Segregation of Avirulences and Genetic Basis of Infection Types in *Erysiphe graminis* f. sp. *hordei*

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ABSTRACT

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The genetics of avirulence were studied in crosses of the barley powdery mildew fungus *Erysiphe graminis* f. sp. *hordei* (synamorph *Blumeria graminis* f. sp. *hordei*). The number of genes controlling avirulence on 10 near-isogenic lines of Pallas carrying different resistance genes were determined, as were the incompatible infection types (ITs) they conferred. Four crosses were made between isolates of different origins, and one cross, CC52 × DH14, was analyzed further with backcrosses and sibcrosses. Avirulence on P11 (*Mla13*) was controlled by two genes giving different ITs: $Avr_{a13}1$ with ITs 0 or 0-1 and $Avr_{a13}2$ with ITs 1-2 or 2. Two hypotheses can be suggested: (i) P11 carries a second gene, *Mla*(*Ru3*), in addition to *Mla13*, and each resistance gene matches one avirulence gene; or (ii) P11 carries the single resistance gene *Mla13*, which matches the two avirulence genes. Avirulences on P01 (*Mla1*), P08B (*Mla9*), P09 (*Mla10*), P10 (*Mla12*), P12 (*Mla22*), P16 (*Mlk1*), and P21 (*Mlg*) were controlled by one gene in all crosses in which phenotypes segregated. It could not be concluded whether avirulence on P04B (*Mla7*) and P23 (*MlLa*) was controlled by one or two genes. One linkage group, including Avr_{a9}, Avr_{a10}, Avr_{a22}, Avr_{k1}, and probably $Avr_{a13}1$, was detected.

Erysiphe graminis DC. f. sp. *hordei* Em. Marchal (synamorph *Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei*), the causal agent of powdery mildew, interacts specifically with barley cultivars. Incompatible reactions occur through diverse infection types (ITs), from no easily visible symptoms (IT 0) to the presence of necrosis or chlorosis with more or less sporulation (ITs 1 to 3). In a compatible reaction, there is profuse sporulation without necrosis and chlorosis (IT 4) (26). Genetic analyses in the pathogen and the host have shown that, on the whole, single avirulence genes match single resistance genes, giving an incompatible reaction, which is in agreement with the gene-for-gene relationship (12). More than 25 avirulence genes have been discovered in this haploid and heterothallic pathogen (3,6,9,16,23,24).

Locus *Mla*, comprising at least 28 resistance alleles (18), is complex and constitutes a multigene family (15,17,21). Based on the gene-for-gene relationship, two cultivars that differ in their resistance reactions (compatible or incompatible) with a set of powdery mildew isolates carry different resistance genes. An extension of the gene-for-gene relationship was proposed in the barley-powdery mildew interaction, taking into account differences in the expression of incompatible ITs (14): one cultivar that gives two different incompatible ITs with two different avirulent isolates carries two resistance genes. Based on this hypothesis, additional resistance genes have been postulated in barley cultivars, as well as in the near-isogenic lines (NILs) of Pallas (18,19), which were initially considered as carrying single resistance genes (Table 1). The genetic basis of ITs is not well understood, however. With the exception of *Mla6* (21), genetic analysis of the host has not allowed the additional resistance genes, which may be closely linked to the main gene, to be detected (18). In a recent genetic analysis of avirulence (6), a cross between an avirulent (IT 0) and a virulent isolate (IT 4) segregated in a 3:1 avirulent/virulent ratio on a cultivar carrying *Mla13*. The segregation ratio was consistent with avirulence controlled by two genes, one of which conferred IT 0 and the other IT 1 or 2. Based on the gene-for-gene relationship, a second resistance gene, *Mla*(*Ru3*), was postulated in this cultivar in addition to *Mla13* (19). Similar results suggesting segregation of two or three avirulence genes were observed for *Mla6* (5), *Mla7* (3,17), and *Mla9* (17). However, the analysis of F_1 progenies is not sufficient to exclude other hypotheses, such as the existence of modifier genes or polygenic control of ITs (1,8).

The objective of our study was to determine the number of avirulence genes and the incompatible ITs conferred by each of them, matching the resistances in 10 different NILs of Pallas spring barley. Progenies from four crosses between *E. graminis* f. sp. *hordei* isolates of different origins were initially analyzed to compare the range of ITs. Subsequently, backcrosses and sibcrosses with progeny isolates of cross CC52 × DH14, which was described by Brown and Simpson (6), were carried out to test hypotheses concerning the genetic basis of ITs.

MATERIALS AND METHODS

Experimental procedures. *Isolates and crosses.* Single conidial isolates of different origins were used as parents. Isolates CC52 and DH14 came from the United Kingdom (6). Isolates GF1, GF3, GF4, and GF13 were collected in France in 1991 and 1992. The isolates had diverse combinations of virulence phenotypes (Table 1) on 10 NILs of Pallas, each having one of the resistance alleles *Mla1*, *Mla7*, *Mla9*, *Mla10*, *Mla12*, *Mla13*, *Mla22*, *Mlk1*, *Mlg*, and *MlLa* (19). Seed of the Pallas lines was provided by L. Munk (Royal Agricultural and Veterinary University, Copenhagen).

Initially, four isolate crosses were made: CC52 × DH14, GF13 × DH14, GF1 × GF3, and GF13 × GF4. Eighteen progeny isolates derived from CC52 × DH14, designated D1/x, were backcrossed

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to determine their mating type. The development of cleistothecia 2 to 3 weeks after the cross indicated that the tested isolate's mating type was opposite of that of the tester parent. Sibcrosses between progeny isolates of opposite mating types were carried out.

Crosses were carried out in the greenhouse (10 to 20°C) during autumn or winter on the moderately susceptible spring cv. Proctor by the method of Brown et al. (4). Each time an isolate was inoculated as a parent of a cross, it was tested on the Pallas differential lines to confirm its virulence spectrum.

Recovery of ascospore progeny. Progeny isolates were recovered, as described by Brown et al. (4), on Manchuria leaf segments, which were changed twice a day. After 10 days of incubation, conidiospores from sporulating colonies were transferred to leaf segments of cv. Igri, on 4 g of agar with 30 mg of benzimidazole per liter.

The numbers of cleistothecia and colonies developed from ascospores were counted to assess the efficiency of the recovery of progeny. For one backcross and two sibcrosses, the germination rate of the ascospores was assessed by the method of de Vallavieille-Pope et al. (10). A leaf segment bearing cleistothecia was suspended over Manchuria leaf segments for 24 h. After 24 additional hours of incubation, the Manchuria leaf segments were laid on filter paper and cleared with lactophenol. After 1 week, the ascospores were stained in lactophenol-trypan blue acid fuschin, and nongerminated and germinated ascospores were counted with a light microscope. At least 50 ascospores were counted for each of the three crosses.

Virulence tests. Twelve to fifteen days after the transfer of the progeny isolates to cv. Igri, the isolates were tested on the Pallas lines. Each isolate was inoculated with a settling tower on 1.5-cm segments cut from the middle of the first leaf of 10-day-old plants and maintained in subdivided clear plastic boxes, on 4 g of agar with 30 mg of benzimidazole per liter. A first test with three replicates (three leaf fragments inoculated independently) was done for each progeny isolate. The progeny isolates were transferred again to cv. Igri leaves, and 12 to 15 days later a second test with three replicates was carried out. The boxes were incubated in a controlled environment cabinet (16°C, continuous light, 10 μE m^{-2} s^{-1}) for 8 days, and ITs were scored on a 0 to 4 scale (26). For most progeny isolates from CC52 × DH14, two to four additional tests were carried out.

Genetic analyses. *Control of avirulence.* Each progeny isolate was characterized by two scores, one for each test. The score was the most frequent or the middle IT among the three replicates of each test. When the scores were the same for the two tests, the progeny isolate was characterized by a single score (for instance IT 0). When the two scores were different, the progeny isolate was characterized by two scores (for instance IT 0-1). Thus, ITs were assigned to nine classes (0, 0-1, 1, 1-2, 2, 2-3, 3, 3-4, or 4). In a few cases, which were sufficiently rare as not to affect the analysis, the two scores differed by two classes of ITs; in these cases, the isolate was characterized by the mean score. In most cases, progeny isolates were classified as avirulent (ITs 0 to 3) or virulent (IT 4).

