Genetic Analysis of an Interspecific Hybrid Swarm of Populus: Occurrence of Unidirectional Introgression

Paul Keim,*[,1] Ken N. Paige,*[,†,2] Thomas G. Whitham[†] and Karl G. Lark*

**Department of Biology, University of Utah, Salt Lake City, Utah 84112, and [†]Department of Biology, Northern Arizona State University, Flagstaff, Arizona 86011*

Manuscript received March 17, 1989
Accepted for publication July 10, 1989

ABSTRACT

Restriction fragment length polymorphisms were used to distinguish genotypes of two species of *Populus*, *P. fremontii* ('Fremont') and *P. angustifolia* ('narrowleaf'). Both *inter-* and *intra*specific polymorphisms were detected in these cottonwood trees. The interspecific variation was much greater than the intraspecific variation. This permitted identification of parental genotypes within individual trees of a hybrid swarm which exists in an overlap zone between the two species. Within this hybrid swarm, individual trees are either F_1 hybrids or backcrosses with a pure 'narrowleaf' parent; no progeny were found that could be attributed to crossing between F_1 hybrid trees, or to backcrossing between F_1 hybrid trees and 'Fremont'.

THE recent development of molecular genetic markers, especially restriction fragment length polymorphisms (RFLP), provide an almost unlimited number of qualitative genetic markers which are easily characterized, and are completely penetrant (BOTSTEIN *et al.* 1980; BECKMANN and SOLLER 1983). The availability of such markers should allow the genetic study of natural populations which are difficult to analyze by classical segregation studies. We have investigated whether it is possible to use RFLP markers to establish a genetic data base for studying natural populations of long-lived organisms, whose reproductive cycles require many years.

We present here the initial genetic description of natural populations of two species of cottonwood trees, *Populus fremontii* ('Fremont') and *Populus angustifolia* ('narrowleaf'). These trees have several interesting characteristics: a) Individuals can live for 100 or more years, and dendrochronological (growth ring) analysis of any individual reveals its age and the history of variation in its growth; b) in nature, the trees reproduce sexually (dioecious, male and female trees) as well as by vegetative reproduction (leading to clones of as many as 60 trees) (WHITHAM *et al.* 1984); c) trees can be hand pollinated and seedlings grown to sexual maturity within 4–5 yr. Cuttings can be propagated vegetatively in different environments, again reaching sexual maturity within 3–4 yr (T. G. WHITHAM, unpublished data); d) previous studies have established that selective interactions occur between these trees and numerous insect and vertebrate herbivores (WHITHAM *et al.* 1990).

We are studying a natural population in the Weber canyon, near Ogden, Utah (see Figure 1). Pure populations of 'Fremont' occur at the lower elevations (below 1300 meters); 'narrowleaf' is found at higher elevations (above 1470 meters). Their ranges overlap, and in this overlap zone (Figure 1, shaded area) trees are observed with intermediate morphology (WHITHAM 1989). These intermediate trees make up a substantial portion of the population within the overlap zone and it has been proposed that they form a hybrid swarm (based upon morphology and phenolic chemistry), *i.e.*, are the result of interspecific hybridization (CRAWFORD 1974; JONES and SEIGLER 1976).

In this study we have used RFLP markers to study the genetic structure of individual trees within the Weber River drainage. We wished to establish a genetic data base for ongoing studies of interactions between these trees and their herbivores and to establish the pattern of hybridization within the swarm. (Do F_1 offspring interbreed and do they backcross freely with parental trees? What is the evolutionary future of the hybrid swarm?) Our results indicate that the distribution of 'Fremont' and 'narrowleaf' alleles in the hybrid swarm is not symmetrical. The genetic structure is consistent with unilateral introgression of the 'Fremont' genome into the 'narrowleaf' population.

MATERIALS AND METHODS

Differentiation of *P. fremontii* from *P. angustifolia* trees: 'Fremont' trees characteristically have wide leaves, long

[1] Current address: Department of Biology, Northern Arizona State University, Flagstaff, Arizona 86011.

[2] Current address: Institute for Environmental Studies and Department of Ecology, Ethology and Evolution, University of Illinois, Urbana, Illinois 61801.

The publication costs of this article were partly defrayed by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

petioles and a few (10–12) large teeth on the serrated edge of the leaf. In contrast, 'narrowleaf' trees have narrow leaves with short petioles and a serrated leaf edge comprising very many, small teeth. An example of a 'Fremont' leaf is shown on the left of the array of leaves in Figure 2. A 'narrowleaf' leaf is shown on the right of the array. Between these two extremes are other leaves representing morphologies found on trees in the overlap zone (Figure 1). Parental trees were chosen on the basis of their location (outside of the overlap zone) or from within the overlap zone on the basis of their leaf and branch morphology (*e.g.*, trees F, 996, and 9–17, see below).

Isolation of DNA: For isolation of DNA, one gram of young leaves were frozen in liquid nitrogen and then ground with a mortar and pestle. The cold leaf powder was thawed in 65° extraction buffer (0.005 M 1,10-phenanthroline (Sigma Chemical Co., St. Louis), 0.05 M Tris (pH 8), 0.02 M EDTA, 0.25 M NaCl, 1.0% w/v SDS, and 1% w/v PVP-40 (Sigma)) with 20 mg/liter of proteinase K and incubated at 65° for 1 hr. This was cooled and centrifuged to remove insoluble debris. The supernatant was mixed with 0.65 volume of isopropanol to precipitate the DNA. The precipitate was collected by centrifugation, dried, and then resuspended in 3 ml of TE (10 mM Tris (pH 8), 1 mM EDTA). The DNA was further purified by cesium chloride equilibrium centrifugation (APUYA *et al.* 1988).

Preparation of probes: Recombinant DNA clones were obtained using two cloning strategies both of which have been reported in detail elsewhere. In one case we digested cottonwood DNA with *Sau*3AI to generate small fragments and then ligated the fragments into the *Bam*HI restriction site of the M13 vector MP9 [seeAPUYA *et al.* (1988) for details]. In the other, we used *Pst*I (a methylation sensitive restriction enzyme) to enrich for single copy DNA [see KEIM and SHOEMAKER (1988) for details]. The *Pst*I digested cottonwood DNA was ligated into the plasmid pBS+ (Stratagene Inc.). Both nuclear and cytoplasmic (organelle) DNA clones were represented in these libraries. In Southern hybridization, organelle DNA sequences produce a *ca.* 1000-fold stronger signal than that obtained with nuclear DNA sequences. This allows the discrimination between nuclear and cytoplasmic sequences. Only probes against nuclear sequences were used in this study.

