A Single Gene Cluster Controls Incompatibility and Partial Resistance to Various *Melampsora larici-populina* Races in Hybrid Poplars

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ABSTRACT

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Complete cosegregation for race-specific incompatibility with three *Melampsora larici-populina* rust races was observed in five F_1 hybrid progenies of *Populus*, with different patterns among the various progenies. A single gene cluster could explain these segregations: one locus with multiple alleles or two tightly linked loci controlling complete resistance to E1 and E3, and two tightly linked loci for E2. The random amplified polymorphic DNA marker OPM03/04_480 was linked to that cluster in all families (<1 cM). This marker accounted for more than 70% of the genetic variation for field resistance in each family (heritability $\approx$

0.40). The same marker accounted for up to 64% of the clonal variation for growth in the nursery under natural inoculum pressure; the weak tolerance to rust of F_1 interspecific hybrids was attributed to a genetic background effect. Partial resistance was split into epidemiological components (heritability ranged from 0.35 to 0.87). Genotypic correlations among resistance traits for the different races were high (0.73 to 0.90). However, correlations among different resistance components for a single race were not all significant. A major quantitative trait locus for all components of partial resistance to E2 was associated to the cluster controlling incompatibility to E1 and E3 and marked by OPM03/04_480 (R^2 from 48 to 68%).

Additional keywords: leaf-disk bioassay, quantitative resistance.

Poplar wood, in Europe, is traditionally produced through monoclonal plantations of exotic interspecific hybrids. Combining complementary traits of *Populus* species, poplar breeders have initiated a domestication program. Modern clones have been selected for productivity, stability across various environmental conditions, disease resistance, and high-quality timber wood (1). Concerning disease resistance, some pathogens have been efficiently managed by the selection of resistant cultivars (e.g., bacterial canker due to *Xanthomonas populi* Ridé & Ridé). However, in other cases, the situation has become more problematic: new *Melampsora larici-populina* Kleb. races are regularly emerging that have overcome the resistance of recently selected clones (26,28,31). Thus, there is no longer a cultivar showing complete resistance to *Melampsora* leaf rusts in the European catalog (27). We can assume that poplar breeders have never had a deliberate strategy of selection for race-specific resistance, since their objective was rather durable resistance; poplar clones remained resistant to natural inoculum in the field during selection and testing (over 10 or 15 years), but resistance was overcome years later by new races. The question is thus: how could long-term selection in the field lead to race-specific and nondurable resistance? Now *M. larici-populina* may represent a major risk for poplar stands in Europe. *M. larici-populina* was also recently introduced in Northern America (20), but only the race E1 (25).

An integrated study of poplar-rust interaction has been developed by the French National Institute for Agronomic Research (INRA) that involves the three main poplar species used in the breeding programs in Europe: *Populus deltoides* Bartr., *P. tricho-*

carpa Torr. & Gray, and *P. nigra* L. With *M. larici-populina*, two kinds of resistance can be distinguished: (i) some poplar genotypes show complete resistance to some races (this is called qualitative resistance, and it is how rust races are defined), and (ii) there is also genetic variation for the level of susceptibility in the compatible cases (quantitative or partial resistance). This quantitative variation can be observed either in the field under natural inoculum pressure or in controlled conditions. In the pure species *P. deltoides*, both qualitative and quantitative resistance are simultaneously operating (21,22). However, only quantitative variation was found in *P. trichocarpa* and *P. nigra*, and no complete race-specific resistance has yet been observed in these species (13, 14). Interspecific F_1 progenies between *P. deltoides* and *P. trichocarpa* generally segregate for race-specific resistance, and they display a lower level of quantitative resistance in the field than the related pure species (13). In a 2×2 factorial mating design, complete resistance to *M. larici-populina* race E1 inherited from the *P. deltoides* parent as a single dominant gene and a random amplified polymorphic DNA (RAPD) marker tightly linked to that locus was identified using a bulked segregant analysis (33). The segregation for complete resistance was also interpreted as monogenic in a *P. deltoides* $\times$ *P. nigra* progeny, and three markers could be identified using amplified fragment length polymorphism (3).

Poplar populations, as monoclonal stands, interact with complex rust populations or mixtures of undetermined races. Thus, breakdown of resistance into epidemiological components includes the compatibility pattern towards different races and quantitative traits for partial resistance (latent period, infection, and sporulation). The level of tolerance is the response in terms of growth to the level of infection. According to this model, breeding for durable resistance should not rely on a single selection criterion, because (i) complete resistance alone is race-specific and it masks the variation for other components of partial resistance, making it

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impossible to predict the behavior of the selected clones when new races arise; and (ii) field resistance alone depends on the local rust population, and it might even only reflect specific resistance to a particular set of races.

In this work, we analyzed the relationship among various components of the resistance to rust, with special emphasis on inter-specific F_1 progenies. Poplar-rust interaction was analyzed within five full-sib families in an incomplete factorial mating design, in which each progeny was vegetatively propagated. The material was inoculated with three *M. larici-populina* races. The quantitative components of partial resistance, either in the field or under controlled conditions, were related to the complete resistance patterns and to the marker genotypes.

MATERIALS AND METHODS

Plant materials and rust races. The progenies used in this study were partly presented elsewhere (33). They were produced in a multiparental F_1 mating design and include five families from six parents, three *P. trichocarpa* and three *P. deltoides*. Family 23B is an intraspecific *P. trichocarpa* family and family 87B is an intraspecific *P. deltoides* family, whereas families 27B, 83B, and 54B are interspecific F_1 progenies (Table 1).