The avirulent/virulent ratio was compared by a χ^2 test to the expected 1:1 segregation ratios, indicating the presence of one gene, and 3:1, indicating the presence of two genes. For consistency with the existing nomenclature of *E. graminis* f. sp. *hordei* avirulence genes (3,6), each gene was named as Avr_Rn: in which *Avr* characterizes an avirulence gene, R is the suffix (following *Ml*) of the name of the resistance gene in the Pallas line, and *n* is a serial number required when two *Avr* genes matched the resistance on one Pallas line. For simplicity, we propose that two avirulence genes detected from different crosses on the same Pallas line should be considered the same gene, unless it is shown that they are different by a test of allelism.

Linkages between avirulence genes. The independence of the segregation of two avirulence genes was tested with a χ^2 test on a contingency table with four classes: AA, AV, VA, and VV, with avirulent (A) and virulent (V) alleles. When a linkage was observed, the recombination frequency (r) was estimated and converted to map distances (m) by Kosambi's function (20):

$$m = 0.25\log_e[(1 + 2r)/(1 - 2r)]$$

If avirulence was controlled by one gene on each of two Pallas lines (genes Avr_X and Avr_Y), the recombination frequency between Avr_X and Avr_Y was estimated as the frequency of recombinant progeny. If avirulence was controlled by one gene (Avr_X) on one Pallas line and by two unlinked genes (Avr_Y1 and Avr_Y2) on another Pallas line, the recombination frequency between Avr_X and Avr_Y1 could be estimated in two ways, depending on the number of genes segregating. In crosses in which Avr_Y1 segregated without Avr_Y2, the recombination frequency was estimated as above. In crosses in which both Avr_Y1 and Avr_Y2 segregated, the maximum likelihood estimate of the recombination frequency was calculated by the method of Mather (22):

$$AA/(s - 2) + AV/(1 + s) + VA/s + VV/(s - 1) = 0$$

with $s = r$ if Avr_X and Avr_Y1 are linked in coupling and $s = r - 1$ if Avr_X and Avr_Y1 are linked in repulsion.

For cross CC52 × DH14, backcrosses and sibcrosses, the distributions of the four classes, AA, AV, VA, and VV, were compared by a χ^2 test of homogeneity. If the frequencies of the classes in

TABLE 1. Virulences of parental *Erysiphe graminis* f. sp. *hordei* isolates on 10 near-isogenic barley lines of Pallas with different genes for powdery mildew resistance

Pallas isoline	Main[a] R allele	Additional R allele	Isolate (infection type)[b]					
			DH14	CC52	GF1	GF3	GF4	GF13
P01	*Mla1*[c]	*Mla(Al2)*[d]	0-1	0	0-1	4	0-1	4
P04B	*Mla7*	*Mla(No3)*[d]	2-3	4	2-3	4	4	4
P08B	*Mla9*	*Mla(MC4)*[d]	0-1	4	0	4	0	0
P09	*Mla10*	*Mla(Du2)*[a]	4	4	1	4	4	1-2
P10	*Mla12*	*Mla(Em2)*[d]	1	4	1	1	4	4
P11	*Mla13*	*Mla(Ru3)*[a]	0	4	0	4	0	0
P12	*Mla22*[e]		4	0	4	1	0-1	4
P16	*Mlk1*[e]		4	4	2-3	4	4	2-3
P21	*Mlg*	*Ml(CP)*[f]	4	4	0	4	4	4
P23	*MlLa*[e]		2	2-3	2-3	1-2	3	4

[a] Kølster et al. (19).

[b] Infection types were scored as follows: 0 = no visible symptoms; 1 = necrotic flecks, no sporulation; 2 = necrotic flecks, scarce sporulation; 3 = necrotic flecks or chlorosis, moderate sporulation; and 4 = no necrosis or chlorosis, profuse sporulation.

[c] Alleles *Mla1* to *Mla22* and their additional postulated alleles are at the *Mla* locus or at closely linked loci. Alleles *Mlk1*, *Mlg*, *MlLa*, and *Ml(CP)* map to other, different loci.

[d] Postulated by Jørgensen (18).

[e] *Mla22*, *Mlk1*, and *MlLa* were formerly known as *Mlc*, *Mlk*, and *Ml(La)*, respectively (18).

[f] Wiberg (30).

different crosses did not deviate significantly from homogeneity, parental and recombinant types were summed over all crosses to obtain a more accurate estimation of the recombination frequency. When there was significant linkage among three avirulence genes, the gene order was estimated by analyzing double crossovers.

Variation in the expression of avirulence. On each Pallas line, the ITs of the progeny isolates from cross CC52 × DH14 that were classified as avirulent by the method described above were analyzed further. For each avirulent isolate, the ITs obtained from the 6 to 18 replicates were taken into account, and variation in the IT was studied by generalized linear modeling (GLM), using the Genstat package (13), to analyze contingency tables of numbers of isolates with different ITs. When some expected values in the contingency table were below 0.5, data from close ITs (for instance ITs 0 and 1) were pooled (11). When only two classes of avirulent ITs could be distinguished, the interaction between isolate and IT was subjected to a χ^2 test, which indicated whether there was a significant effect of the isolate genotype on IT. When at least three classes of avirulent ITs could be distinguished, a linear effect of isolate on IT was fitted first to test whether progeny varied significantly in their mean IT, and then the IT was fitted as a factor, to test whether there was significant residual variation in the numbers of leaves of each isolate with each IT, possibly because some isolates were more variable than others in their expression of IT. This kind of analysis was performed only on the expression of avirulence, not on the expression of virulence, because virulence was expressed mostly by one IT (IT 4), and in cases in which more than one virulent IT was observed, it was not possible to pool ITs to raise all expected frequencies above 0.5.

