval[a]	Additive effect[b]	Variance explained[b] (%)
ju-*LSI1*	LAN3	*nga59*	4.27	1, 2.9 (0–9.7)	2.508	6.6
	LPN3	*nga59*	4.30	1, 2.9 (0–9.8)	0.628	6.5
	LLN3	*nga59*	3.95	1, 2.9 (0–9.3)	0.178	5.9
	LWN3	*nga59*	5.30	1, 2.9 (0–8.7)	0.236	9.6
	PLN3	*nga59*	3.64	1, 2.9 (0–17.6)	0.342	5.1
	PWN3	*0846A*	3.48	1, 5.9 (0–18.6)	0.043	6.2
ju-*LSI2*	LAN3	*er*	8.73	2, 50.6 (48.4–54.6)	3.925	15.3
	LPN3	*er*	9.19	2, 50.6 (48.0–54.5)	1.010	15.9
	LLN3	*er*	9.77	2, 50.6 (49.1–54.8)	0.313	17.1
	LWN3	*er*	7.24	2, 50.6 (48.9–55.6)	0.292	13.7
	PLN3	*er*	14.45	2, 50.6 (48.9–53.6)	0.917	26.7
	PWN3	*er*	9.36	2, 50.6 (46.8–58.3)	−0.086	19.1
ju-*LSI3*	LAN3	*mi330*	3.23	4, 58.2 (50.9–63.2)	−3.549	4.9
	LPN3	*mi330*	3.31	4, 58.2 (51.9–62.9)	−0.910	4.9
	LLN3	*mi330*	3.23	4, 58.2 (53.2–62.5)	−0.317	4.9
	LWN3	*mi32*	4.43	4, 60.9 (57.0–62.9)	−0.444	7.7
	PWN3	*m226*	3.26	4, 57.0 (48.7–72.2)	−0.041	5.5
ju-*LSI4*	LAN3	*mi422*	5.58	4, 69.4 (64.1–72.0)	4.396	9.1
	LPN3	*mi422*	5.76	4, 69.4 (64.2–72.2)	1.116	9.2
	LLN3	*JGB9*	5.06	4, 65.7 (63.2–71.4)	0379	8.0
	LWN3	*JGB9*	5.21	4, 65.7 (63.2–72.2)	0.472	9.3
	PLN3	*mi422*	2.79	4, 69.4 (60.9–76.8)	0.289	3.9
ju-*LSI5*	LAN3	*g4028*	10.26	5, 86.2 (84.3–88.7)	−4.728	18.2
	LPN3	*g4028*	10.57	5, 86.2 (84.2–89.0)	−1.202	18.6
	LLN3	*g4028*	11.77	5, 86.2 (83.9–89.2)	−0.326	21.5
	LWN3	*g4028*	8.33	5, 86.2 (84.2–90.5)	−0.327	15.6
	PLN3	*g4028*	4.46	5, 86.2 (81.9–90.6)	−0.619	6.3
ju-*LaSI1*	LAN3	*pCITf3*	3.11	4, 30.8 (25.9–32.8)	3.808	4.8
	LPN3	*pCITf3*	3.58	4, 30.8 (26.7–32.6)	1.028	5.5
	LLN3	*pCITf3*	4.50	4, 30.8 (26.2–31.3)	0.346	7.2
	LWN3	*pCITf3*	4.91	4, 30.8 (27.6–32.8)	0.404	9.1
ju-*LaSI2*	LAN3	*pCITd23*	3.65	4, 40.3 (32.8–45.3)	−4.354	5.7
	LPN3	*pCITd23*	4.13	4, 40.3 (33.2–44.5)	−1.165	6.4
	LLN3	*pCITd23*	5.11	4, 40.3 (33.8–44.3)	−0.398	8.2
	LWN3	*pCITd23*	4.21	4, 40.3 (33.1–45-0)	−0.393	7.5
ju-*LaSI3*	LAN3	*g3715*	3.89	5, 7.2 (0–12.2)	2.561	6.1
	LPN3	*g3715*	3.88	5, 7.2 (0–12.3)	0.638	6.0
	LLN3	*g3715*	7.13	5, 7.2 (4.7–10.3)	0.437	11.7
	LWN3	*pAtT80*	4.90	5, 2.5 (0–10.3)	0.237	8.9
ju-*PSI1*	PLN3	*nga168*	2.45	2, 73.8 (67.4–109.9)	−0.306	3.4
	PWN3	*nga168*	4.59	2, 73.8 (67.4–109.9)	−0.055	7.8
ju-*LLE1*	LAN3	*mi425*	2.72	1, 117.2 (88.4–134.9)	−2.035	4.2
	LPN3	*mi425*	2.61	1, 117.2 (86.4–134.9)	−0.497	4.0
	LLN3	*mi425*	4.98	1, 117.2 (112.0–124.2)	−0.208	7.6
	PLN3	*mi425*	6.45	1, 117.2 (111.8–122.7)	−0.706	9.6

