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A first linkage map of pecan cultivars based on RAPD and AFLP markers

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Abstract We report here the first genetic linkage maps of pecan [*Carya illinoinensis* (Wangenh.) K. Koch], using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. Independent maps were constructed for the cultivars 'Pawnee' and 'Elliot' using the double pseudo-testcross mapping strategy and 120 F_1 seedlings from a full-sib family. A total of 477 markers, including 217 RAPD, 258 AFLP, and two morphological markers were used in linkage analysis. The 'Pawnee' linkage map has 218 markers, comprising 176 testcross and 42 intercross markers placed in 16 major and 13 minor (doublets and triplets) linkage groups. The 'Pawnee' linkage map covered 2,227 cM with an average map distance of 12.7 cM between adjacent markers. The 'Elliot' linkage map has 174 markers comprising 150 testcross and 22 intercross markers placed in 17 major and nine minor linkage groups. The 'Elliot' map covered 1,698 cM with an average map distance of 11.2 cM between adjacent markers. Segregation ratios for dichogamy type and stigma color were not significantly different from 1:1, suggesting that both traits are controlled by single loci with protogyny and green stigmas dominant to protandry and red stigmas. These loci were tightly linked (1.9 cM) and were placed in 'Elliot' linkage group 16. These linkage maps are an important first step towards the detection of genes controlling horticulturally important traits such as nut size, nut maturity date, kernel quality, and disease resistance.

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Introduction

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is one of the most commercially important nut crops grown in the United States and the most economically important member of the genus *Carya* Nutt. (hickory) (Wood 1994). Most pecan cultivars are diploid, with a chromosome number of $2n = 2x = 32$. Pecan trees are very large and long-lived trees with a juvenile period of 5–10 years. These characteristics, in combination with a high degree of heterozygosity maintained through dichogamy, have limited genetic studies in this crop. Consequently, the genetic control of important traits, including disease resistance and nut quality, are poorly understood.

Pecan is monoecious, with male and female flowers produced on the same individual. The staminate flowers are organized into an ament or catkin, and the female flowers are borne on a spike (Wetzstein and Sparks 1986). Self-pollination in pecan is usually limited by dichogamy, whereby the male and female flowers mature at different times. Pecan cultivars differ with respect to maturity of the staminate and pistillate flowers, leading to both protandrous and protogynous patterns of flowering, termed heterodichogamy. In protogynous types, stigmas become receptive prior to pollen shed, and in protandrous types, pollen begins to shed before the stigmas are receptive. The only trait in pecan for which the genetics has been established is dichogamy,

which is controlled by a single locus such that protandry (*pp*) is recessive to protogyny (*PP* or *Pp*) (Thompson and Romberg 1985).

The potential of molecular markers to increase our understanding of the pecan genome has been demonstrated in several studies. Isozyme systems have been used to study the genetic diversity of pecan populations (Marquard 1987, 1989, 1991; Marquard et al. 1995; Ruter et al. 2000, 2001). Conner and Wood (2001) used random amplified polymorphic DNAs (RAPDs) to identify pecan cultivars and estimate their genetic relatedness. Vendrame et al. (2000) used amplified fragment length polymorphisms (AFLPs) for the molecular evaluation of pecan trees regenerated from somatic embryogenic cultures, and Grauke et al. (2003) evaluated simple sequence repeat (SSR) markers for genetic studies. Grauke et al. (2001) determined the mean 2C genome size of pecan to be approximately 1.7 pg. Aside from these studies, little information exists on the pecan genome.

With the advent of several PCR-based methodologies, molecular markers became common tools for genetic studies. Molecular markers are particularly valuable in perennial crops for linkage map construction and the mapping of qualitative and quantitative traits (Crespel et al. 2002). Molecular linkage maps are also being used successfully in many crop species for directed germplasm improvement (Pearl et al. 2004). Though most agronomically important crops have saturated, or nearly saturated, linkage maps, mapping studies in perennial fruit and nut crops have lagged behind. Molecular linkage maps of several tree fruit and nut crops have been recently produced, including pear (*Pyrus communis*) (Yamamoto et al. 2002), apricot (*Prunus armeniaca*) (Vilanova et al. 2003; Lambert et al. 2004), *Citrus* (Sankar and Moore 2001), macadamia (*Macadamia* sp.) (Peace et al. 2003), apple, (*Malus* × *domestica*) (Conner et al. 1997), and walnut, (*Juglans* sp.) (Fjellstrom and Parfitt 1994). Linkage maps facilitate the identification and localization of genes controlling important traits, subsequently allowing marker-assisted selection and positional cloning of genes (Staub et al. 1996; La Rosa et al. 2003).

Like many tree fruit and nut crops, pecan is an outbreeding heterozygous crop and inbreeding depression limits the ability to produce F_2 or backcross populations for mapping. Instead, F_1 progenies obtained from crossing two highly heterozygous parent clones and the double pseudo-testcross strategy are used to generate linkage maps. This method produces two independent maps, one for each of the parents (Weeden 1994; Atienza et al. 2002; Yin et al. 2002; La Rosa et al. 2003).