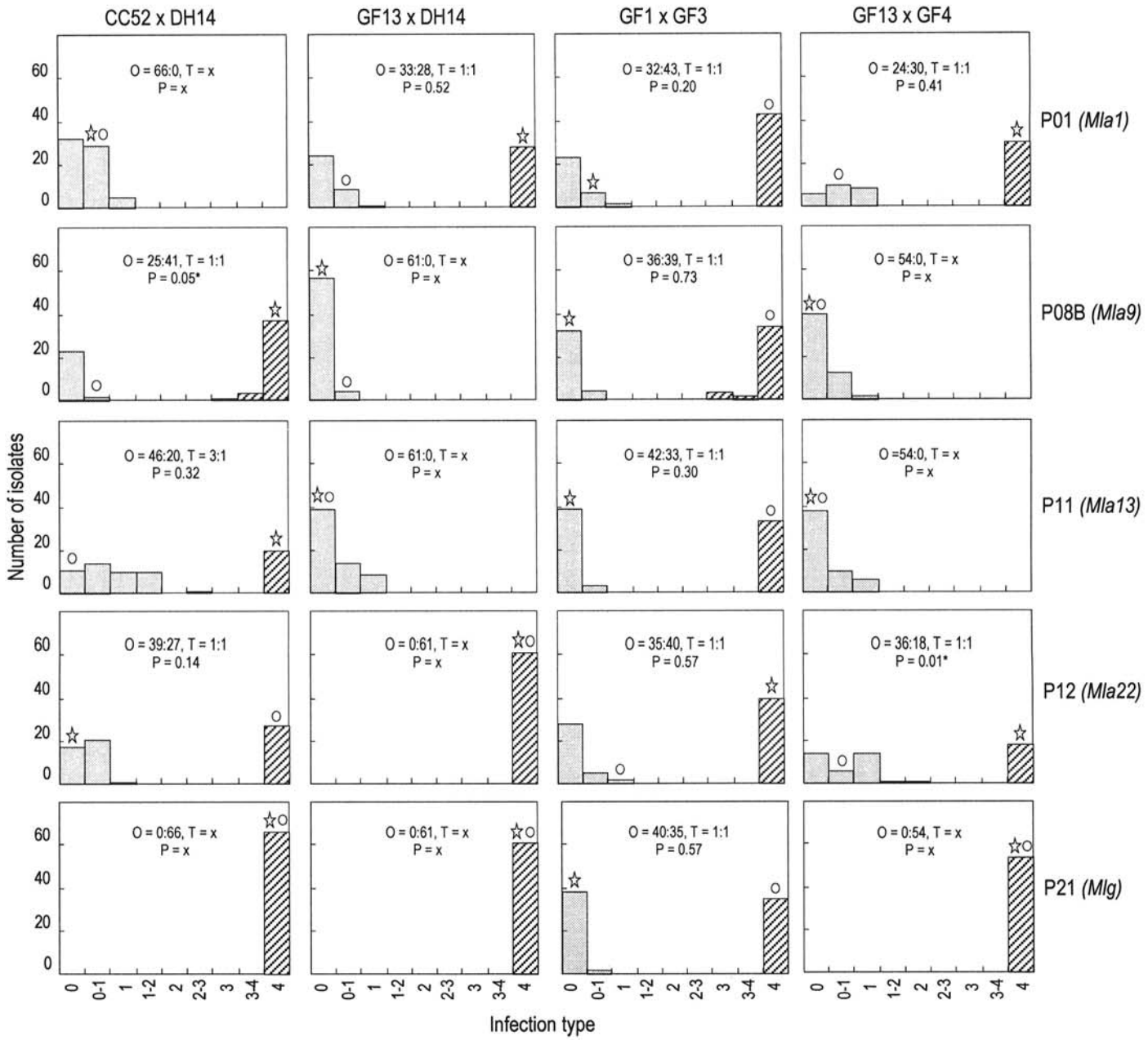

Fig. 1. Distribution of infection types (ITs) among ascospore progeny in four crosses of *Erysiphe graminis* f. sp. *hordei* isolates—CC52 × DH14, GF13 × DH14, GF1 × GF3, and GF13 × GF4—on five near-isogenic barley lines of Pallas that give strong resistance reactions (ITs of 0 to 1). Columns with dot patterns contain avirulent isolates; columns with bar patterns contain virulent isolates. In each cross, P1 × P2, the P1 parent has the same mating type as CC52, and the P2 parent has the same mating type as DH14; a star above a column shows the IT of P1; and a circle above a column shows the IT of P2. O = observed segregation ratio of avirulent/virulent progeny isolates; T = theoretical segregation ratio of avirulent/virulent progeny isolates; P = *P* value of the χ^2 test (1 df) for adequation of data to the theoretical ratio; * = significant difference between the observed and the theoretical ratio, with $P < 0.05$; and x = no segregation of avirulence and virulence.

RESULTS

Control of avirulence. *Initial crosses.* The range of variation of incompatible ITs on each Pallas line was, on the whole, wider in the F_1 progeny than in the avirulent parents. Incompatible ITs, ranging from 0 to 1, were observed on P01, P08B, P11, P12, and P21 (Fig. 1), except for cross CC52 × DH14 on P11, in which four classes of incompatible ITs (0, 0-1, 1, and 1-2) were observed. On P01, P11, P12 and P21, virulent progeny isolates all gave IT 4, as did the virulent parent. On P08B, the range of compatible ITs was wider (ITs 3 to 4) than in the virulent parents (IT 4), but there was a clear separation between avirulent and virulent progeny isolates, and few isolates had IT 3 or 3-4.

Incompatible ITs, ranging from 0-1 to 3, were observed on P09, P10, P16, P23, and P04B (Fig. 2). In most cases, incompatible ITs were distributed around a most frequent IT. From one cross to another, only small differences were observed in the range of variation of incompatible ITs on the same Pallas line. On P09 and P10, the range of compatible ITs was wider (ITs 3 to 4) than in the virulent parents (IT 4), but there was a clear separation between avirulent and virulent progeny isolates, and few isolates had IT 3 or 3-4. On P16, P23, and P04B, however, the separation between avirulent and virulent progeny isolates was more difficult to discern, with ITs from 1 to 4 appearing among the progeny. On P16 and P23, isolates with IT 3-4 were infrequent, but there was no indication as to whether these isolates should be considered virulent or