RPLPs: The techniques for producing Southern transfers and for radioactively labeling DNA clones, have been discussed in detail elsewhere (APUYA *et al.* 1988). Briefly, we digested cottonwood DNA with restriction enzymes according to the supplier's instructions, and then separated the fragments according to size by agarose gel electrophoresis. The separated DNA fragments were transferred to nylon membranes (SOUTHERN 1975) and then hybridized with radioactive DNA from individual recombinant DNA (APUYA *et al.* 1988, MANIATIS, FRITSCH and SAMBROOK 1982) clones. The membranes were washed and autoradiographs prepared. Membranes were reused by stripping them of radioactivity using NaOH and then probing with a new DNA clone.

Linkage cluster analysis: The dendrogram in Figure 5 was generated by average linkage cluster analysis (unweighted pair groups) of the genetic distances (Mahatten) between 13 'Fremont' and 'narrowleaf' trees (SNEATH and SOKAL 1973). A computer program for this analysis (MVSP) was kindly provided by WARREN L. KOVACH (Indiana University).

Greenhouse crosses: Three foot branch cuttings, bearing floral buds, were taken from each parental plant. Branches were wrapped in moist plastic bags and stored at 5° prior to breeding (STETTLER and BAWA 1971). In the greenhouse, the branches were placed in aerated buckets of water (water was replaced approximately every 3 days to avoid contamination). Greenhouse temperatures ranged from approximately 15–25°. Male cuttings were brought into the greenhouse prior to females to collect pollen. Catkins were collected just prior to dehiscence and air-dried in small cardboard containers. Pollen was stored in glass vials at 4° until used. Females were brought into the greenhouse following pollen collection. Following floral bud break female plants were temporarily isolated and pollen was dusted evenly over each receptive catkin. Most catkins were pollinated at least twice on consecutive days, but those with asynchronous flower development were pollinated over a several day period. Mature seeds were obtained 30–40 days following pollination. Mature catkins were collected upon dehiscence, air dried and the seeds were collected. Seeds were stored in plastic bags at room temperature until germination. For germination, seeds were placed on moist filter paper in sterile Petri plates and germinated at room temperature. Within 48 hr after germination seedlings were transferred to sterile soil and later transplanted to one gallon pots.

RESULTS

Comparisons of tree morphologies in the Weber River drainage system indicate the presence of an apparent hybrid zone (Figure 1, shaded region). Trees with intermediate phenotype are characterized by intermediate leaf morphologies (Figure 2). 'Fremont' cottonwoods are characterized by broad leaf blades and long petioles, while 'narrowleaf' cottonwoods are characterized by narrow leaf blades and short petioles. Apparent hybrids are intermediate in these traits. The genetic compositions of individual trees in all three zones were analyzed using restriction fragment length polymorphisms as markers to confirm the apparent genotype of individual plants.

Probes: RFLPs between the 'Fremont' and 'narrowleaf' species were detected by using cloned DNA inserts as probes to screen DNA from each species. Three to five restriction enzymes were used to digest the genomic DNA. Polymorphisms were identified by hybridizing these probes with Southern transfers of restriction fragments of genomic DNA separated according to size by electrophoresis. Figure 3 presents typical examples of fragments identified by *Pst*I and *Sau*3AI probes. As had been found previously with soybean (Keim *et al.* 1989), probes prepared from *Sau*3AI digests hybridized with, and revealed, many more DNA fragments than did those from *Pst*I digests. As a result, the patterns of fragments from the *Sau*3AI digests were often very complex. In Figure 3, A, B and D, we show examples of polymorphisms identified with *Sau*3AI. They vary from very large numbers of fragments to much simpler patterns. *Pst*I probes yielded patterns which usually were simple (*e.g.*, Figure 3C). In other plants (YOUNG, MILLER and TANKSLEY 1987, BURR *et al.* 1988) this simplicity has been attributed to the tendency of single copy sequences to be under methylated and therefore se-