The original *M. larici-populina* rust isolates were kindly provided by J. Pinon (INRA-Nancy) and maintained on specific poplar clones: 'Robusta', susceptible to races E1, E2, and E3; 'Ogy', susceptible only to race E2; and 'Candicans', susceptible only to race E3 (26,28). These poplar clones were also included in each inoculation experiment as controls, and no contamination was ever found.

Assessment of race-specific compatibility. The data concerning the complete resistance to E1 in families 23B, 27B, 83B, and 87B were taken from another study (33). Leaf-disk bioassay was used in the same way to screen these families with races E2 and E3, and family 54B with all three races. The leaf disks (3-cm diameter) were taken from plants grown in a greenhouse protected from natural contamination; leaves were in the fifth to tenth position from the apex. Leaf disks were sprayed with inoculum suspension, and maintained on distilled water for 15 days in a room under controlled temperature ($19 \pm 1^\circ\text{C}$) and photoperiod (16 h of light).

A particular clone-race interaction was declared compatible as soon as at least one sporulating uredium was observed on one leaf disk. Each clone has been repeatedly inoculated every year since 1992, and the results were perfectly reproducible; the compatible combinations were always freely sporulating, whereas the incompatible combinations showed no symptoms at all.

Evaluation of quantitative resistance components. Seventy-six clones from family 54B were tested in controlled conditions

for their quantitative response to artificial inoculation with the three races. A specific experimental design was used, and different factors of variation were controlled according to a previous publication (13). The leaf disks were taken as previously described. The concentration of inoculum (5,000 to 10,000 spores per ml) and germination rate (above 80%) were checked. A complete randomized block experiment was used in the growth chamber with four disks per clone, plus clone 'Robusta' as a control.

Four quantitative components were evaluated for each disk: the latent period (LAT, number of days between inoculation and the first sporulating uredinia); the level of infection (INF, total number of uredinia 15 days after inoculation, which was log-transformed for homoscedasticity); the sporulation (SPO, approximate mean size of uredinia on a 1 to 3 scale as compared with the control clone 'Robusta', which was scored 2); and the number of nonsporulating necrotic flecks found after 15 days (NEC, log-transformed). A global index of susceptibility (IND) taking into account the length of each cycle and its "productivity" was computed as follows: $\text{IND} = \text{INF} \times \text{SPO}/\text{LAT}$ (J. Pinon, personal communication).

Nursery trials. To assess the levels of field susceptibility and tolerance, two nursery trials were established in contrasting sites, Orléans (central part of France) and Guémené (western part of the country). The two sites differ in local rust populations (J. Pinon, personal communication) and in climatic conditions, with a much longer growing season in Guémené. In these trials, each family was represented by 30 to 40 clones. A complete randomized block design was used, with six replications per clone, plus common control clones. The level of infection by rust was observed during the first year and measured on a 1 to 9 scale (13). Total stem height and stem diameter were measured at the end of the second year.

DNA analysis. The DNA analysis proceeded as previously described (33), and 79 clones from family 54B were genotyped for the RAPD marker OPM03/04_480 that was previously found linked to complete resistance to race E1 in the four other families.

Statistical analysis. Mendelian segregations for complete resistance were tested with a chi-square test. A 95% confidence interval for the proportion of susceptible clones within each family was computed.

Quantitative data from the nursery and the laboratory trials were analyzed with the following mixed models within each progeny

$$\begin{aligned}\text{var} &= \mu + \text{block} + \text{clone} + \varepsilon \text{ or} \\ \text{var} &= \mu + \text{block} + \text{group} + \text{group/clone} + \varepsilon\end{aligned}$$

in which block was a fixed effect, group was a fixed effect for groups of clones within each family (this will be justified later), and clone was a random effect. Restricted maximum likelihood estimates of the clonal and the residual components of

TABLE 1. Segregation for complete resistance to three *Melampsora larici-populina* races in the five controlled progenies

Female parent	Male parent		
	19-77 (T) ^a , Oregon, + + + ^b	101-74 (T), Washington, + + +	L123-011 (D), Illinois, - - - ^c
36-134 (T), Oregon, + + +	Family 23B; + + +, 40; <i>P</i> ^d (0.89,1.00)		Family 27B; + + +, 20; - - -, 16; <i>P</i> (0.38,0.72)
73028-62 (D), Illinois, - - -		Family 54B; + + +, 35; - + - ^e , 50; <i>P</i> (0.31,0.52) E1, E3; <i>P</i> (0.95,1.00) E2	
L155-79 (D), Illinois, - - -	Family 83B; + + - ^f , 19; - - -, 12; <i>P</i> (0.42,0.78) E1, E2; <i>P</i> (0.00,0.14) E3		Family 87B; ? ? ? + ^g , 10; - - - ^h , 28; <i>P</i> (0.14,0.43)

^a T = *Populus trichocarpa* and D = *P. deltoides*.

^b + + + = individuals susceptible to all three races.

^c - - - = individuals resistant to all three races.

^d *P* = 95% confidence interval for the proportion of susceptible genotypes in the progeny.

^e - + - = individuals resistant to E1 and E3, susceptible to E2 (equivalent to 'Ogy').

^f + + - = individuals resistant to E3, susceptible to E1 and E2.

^g ? ? ? + = undetermined resistance to races E1, E2, and E3, but repeated infection in the nursery.