In this report we describe the construction of genetic linkage maps for the pecan cultivars 'Pawnee' and 'Elliot' using a combination of RAPD and AFLP markers. Both 'Pawnee' and 'Elliot' are important cultivars to the breeding program. 'Pawnee' is one of the most widely planted pecan cultivars and combines the desirable characteristics of large nut size, high quality, and early nut maturity (Thompson and Grauke 2000). 'Elliot' has a high degree of resistance to pecan scab (Goff et al. 2003) caused by the fungus *Cladosporium caryigenum*. These maps are a first step towards the identification of loci controlling horticulturally important quality traits such as nut size, nut maturity date, and disease and insect resistance (Conner 1999).

Materials and methods

Plant material

The mapping population consisted of 120 progeny of pecan [*Caryl illnoinensis* (Wagnehn) K. Koch] obtained from the cross 'Pawnee' × 'Elliot'. The first progeny set consisted of 55 trees in Byron, Ga. (USA) produced from a cross made in 1986. The second progeny set consisted of 65 seedlings in Tifton, Ga. produced from the same cross in 1999. Trees were grown at a spacing of approximately 3.1/4.6 m in both locations.

The dichogamy type of the Byron progeny was determined in the spring of 2003. Pistil receptivity was determined by first dusting pollen onto the stigmas and then gently blowing off the excess pollen (Thompson and Romberg 1985). A receptive stigma has a sticky surface to which a large number of pollen grains adhere. Anther dehiscence was assessed biweekly by shaking the catkins and observing whether pollen was released. Stigma color was determined visually at peak receptivity; any sign of red or purple coloration was scored as "red" and the lack of such coloration was scored as "green".

Marker analysis

Total DNA was extracted from fresh mature leaves of parental lines and seedlings according to the protocol established for olive (Claros et al. 2000), which was a modification of the method of Doyle and Doyle (1987). The DNA concentration was measured by comparison to known concentrations of λ-DNA by electrophoresis on agarose gels.

A total of 600 RAPD primers were screened using DNA from three progeny and both parents. Primers used for RAPD analysis were synthesized by the University of British Columbia, Vancouver (Canada). Amplifications were carried out in 25-µl aliquots of the solution containing 11.4 µl H_20, 2.5 µl 10× buffer (20 m*M* Tris-HCl pH 8.0, 100 m*M* KCl, 0.1 m*M* EDTA, 1 m*M* DTT, 50% glycerol, 0.5% Tween 20, and 0.5% Nonidet-p40), 2.25 µl 25 m*M* $MgCl_2$, 2 µl 2.5 m*M* solution of each dNTP (Promega, Madison, Wis.) 1.5 µl 10 μM primer, 5 µl of a 5 ng/µl DNA solution, and 0.4 µl *Taq* DNA polymerase (Promega). Amplifications were performed in an Eppendorf scientific thermal cycler (Eppendorf Scientific, Westbury, N.Y.) programmed for 40 amplification cycles (94°C for 1 min, 35°C for 2 min,

and 72°C for 2 min) followed by an 8-min extension cycle at 72°C, with a maximum ramping speed between temperatures. Amplification products were resolved by electrophoresis on a gel of 0.7% agarose and 0.3% synergel (Diversified Biotech, Boston, Mass.) run at 110 V for 4 h in 0.5× TBE buffer (Sambrook et al. 1989). Band sizes were estimated by comparison to a 100-bp DNA ladder (Promega). Gels were stained with ethidium bromide and photographed on a transilluminator, and the segregation patterns were scored manually as a band being present or absent from a computer printout. The RAPD markers were denoted by their primer code followed by the size of the band scored (e.g. B199-1400).

The amplified fragment length polymorphism (AFLP) analyses were carried out as described in Vos et al. (1995) using fluorescently tagged primers. The AFLP core reagent and starter primer kit were purchased from PE-Applied Biosystems (ABI, Foster City, Calif.). A 200-ng aliquot of genomic DNA was digested with the restriction enzymes *Eco*RI and *Mse*I, and DNA fragments were then ligated to adaptors. The pre-selective amplification cycle was carried out with one selective nucleotide (E-A, M-C), followed by a selective amplification cycle using two additional selective nucleotides. The amplification products thus obtained by using three selective nucleotides for both *Eco*RI and *Mse*I were run on a 6% polyacrylamide gel along with size-standard Gene Scan-500 (ROX) and deionized formamide for 45 h on an ABI 377 DNA sequencer using one-time TBE buffer. Digital gel images were obtained using GENESCAN software (Applied Biosystems) and were manually scored for the presence or absence of bands. The AFLP bands were named with the three selective nucleotides of *Eco*RI and the three selective nucleotides of *Mse*I followed by the size of the band scored (e.g., AGC/CTG 275).

Mapping and linkage analysis

Linkage maps were generated for each parent independently using the double pseudo-testcross mapping method (Weeden 1994). This mapping method utilizes the segregation data for polymorphic markers heterozygous in one or both parents and is particularly useful in mapping dominant markers like RAPDs and AFLPs. Testcross markers are heterozygous (band present) in one parent and homozygous recessive (band absent) in the other, and they segregate in a 1:1 present/absent ratio in the progeny. Intercross markers are heterozygous in both parents and segregate in a 3:1 present/absent ratio in the progeny. All testcross markers were tested for a Mendelian segregation ratio of 1:1 using Chi-square analysis ($P < 0.05$). Linkage maps were constructed using the mapping program MAP MANAGER QTXB17 (Meer et al. 2002). Initial linkage groups were constructed from testcross markers using the command MAKE LINKAGE GROUPS. The criteria for linkage was a LOD threshold of 3.0 and a recombination fraction ≤ 0.35. Then, linkages in repulsion phase of all loci were tested using the commands FLIP PHASE and LINKS. The order of loci within linkage groups was refined as necessary by using RIPPLE command and by manual rearrangement of the loci. Linkage distances were computed using the Kosambi mapping function and the allow for SEGREGATION DISTORTION OPTION. Once the framework maps composed of testcross markers were established, intercross markers showing 3:1 ratio were added using the cross-type designation ARBITRARY and the LINKS command (Tsarouhas et al. 2002). Intercross markers were paired with the testcross marker with which they had the most significant linkage (LOD threshold of 3.0). Intercross markers were only considered accessory markers and were not used in the estimation of distances.