(*continued*)

PWN7 analysis) and the 2-LOD intervals ranged from 42.3 cM (ad-*PLE2*, identified in the PLN7 analysis) to 4.9 cM (ad-*PLE3*, identified in the PLN7 analysis) with an average of 17.1 cM. Due to the high phenotypic correlations between juvenile and adult leaves, we expected to find similar QTL affecting the same trait in different leaves. However, only 8 QTL were found to be apparently common to juvenile and adult leaves.

TABLE 9

(Continued)

QTL	Trait	Cofactor marker	LOD	Map interval[a]	Additive effect[b]	Variance explained[b]
ju-*PLE1*	PLN3	*Tag1*	3.78	1, 109.8 (106.2–111.8)	0.517	5.2
ju-*PLE2*	PLN3	*nga162*	2.59	3, 20.6 (6.2–50.6)	−0.291	3.6
ju-*PLE3*	PLN3	*mi83*	4.09	5, 93.7 (90.6–96.7)	0.584	5.7
ju-*LaWI1*	LWN3	*mi133*	2.47	1, 61.2 (44.1–76.0)	−0.154	4.4
ju-*LaWI2*	LAN3	*TSL*	4.93	5, 37.8 (33.6–41.6)	−3.319	8.1
	LPN3	*TSL*	4.57	5, 37.8 (32.4–41.9)	−0.794	7.3
	LWN3	*TSL*	4.92	5, 37.8 (34.3–49.5)	−0.269	9.1
ju-*LaWI3*	LWN3	*mi125*	2.94	5, 65.2 (55.0–70.3)	0.199	5.1

LSI, leaf size; LLE, leaf length; LaSI, lamina size; LaWI, lamina width; PSI, petiole size; PLE, petiole length; ju, juvenile; ad, adult.

[a] Map positions (in centimorgans) and 2-LOD intervals (in parentheses) calculated as described in MATERIALS AND METHODS are shown.

[b] The allele additive effect was estimated using the MQM option of the MapQTL 4.0 program as the difference between the estimated mean value of the RILs homozygous for the Col-4 allele minus the mean value of the RILs homozygous for the L*er*-0 allele, divided by 2, and the percentage of explained variance was calculated as explained in MATERIALS AND METHODS.

Altogether, 21 QTL affecting leaf morphology were found, 8 of which were shared by juvenile and adult leaves, in the genome of *A. thaliana* (4, 2, 3, 5, and 7 QTL were found in chromosomes 1, 2, 3, 4, and 5, respectively). The proportion of the phenotypic variance explained by individual QTL ranged from 3.4% (ju-*PSI1*, identified in the PLN3 analysis) to 26.7% (ju-*LSI2*, identified in the PLN3 analysis) in juvenile leaves and 2.3% (ad-*LSI2*, identified in the PWN7 analysis) to 44.1% (ad-*PSI2*, identified in the PWN7 analysis) in the adult ones.

DISCUSSION

Substantial variability exists in leaf architectural traits among *A. thaliana* accessions: The AIS collection of accessions is one of the largest sets of wild-type races of *A. thaliana*. Maintained for years by A. R. Kranz, it was created by F. Laibach and enlarged later with the addition of new accessions by other researchers such as G. Röbbelen, D. Ratcliffe, and C. Gómez-Campo (KRANZ 1978). This collection of accessions constitutes an excellent sample of the variability existing in leaf shape and size as well as in rosette structure among *A. thaliana* natural races. Some of this variability has probably arisen because of flowering time diversity, which causes differences in the number of rosette leaves, which are continuously generated until bolting. Differences in the levels of activity of genes controlling leaf morphogenesis could also contribute to natural variations in vegetative leaf shape and size, traits that are traditionally assumed to be quantitative, due to the joint action of several genes whose expression is probably influenced by the environment. Controlled conditions of substrate, light, temperature, and humidity should reduce such environmental phenotypic variation, allowing the genetic component of the traits under study to be unraveled.