^h - - - - = individuals resistant to E1, E2, and E3, and not infected in the nursery.

variance were computed. Within each family, the following ratio was computed

$$h^2 = 2\sigma_{\text{clone}}^2 / (2\sigma_{\text{clone}}^2 + \sigma_{\text{residual}}^2)$$

which related to broad-sense heritability, given that within a full-sib family vegetatively propagated

$$\sigma_{\text{clone}}^2 = (Va + Vd) - \text{Cov}_{\text{full-sib}} = 1/2Va + 3/4Vd$$

in which Va and Vd stand for additive and dominance variance, and $\text{Cov}_{\text{full-sib}}$ stands for the variance between full-sib families (13). When dominance variance is ignored, then σ_{clone}^2 equals half the genetic variance.

Under the polygenic model, clonal means were expected to follow a normal distribution within each progeny. This hypothesis was tested by the nonparametric Lilliefors test (30). All computations were made with S-plus 3.2 (Mathsoft Inc., Seattle, WA).

RESULTS

Race-specific compatibility. Incompatibility was defined as a complete lack of any symptoms on the leaf disks. Necrotic flecks were occasionally observed, mainly in the interspecific progenies. However, these necroses were always found in compatible situations, i.e., when free sporulation occurred either on the same leaf disk or other leaf disks from the same genotype. This form of necrosis was therefore not related to incompatibility, but to some quantitative reaction; this question is treated later on.

After inoculation with the three *M. larici-populina* races (E1, E2, and E3), four different phenotypes were found among the total set of genotypes, out of the eight possible resistance patterns (Table 1). The *P. trichocarpa* parents were compatible with (susceptible to) all races, whereas the *P. deltoides* parents were completely resistant to the three races. Moreover, all *P. trichocarpa* parents lacked the OPM03/04_480 band, whereas the *P. deltoides* parents had this marker.

Within each interspecific F_1 family, the segregation involved only two phenotypes. Complete cosegregation for the three races was observed in family 27B. Within family 54B, all the genotypes were susceptible to E2 and there was cosegregation for resistance to E1 and E3. Within family 83B, all the genotypes were resistant to E3 and cosegregated for resistance to races E1 and E2.

In a previous study, we have shown that the RAPD marker OPM03/04_480 cosegregated with resistance to E1 in families 27B and 83B, and no recombinant genotype was found (33). Thus, in this study, the same marker was also tightly linked to resistance

to E2 and E3 in these two progenies. Moreover, family 54B was screened here for the first time with this marker, and a close linkage with resistance to E1 and E3 was also found: from the 79 full-sibs that have been genotyped, 32 susceptible clones (+ + +) had the band, 47 resistant clones (- + -) lacked the marker, and no recombinant genotype was found. Finally, the three interspecific progenies revealed a close linkage between the marker and race-specific resistance, and, since no recombinant genotype was found, an overall estimate of the distance would be less than 1 cM. This linkage was inferred from three different double-heterozygous *P. deltoides* parents; the marker was linked to the resistance allele in two cases (27B and 83B) and to the susceptible allele in one case (54B).

No segregation was observed in the intraspecific *P. trichocarpa* family; all the genotypes were susceptible to the three races in that progeny. In the intraspecific *P. deltoides* family 87B, clear segregation was repeatedly observed in the nursery, but clones that were regularly infected in the nursery gave inconsistent results in vitro, probably because of a high level of partial resistance to the races E1, E2, and E3; their infection in the nursery involved a different race (24), and resistance to this new pathotype was also in tight linkage with the OPM03/04_480 marker (33).

These patterns led us to consider two groups of clones within each family (except family 23B). These groups were defined equally by the race-specific compatibility pattern or by the genotype at the OPM03/04_480 marker.

Field trials. Genetic effects were highly significant for all variables within each family in the two nurseries (Table 2). The genetic variance was higher in the three interspecific families than in the pure species for all traits under study.

For rust infection, most of the genetic variation was associated to the OPM03/04_480 marker (or compatibility pattern); R^2 ranged from 72% for the intraspecific family 87B in Guémené to 94% for the interspecific family 83B in Orléans. Moreover, part of the genetic variation for growth traits was also associated with the same marker in the interspecific families. This was particularly obvious in the interspecific family 54B, for which more than 50% of the variation for total height and diameter is explained by this marker.

Within each family, heritabilities were computed on the basis of clone within group variance. Concerning rust infection, the clone-within-group effect was significant in at least four progenies, but not for both test sites, suggesting the existence of genetic variation beyond the major effect of race-specific compatibility. For resistance and growth traits, these heritabilities were not higher in the interspecific than the intraspecific progenies, illustrating the major

TABLE 2. Genetic effects at the clone within-family level for nursery traits

	T × T ^a family 23B Orléans ^b	D × D family 87B Orléans	D × D family 87B Guémené	Inter family 27B Guémené	Inter family 54B Orléans	Inter family 54B Guémené	Inter family 83B Orléans	Inter family 83B Guémené
RO ^c								
H^2 ^d	0.46	0.57	***	***	0.87	***	0.93	***
R^2 ^e	...	82%	72%	90%	82%	77%	94%	76%
h^2 ^f	0.46	Ns	***	Ns	0.39	Ns	0.30	Ns
HT2 ^c								
H^2	0.37	0.53	0.22	0.87	0.64	0.62	0.64	0.69
R^2	...	Ns	Ns	Ns	61%	49%	Ns	17%
h^2	0.37	0.54	0.23	0.84	0.27	0.38	0.50	0.56
DI2 ^c								
H^2	0.29	0.49	0.20	0.88	0.71	0.70	0.61	0.75
R^2	...	Ns	Ns	Ns	64%	56%	Ns	28%
h^2	0.29	0.50	0.22	0.85	0.30	0.42	0.48	0.64

^a T × T = *Populus trichocarpa* family, D × D = *P. deltoides* family, and Inter = interspecific hybrid family.