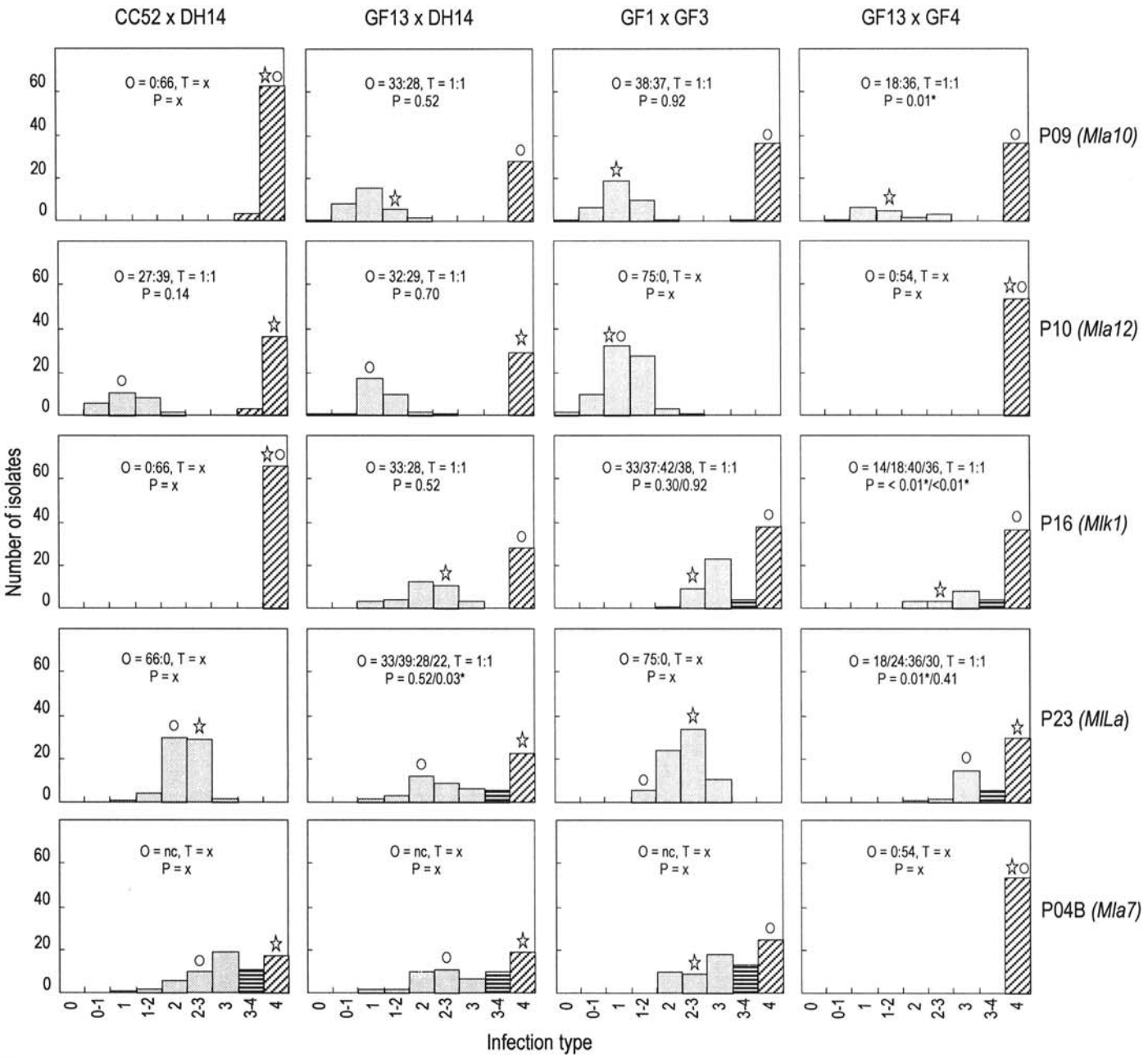

Fig. 2. Distribution of infection types (ITs) among ascospore progeny in four crosses of *Erysiphe graminis* f. sp. *hordei* isolates—CC52 × DH14, GF13 × DH14, GF1 × GF3, and GF13 x GF4—on five near-isogenic barley lines of Pallas that give moderate resistance reactions (ITs of 0-1 to 3). Columns with dot patterns contain avirulent isolates; columns with diagonal bar patterns contain virulent isolates; and columns with horizontal bar patterns contain isolates that cannot be classified as avirulent or virulent. In each cross, P1 × P2, the P1 parent has the same mating type as CC52, and the P2 parent has the same mating type as DH14; a star above a column shows the IT of P1; and a circle above a column shows the IT of P2. O = observed segregation ratio of avirulent/virulent progeny isolates; T = theoretical segregation ratio of avirulent/virulent progeny isolates; P = *P* value of the χ^2 test (1 df) for adequation of data to the theoretical ratio; * = significant difference between the observed and the theoretical ratio, with $P < 0.05$; x = no segregation of avirulence and virulence; and nc = segregation ratio not calculated because there was no reliable classification of avirulent and virulent. On P16 and P23, the observed ratio of avirulent/virulent progeny isolates and the *P* value were calculated in two ways (i)/(ii): (i) isolates with IT 3-4 were considered virulent, and (ii) isolates with IT 3-4 were considered avirulent.

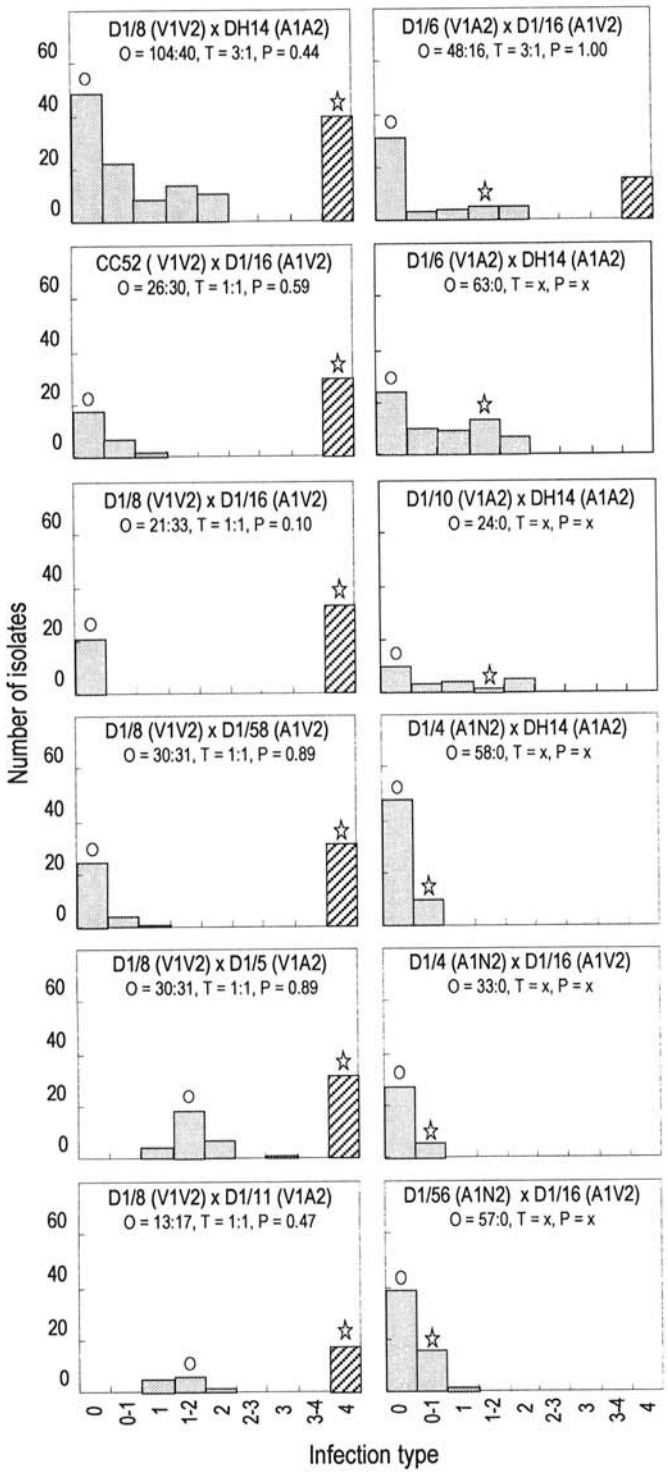

Fig. 3. Distribution of infection types (ITs) among ascospore progeny in backcrosses and sibcrosses of *Erysiphe graminis* f. sp. *hordei* progeny isolates from cross CC52 × DH14 on the near-isogenic barley line of Pallas P11 (*Mla13*). Columns with dot patterns contain avirulent isolates; columns with bar patterns contain virulent isolates. In each cross, P1 × P2, the P1 parent has the same mating type as CC52, and the P2 parent has the same mating type as DH14; a star above a column shows the IT of P1; a circle above a column shows the IT of P2; and the genotypes of P1 and P2 are in brackets, with A1/V1 and A2/V2 for the avirulence/virulence alleles of $Avr_{a13}1$ and $Avr_{a13}2$, respectively. N = genotype not determined; O = observed segregation ratio of avirulent/virulent progeny isolates; T = theoretical segregation ratio of avirulent/virulent progeny isolates; P = *P* value of the χ^2 test (1 df) for adequation of data to the theoretical ratio; and x = no segregation of avirulence and virulence.

avirulent. The analyses of segregation were performed with IT 3-4 considered both as an avirulent IT, and as a virulent IT. On P04B, isolates producing IT 3-4 were frequent, and a clear separation between avirulent and virulent isolates could not be established.

All F_1 progeny isolates derived from crosses in which both parents were avirulent on a particular Pallas line also were avirulent on that line, and all progeny isolates of crosses between two virulent parents were virulent. On each of the 10 Pallas lines, the progeny segregated for avirulence in at least one of the initial crosses. On P11 (Fig. 1), the progeny of CC52 × DH14 had a segregation of 3:1 avirulent/virulent, suggesting the presence of two avirulence genes, $Avr_{a13}1$ and $Avr_{a13}2$, whereas the progeny of GF1 × GF3 had a segregation of 1:1 avirulent/virulent, indicating that a single gene was segregating in this cross. For crosses CC52 × DH14, GF13 × DH14, and GF1 × GF3, segregations of 1:1 avirulent/virulent were observed on P01, P08B, P12, and P21 (Fig. 1) and on P09, P10, and P16 (Fig. 2) in all crosses in which phenotypes segregated, suggesting the presence of one avirulence gene, Avr_{a1}, Avr_{a9}, Avr_{a22}, Avr_g, Avr_{a10}, Avr_{a12}, and Avr_{k1}, respectively, matching the resistance gene in each Pallas line. On P16, whether isolates with IT 3-4 were classified as avirulent or virulent did not significantly alter the fit to a theoretical segregation ratio. On P23, however, the segregation was either 1:1 or 3:1 avirulent/virulent, depending on the classification of avirulence and virulence. Therefore, we could not reach a definite conclusion about the presence of one or two avirulence genes. On P01, the 1:1 segregation ratio was confirmed in cross GF13 × GF4. On P09, P12, and P16, deviations from the 1:1 avirulent/virulent ratio were observed, however, in this cross, with an excess of isolates virulent on P09 and P16 and avirulent on P12. The isolates in excess had the same phenotypes as parent GF4 on P09, P16, and P12.

Backcrosses and sibcrosses of progeny of CC52 × DH14. In the progeny of CC52 × DH14, mating type segregated in a 1:1 ratio, with 9 of the 18 progeny tested being of each mating type. Five backcrosses and eight sibcrosses were analyzed. The cleistothecia of *E. graminis* f. sp. *hordei* produced in the greenhouse contained 10 to 20 asci, and each ascus contained 8 ascospores, as observed in cleistothecia from the field. The efficiency of the recovery of progeny varied from 0.4 to 4.5 ascospores per cleistothecium, with a mean of 1 ascospore per cleistothecium. Variation in the fertility of cleistothecia among crosses was not related to the segregation of avirulence genes. The germination rate of ascospores was 72% in cross D1/8 × D1/16, 77% in cross D1/8 × DH14, and 82% in cross D1/4 × D1/16. The distribution of ITs was analyzed on the five Pallas isolines that showed a segregation of avirulence in the initial cross, CC52 × DH14.