Very few studies have been published on the inter- and intraecotypic variability of life history traits, such as the timing of leaf primordia initiation and the number of leaves in *A. thaliana* plants grown under controlled culture conditions (DOBROVOLNA 1967; ZWAN *et al.* 2000). Developmental traits were shown to be the most variable, not only between different accessions but also within a given accession. Similar results were obtained by KARBE and RÖBBELEN (1968), who studied rosette height and leaf number, shape, marginal configuration, and color. These results indicate apparent heterozygosity, at least for the studied traits (DOBROVOLNA 1967), in contrast with the high level of autogamy that *A. thaliana* is known to present (RÖBBELEN 1971).

We have studied the variability of a large group of accessions from the AIS collection under controlled culture conditions, classifying them into 14 phenotypic groups according to overall leaf shape, leaf marginal configuration, and rosette structure. Only minor intraecotypic variability was found for the studied traits, all the individuals of a given accession being unambiguously assigned to the same phenotypic group, the only exceptions being JI-5 and Li-5-3. Given that these two accessions included two clearly distinguishable subpopulations, we chose plants belonging to the subpopulation displaying the trait of interest for further analysis. We also studied leaf initiation rates and found that only a few accessions deviated from the predominant pattern. Therefore, substantial variability exists in the shape and

TABLE 10

QTL involved in adult leaf morphology

QTL	Trait	Cofactor marker	LOD	Map interval[a]	Additive effect[b]	Variance explained[b] (%)
ad-*LSI1*	LAN7	*mi424*	2.40	1, 92.5 (62.7–104.2)	2.442	5.7
	LPN7	*mi424*	2.88	1, 92.5 (69.6–102.6)	0.821	6.6
	LLN7	*mi424*	2.94	1, 92.5 (71.9–103.2)	0.300	7.3
	PWN7	*mi424*	1.68	1, 92.5 (86.4–104.2)	0.038	2.8
ad-*LSI2*	LAN7	*CDs5*	3.51	5, 26.3 (20.1–29.6)	4.531	8.6
	LPN7	*CDs5*	3.19	5, 26.3 (20.5–29.4)	1.311	7.4