^b Location of the nursery test.

^c RO = rust infection; HT2 = stem height (end of second year); DI2 = stem diameter (end of second year). For variable RO in Guémené, the results of the *F* test of differences among compatible clones only is provided (Ns = $P > 0.05$; *** = $P < 0.001$).

^d H^2 = total genetic effect estimated as $2\sigma_{\text{Tclone}}^2 / (2\sigma_{\text{Tclone}}^2 + \sigma_{\text{residual}}^2)$, in which σ_{Tclone}^2 is the total clonal variance.

^e R^2 = effect associated to the marker, or compatibility group, estimated as $\text{SS}_{\text{group}} / (\text{SS}_{\text{group}} + \text{SS}_{\text{clone/group}})$.

^f h^2 = heritability estimated as $2\sigma_{\text{clone}}^2 / (2\sigma_{\text{clone}}^2 + \sigma_{\text{residual}}^2)$, in which σ_{clone}^2 is the clonal variance within compatibility group.

effect of compatibility pattern on the genetic variation found within interspecific F_1 progenies. One particular case concerned family 27B; in that family, the compatibility pattern accounted for almost all the variation in field susceptibility, but not for growth traits. Therefore, another undetermined factor of variation should be invoked to explain the highest total clone variance in this family.

Considering the distributions of clonal means, normality was not rejected for any variable in *P. trichocarpa* family 23B. For rust infection, the distribution was significantly non-normal in the four other families ($P < 0.01$). This could be attributed to the major effect associated with complete resistance. For growth traits, normality was also rejected for two interspecific families ($P < 0.01$).

Quantitative components of partial resistance. The interspecific F_1 progeny 54B offered a good model for studying the genetic relationship between quantitative resistance (all genotypes compatible with E2) and complete race-specific resistance (segregation for E1 and E3).

The quantitative components of resistance were highly variable. In compatible situations, the latent period varied from 7 to 14 days, the level of infection from 0 to 50 uredinia per disk, the sporulation score from 1 to 3, the number of necroses from 0 to 15, and the global index from 0.05 to 1.70, depending on the race and the clone. Genetic effects were highly significant for all traits (Table 3). The complete resistance to E1 and E3 accounted for most of the genetic variation found in quantitative resistance to E2. R^2 ranged from 48% for sporulation to 68% for the latent period. The distributions of clonal means for the components of the resistance to race E2 are given in Figure 1; significant deviation to normality was found for latent period, sporulation, and susceptibility index. This was fully explained by the major effect associated with the compatibility to races E1 and E3.

The genotypic correlations between quantitative resistance components within family 54B are given in Table 4. The same tendency was found for each race, with two sets of variables. The level of infection, the sporulation, and the global index were positively correlated and opposed to the latent period and the number of necroses (NEC). Thus, resistant clones tended to combine all kinds of resistance. However, some correlations were not significant, and in particular sporulation was less correlated to latent period or infection. Part of the correlations among quantitative components of the resistance to E2 could be associated to the pattern

TABLE 3. Genetic effects for quantitative resistance components within family 54B

Race	Resistance component	H^2 ^a	R^2 ^b	h^2 ^c
E2	LAT ^d	0.81	68%	0.43
	INF	0.79	57%	0.56
	SPO	0.74	48%	0.55
	NEC	0.72	55%	0.46
	IND	0.82	61%	0.59
E1	LAT	...	...	0.67
	INF	...	...	0.80
	SPO	...	...	0.87
	NEC	...	...	0.71
	IND	...	...	0.86
E3	LAT	...	...	0.35
	INF	...	...	0.58
	SPO	...	...	0.70
	NEC	...	...	0.61
	IND	...	...	0.75

^a H^2 = total genetic effect estimated as $2\sigma^2_{Tclone}/(2\sigma^2_{Tclone} + \sigma^2_{residual})$, in which σ^2_{Tclone} is the total clonal variance (only for race E2).

^b R^2 = effect associated to the marker, or compatibility group, estimated as: $SS_{group}/(SS_{group} + SS_{clone/group})$.

^c h^2 = heritability estimated as $2\sigma^2_{clone}/(2\sigma^2_{clone} + \sigma^2_{residual})$, in which σ^2_{clone} is the clonal variance within group.

^d LAT = latent period, INF = level of infection, SPO = sporulation, NEC = necrosis, and IND = susceptibility index.

of compatibility with E1 and E3: all correlations were higher for the whole progeny than for the subset of clones compatible to E1 and E3. Within the subset of genotypes compatible with all races, the clonal values obtained for a single parameter evaluated on different races were highly correlated; for the susceptibility index, the correlation ranged from 0.73 (between E2 and E3) to 0.90 (between E1 and E3).

Necrotic symptoms were only observed in compatible interactions, as previously mentioned. For each race, the number of flecks was negatively correlated to the susceptibility index. This

Fig. 1. Distribution of clonal means for quantitative components of the resistance to race E2, within family 54B. Clones susceptible to E1 and E3 appear in the black bars and those completely resistant appear in the white bars. (Results of Lilliefors' test for normality.)

was due to a triangular relationship with more frequent necroses in the less-infected clones. In the case of necrotic reactions with race E2, the major effect associated with the compatibility to other races was still found (Fig. 2).