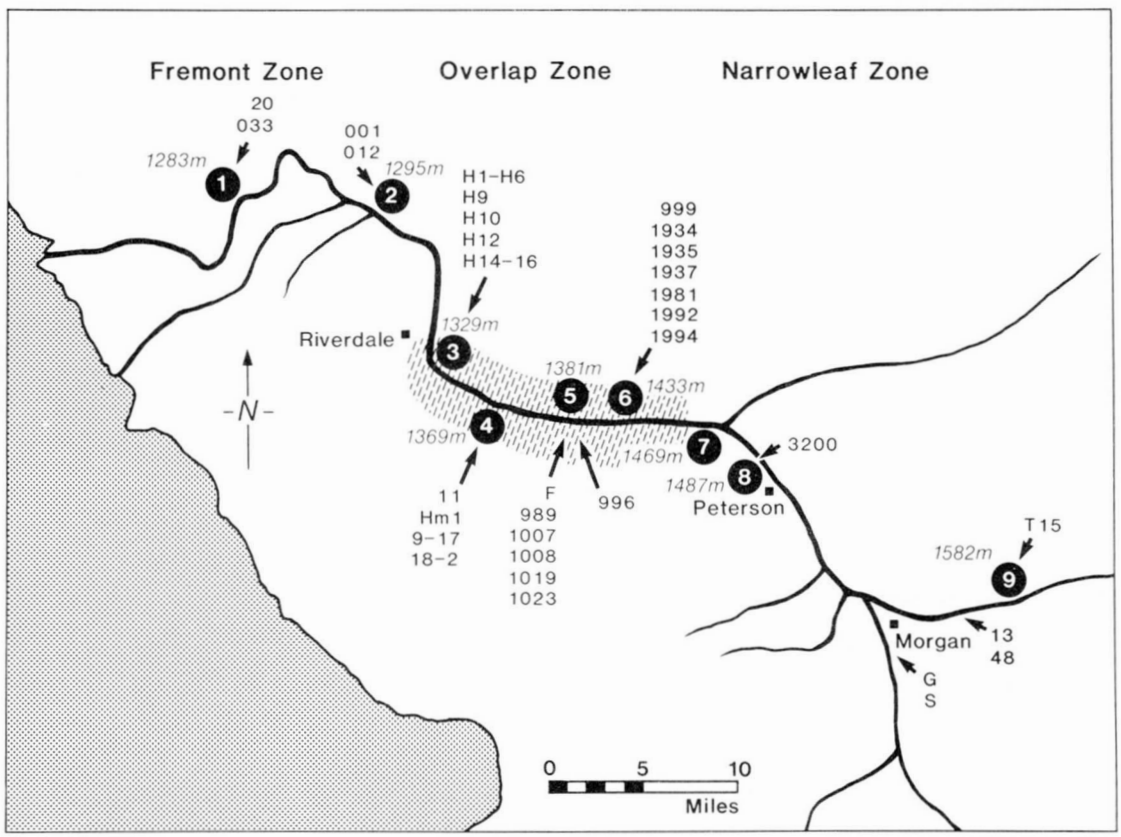

FIGURE 1.—The geographical distribution of cottonwood trees within the study area along the Weber River Drainage in Utah. Sites of trees are indicated by arrows. Altitudes (in meters) are shown at intervals. The overlap zone is indicated by the shaded area between Riverdale and Peterson and includes sites 3 through 7. (One mile = 1.6 km.)

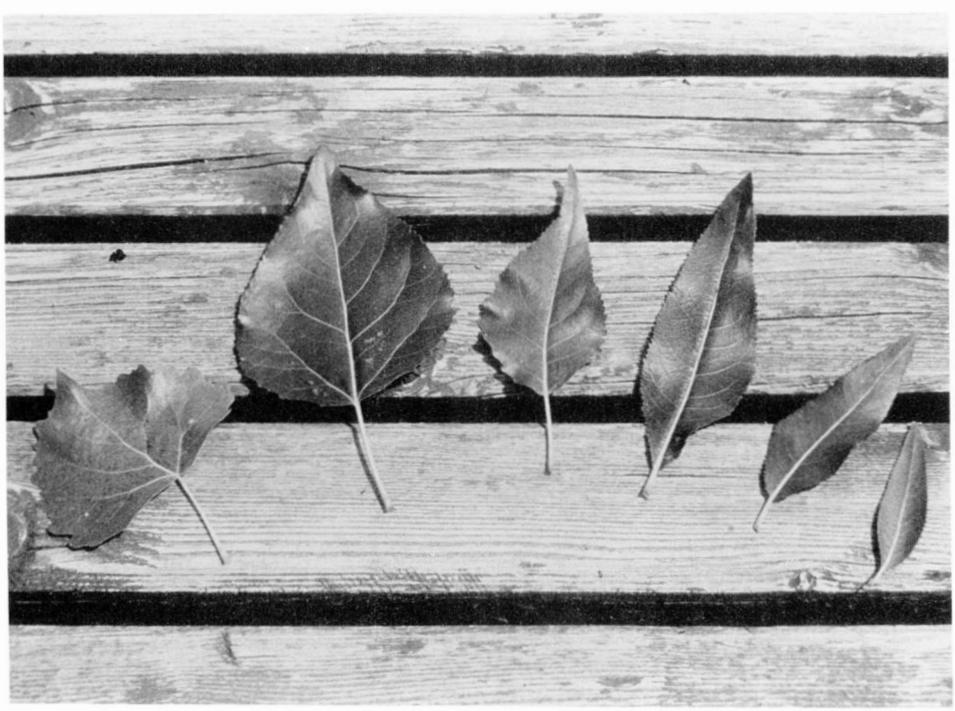

FIGURE 2.—Characteristic leaf morphology of cottonwoods found in the overlap zone, ranging from 'narrowleaf' on the right, to 'Fremont' on the left.

lected by *Pst*I, an enzyme which is inhibited by methylation of DNA (NELSON and MCCELLAND 1987). Single copy sequences appear to be hypomethylated in Populus as well.

Tree 989 is an F_1 hybrid tree. This tree (with intermediate morphology) had previously been identified by T. WHITHAM as possibly having a hybrid genotype (on the basis of branch and leaf structure). DNA from tree 989 was therefore included in the screening process. Tree 989 did, indeed, prove to be

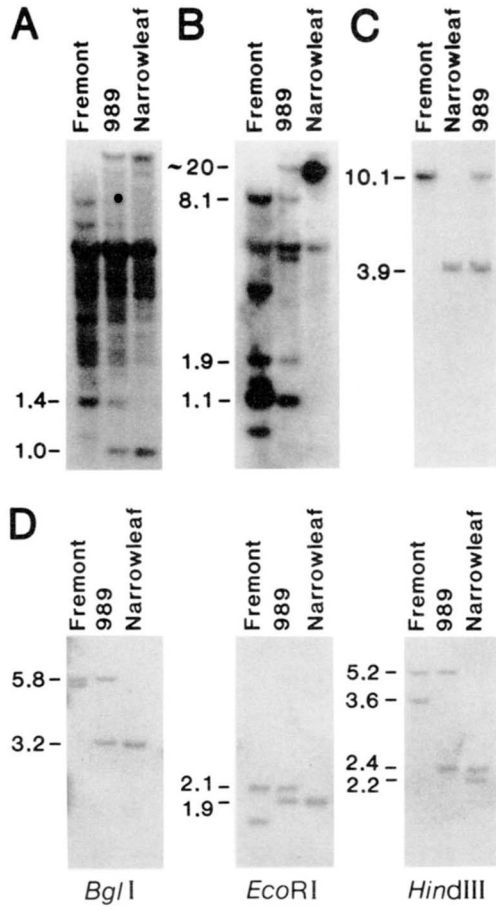

FIGURE 3.—Examples of genomic DNA fragments hybridized with *Sau*3A (A, B and D) or *Pst*I (C) radioactive DNA probes. Probes CW 28 (A) and MR 43 (B) were hybridized with fragments of DNA prepared by digesting genomic DNA with *Bgl*II (A) or *Eco*RI, respectively. The *Pst*I probe 35 was hybridized with fragments of DNA prepared by digesting genomic DNA with *Hin*dIII (C). The enzymes *Bgl*II, *Eco*RI and *Hin*dIII were used to digest genomic DNA to produce the fragments in (D) for hybridization with N-17. 'Fremont' DNA was from tree F; 'narrowleaf' DNA was from tree S.

an F_1 hybrid, containing restriction fragments characteristic of both parent species for every marker examined (*i.e.*, every marker appeared to be heterozygous for fragments from the two species).