Estimation of genome length

The genome length estimation was determined by "method no. 3" of Chakravarti et al. (1991): $G = N(N-1)\ X/K$, where G is the estimate of genome length; N, the number of markers analyzed; X, the largest distance value in centiMorgans between linked markers, and K, the number of linked markers at LOD value 3.

Results

Molecular markers

From the 600 primers initially screened, 90 (15.0%) were chosen based upon the number of strong polymorphic markers they produced (Table 1). Using these 90 primers, RAPD analysis was performed on the entire 120 progeny, generating a total of 217 markers. The number of polymorphic RAPD markers produced per primer ranged from one to six, with an average of 2.4. Of the 217 RAPD markers, 174 (80.2%) were testcross markers and the remaining 43 (19.8%) were intercross markers. Out of 174 testcross RAPD markers generated, 82 (47.1%) and 92 (52.9%) markers were heterozygous in 'Pawnee' and 'Elliot', respectively. Distorted segregation was seen in 25 (14.4%) markers, 11 from 'Pawnee' and 14 from 'Elliot'.

The AFLP selective primer screening was carried out using all 64 possible combinations of primers provided in the starter kit, which includes eight *Eco*RI + 3 primers and eight *Mse*I + 3 primers (Applied Biosystems 2000). Based on the number of polymorphic markers produced on a subset of ten progeny and both parents, 20 highly polymorphic primer pairs were chosen for mapping (Table 1). Using these 20 primer pairs, we carried out AFLP analysis on the entire progeny,

Table 1 Markers scored in the pecan [*Carya illnoinensis* (Wagnehn) K. Koch] progeny using RAPD and AFLP analysis

	RAPD markers	AFLP markers
Number of primers/ primer pairs screened	600	64
Number of primers/ primer pairs used for mapping	90	20
Number of testcross markers (1:1) present in 'Pawnee'	82	116
Number of testcross markers (1:1) present in 'Elliot'	92	96
Number of intercross markers (3:1)	43	46
Total number of markers produced	217	258
Average number of markers obtained per primer/primer pair[a]	2.41	12.9

[a] Includes both intercross and testcross markers obtained for each primer/primer pair

Table 2 Primer combinations used for AFLP marker analysis in pecan mapping and bands scored for linkage analysis

Markers segregating 1:1				
Primer pair	'Pawnee'[a]	'Elliot'[b]	Total	Markers segregating 3:1
E-AAC/M-CAC	6	6	12	1
E-AAC/M-CAG	7	4	11	3
E-AAC/M-CAT	6	2	8	5
E-AAC/M-CTG	10	9	19	5
E-ACA/M-CAC	12	11	23	1
E-ACA/M-CAG	5	2	7	1
E-ACA/M-CAT	8	5	13	5
E-ACA/M-CTA	1	5	6	2
E-ACC/M-CAA	8	4	12	1
E-ACC/M-CAG	5	3	8	2
E-ACC/M-CAT	5	4	9	0
E-ACG/M-CAA	5	6	11	0
E-ACG/M-CTA	0	6	6	2
E-AGC/M-CAG	12	7	19	4
E-AGC/M-CAT	3	3	6	0
E-AGC/M-CTA	6	3	9	2
E-AGC/M-CTG	0	2	2	0
E-AGG/M-CAA	7	4	11	4
E-AGG/M-CAG	6	7	13	2
E-AGG/M-CTA	4	3	7	6
Total	116	96	212	46

[a] Band present (heterozygous) in 'Pawnee', null in 'Elliot' and segregating in 1:1 in F_1progeny

[b] Band present (heterozygous) in 'Elliot', null in 'Pawnee' and segregating in 1:1 in F_1progeny

generating a total of 258 markers, of which 212 (82.2%) were testcross markers and 46 (17.8%) were intercross markers. Out of the 212 testcross AFLP markers generated, 116 (54.7%) and 96 (45.3%) markers were heterozygous in 'Pawnee' and 'Elliot', respectively. The number of polymorphic AFLP markers generated per primer pair ranged from 2 (E-AGC/M-CTG) to 24 (E-ACA/M-CAC) with an average of 12.9 markers (Table 2). In 'Pawnee' the number ranged from 0 to 12 with an average of 5.8, and in 'Elliot' ranged from 2 to 11, with an average of 4.8 (Table 2). Out of the 212 testcross AFLP markers, 35 (16.5%) showed distorted segregation, 22 from 'Pawnee' and 13 from 'Elliot'.