	LLN7	*CDs5*	2.36	5, 26.3 (12.8–29.4)	0.404	5.8
	LWN7	*CDs5*	1.96	5, 26.3 (20.0–29.7)	0.196	4.9
	PLN7	*CDs5*	3.38	5, 26.3 (15.6–32.8)	0.423	5.2
	PWN7	*CDs5*	1.47	5, 26.3 (13.8–32.6)	0.033	2.3
ad-*LSI3*	LAN7	*nga106*	4.09	5, 33.3 (30.1–49.8)	−5.299	8.6
	LPN7	*nga106*	3.76	5, 33.3 (30.2–51.5)	−1.542	8.8
	LLN7	*nga106*	3.16	5, 33.3 (30.1–52.7)	−0.508	8.0
	LWN7	*mi138*	4.32	5, 39.6 (29.8–46.7)	−0.308	11.5
	PLN7	*mi138*	4.64	5, 39.6 (34.1–50.0)	−0.565	7.3
ad-*LSI4*	LAN7	*g4028*	5.78	5, 86.2 (82.6–95.8)	−3.908	14.6
	LPN7	*g4028*	6.23	5, 86.2 (81.9–91.6)	−1.245	15.2
	LLN7	*g4028*	5.83	5, 86.2 (82.0–90.1)	−0.434	15.5
	LWN7	*mil194*	3.46	5, 90.6 (83.9–96.9)	−0.220	9.0
	PLN7	*g4028*	5.20	5, 86.2 (81.4–90.2)	−0.482	8.2
	PWN7	*g4028*	3.29	5, 86.2 (80.9–90.6)	−0.078	5.4
ad-*LaSI1*	LAN7	*ve018*	4.89	2, 69.7 (59.6–76.4)	3.467	12.3
	LPN7	*ve018*	6.42	2, 69.7 (58.5–73.7)	1.234	16.0
	LLN7	*ve018*	3.62	2, 69.7 (55.5–78.2)	0.324	9.2
	LWN7	*ve018*	2.87	2, 69.7 (52.6–109.9)	0.190	7.4
ad-*LaSI2*	LAN7	*mi456*	1.69	3, 72.7 (66.6–76.8)	3.045	3.9
	LPN7	*mi456*	1.71	3, 72.7 (64.4–76.7)	0.935	3.7
	LLN7	*mi456*	1.74	3, 72.7 (67.0–76.8)	0.341	4.1
	LWN7	*mi456*	2.32	3, 72.7 (63.3–76.8)	0.273	5.9
ad-*LaSI3*	LAN7	*g2778*	2.83	3, 78.1 (77.2–85.5)	−4.034	6.7
	LPN7	*g2778*	2.85	3, 78.1 (77.4–85.7)	−1.234	6.4
	LLN7	*g2778*	3.43	3, 78.1 (76.8–85.9)	−0.493	8.5
	LWN7	*g2778*	2.52	3, 78.1 (76.8–85.9)	−0.286	6.5
ad-*LaSI4*	LAN7	*mi465*	3.75	4, 46.0 (34.3–50.9)	−3.149	9.3
	LPN7	*mi465*	3.32	4, 46.0 (28.4–52.2)	−0.897	7.7
	LLN7	*mi465*	2.68	4, 46.0 (26.8–52.9)	−0.289	6.7
	LWN7	*mi465*	2.19	4, 46.0 (35.3–51.9)	−0.158	5.6
ad-*LaSI5*	LAN7	*mi232*	2.41	4, 76.8 (60.9–95.4)	2.680	5.8
	LPN7	*mi232*	2.87	4, 76.8 (66.3–95.7)	0.895	6.6
	LLN7	*mi232*	2.87	4, 76.8 (60.9–95.4)	0.895	6.6
ad-*PSI1*	PLN7	*er*	11.69	2, 50.6 (48.7–54.4)	0.752	21.9
	PWN7	*er*	18.30	2, 50.6 (48.9–55.6)	−0.143	44.1
ad-*PLE1*	PLN7	*apx1A*	2.49	1, 9.3 (0–39.3)	0.292	3.7
ad-*PLE2*	PLN7	*mi330*	2.90	4, 58.2 (21.9–64.2)	−0.466	4.5
ad-*PLE3*	PLN7	*mi422*	9.30	4, 69.4 (66.2–71.1)	0.958	16.4