DNA rearrangements may account for a large percentage of the polymorphisms observed in cottonwood. A single rearrangement would alter the pattern of digestion by several restriction enzymes, whereas a point mutation would not. More than 50% of the probes detected polymorphisms with more than one restriction enzyme. Probe N-17 (Figure 3D) illustrates this type of variation using DNA digested by *Hin*dIII *Eco*RI and *Bgl*II.

Another characteristic of many of these probes is that fragments present in one or the other of the parental trees is often not present in the hybrid tree, 989. This would occur if individuals within the parental populations were themselves polymorphic and parental trees used in screening probes for polymorphisms differed from the trees which served as parents for tree 989. Thus, in the *Hin*dIII digest of N-17 (Figure 3D) there are two fragments in the 'narrowleaf' DNA (2.2 and 2.4 kb) not present in the 'Fremont' DNA. Conversely, two fragments are present in the 'Fremont' DNA (3.6 and 5.2 kb) which are absent in the 'narrowleaf.' Tree 989 has two fragments, one from each parent (2.2 and 3.6 kb), but also lacks two fragments, one each found in the parental trees. This situation (which occurs frequently) would result if the F_1 hybrid '989' was derived from different individual parents than the trees 'S' and 'F' (see legend to Figure 3) used in screening the probe (which is highly likely since these trees are far removed from one another).

Parental trees: Variation among parental trees was investigated to determine genetic relatedness of individual trees, both within a species and between species. Individual trees representing the two parental populations were chosen by virtue of their location within one of the pure zones or on the basis of their leaf and branch morphology. Examples of how these data were generated are presented in Figure 4, where DNA restriction fragments from different parental trees were hybridized with the *Sau*3AI probes, CW76–10, CW-16 and CW-23. Some fragments are present in all trees of both parents (*e.g.*, with the CW 76–10 probe, the 9.3, 2.8, and 0.72 kb fragments). Others appear to characterize one or the other parental population (*e.g.*, the 1.2 and 3.9 kb fragments characterize *P. fremontii;* the 4.1- and 7.1-kb fragments characterize *P. angustifolia*). Yet other fragments are found in only some individuals (*e.g.*, the 5.7 kb in the *P. fremontii* population, the 4.9 kb in the *P. angustifolia*).

Cluster analysis of 50 variant fragments (10 different probes) allowed us to clearly separate 'narrowleaf' and 'Fremont' cottonwood species (Figure 5). A dissimilarity matrix was calculated using Mahatten's distance (equivalent to the number of variant fragments between two individuals (SNEATH and SOKAL 1973)). Cluster analysis of the data is represented in the dendrogram in Figure 5. Although variation within each species occurred, the much larger differences between the two parental populations made an analysis of the hybrid swarm possible.

The hybrid swarm: We have analyzed the genotypes of individual trees within the hybrid swarm to determine the pattern of gene flow between the two parental species. To do this, individual trees in the overlap zone were selected and surveyed with probes and enzyme combinations known to reveal interspecific polymorphisms. A discussion of the data in Figure 6 will clarify how such data were analyzed.

*Pst*I probes gave expected simple patterns revealing parental or heterozygote genotypes (Figure 6, A and B), as well as new fragments. For example, trees 1934

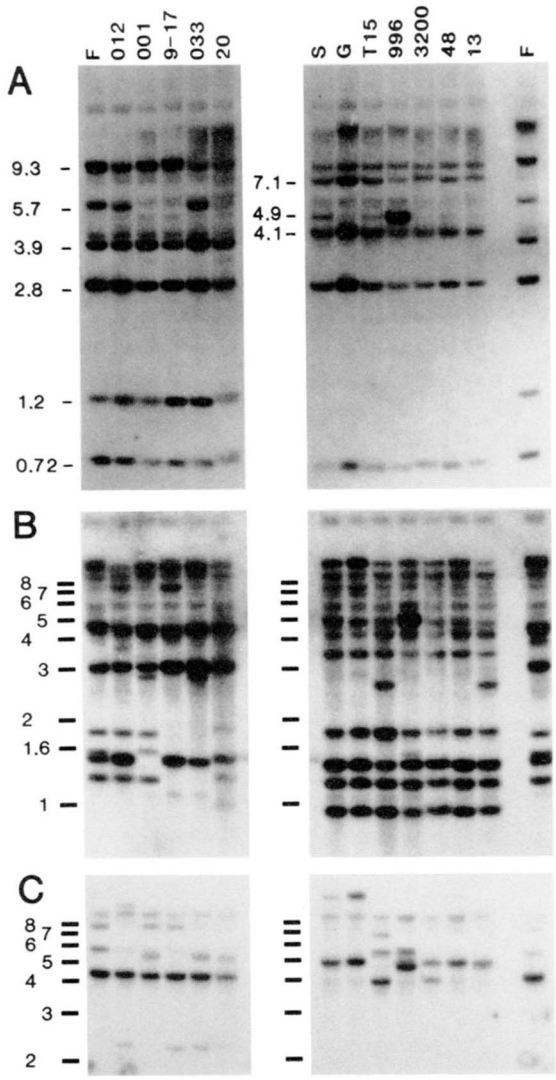

FIGURE 4.—Examples of fragment variation between parental 'Fremont' or 'narrowleaf' trees. DNA was isolated from the 'Fremont' trees F, 012, 001, 9–17, 033 and 20; or from the 'narrowleaf' trees S, G, T15, 996, 3200, 48 and 13. After digestion with *Hin*dIII the fragments were separated and hybridized with the radioactive *Sau*3AI probes CW 76–10 (A), CW 16 (B) or CW 23 (C).