Finally, Figure 3 illustrates the effect of rust resistance on growth traits in the interspecific family 54B. It also shows the correlation between quantitative resistance estimated in the field and in controlled conditions, and it shows the major effect associated with the incompatibility to races E1 and E3 (i.e., associated with the OPM03/04_480 marker) on all traits.

DISCUSSION

Phenotypic evaluation of the resistance components. There was a clear difference between complete resistance and partial resistance in the segregating progenies. This was not just a matter of threshold. In the interspecific progenies, incompatibility was not related to macroscopic necrotic flecks as observed with the same pathogen on *P. deltoides* (21) or with *M. medusae* f. sp. *deltoidae* on interspecific F_2 genotypes (19). A detailed histological study of poplar-*M. larici-populina* interaction showed that cellular necrosis often occurred at the microscopic level (11).

For the quantitative components of resistance, strong genetic effects could be detected, thanks to the designs used in the nursery and the laboratory experiments. The heritability estimates of field resistance within each family (0.30 to 0.46) corresponded to previous literature (32,35). For in vitro experiments, environmental effects can be enhanced by the high physiological variations among leaf disks and the effect of wounding (19). Nevertheless, the heritabilities of resistance components under controlled conditions were also high (0.59 to 0.86 for global indices) and corresponded to the estimates obtained for other *Melampsora* species and other *Populus* pedigrees (13,19,22,29).

Partial resistance was a priori split into epidemiological components. Although these components were generally correlated, correlations were not all significant; this showed that splitting up the resistance was not just an artificial view of a single phenomenon. The same validation of the breakdown of resistance had been found within *P. trichocarpa* (13).

Genetic model for race-specific incompatibility. Different strategies may be used for genetic studies in allogamous tree species: three-generation pedigrees generally descending from only

two ancestors (2) or F_1 mating designs from several parents (8, 17). These approaches are complementary. This study focused on the particular situation of interspecific F_1 genotypes because they corresponded to actually planted cultivars. The multiple-family approach was used to compensate for the lack in fully heterozygous parents and to validate the genetic hypothesis among the whole set of progenies.

Forgetting the different rust races, phenotypic segregations in the interspecific progenies fit a 1:1 ratio, and a 1:3 ratio for the *P. deltoides* family. This could be interpreted as a monogenic model of inheritance, in which the resistance alleles coming from *P. deltoides* would be dominant. The *P. trichocarpa* parents were inferred to be recessive homozygous and the *P. deltoides* heterozygous in most cases. This corresponded to our previous interpretation for race E1 (33), and the same conclusion was obtained in a *P. deltoides* $\times$ *P. nigra* family (3). A single dominant gene for necrotic flecking with *M. medusae* f. sp. *deltoidae* was also inferred in a F_2 pedigree (19), but, in that case, the resistance gene originated from *P. trichocarpa*.

Interpretation became more complex when considering each race individually, since the phenotypes differed from one segregating progeny to another. Segregation in family 27B suggested that a single cluster of loci, or even a single locus, controlled complete resistance to all three races. Resistance genes to E1 and E2 were also clustered in family 83B, as for E1 and E3 in family 54B. Since the segregations in the different progenies were tightly linked to the same marker (OPM03/04_480) and this also concerned the segregation to another pathotype in the *P. deltoides* family 87B, it became obvious that a common genomic region was involved in all race-specific incompatibility systems. However, two particular cases still needed to be explained: the lack of segregation for E3 within family 83B and for E2 within family 54B.

Within family 83B, complete resistance of all genotypes to E3 could still fit a monogenic model, assuming that the *P. deltoides* parent was homozygous. This hypothesis was checked in another interspecific family (84B) from the same *P. deltoides* mother L155-79, in which no segregation and complete resistance to E3 was also found (data not shown). Therefore, L155-79 behaved like a homozygous genotype for resistance to E3 and, like a heterozygous genotype for resistance to E1, E2, another pathotype (family 87B), and the RAPD marker, all tightly linked with no observed recombinant. This could still be interpreted with a single gene

TABLE 4. Genotypic correlations between quantitative resistance components within family 54B

	Race													
	E2					E1					E3			
Race	LAT	INF	SPO	IND	NEC	LAT	INF	SPO	IND	NEC	LAT	INF	SPO	IND
E2														
LAT ^a	1	−0.87	−0.57	−0.81	0.61									
INF	−0.51	1	0.53	0.79	−0.46									
SPO	Ns	Ns	1	0.87	−0.55									
IND	−0.56	0.54	0.85	1	−0.60									
NEC	0.37	Ns	−0.55	−0.49	1									
E1														
LAT	0.84					1								
INF		0.49				−0.76	1							
SPO			0.52			Ns	Ns	1						
IND				0.74		−0.56	0.60	0.87	1					
NEC					0.53	0.62	−0.72	−0.44	−0.69	1				
E3														
LAT	0.76					0.81					1			
INF		0.58					0.72				−0.62	1		
SPO			0.52					0.83			−0.36	0.35	1	
IND				0.73					0.90		−0.57	0.60	0.82	1
NEC					0.35					0.77	0.69	−0.69	−0.40	−0.57

^a LAT = latent period; INF = level of infection; SPO = sporulation; NEC = necrosis; and IND = susceptibility index. Above diagonal = genotypic correlations for all clones (race E2 only) and below diagonal = genotypic correlations among the subset of clones susceptible to E1 and E3; Ns = $P > 0.05$.

cluster: either a single locus with pleiotropic action on the three races and different resistance alleles or several tightly linked loci, at least one that controls resistance to E1 and E2 and a second that controls resistance to E3.