Map construction

Independent linkage maps were constructed for each parent using the double pseudo-testcross mapping strategy (Weeden 1994). A total of 477 (Table 1) markers, including 217 RAPD, 258 AFLP and two morphological markers were available for map construction. Framework maps were created using only markers in the testcross configuration. For the 'Pawnee' map, 176 testcross markers were assigned to 29 linkage groups, leaving 22 markers unlinked. The 29 linkage groups include 16 major groups, two triplets, and 11 pairs (Fig. 1). Once the framework maps had been completed, 42 intercross markers were added to the map as accessory markers, while 47 intercross markers remained unlinked. The final 'Pawnee' linkage map consists of 218 markers, including 176 testcross markers (80.7%) and 42 (19.3%) intercross markers. The 'Pawnee' map covered a total distance of 2,228 cM, with an average distance of 12.6 cM between adjacent markers (Fig. 1). The groups ranged in size from 15 cM to 303 cM, and the total number of markers per linkage group ranged from 2 to 23.

The 'Elliot' framework map was created using 152 testcross markers, which were assigned to 26 linkage groups, leaving 36 markers unlinked. The 26 linkage groups include 15 major linkage groups, five triplets, and six pairs (Fig. 2). Once the framework maps were completed, 25 intercross markers were added to the map as accessory markers, while 64 remained unlinked. The 'Elliot' map covered a total of 1,698 cM with an average of 11.2 cM between adjacent markers. The size of the linkage groups ranged from 1.3 cM to 257 cM, and the total number of markers per linkage group ranged from 2 to 17.

Intercross markers are useful in finding homologous linkage groups common to both the maps. The 'Pawnee' and 'Elliot' maps shared 14 of the 89 (15.7%) of the intercross markers. Using intercross markers placed on both maps, ten 'Pawnee' linkage groups could be paired with one or more 'Elliot' linkage groups (Table 3).

Phenotypic traits

Dichogamy type and stigma color were evaluated in the 55 trees from the cross made in 1986. The trees

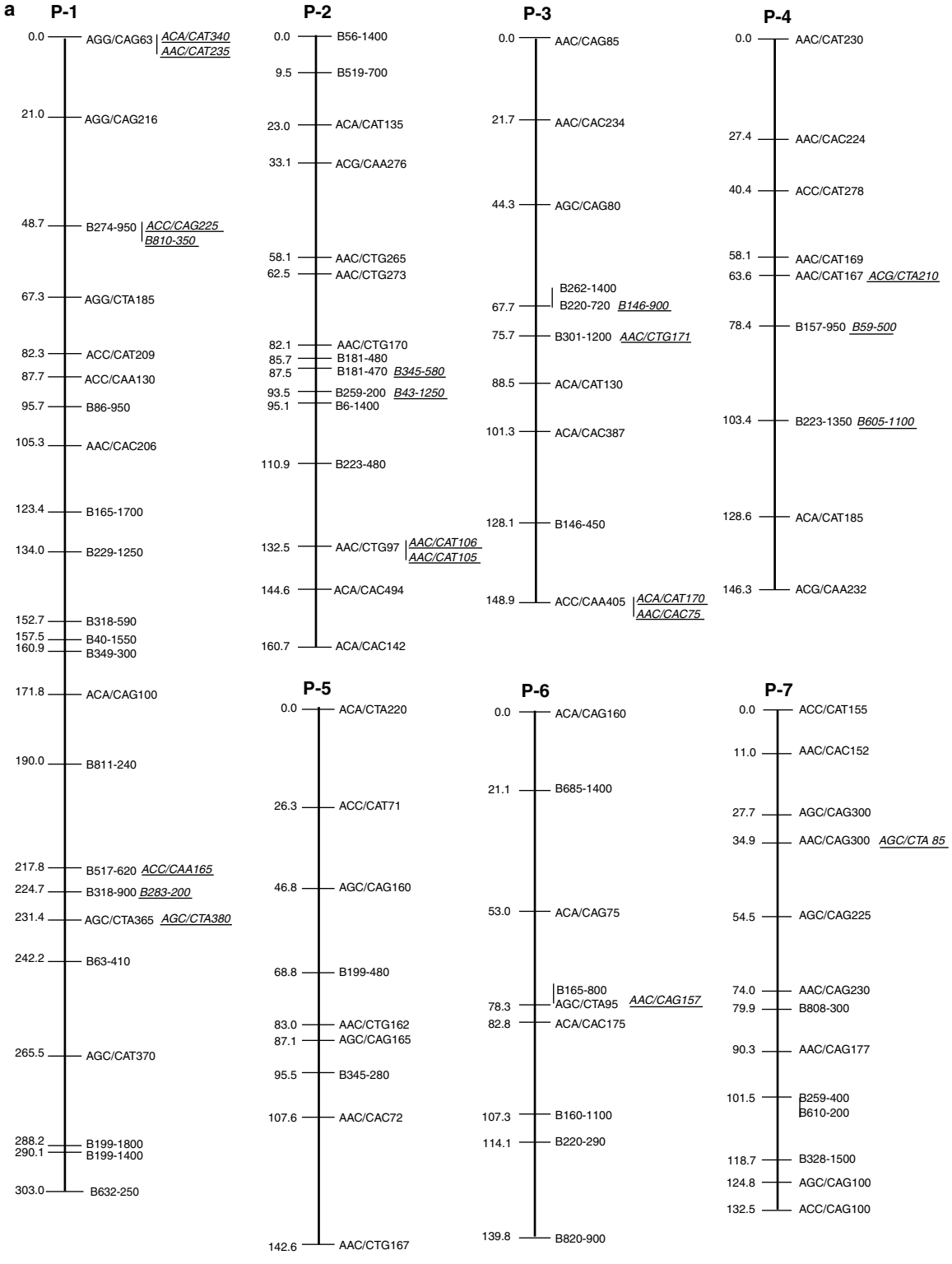

Fig. 1 Genetic linkage map of pecan *Carya illinoinensis* (Wangenh) K. Koch cv. 'Pawnee' (P) consisting of RAPD and AFLP markers. Map distances in centiMorgans are indicated to the *left* and loci to the *right* of each linkage group. Testcross markers are located to the immediate *right* of the linkage group in *normal typeface*. Intercross markers are located to the *right* of the testcross marker with which they are most closely linked and are *italicized* and *underlined*. Linkage groups consisting of only two markers are not presented