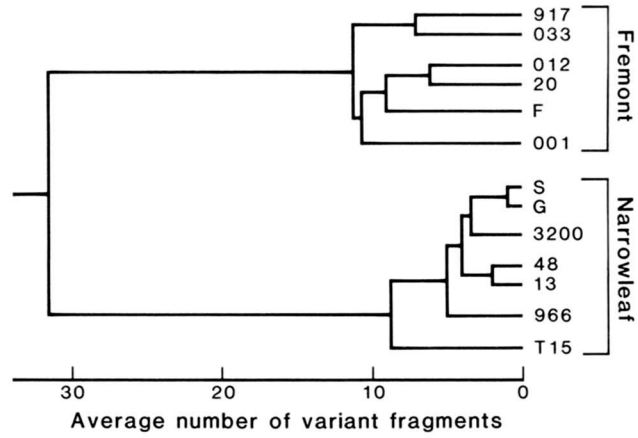

FIGURE 5.—Relatedness of trees in parental 'Fremont' and 'narrowleaf' populations. The ordinate presents the average number of fragments by which trees or clusters of trees differ. Thus, the 'Fremont' population differs from the 'narrowleaf' population by an average value of 31.6 fragments; within the 'Fremont' population, trees 20 and 012 differ by 6 fragments from each other and these two differ from F by an average of 9 fragments. Note that the spread of values in the 'Fremont' population is greater than in the 'narrowleaf' population.

and 1937 in Figure 6, A and B, differ from the other trees, suggesting that at least one parent belonged to a different intraspecific polymorphic group. The *Sau*3AI probes reveal more complex patterns of fragments (Figure 6C), but consistent interspecific polymorphisms can be identified by hybridization of radioactivity to particular fragments—*i.e.*, the 1.2-kb 'Fremont' fragment and the 4.1-kb 'narrowleaf' fragment (see also Figure 4). Again, new fragments (*e.g.*, the 4.9- and 3.3-kb fragments found in trees H1 and H3 and in H12, 18–2 and 18–17, respectively) suggest intraspecific polymorphisms within the 'narrowleaf' parents. The 4.9-kb fragments in trees H1 and H3 were found in one of the *P. angustifolia* parental trees (Figure 4 trees S and 996), but the 3.3-kb fragment has not yet been found in any of the parental trees surveyed in Figure 4. Eventually it should be possible to use intraspecific polymorphisms to determine the degree of relatedness between individual trees in the parental populations, as well as in the hybrid swarm.

Based on the *interspecific* polymorphisms we are able to discriminate between loci with parental or hybrid genotypes. 'Narrowleaf' genotypes were assigned to trees 999, 1019 and 1023 in Figure 6a, to trees 999, 1019, 1023 and 1992 in Figure 6B and to trees H12, H10, H9, H3, 18–2 and 18–17 in Figure 6C. Interspecific heterozygote genotypes are clearly evident in trees 1981, 1994 and 1997 in Figure 6A, in trees 1934, 1937, 1981, 1994 and 1997 in Figure 6B, and in trees H1, H2 and H6 in Figure 6C. Because of possible intraspecific polymorphisms, trees 1934 and 1937 in Figure 6A could be either heterozygous or homozygous 'Fremont'; tree 1992 could be either heterozygous or homozygous 'narrowleaf.' Only further examination of intraspecific parental polymorphisms will clarify these genotypes.

Within the hybrid swarm, confirmed genotypes of individual trees were either hybrid or backcrosses to *P. angustifolia*. Surprisingly, no examples of progeny from crosses between different hybrid trees or between hybrids and *P. fremontii* were found. Table 1 summarizes the genotppes of different trees which have been analyzed at this stage of our study. These trees are located on the map in Figure 1. The trees in Table 1 are listed in the order in which they occur geographically (progressing upstream or west to east from the pure 'Fremont' to the pure 'narrowleaf' populations). Only loci with unambiguous interspecific polymorphisms were included in each tree's genotype. Indeterminate genotypes such as those of trees 1934, 1937 or 1992 in Figure 6A were not included

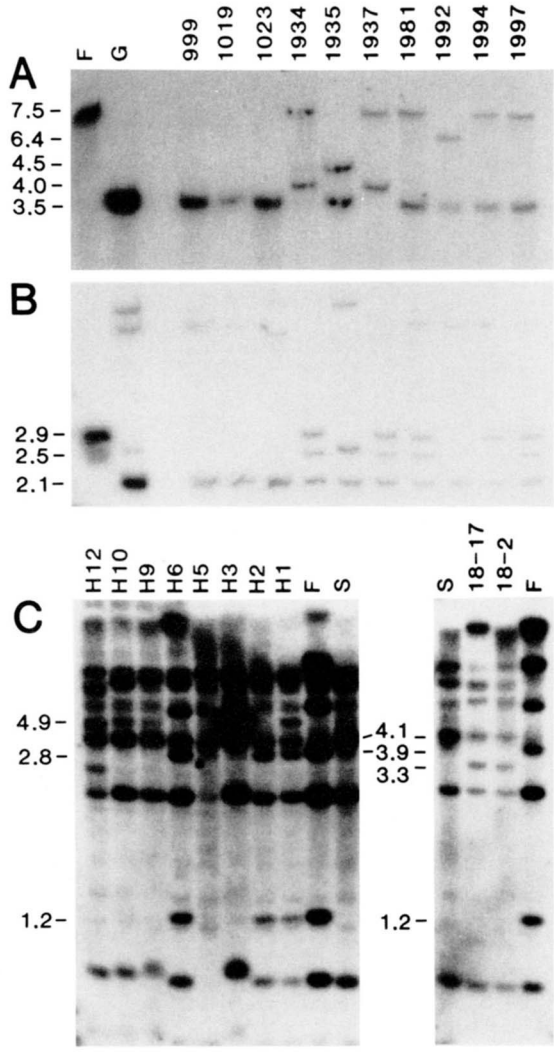

FIGURE 6.—Examples of fragments found in trees from the "hybrid swarm" in the overlap zone in Figure 1. (A) and (B): *Hin*dIII DNA fragments from trees hybridizing with the radioactive *Pst*I probes 35 (A) and 48 (C) *Hin*dIII DNA fragments from trees hybridizing with the radioactive *Sau*3AI probe CW 76–10.

in this summary. Inspection of the data in this table shows that interspecific genotypes are consistent with trees being either F_1 hybrid or backcrosses to *P. angustifolia*. In fact, no backcrosses to *P. fremontii* were found even in areas bordering the pure 'Fremont' population (*e.g.*, site 3, Figure 1), although many parental 'Fremont' genotypes were found in this border region (such as H14, H15 and H16). In an equilibrium population, the hybrid zone allele frequencies would predict 18 homozygous 'Fremont' loci (Table 2). Data summarized in Table 1 were used to calculate allele frequencies for Hardy-Weinburg prediction of homozygous or heterozygous classes of loci. The predicted classes were compared with the observed, using a chi-square test. The lack of homozygous 'Fremont' loci was highly significant.