Within family 54B, the generalized susceptibility to E2 was more surprising, since the *P. deltoides* parent showed complete resistance. Another interspecific family (53B) from the same *P. deltoides* mother was checked; all genotypes were also compatible with E2 (data not shown). No monogenic inheritance with dominant resistance allele could account for this result. The only possible monogenic model would assume a recessive resistance allele for that race and heterozygosity of the *P. trichocarpa* parent at that locus; this interpretation was rejected, since no case of incompatibility to *M. larici-populina* spp. has ever been found in this species. Assuming that alleles for incompatibility were dominant, a digenic model could explain the compatibility with E2 in the whole progenies 54B and 53B, but with restrictive conditions as follow: (i) the two loci should be tightly linked in a single cluster so that no recombinant genotype was observed in both progenies; (ii) genes would interact in such a way that dominant alleles are needed at both loci to obtain a resistant phenotype; and (iii) the *P. deltoides* parent should be double heterozygous in repulsing phase, and the *P. trichocarpa* parents should be double recessive homozygous. Thus, the nonrecombinant gametes produced by that particular *P. deltoides* genotype would transmit only part of the alleles needed for incompatibility. These assumptions seem rather restrictive, but no simpler digenic model could be found. The two loci would be clustered, and, moreover, our set of data would allow one of these loci to be the one involved in the resistance to E1 and E3, all of them being located in the region marked by OPM03/04_480. More complex models with three genes or suppressors of resistance might also be invoked. A digenic model has previously been proposed to account for the whole set of segregations in a 6×6 factorial mating design within *P. deltoides* (21). For the quantitative resistance to leaf spot, a similar model featuring two quantitative trait loci (QTL) was invoked (18).

Finally, the interpretation of the whole set of data suggested the existence of a key region in the genome involved in the control of specific incompatibility to three different races and to which the marker OPM03/04_480 was tightly linked. Polymorphism at both the marker locus and the incompatibility cluster originated in the *P. deltoides* genome.

From an evolutionary point of view, it was noticeable that major genes for resistance were dominant and came from a species that did not co-evolve with the pathogen; *P. deltoides* is a North American poplar species, whereas *M. larici-populina* is a Eurasian pathogen. In another study, the polymorphism at one locus controlling flecking with *M. medusae* f. sp. *deltoidae* in the interspecific F_2 originated from *P. trichocarpa*, although the pathogen was specific to *P. deltoides* (19). In the interaction of hybrid poplar with *Septoria populicola*, the resistant alleles at the different

Fig. 2. Relationship between susceptibility index and necrosis after inoculation with race E2, within family 54B. Clones showing complete resistance to races E1 and E3 appear as "o" and those susceptible appear as "+."

QTL were also inherited from the non-native *P. deltoides* parent (18). The marker OPM03/04_480 is currently mapped on the *P. deltoides* genome to check if it is not located in the vicinity of a QTL for resistance to another pathogen of that species.

Nature of the resistance genes. Complete resistance was clearly race-specific; even if there was cosegregation for all races in each individual progeny, the phenotypes differed from one family to the other, showing that various alleles (on a single locus or several clustered genes) were involved. The genetics of specificity has not been fully resolved yet, but evidence is emerging that recombination or gene conversion in complex loci might be involved, and colocalization of genes involved in the different race-specific patterns is often observed (4). Demonstration of allelism or, by contrast, clustering of genes would require a large number of individuals within each progeny. However, in this study, the hypothesis of allelism at a single locus was rejected for the 54B-E2 interaction, and a model of at least two loci in a close cluster was proposed.

A very tight clustering of genes responsible for race-specific incompatibility and partial resistance was detected in these progenies; thus, simply by using different rust races on progeny 54B, qualitative and quantitative resistance to the same pathogen could

Fig. 3. Relationship between susceptibility index with race E2, field resistance, and growth, within family 54B. Nursery traits concern the experiment in Orléans. Clones showing complete resistance to races E1 and E3 appear as "o" and those susceptible appear as "+."

be related, and the DNA marker cosegregating with both traits was an extra argument. A locus involved in the necrotic flecking due to *M. medusae* was found linked to a QTL for field resistance to the same disease (19). The development of genome mapping has recently provided many examples of clustering of genes involved in qualitative and quantitative resistance to a single pathogen (5,7,34) or different pathogens (10,15,16). However, in a *Populus* F₂ pedigree, no clustering of the three QTL involved in the resistance to *S. populicola* was found, neither with the QTL involved in the resistance to *M. medusae* nor with the major QTL for growth or phenological traits (18).

Partial resistance seemed to be general rather than race-specific, since epidemiological components for the different races were highly correlated. Thus, poplar clones that showed a high level of partial resistance to the three races are expected to behave well with other isolates. However, the final question is the durability of resistance rather than just specificity or apparent nonspecificity (9). Durability does not only concern the evolution of pathogen populations, but also the stability of resistance genes. Meiotic instability in clusters of genes from multigene families (4) should be further investigated in this particular case, in which partial resistance is controlled by few major QTL clustered with genes of complete race-specific resistance.

In this study, race-specific compatibility, as opposed to partial resistance, had a major effect in the interspecific F₁ progenies. Indeed, *M. larici-populina* rust races had been identified on cultivated F₁ hybrids (26,28,31), and only one case of race identification on pure *P. deltoides* was reported (24). These races clearly emerged due to poplar cultivation, and they were rarely found in the wild stands of *P. nigra* (23). The study of co-evolution between wild or cultivated *Populus* and *Melampsora* rusts is an important field of investigation as far as the durability of resistance to rust is concerned.