The 3:1 segregation of avirulence/virulence toward P11 in cross CC52 × DH14 was confirmed with the backcross of the virulent isolate, D1/8 (IT 4), to the avirulent parent, DH14 (IT 0), which segregated with ITs 0 to 2 and IT 4 (Fig. 3). This suggested that there are indeed two independent genes that control avirulence toward *Mla13* resistance in this line. One gene, named $Avr_{a13}1$ by us and Avr_{a13} by Brown and Simpson (6), conferred IT 0 or 0-1 in an incompatible interaction. The second, named $Avr_{a13}2$ by us and Avr_{Ru3} by Brown and Simpson (6), conferred IT 1-2 or 2 in the absence of $Avr_{a13}1$. This hypothesis was tested by other backcrosses and sibcrosses.

In the backcross of isolate D1/16, which had IT 0, to virulent parent CC52, and in the sibcross of D1/16 to virulent isolate D1/8, the progeny segregated with ITs 0 to 1 and IT 4, with an avirulent/virulent ratio that was not significantly different from 1:1. The same segregation was observed in the sibcross of avirulent isolate D1/58 (IT 0) to virulent isolate D1/8. One gene, presumed to be $Avr_{a13}1$, segregated in these three crosses. In two sibcrosses, D1/8 × D1/5 and D1/8 × D1/11, in which one parent had IT 1-2 and the other had IT 4, progeny segregated with ITs 1 to 2 and IT 4, with an avirulent/virulent ratio that was not significantly different from 1:1. One gene, presumably $Avr_{a13}2$, therefore, segregated

in these sibcrosses. D1/16, with IT 0 presumably conferred by avirulence allele $Avr_{a13}1$, was crossed with D1/6, with IT 1-2 presumably conferred by avirulence allele $Avr_{a13}2$. The progeny of this cross between two avirulent parents were either avirulent, with ITs from 0 to 2, or virulent, with IT 4, and fitted a 3:1 avirulent/virulent ratio. This result definitively proved that two independent genes were segregating, giving two ITs. D1/16 carried the single avirulence allele $Avr_{a13}1$ and D1/6 carried the single avirulence allele $Avr_{a13}2$.

In backcrosses of isolates D1/6 and D1/10, which had IT 1-2, to avirulent parent DH14, which had IT 0, progeny isolates had ITs 0 to 2. No virulent progeny isolate was recovered, suggesting that at least one avirulence gene was not segregating. The segregation of one avirulence gene could explain progeny isolates that had the same ITs as their parents (1:1 ratio of ITs 0 and 0-1:1-2 and 2) but with a larger range of variation (presence of isolates with IT 1, which cannot be classified in the two parental ITs) due to variation in the expression of avirulence. Because DH14 is presumed to have both avirulence alleles $Avr_{a13}1$ and $Avr_{a13}2$, isolates D1/6 and D1/10, which were backcrossed to DH14, also may have the avirulence allele $Avr_{a13}2$, which conferred IT 2, but lack the avirulence allele $Avr_{a13}1$. In backcross D1/4 × DH14 and two sibcrosses, D1/4 × D1/16 and D1/56 × D1/16, in which both parents had IT 0 or 0-1, all progeny had ITs ranging from 0 to 1. This suggests that all parents of this crosses had the avirulence allele $Avr_{a13}1$, which conferred the low IT. In addition, we checked that the progeny of the sibcross between the two virulent isolates, D1/8 × D1/13, did not segregate, with all progeny isolates having IT 4 (data not shown). Avr_{a13} genotypes of the parental and progeny isolates are shown in Figure 3.

We tried to follow the same procedure with P04B as with P11 (Fig. 4). In the sibcrosses of avirulent isolates D1/6 (IT 2), D1/4 (IT 2), D1/56 (IT 2), and D1/8 (IT 3) to virulent isolate D1/16 (IT 4) and in the sibcross of D1/8 (IT 3) to virulent isolate D1/58 (IT 4), the progeny had ITs from 1-2, 2, 2-3, or 3 to 4, depending on the cross. The progeny of sibcrosses with D1/8 had the higher ITs, from 3 to 4. This result suggested that at least one avirulence gene segregated. In all of these sibcrosses, the number of isolates with IT 3-4 was high compared to the number of isolates with ITs 1-2 to 3 or IT 4. Therefore, it was not possible to distinguish two classes of avirulence and virulence or to determine whether one or two avirulence genes were segregating. In backcrosses of avirulent isolates D1/4 (IT 2), D1/6 (IT 2), D1/10 (IT 2-3), and D1/8 (IT 3) to the avirulent parent, DH14 (IT 2), the progeny isolates had ITs 1 or 2 to 2-3 or 3-4. No progeny isolate was virulent, suggesting at least one avirulence gene was not segregating. Again, the progeny of the backcross with D1/8 had the higher ITs, with some isolates having IT 3-4, which were not found in the other backcrosses.

In two sibcrosses of D1/8 to avirulent isolates D1/11 (IT 2-3) and D1/13 (IT 3), which had a higher IT than DH14, progeny isolates had ITs 1-2 or 2 to 4, distributed around IT 3. The number of isolates with IT 4 was low. This distribution of ITs among the progeny suggested, as in the backcross of D1/8 to DH14, that at least one avirulence gene was not segregating. In this case, IT 4 did not result from the expression of virulence but could be explained as variation in the expression of avirulence. In the sibcross D1/8 (IT 3) × D1/5 (IT 3-4), the progeny isolates had ITs 2 to 4. The three classes, ITs 3, 3-4, and 4 occurred at high frequencies, so it was not possible to determine whether IT 4 resulted from the expression of avirulence or virulence, i.e., to determine whether an avirulence gene was segregating. The backcross of D1/16 (IT 4) to virulent parent CC52 (IT 4) produced only virulent (IT 4) progeny isolates (data not shown).

On P08B, P10, and P12, the distributions of ITs among ascospore progeny were similar to the distributions observed in the initial crosses (data not shown). On P08B and P12, the incompatible ITs varied from 0 to 1 or 1-2. Rare progeny with ITs 3 and 3-4 were recovered and were considered virulent. On P10, the range of incompatible ITs was from 0-1 to 2-3. All virulent progeny isolates had IT 4. On P08B, P10, and P12 (Table 2), the progeny of crosses between virulent parents were virulent, and the progeny