The absence of backcrosses to the *P. fremontii* parent, or of homozygous 'Fremont' loci in genetic backgrounds with 'narrowleaf' alleles (see Table 1), led us to question whether such crosses were infertile or whether, if fertile, they were inviable. Some hybrid trees appear sterile (data not presented). However, hybridization and backcrossing must be occurring to achieve the genotypic patterns we have observed and the morphology types common in the hybrid zone. In preliminary experiments, we attempted to carry out crosses in the greenhouse (see MATERIALS AND METHODS). A high yield of seed was obtained from backcrosses between trees 1007 or 1008 ('narrowleaf' backcrosses) and a 'narrowleaf' parent. A much lower yield of seed was obtained when these two trees were crossed with a 'Fremont' parent. Seeds from these two crosses germinated with about equal success ('Fremont': 8/10 (80%); 'narrowleaf': 55/65 (85%)), but most seedlings did not survive beyond the first few days. However, in backcrosses to each parent, three plants of each cross survived (Figure 7). While backcrosses with 'narrowleaf' survived, backcrosses with 'Fremont' ultimately died. Those from the backcross to 'narrowleaf' gave rise to typical juvenile plants

TABLE 1

Genotypes of individual cottonwood trees in the Weber River drainage study area (see Figure 1)

Tree	Genotype (*F:H:N*)	Tree	Genotype (*F:H:N*)
20	16:00:00	F	37:00:00
033	16:00:00	989	00:26:00
001	16:00:00	1007	00:04:11
012	16:00:00	1008	00:02:13
H1	00:09:02	1019	00:01:10
H2	00:10:03	1023	00:01:08
H3	00:04:08	996	00:00:19
H4	00:01:01	999	00:02:09
H5	00:03:03	1981	00:13:02
H6	00:08:04	1934	00:03:01
H9	00:02:00	1937	00:03:00
H10	00:04:07	1935	00:05:00
H12	00:05:04	1992	00:03:01
H14	05:00:00	1994	00:05:00
H15	05:00:00	3200	00:00:16
H16	05:00:00	G	00:00:23
11	00:07:19	S	00:00:29
HM1	00:17:00	13	00:00:16
9-17	18:00:00	48	00:00:16
18-2	00:07:13	T-15	00:00:16

The DNA from each tree was characterized for RFLP markers ('Fremont' or 'narrowleaf') by hybridizing fragments with different radioactive probes. The trees are listed in the columns on the left, in order of their location (from west to east) on the map in Figure 1. The genotype of each tree is presented as the number of markers examined and classified as 'Fremont' only (F); Hybrid or Heterozygotic (H); or 'narrowleaf' (N). Among a total of 24 trees which had both 'Fremont' and 'narrowleaf' alleles, a total of 264 loci were examined (an average of 11 markers/individual tree). In these 24 trees, only homozygous 'narrowleaf' loci (N) or heterozygous loci (H) were found. No instance of a homozygous 'Fremont' locus (F) was observed. The high number of markers tested in trees 11, 18-2, F, 989, S and G is due to their frequent inclusion in Southern transfers when screening for polymorphisms.

TABLE 2

Observed and predicted classes of loci in the hybrid swarm

Classes of Loci	Observed	Predicted[a]
Homozygous 'Fremont'	0	18
Heterozygous	141	106
Homozygous 'narrowleaf'	123	140

[a] The loci classes were predicted by assuming a population at genetic equilibrium. Then, using the allele frequencies observed in hybrid trees and the Hardy-Weinburg equation, classes were calculated. The chi-square value equaled 31.7 which is highly significant (df = 2, $P \ll 0.001$).

(Figure 7, A and C) and now, after more than a year, continue to grow as healthy individuals. The three progeny of the 'Fremont' backcross (Figure 7B) quickly produced woody stems (Figure 7D) and leaves accumulated in a rosette at the stem apex (Figure 7E). The young plants from the 'Fremont' cross died when they were 3 months old. Material from the few leaves in the crown rosette was insufficient to allow us to prepare nuclear DNA for analysis. Further experiments of this type must be carried out. However, the results demonstrate that while the seed produced from backcrosses to *P. fremontii* can be viable, the progeny, at least in this cross, later developed abnormalities and died.

DISCUSSION

RFLPs can be used to determine genetic relatedness: Random cottonwood DNA fragments, cloned into plasmid (pBS) or phage (M13) vectors, identify specific DNA sequences within the cottonwood genome. The position of these sequences relative to internal or neighboring restriction sites define the length of restriction fragments containing the identifying sequences. For most markers, these lengths differ between the 'Fremont' and 'narrowleaf' species (Figure 3) constituting RFLP markers which allow us to distinguish the two species. We have used these to estimate the genetic contribution of 'Fremont' and 'narrowleaf' parents to genomes of trees in the hybrid swarm (Figure 6 and Table 1) which exists in the overlap zone (Figure 1) between the two parental populations.

We have also surveyed the parental populations for the existence of polymorphic variation within 'Fremont' or 'narrowleaf' populations. We have found variation within each of these parental populations (Figure 4 and Figure 5). Although this variation is less than that between species, it is significant. This is to be expected considering that in a dioecious genus, such as Populus, outcrossing between individuals will maintain a high level of heterozygosity. This, in turn, will allow deleterious recessive mutations (produced for example by DNA rearrangements) to be maintained in the population. If this is occurring to a large extent, we may expect that such populations carry a large genetic load. This may, in part, be offset by the longevity of individual trees which over the years have repeated opportunities to outcross and reproduce successfully.