See Table 9 legend.

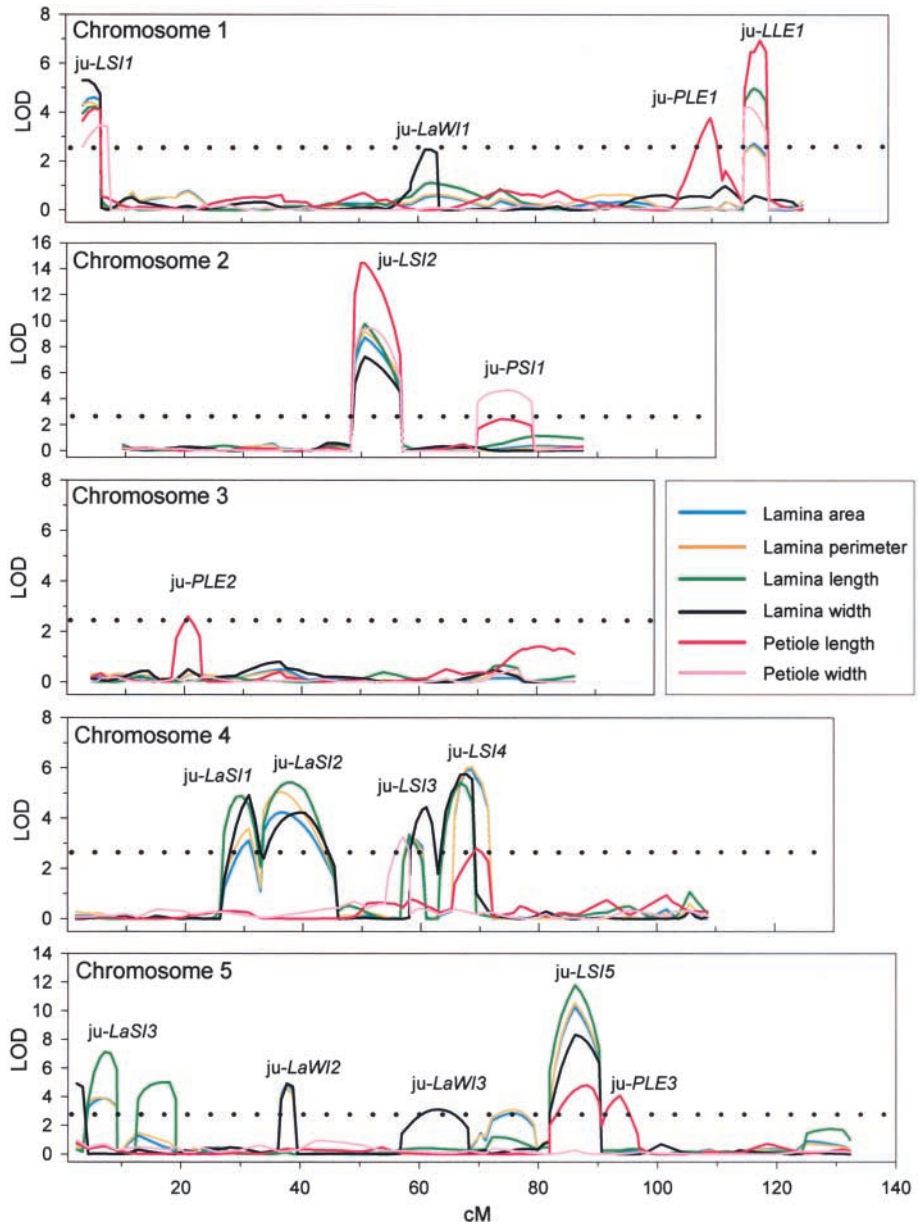

FIGURE 7.—Likelihood plots for QTL affecting juvenile leaf architectural traits. LOD scores are indicated along the ordinate and map positions along the abscissa in centimorgans. The dotted line at LOD 2.7 indicates the significance threshold for QTL identification.

size of *A. thaliana* vegetative leaves as well as in rosette structure, which is unlikely to be related with differences between vegetative growth rates.

Leaf variants among accessions represented variations less extreme than those found in mutant searches. Many of the leaf mutant lines included in the AIS form mutants collection display phenotypes that are more extreme than those of any of the natural variants studied here (BÜRGER 1971; KRANZ 1978; SERRANO-CARTAGENA *et al.* 1999, 2000). Likewise, most of the lines that we have isolated in a large-scale screen for EMS-induced leaf mutants (BERNÁ *et al.* 1999) presented phenotypes that are more extreme than those of AIS ecotypes. Genetic analysis of two such collections of mutants has shown that almost all the studied traits were monogenic and that mutations affecting at least 57 different genes in the former (SERRANO-CARTAGENA *et al.* 1999) and 94 in the latter group of lines (BERNÁ *et al.* 1999) were responsible for the leaf phenotypes.

Quantitative analysis of the expansion of En-2 rosette leaves: To analyze mutants displaying morphological aberrations, criteria are required to determine the nature of their differences with wild-type individuals. A considerable amount of information on wild-type leaf growth has been obtained in different plant species (reviewed in CUSSET 1986; DALE 1988), some of them dicotyledonous, such as *Phaseolus vulgaris* (DALE 1964), *Vicia faba* (DENNET *et al.* 1978), *Glycine max* (BARTHOU and BUIS 1988), *Lycopersicon esculentum* (DENGLER 1984), *Nicotiana tabacum* (POETHIG and SUSSEX 1985), *Vitis riparia* (LACROIX and POSLUSZNY 1990), and *Cucurbita argyrosperma* (JONES 1993), and others monocotyledonous, such as *Festuca arundinacea* (SKINNER and NELSON 1994). Studies on the expansion of *A. thaliana* cotyledons (TSU-