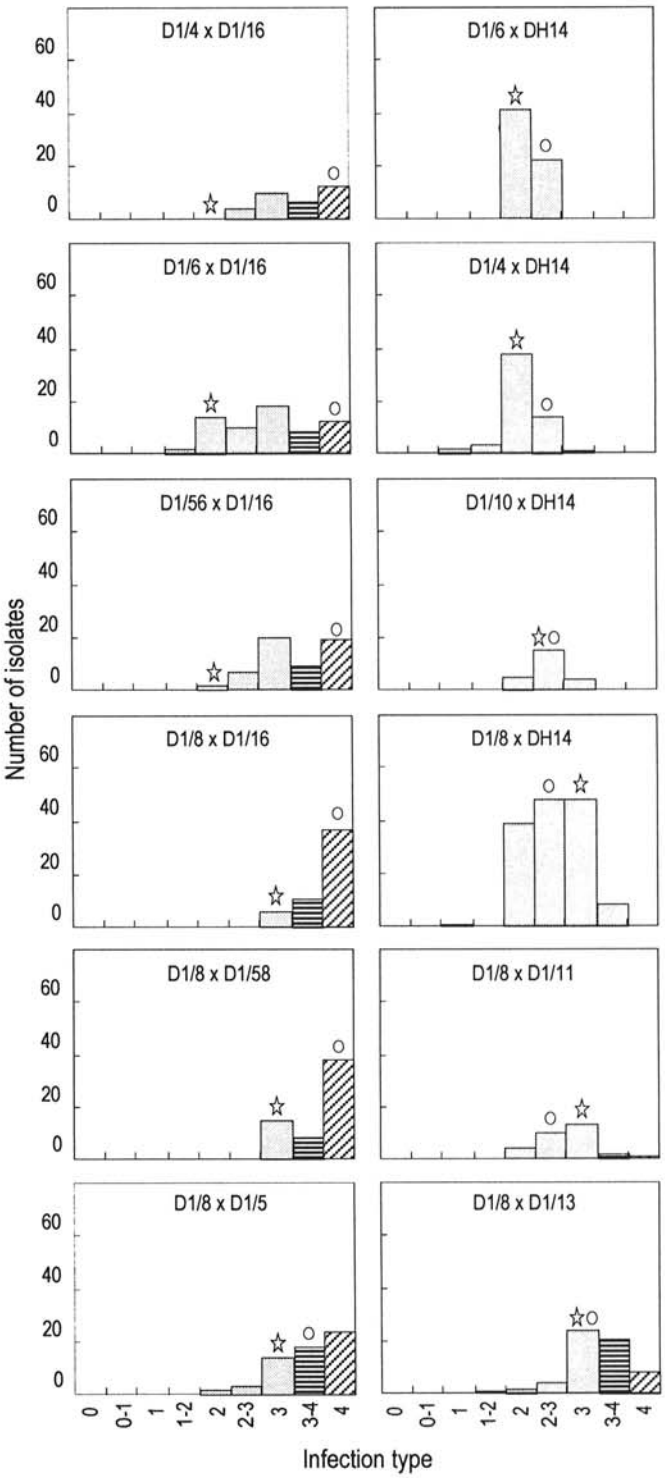

Fig. 4. Distribution of infection types (ITs) among ascospore progeny in backcrosses and sibcrosses of *Erysiphe graminis* f. sp. *hordei* progeny isolates from cross CC52 × DH14 on the near-isogenic barley line of Pallas P04B (*Mla7*). Columns with dot patterns contain avirulent isolates; columns with diagonal bar patterns contain virulent isolates; and columns with horizontal bar patterns contain isolates that cannot be classified as avirulent or virulent. In each cross, P1 × P2, the P1 parent has the same mating type as CC52, and the P2 parent has the same mating type as DH14; a star above a column shows the IT of P1; and a circle above a column shows the IT of P2. The segregation ratio was not calculated because there was no reliable classification of avirulent and virulent.

of crosses between avirulent parents were avirulent. The progeny of crosses between avirulent and virulent parents segregated in a 1:1 ratio of avirulent/virulent, as in the progeny of parental cross CC52 × DH14, confirming the existence of one gene for avirulence on each line: Avr_{a9}, Avr_{a12}, or Avr_{a22}. Two exceptions were found, with segregations that deviated from the 1:1 ratio—one on P08B and the other on P12—but such exceptions were not sufficient to reject the hypothesis of one avirulence gene.

Linkage between avirulence genes. The independence of each pair of avirulence genes was tested for each cross (7). Significant linkages were observed for seven pairs of virulence genes (Table 3). Among the six segregating avirulence genes in CC52 × DH14, significant linkage was observed between Avr_{a9} and Avr_{a22}, as in the five backcrosses and four sibcrosses in which the two genes segregated. The distributions of classes AA, AV, VA, and VV were homogeneous ($P > 0.05$) in all the crosses, and the estimated recombination frequency was 12% based on the pooled data.

Linkage between $Avr_{a13}1$ and Avr_{a9} was significant in crosses in which $Avr_{a13}1$ segregated without $Avr_{a13}2$, with an estimated recombination frequency of 41% based on the pooled data (CC52 × D1/16, D1/8 × D1/16, and D1/8 × D1/58). The analysis of the gene order from cross D1/8 × D1/16, in which three genes, $Avr_{a13}1$, Avr_{a9}, and Avr_{a22}, segregated, indicated that Avr_{a9} was situated between $Avr_{a13}1$ and Avr_{a22}. The linkage between $Avr_{a13}1$ and Avr_{a9}, however, was not significant ($P = 0.27$) in crosses CC52 × DH14, D1/8 × DH14, and D1/16 × DH14, in which both $Avr_{a13}1$ and $Avr_{a13}2$ segregated. The estimated recombination frequency based on the pooled data of the six crosses was 45%, with Avr_{a9} and $Avr_{a13}1$ linked in coupling in all crosses.

In cross GF13 × DH14, no recombinant was found between Avr_{a10} and Avr_{k1}. There was an excess of recombinant progeny isolates for gene pairs Avr_{a1}/Avr_{a10} and Avr_{a1}/Avr_{k1} ($\chi^2 = 4.57$, 1 df, $P = 0.03$). In cross GF1 × GF3, genes Avr_{a10}, Avr_{a22}, and Avr_{k1} were very closely linked. There also was linkage between Avr_{a9} and these

TABLE 2. Segregation of avirulence in backcrosses and sibcrosses of *Erysiphe graminis* f. sp. *hordei* progeny isolates of CC52 × DH14 on Pallas barley lines P08B (*Mla9*), P10 (*Mla12*), and P12 (*Mla22*)

Crosses		P08B					P10					P12				
		IT[b]		Genetic ratio			IT		Genetic ratio			IT		Genetic ratio		
P1[a]	P2[a]	P1	P2	A[c]	V[c]	*P*[d]	P1	P2	A	V	*P*	P1	P2	A	V	*P*
D1/4	DH14	0	0-1	58	0	...[e]	2	1	58	0	...	0	4	25	33	0.29
D1/6	DH14	4	0-1	24	39	0.06	1-2	1	63	0	...	0-1	4	39	24	0.06
D1/8	DH14	4	0-1	71	73	0.86	4	1	65	79	0.24	0-1	4	65	79	0.24
D1/10	DH14	4	0-1	13	11	0.68	1-2	1	24	0	...	0-1	4	10	14	0.41
CC52	D1/16	4	0-1	25	31	0.42	4	4	0	56	...	0	4	30	26	0.59
D1/4	D1/16	0	0-1	33	0	...	2	4	14	19	0.38	0	4	10	23	0.02*
D1/6	D1/16	4	0-1	36	28	0.32	1-2	4	34	30	0.62	0-1	4	27	37	0.21
D1/8	D1/5	4	4	0	61	...	4	4	0	61	...	0-1	4	29	32	0.70
D1/8	D1/11	4	4	0	30	...	4	4	0	30	...	0-1	0	30	0	...
D1/8	D1/13	4	0	22	38	0.04*	4	2	28	32	0.60	0-1	4	35	25	0.20
D1/8	D1/16	4	0-1	21	33	0.10	4	4	0	54	...	0-1	4	30	24	0.41
D1/8	D1/58	4	0	23	38	0.05	4	4	0	61	...	0-1	4	33	28	0.52
D1/56	D1/16	0	0-1	57	0	...	4	4	0	57	...	4	4	0	57	...

[a] P1: parent 1, with same mating type as CC52. P2: parent 2, with same mating type as DH14.
[b] Infection type.
[c] A = avirulent; V = virulent; on P08B, A = IT 0 to 1 and V = IT 3 to 4; on P10, A = IT 0 to 2-3 and V = IT 3 to 4; and on P12, A = IT 0 to 1-2 and V = IT 4.
[d] *P* value of the χ^2 test (1 df) for a 1:1 segregation ratio of avirulent/virulent; * indicates $P < 0.05$.
[e] Not calculated because there was no segregation of avirulence or virulence.