from the cross made in 1999 could not be evaluated because the trees had not yet flowered. In the spring of 2003 nearly all of the 1986 progeny flowered and were evaluated for these two traits. One tree did not produce any flowers and another tree was scored for stigma color, but dichogamy type was not clear; thus,

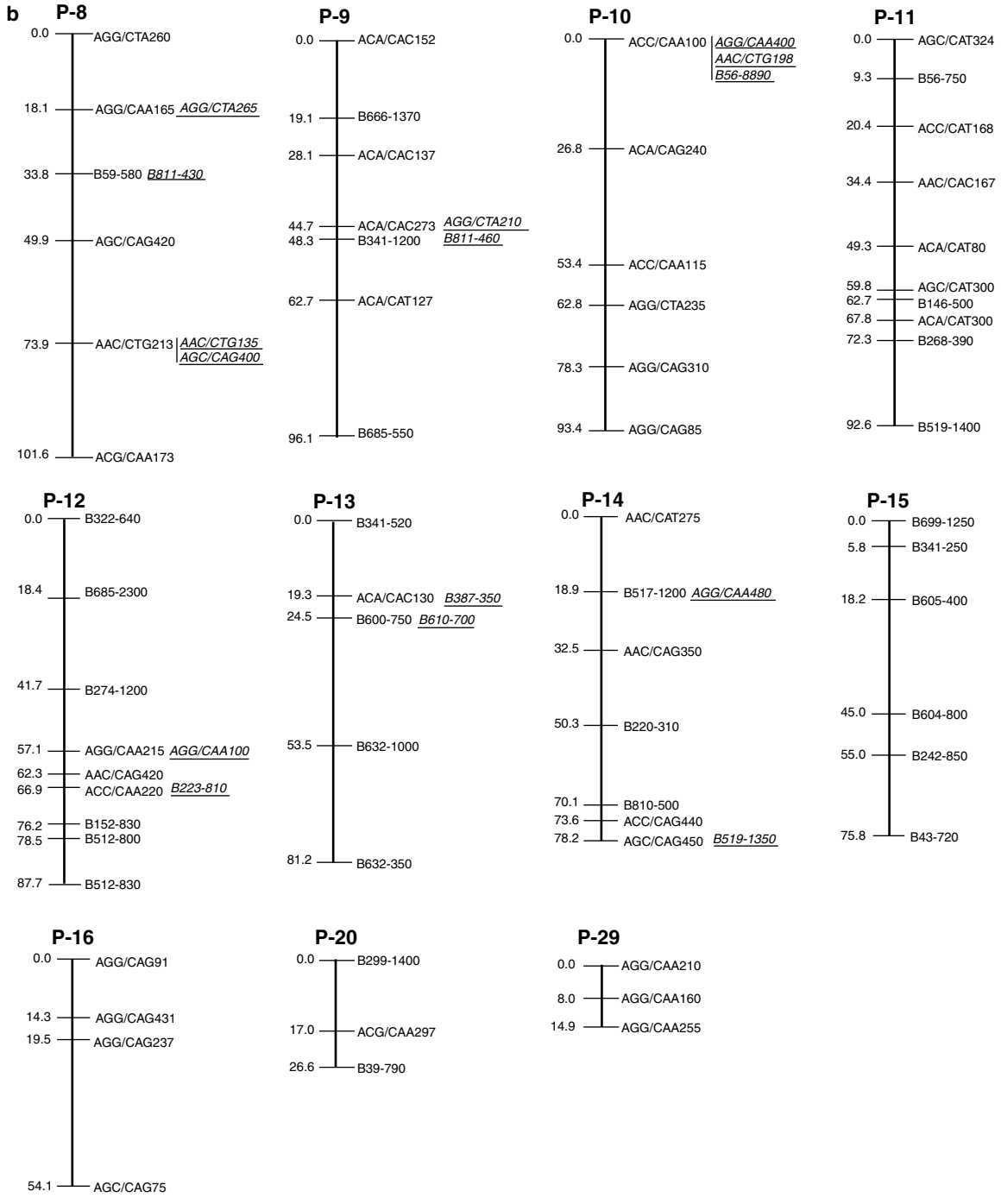

Fig. 1 (Contd.)

54 trees were scored for stigma color and 53 trees were scored for dichogamy type. Out of the 53 trees scored for dichogamy type, the ratio of protandrous to protogynous was 22:31, which fit a 1:1 ratio ($\chi^2 = 1.52$, $P \cong 0.27$), thereby confirming the monogenic control established by Thompson and Romberg (1985). Out of the 54 trees evaluated for stigma color, 21 produced green stigmas and 33 produced red stigmas, which also fit a 1:1 ratio ($\chi^2 = 2.67$, $P \cong 0.09$), suggesting monogenic inheritance.