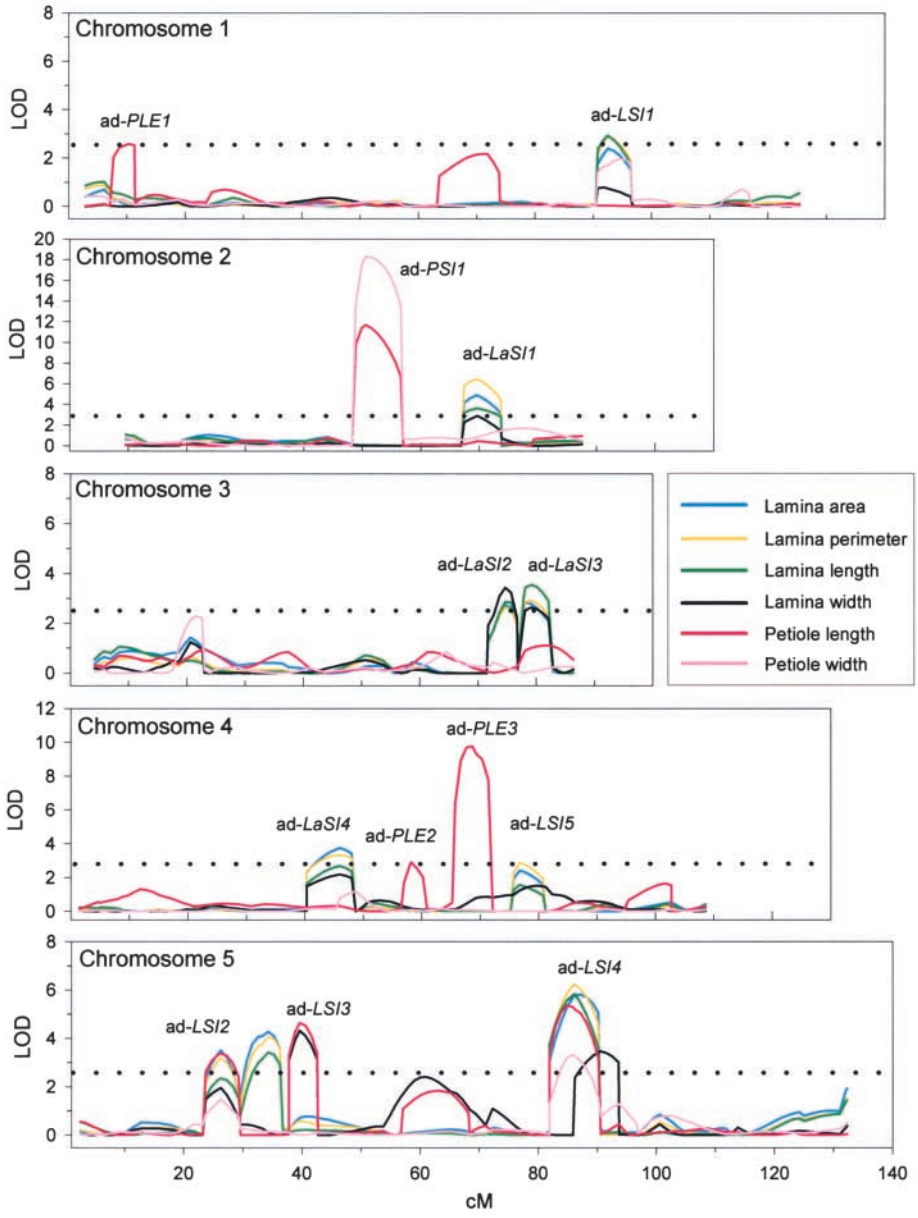

FIGURE 8.—Likelihood plots for QTL affecting adult leaf architectural traits. See Figure 7 legend.

KAYA *et al.* 1994), the first vegetative leaf (PYKE *et al.* 1991), and all rosette leaves (TSUGE *et al.* 1996) have been published. Morphometric analysis has been performed in only some of the above-mentioned cases.

We performed a morphometric analysis of the expansion of the rosette leaves of the Enkheim-2 accession, which was chosen because it represents the genetic background for most of the mutant lines belonging to the large AIS form mutants collection. Our quantitative results agree with the qualitative observations that previous authors made on other accessions and provide a framework for the phenotypic characterization of AIS leaf form mutants, making possible their precise comparison with the wild-type pattern of leaf organogenesis. Our data on the growth of all the vegetative leaves of En-2 confirm and extend previous studies on the first leaf of L*er* (PYKE *et al.* 1991) and the cotyledon of Col-0 (TSUKAYA *et al.* 1994). The results obtained on the area, perimeter, length, and width of all the rosette leaves, collected from the 10th to the 32nd day after sowing, provide information on the morphological differences between successive leaves as well as on the variation with time of the shape and size of each leaf and will facilitate quantitative comparisons between the En-2 accession and leaf mutants with an En-2 background, such as those of the AIS collection. The leaf parameters studied in this work in En-2 allowed us to reach conclusions similar to those made by previous authors studying different genetic backgrounds on the heteroblastic differences between *A. thaliana* vegetative leaves (TSUKAYA *et al.* 2000) and the proportionality of leaf expansion in the dimensions of width and length (TSUGE *et al.* 1996).

Natural variation in leaf architecture is multifactorial in *A. thaliana*: After the classification of wild-type strains according to leaf architectural traits, we tried to determine the genetic basis of the observed variations. For that purpose, we chose accessions displaying extremes of the most representative trait of each phenotypic group and performed crosses involving accessions displaying a given trait in two extreme and opposite ways, their F_1 and F_2 progenies being analyzed. Of note was the high number of unsuccessful crosses, suggesting that accession diversification has led to some degree of incompatibility.