TABLE 3. Linkages between two avirulence genes, significant at $P < 0.05$ by a χ^2 test, for crosses between *Erysiphe graminis* f. sp. *hordei* isolates CC52 × DH14, GF13 × DH14, and GF3 × GF1

Gene 1[a]	Gene 2	AA	AV	VA	VV	*P*[b]	*r*(%)[c]	m(cM)[d]	CI[e] (±)
CC52 × DH14									
Avr_{a9}[f]	Avr_{a22}	25	235	283	49	<0.01	12.5	12.7	2.7
Avr_{a9}[g]	$Avr_{a13}1$	38	39	31	63	0.03	40.9	57.5	7.6
GF13 × DH14									
Avr_{a10}	Avr_{k1}	33	0	0	28	<0.01	0.0	...[h]	...
GF1 × GF3									
Avr_{a9}	Avr_{a10}	30	6	8	31	<0.01	18.7	19.6	9.9
Avr_{a9}	Avr_{a22}	6	30	29	10	<0.01	21.3	22.8	10.5
Avr_{a9}	Avr_{k1}	25/29	11/7	8	31	<0.01	25.3/20.0	27.9/21.2	10.2/9.3
Avr_{a10}	Avr_{a22}	0	38	35	2	<0.01	2.7	2.7	3.9
Avr_{a10}	Avr_{k1}	33/37	5/1	0	37	<0.01	6.7/1.3	6.7/1.3	6.1/2.6
Avr_{a22}	Avr_{k1}	0	35	33/37	7/3	<0.01	4.0/9.3	4.0/9.4	4.7/7.1

[a] In all crosses in which the two avirulence genes segregated, there was no significant linkage for the following pairs: Avr_{a1}/Avr_{a9}, Avr_{a1}/Avr_{a12}, $Avr_{a1}/Avr_{a13}1$, Avr_{a1}/Avr_{a22}, Avr_{a1}/Avr_{k1}, Avr_{a1}/Avr_{g}, Avr_{a9}/Avr_{a12}, Avr_{a9}/Avr_{g}, Avr_{a10}/Avr_{a12}, $Avr_{a10}/Avr_{a13}1$, Avr_{a10}/Avr_{g}, Avr_{a12}/Avr_{a22}, Avr_{a12}/Avr_{k1}, $Avr_{a13}1/Avr_{a22}$, $Avr_{a13}1/Avr_{k1}$, $Avr_{a13}2/Avr_{a22}$, and Avr_{k1}/Avr_{g}.
[b] *P* value for the χ^2 test of independence on the contingency table: AA, AV, VA, and VV. A = avirulent and V = virulent—defined as in Figures 1 and 2. For pairs including Avr_{k1}, the observed classes AA, AV, VA, and VV, the recombination frequency (*r*), the map distance (m), and the confidence interval (CI) were calculated in two ways (i)/(ii): (i) isolates with IT 3-4 were considered virulent, and (ii) isolates with IT 3-4 were considered avirulent.
[c] Recombination frequency.
[d] Map distance (in centimorgans), calculated by Kosambi's function.
[e] Confidence interval (CI) of the map distance.
[f] Cross CC52 × DH14, five backcrosses, and four sibcrosses of progeny isolates from CC52 × DH14, which segregated for both genes, were grouped.
[g] Backcross CC52 × D1/16 and sibcrosses D1/8 × D1/16 and D1/8 × D1/58, which segregated for Avr_{a9} and $Avr_{a13}1$, but not $Avr_{a13}2$, were grouped.
[h] Not calculated because there was no recombinant progeny isolate.

three genes. The estimation of the gene order indicated that Avr_{a10} was situated between Avr_{a22} and Avr_{a9}. It was not possible to determine the relative position of Avr_{k1}, because the estimation of the recombination frequency between Avr_{k1} and the other genes depended on the classification of isolates with IT 3-4 on *Mlk1*. No linkage was observed between Avr_{a9} and $Avr_{a13}1$. There was an excess of recombinant progeny isolates for gene pairs Avr_g/Avr_{a22} (χ^2 = 4.04, 1 df, P = 0.04) and $Avr_g/Avr_{a13}1$ (χ^2 = 4.21, 1 df, P = 0.04). In cross GF13 × GF4, linkages were not estimated, because biased segregations were suspected for several avirulence genes.

Variation in the expression of avirulence in the progeny isolates from cross CC52 × DH14. Variation in the expression of avirulence on P01 (*Mla1*), P10 (*Mla12*), P11 (*Mla13*), P12 (*Mla22*), and P23 (*MlLa*) was analyzed, taking into account ITs of individual leaf segments and not the score characterizing the isolate as a whole (Table 4). Variation in the expression of avirulence on P04B (*Mla7*) could not be analyzed because it was not possible to classify the isolates as avirulent or virulent. On P08B (*Mla9*), only two avirulent ITs were observed among the progeny isolates (ITs 0 and 1). The expected values for IT 1 were below 0.5, so it was not possible to do an analysis. Two classes of ITs could be analyzed on *Mla1* (ITs 0 and 1) and *Mla22* (IT 0 and pooled ITs 1 and 2). Three classes of ITs could be analyzed on *Mla12* (IT 0; IT 1; and pooled ITs 2 and 3), *Mla13* (IT 0; IT 1; and pooled ITs 2 and 3), and *MlLa* (pooled ITs 0, 1; IT 2; and pooled ITs 3 and 4).

On *Mla13*, there was a significant linear effect of isolate on IT (significant interaction between IT treated as a linear variable and isolate, ITL.ISO) and no significant residual variation (no significant interaction between IT treated as a factor and isolate, ITF.ISO), indicating that some isolates had a higher mean IT than others. This presumably reflected the segregation of the two avirulence genes, $Avr_{a13}1$ and $Avr_{a13}2$, producing different incompatible ITs, even though distinct classes of avirulent progeny could not be discerned. On *Mla12*, there was also a significant linear effect (ITL.ISO), which indicated that some isolates had a higher mean IT than others and, therefore, that a minor gene affecting the expression of the IT might have been segregating. However, there also was significant residual variation (ITF.ISO), so this conclusion should be treated with caution. On *MlLa*, the mean IT of isolates did not vary significantly (nonsignificant ITL.ISO), but some isolates had more variable ITs than others (significant ITF.ISO). On *Mla1* and *Mla22*, the mean ITs of the isolates varied significantly, but because there were only two classes of IT, it was not possible to conclude whether it was because isolates differed in their mean IT or in the variability of expression of their IT.

DISCUSSION

Segregation of avirulence genes toward *Mla13*. On P11, the results of backcrosses and sibcrosses confirmed the existence of the two avirulence genes, as suggested previously by analysis of an F_1 (6). We found that one of the avirulence genes, $Avr_{a13}1$, produced IT 0 or 0-1 and the other, $Avr_{a13}2$, produced IT 1-2 or 2; $Avr_{a13}1$ was epistatic to $Avr_{a13}2$. Brown and Simpson (6) separated avirulent progeny into two distinct classes in cross CC52 × DH14 on cv. Rupal, which carries *Mla13*. In our study, two distinct classes of avirulent progeny could be distinguished only in the backcrosses and sibcrosses, not in the F_1 progeny of CC52 × DH14. This discrepancy might come from differences in environmental conditions or from the use of different cultivars that carry *Mla13*. Isolates of each possible genotype were identified in our study. Isolate DH14 (IT 0) carried both avirulence alleles. Isolate D1/16 (IT 0) carried only avirulence allele $Avr_{a13}1$, whereas isolate D1/6 (IT 2) carried only avirulence allele $Avr_{a13}2$. Isolates CC52 carried none of the avirulence alleles. Isolate GF1 was presumed to have only avirulence allele $Avr_{a13}1$.

Two hypotheses may explain the segregation of two avirulence genes on P11: (i) P11 carries a second gene, *Mla(Ru3)*, in addition to *Mla13*, and each resistance gene matches one avirulence gene; or (ii) P11 carries the single resistance gene *Mla13*, which matches the two avirulence genes. It has been suggested that the existence of two pathogen avirulence genes that match the resistance of barley cultivars with *Mla13* implies that there is a second resistance gene, *Mla(Ru3)*, closely linked to *Mla13* (17). Brown and Simpson (6) gave the two avirulence genes the names Avr_{a13} and Avr_{Ru3}, following the gene-for-gene relationship. However, the possibility that two avirulence genes match a single resistance gene cannot be excluded. Such a situation was demonstrated for *Pseudomonas syringae* on *Arabidopsis thaliana* by molecular cloning (2) and suggested for *Melampsora lini* (28) and *Puccinia recondita* (29). Genetic analysis of the host and tests with isolates of known genotypes may help in distinguishing these hypotheses, as has been done for *Mla6* and *Mla14*, which are two closely linked genes at the *Mla* locus (21). Because the number of resistance genes in P11 is unknown, we prefer to name the avirulence genes $Avr_{a13}1$ and $Avr_{a13}2$.