'Pawnee' is protandrous (*pp*) and has red stigmas while 'Elliot' is protogynous and has a green stigmas. 'Elliot' must be heterozygous (*Pp*) since the progeny segregated for dichogamy type. Indeed, nearly all protogynous cultivars such as 'Elliot' that have come from natural crosses are heterozygous for this trait since

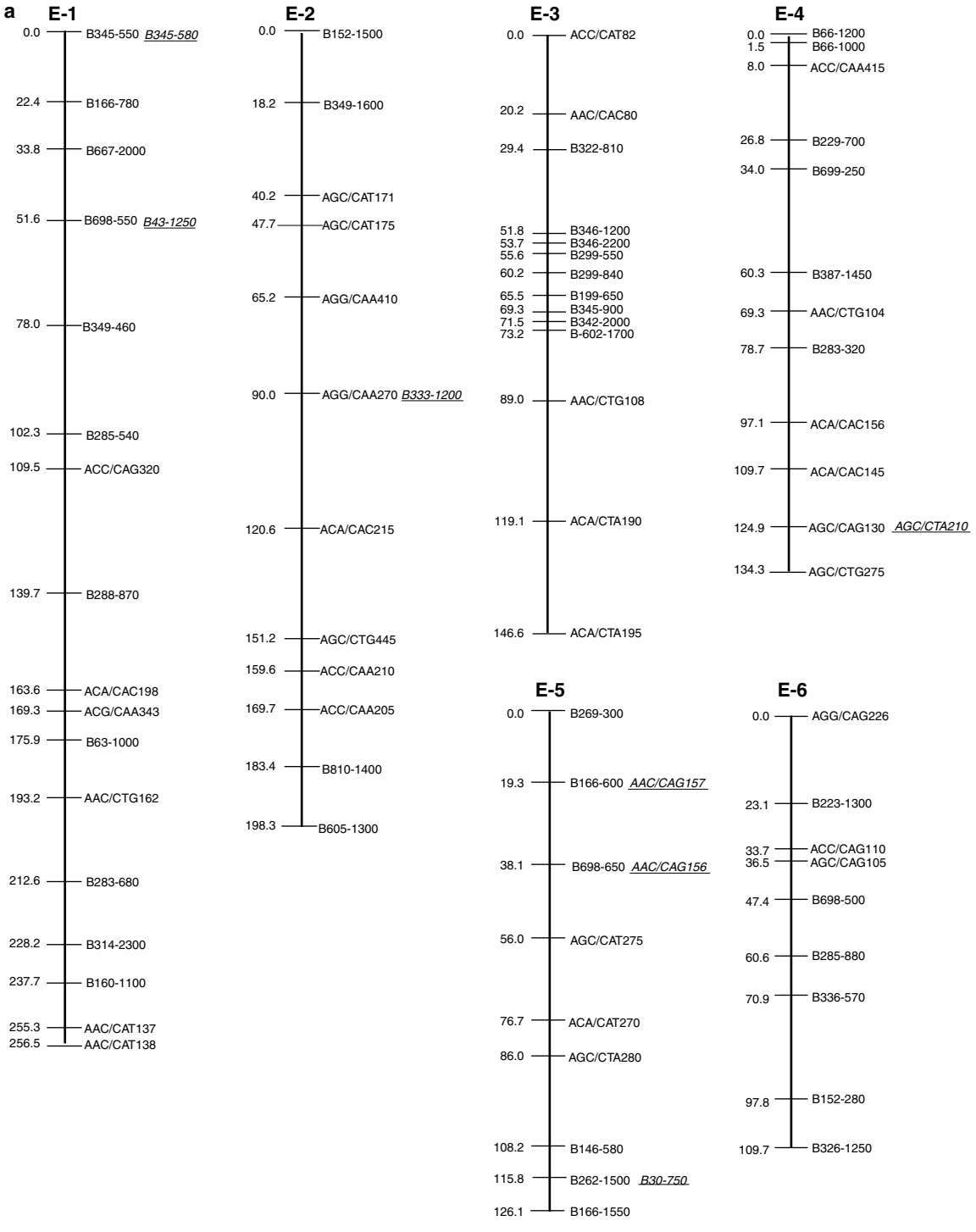

Fig. 2 Genetic linkage map of pecan cv. 'Elliot' (*E*) consisting of RAPD and AFLP markers. Map distances in centiMorgans are indicated to the *left* and loci to the *right* of each linkage group. Testcross markers are located to the immediate *right* of the linkage group in *normal typeface*. Intercross markers are located to the *right* of the testcross marker with which they are most closely linked and are *italicized* and *underlined*. Linkage groups consisting of only two markers are not presented

protogynous types nearly always cross with protandrous types, thereby maintaining a 1:1 phenotypic ratio (Thompson and Romberg 1985). Because only 'Elliot' is heterozygous for this trait, this gene could only be placed on the 'Elliot' map. Dichogamy type mapped to 'Elliot' linkage group 16 (Fig. 2) with linkage to the marker ACA/CAT250 (LOD = 5.6 and 15.4 cM) on one side and B29-340 (LOD = 9.8 and 11.3 cM) on the other.