We expected to obtain, at least in some cases, a discrete number of phenotypic groups among the F_2 progeny of intercrosses, making it possible to estimate the number of genes underlying some of the traits under study. However, despite the substantial number of crosses performed, no obvious phenotypic classes were found in their F_2 progeny, so we could not directly estimate the number of genes controlling the traits under study. Our failure to find natural monogenic variants in petiole length, marginal configuration, and overall lamina shape contrasts with our own results with regard to venation pattern, another leaf architectural trait (Candela *et al.* 1999). In fact, a search for natural variations in venation patterning in the first vegetative leaves of 266 accessions resulted in finding 1, Ei-5, which shows unequivocally different patterning from that of the rest and which is inherited as a monogenic recessive trait.

Polymorphic microsatellites, often referred to as simple sequence repeats (SSR) or simple sequence length polymorphisms (SSLP), have been found in *A. thaliana* (Bell and Ecker 1994), as well as in other eukaryotes (Tautz 1989; Weber and May 1989; Hearne *et al.* 1992). Microsatellites have been proven useful for estimating genetic distances between closely related species, as well as between subpopulations of a single species (Bowcock *et al.* 1994), such as in *A. thaliana*, where they vary greatly among accessions (Innan *et al.* 1997; Zwan *et al.* 2000).

The morphological traits studied allowed us to define clusters of accessions that clearly do not correlate with genetic distances measured according to microsatellite polymorphisms, a type of molecular marker that we used because of their high level of polymorphism and ease of genotyping. Innan *et al.* (1997) determined the level of polymorphism at 20 microsatellite loci in a worldwide sample of 42 *A. thaliana* accessions, while we analyzed 22 microsatellites in 16 accessions. The values of gene diversity found in all the loci studied, 0.794 (Innan *et al.* 1997) and 0.827 (this work), are remarkably close since only 2 accessions (Aa-0 and La-1) and five markers (nga111, nga168, AthCHIB, nga162, and AthCTR1) were used in both studies. As in previous studies (Innan *et al.* 1997; Zwan *et al.* 2000), we found no clear correlations between genetic distances and the geographic origins of *A. thaliana* accessions.

Furthermore, we found that microsatellite-specific distance measurements did not correlate with morphological grouping of the accessions studied. Neighbor-joining phylogenetic trees of three different topologies were obtained from distance matrices calculated using (a) the D_1 and $(\delta\mu)^2$ distance measurements; (b) the D_{KF}, D_{PS}, and D_{FS} distance measurements; and (c) the D_{AD} distance measurement, none of which showed clustering of the accessions studied in relation to the morphological traits under study (Figure 4). The fact that the trait-based leaf morphological clustering and microsatellite-based phylogeny did not correlate in the studied accessions reinforced the hypothesis that intraspecific variability in leaf morphology arises from the accumulation of mutations at quantitative trait loci in *A. thaliana*. This is further shown by the QTL analyses performed.

Although the most likely explanation for our results is that the studied leaf phenotypes are controlled by QTL, the experimental approach required to test such a hypothesis in the F_2 individuals obtained was considered to be beyond the scope of this work, since this would require not only a detailed morphometric and statistical analysis of the F_2 progeny plants obtained, but also their individual genotyping for at least 100 molecular markers, together with that of their parental accessions.

QTL affecting leaf morphology: It has been known for a long time that rosette leaves produced throughout the vegetative development of *A. thaliana* can be distinguished from one another by their size and shape (Tsukaya *et al.* 2000). To undertake a quantitative genetic dissection of leaf morphogenesis, we studied the third and seventh vegetative leaves from plants belonging to a mapping population of 100 RILs derived from a L*er*-0 × Col-4 cross (Lister and Dean 1993). Leaves were collected 25 days after sowing, when third leaves are fully expanded but seventh leaves are not. We used 173 markers covering 519.5 cM of the Arabidopsis genome, which, if randomly chosen, could help to detect a QTL by marker linkage within 3 cM and with a probability of 85%.

We identified 16 QTL affecting highly correlated leaf morphological traits in juvenile leaves: 5 affecting the overall form of the leaf organ, 6 specific for the lamina, 4 affecting only the petiole, and 1 modifying the length but not the width of the whole leaf. In addition, a total of 13 QTL were identified in adult leaves: 4 leaf size QTL, 5 specific for the lamina, and 4 specific for the petiole. In both analyses we found three pairs of linked QTL with opposite effects (ju-*LSI3* and ju-*LSI4*, ju-*LaSI1* and ju-*LaSI2*, and ad-*LSI2* and ad-*LSI3*), which could be discriminated thanks to the high density of markers employed. In juvenile leaves, at least 50% of the variance in leaf size could be explained by two large-effect QTL: ju-*LSI2*, which is linked to *ER*, and ju-*LSI5*.