Segregation of avirulence genes toward *Mla7*. On P04B (*Mla7*), we could not reach a conclusion as to whether there was one or two avirulence genes, because the distributions of compatible and incompatible ITs overlapped, with many progeny isolates having IT 3-4. On cv. Porter, with *Mla7*, Brown and Simpson (6) found

TABLE 4. Variation in the infection types (ITs) of avirulent *Erysiphe graminis* f. sp. *hordei* progeny isolates of CC52 × DH14, analyzed by generalized linear modeling

Pallas barley line (resistance allele)	Linear effect of isolate on avirulent ITs				Residual variation of avirulent ITs			
	Source[a]	df	Deviance	P[b]	Source	df	Deviance	P
P11 (*Mla13*)	ISO	45	43.06					
	ITL	1	27.84		ITF	1	105.71	
	ITL.ISO	45	234.08	<0.01	ITF.ISO	45	45.48	0.45
P10 (*Mla12*)	ISO	26	25.84					
	ITL	1	14.22		ITF	1	71.52	
	ITL.ISO	26	42.60	0.02	ITF.ISO	26	42.81	0.02
P23 (*MlLa*)	ISO	65	67.78					
	ITL	1	24.14		ITF	1	224.33	
	ITL.ISO	65	50.66	0.90	ITF.ISO	65	98.90	<0.01
P01 (*Mla1*)	nt[c]				ISO	65	69.40	
					ITF	1	45.37	
					ITF.ISO	65	115.86	<0.01
P12 (*Mla22*)	nt				ISO	37	68.43	
					ITF	1	58.38	
					ITF.ISO	37	67.31	<0.01

[a] Source of variation: ISO = isolate; ITF = IT treated as a factor; and ITL = IT treated as a linear variable.
[b] P value of the χ^2 test under the hypothesis of no interaction of ITL.ISO or no interaction of ITF.ISO.
[c] The linear effect of the isolate on avirulent ITs could not be tested, because there were only two classes of avirulent ITs.

one avirulence gene segregating in cross CC52 × DH14. However, the analysis of another cross, CC107 × DH14 (3), indicated that DH14 had two avirulence genes, $Avr_{a7}1$ and $Avr_{a7}2$, that conferred distinct classes of incompatible ITs. Cv. Porter conferred higher incompatible ITs than Pallas lines P04A and P04B, which themselves conferred higher incompatible ITs than Pallas lines P05 and P06; these lines all carry the resistance gene *Mla7* but are derived from different sources. It is possible that two avirulence genes segregated in CC52 × DH14, but they were not distinguishable on cv. Porter (6) and produced overlapping classes of avirulence and virulence on line P04B (current study). The comparison of progeny of crosses with isolates differing in the degree of incompatible IT indicated that isolate D1/8 conferred higher incompatible ITs in the progeny than D1/4, D1/6, or D1/56. In addition to the effects of the host genotype in which *Mla7* is included, there might be an effect of the pathogen genotype defined by the Avr_{a7} gene or genes.

Segregation of other avirulence genes and linkages. For each of lines P01, P08B, P09, P10, P12, P16, and P21, there was one class of avirulence, with some variation in the expression of the ITs. Each of these lines carried one resistance allele: *Mla1*, *Mla9*, *Mla10*, *Mla12*, *Mla22*, *Mlk1*, and *Mlg*, respectively. Our results confirmed the existence of a single avirulence gene matching each of these resistance genes (3,6,9,16). The existence of two or three avirulence genes matching *Mla9* and *Mla10* has been suggested (17). The isolates we tested carried only one of the avirulence alleles. On *MlLa*, the segregation of a single avirulence gene was shown previously (3), whereas we could not conclude whether one or two avirulence genes segregated.

In cross GF13 × GF4, we observed biased segregation for the three genes, Avr_{a10}, Avr_{a22}, and Avr_{k1}, that were linked in GF1 × GF3. In some cases, an excess of recombinant progeny isolates also was observed. This suggests a differential viability among parental and recombinant genotypes, which could explain why the linkage was sometimes not consistent among crosses. However, in most cases, genetic linkages were observed in several crosses, which makes the interpretation reliable. One linkage group was described, containing Avr_{a9}, Avr_{a10}, Avr_{a22}, Avr_{k1}, and probably $Avr_{a13}1$.

The linkage between Avr_{a9} and Avr_{a22} observed from backcrosses and sibcrosses with progeny isolates of CC52 × DH14 was consistent with previous results (6). The linkage between Avr_{a9} and Avr_{a22} was confirmed in another cross, GF1 × GF3. Brown and Simpson (6) found linkages between $Avr_{a13}2$ and Avr_{a12} and between $Avr_{a13}1$ and Avr_{a9}, with recombination frequencies of 34 and 39%, respectively. We did not detect any linkage between $Avr_{a13}2$ and Avr_{a12} in the current study. Three of seven crosses in which $Avr_{a13}1$ and Avr_{a9} segregated confirmed the existence of significant linkage between $Avr_{a13}1$ and Avr_{a9}. Our results agree with the gene order estimated by Brown and Simpson (6): Avr_{a22}-Avr_{a9}-$Avr_{a13}1$. This linkage, however, was not observed in all crosses, which suggests that the genetic distance between $Avr_{a13}1$ and Avr_{a9} is at the limit of detection. Other markers situated between both genes will be needed to confirm this linkage.

In crosses GF1 × GF3 and GF13 × GF4, we found linkages that have been described previously, Avr_{a10} and Avr_{k1} (3,16), Avr_{a10} and Avr_{a22} (9), and Avr_{a9} and Avr_{a10} (25). We confirmed that there was no linkage between Avr_{a10} and Avr_{a12} (9). The absence of linkage between Avr_{a1} and Avr_{a12} and between Avr_{a1} and Avr_{a13} has not been reported before. We found, in addition, that Avr_{a10} is situated between Avr_{a22} and Avr_{a9} and confirmed that Avr_{k1} is situated between Avr_{a22} and Avr_{a9}. However, we could not determine the relative position of Avr_{a10} and Avr_{k1}. Thus, the order of the five genes was estimated as Avr_{a22}-(Avr_{a10}, Avr_{k1})-Avr_{a9}-$Avr_{a13}1$.

Variation in expression of ITs and avirulence genes. In barley powdery mildew, it has been suggested that the existence of several ITs on a single cultivar implies the existence of several avirulence genes (14,17). However, there has been no clear proof supporting this hypothesis, because, as far as we are aware, analysis of the backcrosses and sibcrosses needed for testing hypotheses generated by analysis of F_1 generations has not been done previously for powdery mildew, although it was shown to be necessary for other haploid pathogens, such as *Magnaporthe grisea* (27).

In the current study, the analysis of backcrosses and sibcrosses, associated with analysis of variation in the expression of ITs for each progeny isolate, agrees with the hypothesis that one avirulence gene confers one incompatible IT. The only genes that were likely to affect the IT were the avirulence genes, as was shown by GLM analysis of ITs on *Mla13*. Except for the possible existence of a gene with a minor effect on IT on *Mla12*, the variation in the expression of incompatible ITs observed on other Pallas lines did not require the postulation of genes affecting IT other than known *Avr* genes.

As a consequence, our results confirmed that the ITs were under simple genetic control, but that in some cases, the avirulence was controlled by two genes, which might indicate the existence of more complex genetic systems than are implied by the gene-for-gene relationship, with two independent avirulence genes matching either two closely linked resistance genes or a single resistance gene. This has to be taken into account to understand the evolution of virulence frequencies in relation to the selection pressure exerted by resistant cultivars and to predict the effects of sexual reproduction on pathogen population structure.

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