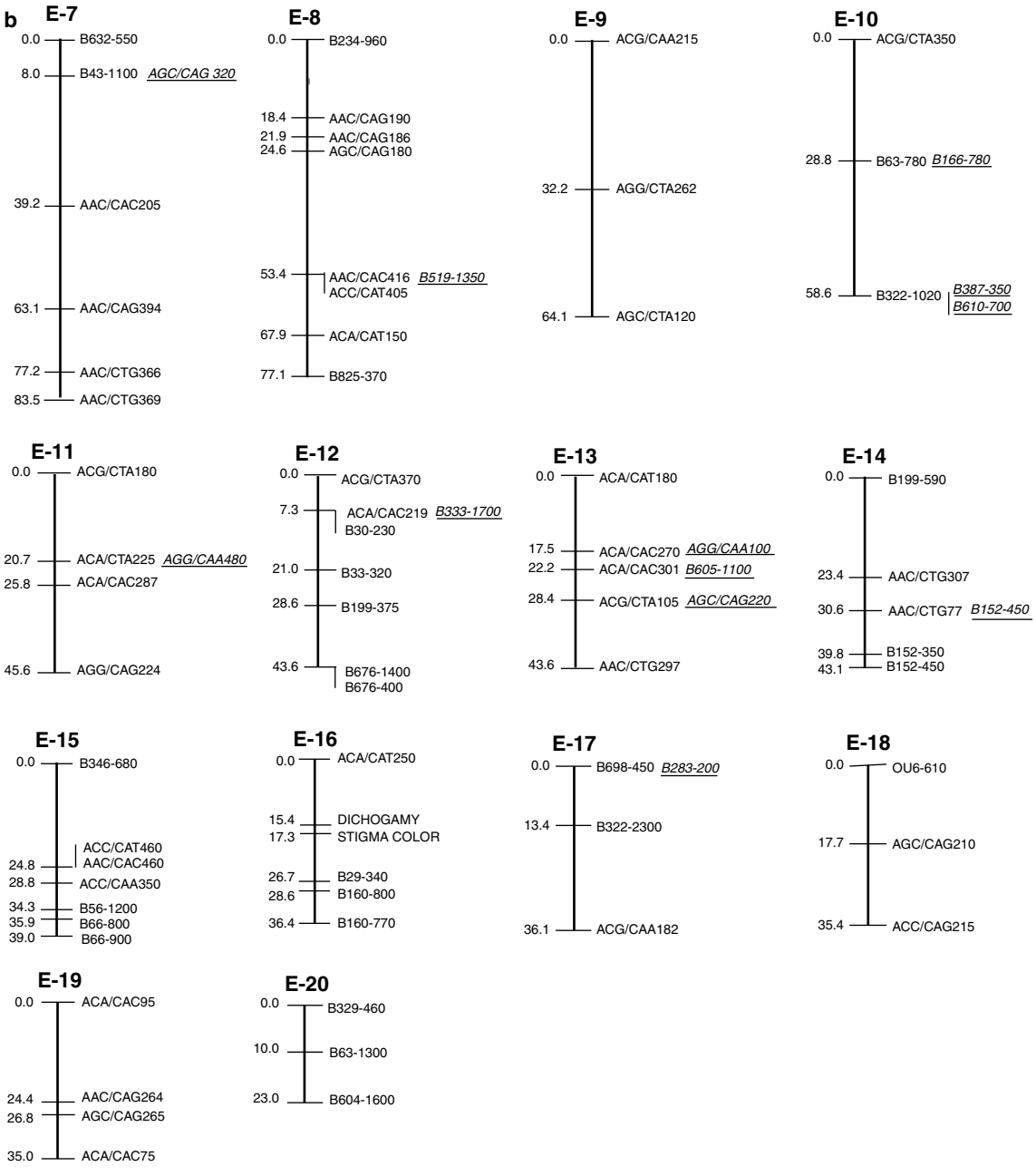

Fig. 2 (Contd.)

Stigma color appears to be tightly linked (LOD = 13.8 and 1.9 cM) with dichogamy type since all protandrous seedlings produced red stigmas and nearly all protogynous seedlings produced green stigmas. Only a single recombinant seedling was detected out of the 53 progeny that could be scored for both traits (Table 4). Assuming green stigma color (*Sc*) is dominant to red stigma color (*sc*), the data suggest that 'Elliot' is heterozygous for this trait (*Scsc*) and that 'Pawnee' is homozygous recessive (*scsc*).

Discussion

For an outcrossing species such as pecan, the double pseudo-testcross mapping strategy is well suited for use with dominant markers. Pecan cultivars are within a couple generations of trees selected from the wild, and most are highly heterozygous. RAPDs and AFLPs are both PCR-based dominant markers. They share many advantages, including a requirement for relatively little

Table 3 Intercross markers placed on linkage maps of both 'Pawnee' and 'Elliot' pecan

'Pawnee' linkage group	'Elliot' linkage group	Marker shared
1	17	B283-200
2	1	B43-1250
2	1	B345-580
6	5	AAC/CAG157
7	24	AGC/CTA85
9	4	AGG/CTA210
10	21	AAC/CTG198
12	18	B223-810
12	13	AGG/CAA100
13	10	B610-700
13	10	B387-350
14	11	AGG/CAA480
14	8	B519-1350
25	23	B345-320

template DNA, the ability to be resolved without the use of radioactivity, and good levels of polymorphism in many species. Perhaps the most important advantage of these markers for use in a minor crop such as pecan is that they can be used without any prior knowledge of the target template DNA sequence. Other marker types such as RFLPs and SSRs require extensive sequencing or cloning efforts before markers can be developed. The AFLP markers require greater inputs in terms of equipment and initial expense than do RAPDs, but the former do have the capability of returning a larger number of markers per primer pair. We found that the AFLP markers were more efficient in terms of the time spent per marker produced, but the need to use a sequencing gel to resolve the bands meant that the AFLP part of the analysis had to be done in a separate facility. In addition, AFLP markers are more difficult to clone and sequence than are RAPD markers, and it will require more effort to convert them to sequence-specific markers such as sequence characterized amplified regions (Paran and Michelmore 1993).