Among the QTL identified for juvenile leaves, those

represented by ju-*LSI3*, ju-*LSI5*, ju-*LaSI2*, ju-*LLE1*, and ju-*LaWI2* had alleles that increased the phenotypic values of the L*er*-0 parental, whereas ju-*LSI1*, ju-*LSI2*, ju-*LSI4*, ju-*LaSI1*, and ju-*LaSI3* had alleles showing a positive effect on the Col-4 parental. For the adult leaves, the Col-4 alleles of ad-*LSI1*, ad-*LSI2*, ad-*LaSI1*, ad-*LaSI2*, ad-*LaSI5*, ad-*PLE1*, and ad-*PLE3* had a positive effect over the variance, whereas the L*er*-0 alleles of ad-*LSI3*, ad-*LSI4*, ad-*LaSI3*, and ad-*LaSI4* increased the variance.

As regards the other parameters analyzed, the total leaf number was scored to detect the QTL responsible for the time profile of production of vegetative leaves, although some flowering-time QTL were expected to be identified in our analyses. We found two major QTL (*TLN1*, linked to the *JGB9* marker in chromosome 4, and *TLN2*, linked to *ve018* in chromosome 2), which were responsible for 25% of the observed variance and whose map positions make them candidates to be alleles of *FWA* and *TOC2*, respectively.

Candidate genes: Although it is assumed that the understanding of the genetic architecture of quantitative traits, which begins by mapping QTL to broad genomic regions, should end with the molecular definition of QTL alleles (Mackay 2001), the resolution of QTL analysis is not sufficient for the positional cloning of candidate genes (Darvasi *et al.* 1993; Boehnke 1994). Consequently, the confirmation that a particular gene is, in fact, a candidate requires narrowing a QTL interval by Mendelization using near-isogenic lines (NILs) to finally achieve the molecular identity of the natural alleles involved in the trait under study. The isolation of near-isogenic lines, which is under progress for some of the major QTL, will help us to narrow the genome interval harboring the QTL identified in this work.

From our results concerning the map positions and phenotypic effects of the identified QTL affecting leaf morphological traits, we looked for candidate genes by using the available genetic and molecular data on leaf mutants. A large leaf mutant collection has been obtained in our laboratory (Berná *et al.* 1999; Robles and Micol 2001), some of whose phenotypic classes correspond to alterations in the size or proportions of leaves, such as those named Rotunda (Ron) and Orbiculata (Orb), with rounded leaves; Exigua (Exi), with small leaves; and Elongata (Elo), with lanceolate leaves and long petioles. Several of the QTL detected in this work had map positions neighboring those of some mutants of the above-mentioned phenotypic classes (Robles and Micol 2001). The ju-*LLE1* QTL maps near the lower telomere of chromosome 1, close to the *EXI6* gene. Another QTL affecting leaf size, in both juvenile and adult leaves, that mapped near the *g4028* marker (ju-*LSI5* and ad-*LSI4*) in chromosome 5, had a strong effect on the phenotypic variance and mapped close to the *EXI2* and *ELO3* genes, whose mutant alleles cause altered proportions of the lamina and petiole size. Another QTL, ju-*LaSI3*, maps close to the *ORB1* gene, in chromosome 5, and both ju-*LaWI2* and ad-*LSI3* are located in the neighborhood of the *ELO2* gene, in chromosome 3. The *EXI1* gene maps in chromosome 4, close to a QTL involved in lamina size in both juvenile and adult leaves (ju-*LaSI2* and ad-*LaSI4*).

Mutant alleles of the *ER* gene, which encodes a leucine-rich repeat (LRR) receptor protein kinase, display a compact inflorescence, blunted fruits, and short petioles (Torii *et al.* 1996). Several QTL mapping close to *ER* have been identified in *A. thaliana* (Alonso-Blanco *et al.* 1999; Swarup *et al.* 1999; Juenger *et al.* 2000; Borevitz *et al.* 2002). It is likely that ju-*LSI2* and ad-*PSI2* are probably caused by the *erecta* mutation carried by the L*er*-0 accession. The effect of *ER*, or that of its linked QTL, was detected both for the laminae and the petioles of juvenile leaves, but only for the petioles of adult leaves. These results are congruent with the described pattern of expression of the *ER* gene, whose transcripts are more abundant in juvenile than in adult leaves (Torii *et al.* 1996; Yokoyama *et al.* 1998).

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