Background effect in the interspecific F₁ hybrids. The distribution of clonal means for resistance and growth traits within interspecific F₁ families generally did not fit the model of polygenic inheritance, but rather fitted a model of major gene effect associated with the genotype at the cluster of incompatibility. Such major gene effect was not found in the pure species *P. trichocarpa* (13). In the F₂ pedigree, variation for resistance to *S. populicola* also appeared to be oligogenic, with two major QTL explaining 70% of the genetic variance (18).

Interspecific F₁ hybrid families differed from the pure species only for their genetic background, not for meiotic abnormalities, since the gametes came from normal meiosis in the parental pure species. Therefore, major gene effects in the F₁ generation were attributed to the particular genetic background. We assume that, in the interspecific F₁ background, the main gene cluster is still controlling incompatibility, but all other minor loci that could be involved in the genetic control of resistance in the pure species are not effective anymore. Furthermore, tolerance was also drastically reduced in the interspecific F₁ hybrids, as revealed by the reduced vigor of the plants in the nursery. This might be related to outbreeding depression (12) due to the breakdown of co-adapted gene complexes in the interspecific F₁ between *P. deltoides* and *P. trichocarpa*. Hybrid breakdown of resistance to herbivores was also suspected as a mechanism limiting natural introgression between *P. angustifolia* and *P. fremontii* (36). However, for another pathogen, *S. populicola*, interspecific hybrids seemed to have a normal, intermediate level of resistance between the resistant *P. deltoides* and the susceptible *P. trichocarpa* parent species (18). Neither singularity was found in *P. tremula* × *P. tremuloides* hybrids for resistance to their own *Melampsora* rust species (6).

For the interspecific progenies involved in this study, the single field evaluation could be confusing. Clearly, when selecting pure species in the nursery, breeders select for a quantitative component; but, in the case of interspecific hybrids, the same procedure may lead to oligogenic resistance. This could be an example of

unintentional selection for complete race-specific resistance. The nursery and laboratory approaches should then be considered complementary to differentiate the various components of resistance.

Within each interspecific progeny, only two phenotypes were observed and no recombination among race-specific compatibilities was found. This might be due to the high heterozygosity in the F₁ genotypes. Only the loci that show polymorphism in the parent species may be detected in such pedigrees. Other genes, monomorphic for different alleles in the various species, would be involved in the control of race-specificity in the F₂ generation. According to the pedigree under study, different polymorphisms may be detected.

Consequences for breeding. Monoclonal plantings are partly responsible for the evolution of poplar rusts in Europe. Multiclonal plantings would require cultivars specifically selected for competing ability. Nevertheless, multiclinal planting does not prevent breeders from selecting for resistance. As a forest tree, a poplar clone should keep a reasonable level of resistance over years; this can be achieved either through pure quantitative resistance or by combining quantitative and race-specific resistance, but probably not with pure race-specific resistance. The origin of the "new" rust races still has to be clarified.

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LITERATURE CITED

1. Bisoffi, S., and Gullberg, U. 1996. Poplar breeding and selection strategies. Pages 139-158 in: *Biology of Populus and Its Implications for Management and Conservation*. R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley, eds. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.
2. Bradshaw, H. D., Jr., Villar, M., Watson, B. D., Otto, K. G., Stewart, S., and Stettler, R. F. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. *Theor. Appl. Genet.* 89:167-178.
3. Cervera, M. T., Gusmao, J., Steenackers, M., Peleman, J., Storme, V., Vanden Broeck, A., Van Montagu, M., and Boerjan, W. 1996. Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. *Theor. Appl. Genet.* 93:733-737.
4. Crute, I. R., and Pink, D. A. C. 1996. Genetics and utilization of pathogen resistance in plants. *Plant Cell* 8:1747-1755.
5. Freymark, P. J., Lee, M., Woodman, W. L., and Martinson, C. A. 1993. Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). *Theor. Appl. Genet.* 87:537-544.
6. Gallo, L. A., Stephan, B. R., and Krusche, D. 1985. Genetic variation of *Melampsora* rust resistance in progenies of crossings between and within *Populus tremula* and *P. tremuloides* clones. *Silvae Genet.* 34:208-214.
7. Gmitter, F. G., Jr., Xiao, S. Y., Huang, S., Hu, X. L., Garnsey, S. M., and Deng, Z. 1996. A localized linkage map of the citrus tristeza virus resistance gene region. *Theor. Appl. Genet.* 92:688-695.
8. Grattapaglia, D., and Sederoff, R. R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross mapping strategy and RAPD markers. *Genetics* 137:1121-1137.
9. Johnson, R. 1992. Reflections of a plant pathologist on breeding for disease resistance, with emphasis on yellow rust and eyespot of wheat. *Plant Pathol.* 41:239-254.
10. Kesseli, R., Witsenboer, H., Stanghellini, M., Vandermark, G., and Michelmore, R. 1993. Recessive resistance to *Plasmopara lactucae-radialis* maps by bulked segregant analysis to a cluster of dominant disease resistance genes in lettuce. *Mol. Plant-Microbe Interact.* 6:722-728.
11. Laurans, F. 1997. Définition histologique et approche moléculaire des interactions peupliers-rouilles. Ph.D. thesis. Orléans University, Orléans, France.