Intercross markers are less informative than testcross markers because the dominant phenotype comprises three indistinguishable genotypes (++, +−, −+) (Crespel et al. 2002). Thus, only in the homozygous recessive progeny is it unambiguous which allele came from each parent. Intercross markers are of interest, however, because they can be used as "locus bridges" to align homologous linkage groups between the two maps (Echt et al. 1994). If enough markers are in common between maps, the maps can be combined into a single integrated linkage map (Conner et al. 1997; Peace et al. 2003). In this study, the percentages of intercross markers for RAPDs (19.8%) and AFLPs (17.8%) were similar to those found for other intraspecific crosses of forest trees [e.g., 19% in *Quercus robur* (Barreneche et al. 1998), 15–18% in *Fagus sylvatica* (Scalfi et al. 2004)].

Table 4 Classification of the 1986 progeny that were scored for stigma color and dichogamy type[a]

	Green stigma	Red stigma	Total
Protogynous	21	1	22
Protandrous	0	31	31
Total	21	32	

[a] Only progeny that were scored for both traits are included in the table

In the present study, the linkage groups were not aligned but were instead reported and numbered individually for both parents. Although several intercross markers were mapped to linkage groups in both parental maps, there were not enough markers in common for the maps to be merged. The difficulty in mapping intercross markers is evident from the low percentage of this type of marker that could be mapped in comparison to testcross markers. In the 'Pawnee' map, 89% of the testcross markers were mapped compared to only 47% of the intercross markers. In the 'Elliot' map, 80% of the testcross markers were mapped in comparison to 40% of the intercross markers. In order to align and compare the homology of both maps effectively, either more intercross markers need to be generated and mapped on a larger progeny set or, preferably, more informative codominant marker types such as SSRs need to be placed on the maps (Powell et al. 1996).

The 'Pawnee' map had 16 major linkage groups, and the 'Elliot' map had 15 major linkage groups, which are close in number to the haploid chromosomal number of pecan ($n = 16$). However, large numbers of minor linkage groups remain in both maps, indicating that the maps are not saturated. Additional markers need to be mapped to resolve the data into 16 linkage groups. Genome length estimates were 2,677 cM for 'Pawnee' and 2,965 for 'Elliot'. Accordingly, the maps cover approximately 83% of the 'Pawnee' genome and 57% of the 'Elliot' genome.

Clustering of AFLP and RAPD markers was sporadically observed in both maps, similar to those reported in other mapping studies (Crespel et al. 2002; Bednarek et al. 2003). This can be explained by a reduced recombination rate around centromeres and also by the tendency of some marker types such as AFLPs to map in clusters (Nilsson et al. 1997).

Two phenotypic markers, dichogamy type and stigma color, were placed on the 'Elliot' map. The genetics of dichogamy type has been established by Thompson and Romberg (1985), but the genetic control of stigma color has not previously been described. Dichogamy type is of importance to pecan culture because it is a major factor in optimizing orchard pollination. The development of cultivars of both dichogamy types is necessary to insure that orchards can incorporate new cultivars that will effectively pollinate each other.

Stigma color is characteristic for each cultivar and can be used to help identify a cultivar (Sparks 1992). In a sample of 82 cultivars from the USDA pecan germplasm collection, 65 had green stigmas and 17 had stigmas with

at least some red coloration (L.J. Grauke, personal communication). Dichogamy type was nearly equally distributed among the cultivars with green stigmas, with 31 protandrous and 34 protogynous. However, among the red stigma cultivars protandry was much more common, with 14 protandrous and three protogynous. Because the pedigree of many of these cultivars is unknown, it is difficult to draw conclusions from these data. The most likely explanation for the preponderance of red stigma protandrous types is the interrelatedness of many of these cultivars. The cultivar 'Success', which is protandrous and has a red stigma, appears in the pedigree of at least 7 of the 17 clones with red stigmas. Several other cultivars with a red stigma have an unknown pedigree but originated near the origin of 'Success' (Ocean Springs, Miss.). The percentage of clones with red stigmas in this sample is thus likely higher than would be found in a random sample of native pecans, and the origin of many of these trace back to a single cultivar. It would be interesting to evaluate a random sample of native trees to determine the ratio of dichogamy type in red stigma individuals.

To our knowledge, we report here the first linkage maps for pecan. These maps will greatly facilitate additional genetic studies in pecan. Most horticulturally important traits in pecan appear to have a complex mode of inheritance, and genetic maps will enable us to tease apart the individual loci in control of these traits and describe their effects. In the short term, molecular markers linked to useful traits will allow marker-assisted selection of these traits. Marker-assisted selection has the potential to improve the efficiency of the breeding program because it will allow inferior seedlings to be removed early in the breeding cycle, thereby eliminating years of costly field maintenance. In the long term, linkage between molecular markers and valuable genes is a necessary first step towards the positional cloning of these genes.

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