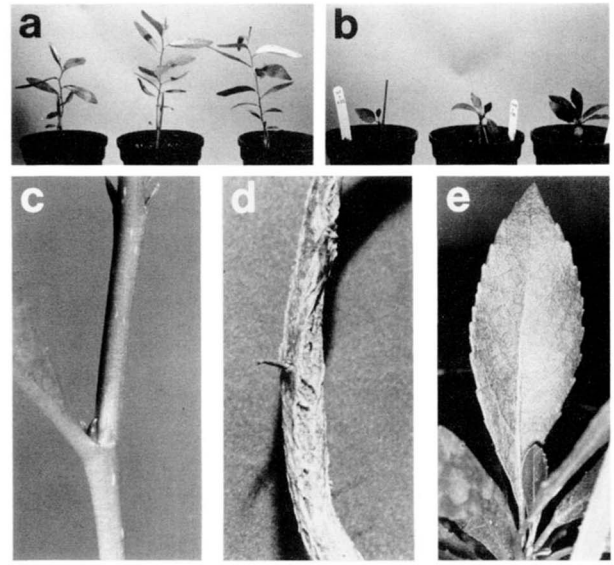

FIGURE 7.—Surviving progeny from the germination of seeds produced by backcrossing trees 1007 or 1008 with (a) 'narrowleaf' or (b) 'Fremont' parental trees. (c) Stem of a plant from (a) above; (d) stem of a plant from (b) above; (e) leaf rosette of a plant from (b) above.

Directional gene flow: In the overlap zone, two mixed genotypes were identified (Table 1): those that appeared to be F_1 hybrids or very close to F_1 hybrids (*e.g.*, 989, HM-1, H1, H2 and 1981) and trees that appeared to be the result of backcrosses between F_1 hybrids and 'narrowleaf' parents (*e.g.*, 18–2, 1007, 1008, 11, H3, H5, H6, H10, H12, 999). No trees were found that could be classified as either backcrosses to 'Fremont' or as progeny of further crossing between hybrids or between hybrids and trees which were themselves the result of backcrossing to 'narrowleaf.' In all, 264 markers were examined in a total of 24 trees with mixed genotypes (an average of *ca.* 11 markers per tree, Table 1). No marker occurred as a homozygous 'Fremont' genotype if a 'narrowleaf' allele was found in the genotype of that tree. That is, *FF* markers never occurred in the same tree together with the genotypes *NF* or *NN*. In this analysis, our estimate of each species' contribution to the genome of an individual is based on our knowledge of parental polymorphisms. In future studies, as our data base is increased, this estimate may be revised as new intraspecific polymorphisms are discovered. However, our main conclusion—that gene flow between the parental populations is assymetric—is based on fragment differences already observed between the parental species. Due to the relatively small number of trees analyzed we cannot rule out the possibility of low

frequency gene flow into the 'Fremont' population. However, when trees were selected from a region of the overlap zone immediately adjacent to the 'Fremont' population (H trees, Figure 1) none contained FF loci.

The simplest explanation for directional gene flow is that crosses between two hybrids, or between 'Fremont' trees and hybrids, are not productive due to genetic incompatibility. The experiment discussed in connection with the plants in Figure 6 supports this idea and suggests that progeny from such crosses are either aborted or yield seed which do not develop normally. An alternate hypothesis is that flowering phenology accounts for the observed distribution of phenotypes. Observation shows a significant overlap in phenologies, inconsistent with this alternative explanation (T. G. WHITHAM, unpublished data).

At least three possible mechanisms could be responsible for the proposed genetic incompatibility. First, this could be due to interactions with cytoplasmically inherited cell functions, but data to be published (K. N. PAIGE, T. G. WHITHAM and K. G. LARK) indicate that this is *not* the case. Second, expression of 'Fremont' information may be suppressed in a background containing 'narrowleaf' alleles. Again, data to be published on insect resistance in hybrid and backcross trees (K. N. PAIGE, T. G. WHITHAM, P. KEIM and K. G. LARK) suggest that this also is not the case. Our results from hand pollination are consistent with a third hypothesis: that genetic barriers to reproduction exist which interfere with plant development (seed production, seed germination or seedling growth). These incompatibilities probably arise when 'narrowleaf' alleles are isolated in a predominantly 'Fremont' genetic background. This would result in the introgression of 'Fremont' genetic material into the 'narrowleaf' population. These new alleles, or allele combinations, will expand the gene pool of the 'narrowleaf' population constituting a selective advantage for this population. The 'Fremont' population will not benefit in a similar manner.

Evolutionary implications: Three evolutionary consequences are implied by the patterns of gene flow described above. First, directional gene flow and introgression of genes from 'Fremont' into 'narrowleaf' cottonwood allows for new 'narrowleaf' genotypes, but not new 'Fremont' genotypes. Because 'narrowleaf' cottonwood is found at higher elevations while 'Fremont' cottonwood occupies lower elevations, the acquisition of genes by directional introgression may allow new 'narrowleaf' genotypes to become better adapted to lower elevations. Thus the hybrid swarm may be the vanguard of advancing 'narrowleaf' genotypes that may compete more effectively with 'Fremont' cottonwood on its home ground.

Second, with directional gene flow and restricted hybridization patterns (*i.e.*, hybrids only backcross with pure 'narrowleaf'), the maintenance of the hybrid zone is more tenuous than in other, more freely, hybridizing species. The complete absence of hybrid × hybrid or hybrid × 'Fremont' crosses means that this hybrid swarm is not self-perpetuating. Presumably, if the 'narrowleaf' population were eliminated, the hybrid zone itself would eventually go extinct unless it was continually rescued by 'narrowleaf' matings. (This could be a transient phase in the formation of the type of "evolutionary relict" described by WIENS *et al.* (1989) in their discussion of developmental failure in the paleoendemic shrub *Dedeckera eurekensis*.) However, the demise of the hybrid swarm is buffered by the propensity of 'narrowleaf' and hybrid cottonwoods to clonally propagate by root sprouts in which over 60 ramets may make up a clone. Thus, even though individual ramets or trunks may live only 100 yr, the clone itself may be thousands of years old (see estimates from KEMPERMAN and BARNES 1976 for *Populus tremuloides*). Such longevity should have a great stabilizing influence on the persistence of hybrids, particularly if the selection coefficients are similar for both the hybrids and their parentals.