12. Ledig, F. T. 1986. Heterozygosity, heterosis, and fitness in outbreeding plants. Pages 77-104 in: Conservation Biology: The Science of Scarcity and Diversity. M. E. Soulé, ed. Sinauer Associates Inc., Sunderland, United Kingdom.
13. Lefèvre, F., Pichot, C., and Pinon, J. 1994. Intra- and interspecific inheritance of some components of the resistance to leaf rust (*Melampsora larici-populina* Kleb.) in poplars. Theor. Appl. Genet. 88:501-507.
14. Legionnet, A. 1996. Diversité et fonctionnement génétique des populations naturelles de *Populus nigra* L., espèce pionnière des ripisylves Européennes. Ph.D. thesis. Univ. Montpellier II, Montpellier, France.
15. Leonard-Schippers, C., Gieffers, W., Schäfer-Pregel, R., Ritter, E., Knapp, S. J., Salamini, F., and Gebhardt, C. 1994. Quantitative resistance to *Phytophthora infestans* in potato: A case study for QTL mapping in an allogamous plant species. Genetics 137:67-77.
16. McMullen, M. D., and Simcox, K. D. 1995. Genomic organization of disease and insect resistance genes in maize. Mol. Plant-Microbe Interact. 8:811-815.
17. Muranty, H. 1996. Power of tests for quantitative trait loci detection using full-sib families in different schemes. Heredity 76:156-165.
18. Newcombe, G., and Bradshaw, H. D., Jr. 1996. Quantitative trait loci conferring resistance in hybrid poplar to *Septoria populicola*, the cause of leaf spot. Can. J. For. Res. 26:1943-1950.
19. Newcombe, G., Bradshaw, H. D., Jr., Chastagner, G. A., and Stettler, R. F. 1996. A major gene for resistance to *Melampsora medusae* f. sp. *deltoidae* in a hybrid poplar pedigree. Phytopathology 86:87-94.
20. Newcombe, G., and Chastagner, G. A. 1993. First report of the Eurasian poplar leaf rust fungus, *Melampsora larici-populina*, in North America. Plant Dis. 77:532-535.
21. Pichot, C., and Teissier du Cros, E. 1993. Susceptibility of *Populus deltoides* Bartr. to *Melampsora larici-populina*. I. Qualitative analysis of a 6 by 6 factorial mating design. Silvae Genet. 42:179-188.
22. Pichot, C., and Teissier du Cros, E. 1993. Susceptibility of *Populus deltoides* Bartr. to *Melampsora larici-populina*. II. Quantitative analysis of a 6 by 6 factorial mating design. Silvae Genet. 42:188-198.
23. Pinon, J., and Frey, P. 1997. Structure of *Melampsora larici-populina* populations on wild and cultivated poplar. Eur. J. Plant Pathol. 103:159-173.
24. Pinon, J., and Lefèvre, F. 1994. A New Virulence Found Among Isolates of *Melampsora larici-populina*. Proc. Int. Poplar Commission and FAO, Working Group on Diseases. FAO (Food Agric. Organ. United Nations), Rome.
25. Pinon, J., Newcombe, G., and Chastagner, G. A. 1994. Identification of races of *Melampsora larici-populina*, the Eurasian rust fungus on *Populus* species in California and Washington. Plant Dis. 78:101.
26. Pinon, J., and Peulon, V. 1989. Mise en évidence d'une troisième race physiologique de *Melampsora larici-populina* Kleb. en Europe. Cryptogam. Mycol. 10:95-106.
27. Pinon, J., and Valadon, A. 1997. Comportement des cultivars de peupliers commercialisables dans l'Union Européenne vis-à-vis de quelques parasites majeurs. Ann. Sci. For. 54:19-38.
28. Pinon, J., Van Dam, B. C., Genetet, I., and de Kam, M. 1987. Two pathogenetic races of *Melampsora larici-populina* in North Western Europe. Eur. J. For. Pathol. 17:47-53.
29. Prakash, C. S., and Heather, W. A. 1989. Inheritance of partial resistance to two races of leaf rust, *Melampsora medusae* in eastern cottonwood, *Populus deltoides*. Silvae Genet. 38:90-94.
30. Sprent, P. 1989. Applied Non Parametric Statistical Methods. Chapman and Hall, New York.
31. Steenackers, M., Steenackers, V., and Delporte, T. 1994. A new physiological race of *Melampsora larici-populina* in Belgium. Proc. Int. Poplar Commission and FAO, Working Group on Diseases. FAO (Food Agric. Organ. United Nations), Rome.
32. Thielges, B. A., Sabdon, A., Rousseau, R. J., and Prakash, C. S. 1989. Genetic variation and heritabilities of growth rate and *Melampsora* leaf rust resistance in a mid-south population of *Populus deltoides* Bartr. Proc. IUFRO (Int. Union For. Res. Organ.) Working Party S2.02.10, Hann. Münden, Germany.
33. Villar, M., Lefèvre, F., Bradshaw, H. D., Jr., and Teissier du Cros, E. 1996. Molecular genetics of rust resistance in poplars (*Melampsora larici-populina* Kleb./*Populus* sp.) by bulked-segregant analysis in a 2 by 2 factorial mating design. Genetics 143:531-536.
34. Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C., and Nelson, R. J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434.
35. Wang, J., and Van der Kamp, B. J. 1992. Resistance, tolerance, and yield of western black cottonwood infected by *Melampsora* rust. Can. J. For. Res. 22:183-192.
36. Whitham, T. G., Floate, K. D., Martinsen, G. D., Driebe, E. M., and Keim, P. 1996. Ecological and evolutionary implications of hybridization: *Populus*-herbivore interactions. Pages 247-275 in: Biology of *Populus* and Its Implications for Management and Conservation. R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley, eds. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.