Third, because hybrid swarms represent regions of unparalleled genetic variation and unique gene combinations, selection should be intense and evolution may be rapid (FISHER 1930). Although natural plant hybrid zones have been largely ignored by ecologists and evolutionary biologists due to their complexity (HEWITT 1988), one might argue that these zones represent the "cutting edge" of selection and should receive much more attention. While some hybrids may be superior to either parent and could speciate through polyploidy (LEWIS 1980; GRANT 1981), other hybrids may be so inferior as to act as "sinks" or reservoirs for pests and pathogens. For example, PAIGE *et al.* (1989) and WHITHAM (1989) showed that inferior hybrids which accounted for less than 3% of the host population supported 85 to 100% of the pest population. The ecological and evolutionary implications of such asymmetrical pest distributions are likely to be important.

At present it is very dilficult to determine how the potential advantages of hybridization might relate to the known and well recognized disadvantages of hybridization (*e.g.*, barriers to gene flow, SAGE *et al.* 1986). Future studies should address such potential advantages, try to determine if there is an optimal hybridization level (*i.e.*, is hybridization an evolved trait?) and examine whether or not hybrids are better adapted to their local environment than either parent.

This research was supported by a grant from the National Science Foundation (BSR 604983) to T.G.W., and by a grant from the National Institute of General Medical Sciences to K.G.L. (GM

42337) and National Institutes of Health (NIH) Biomedical Research Support Grants to K.N.P. (SO7RR07092 and NIH RR7030). We wish to thank MARITZA ARCE-LARETTA, EDWARD MEENEN and DAVID MOONEY for technical assistance.

LITERATURE CITED

APUYA, N., B. FRAZIER, P. KEIM, E. J. ROTH and K. G. LARK, 1988 Restriction length polymorphisms as genetic markers in soybean *Glycine max* (L.) Merr. Theor. Appl. Genet. **75:** 889–901.

BECKMANN, J. S., and M. SOLLER, 1983 Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theor. App. Genet. **67:** 35–43.

BOTSTEIN, D., R. L. WHITE, M. SKOLNICK and R. W. DAVIS, 1980 Construction of a genetic map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. **32:** 314–331.

BURR, B., F. A. BURR, K. H. THOMPSON, M. C. ALBERTSON and C. S. STUBER, 1988 Gene mapping with recombinant inbreds in maize. Genetics **118:** 519–526.

CRAWFORD, D. J., 1974 A morphological and chemical study of *Populus acuminata* Rydberg. Brittonia **26:** 79–89.

FISHER, R. A., 1930 *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

GRANT, V., 1981 *Plant Speciation*, Ed. 2. Columbia University Press, New York.

HEWITT, G. M., 1988 Hybrid zones—natural laboratories for evolutionary studies. Trends Ecol. Evol. **2:** 201–206.

JONES, A. G., and D. S. SEIGLER, 1976 Flavonoid data and populational observations in support of the hybrid status for *Populus acuminata.* Biochem. Syst. Ecol. **2:** 201–206.

KEIM, P., and R. C. SHOEMAKER, 1988 Construction of a random recombinant DNA library that is primarily single copy sequence. Soybean Genet. Newslet. **15:** 147–148.

KEIM, P., B. W. DIERS, R. G. PALMER, R. C. SHOEMAKER, T. MACALMA and K. G. LARK, 1989 Mapping the soybean genome with RFLP markers. Proceedings of the World Soybean Conference (in press).

KEMPERMAN, J. A., and B. V. BARNES, 1976 Clone size in American aspens. Can. J. Bot. **54:** 2603–2607.

LEWIS, W. H., 1980 *Polyploidy.* Plenum Press, New York.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

NELSON, M., and MCCLELLAND, 1987 The effect of site specific methylation on restriction-modification enzymes. Nucleic Acid Res. **15** (suppl): r219.

PAIGE, K. N., P. KEIM, T. G. WHITHAM and K. G. LARK, 1989 The use of restriction fragment length polymorphisms to study the ecology and evolutionary biology of aphid-plant interactions, in *Mechanisms of Aphid-Plant Genotype Interactions*, edited by R. D. EIKENBARY and R. K. CAMPBELL (in press).

SAGE, R. D., D. HEYNEMAN, K. LIMM and A. C. WILSON, 1986 Wormy mice in a hybrid zone. Nature **325:** 60–63.

STETTLER, R. F., and K. S. BAWA, 1971 Experimental induction of haploid parthenogenesis in black cottonwood. Silvae Genet. **20:** 15–25.

SNEATH, P. H. A., and R. R. SOKAL, 1973 *Numerical Taxonomy: The Principles and Practice of Numerical Classification.* W. H. Freeman, San Francisco.

SOUTHERN, E., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

WHITHAM, T. G., 1989 Plant hybrid zones as "sinks" for herbivores. Science (in press).

WHITHAM, T. G., A. G. WILLIAMS and A. M. ROBINSON, 1984 The variation principle: individual plants as temporal and spatial mosaics of resistance to rapidly evolving pests, pp. 15–51 in *A New Ecology: Novel Approaches to Interactive Systems*, edited by P. W. PRICE, C. N. SLOBODCHIKOFF and W. S. GAUD. John Wiley & Sons, New York.

WHITHAM, T. G., J. MASCHINSKI, K. C. LARSON and K. N. PAIGE, 1990 Plant responses to herbivory: the continuum from negative to positive and underlying physiological mechanisms, pp. 00–00 in *Herbivory: Tropical and Temperate Perspectives*, edited by P. W. PRICE, T. W. LEWINSOHN, W. W. BENSON and G. W. FERNANDEZ. John Wiley & Sons, New York.

WIENS, D., D. L. NICKRENT, C. I. DAVERN, C. L. CALVIN and N. J. VIVRETTE, 1989 Developmental failure and loss of reproductive capacity in the rare paleoendemic shrub *Dedeckera eurekensis.* Nature **338:** 65–67.

YOUNG, N.D., J. C. MILLER and S. D. TANKSLEY, 1987 Rapid chromosomal assignment of multiple genomic clones in tomato using primary trisomics. Nucleic Acids Res. **15:** 9339–9348.

Communicating editor: S. D. TANKSLEY