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High-Density Genetic Linkage Maps of *Phytophthora infestans* Reveal Trisomic Progeny and Chromosomal Rearrangements

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ABSTRACT

Detailed analysis of the inheritance of molecular markers was performed in the oomycete plant pathogen *Phytophthora infestans*. Linkage analysis in the sexual progeny of two Dutch field isolates (cross 71) resulted in a high-density map containing 508 markers on 13 major and 10 minor linkage groups. The map showed strong clustering of markers, particularly of markers originating from one parent, and dissimilarity between the parental isolates on linkage group III in the vicinity of the mating-type locus, indicating a chromosomal translocation. A second genetic map, constructed by linkage analysis in sexual progeny of two Mexican isolates (cross 68), contained 363 markers and is thus less dense than the cross 71 map. For some linkage groups the two independent linkage maps could be aligned, but sometimes markers appeared to be in a different order, or not linked at all, indicating chromosomal rearrangements between genotypes. Graphical genotyping showed that some progeny contained three copies of a homologous linkage group. This trisomy was found for several linkage groups in both crosses. Together, these analyses suggest a genome with a high degree of flexibility, which may have implications for evolution of new races and resistance development to crop protection agents.

PHYTOPHTHORA infestans (Mont.) de Bary causes late blight, a highly infectious plant disease that is particularly notorious on potato. It brought about the great famine in Ireland in the 1840s and even now potato growers fear late blight. Under conditions favorable for the pathogen, complete defoliation of potato may occur in just a few weeks. Late blight has also filled breeders with despair because R-gene-based resistance is often lost even before its introduction in commercial cultivars (Garelik 2002). *P. infestans* belongs to the oomycetes, organisms that, despite their fungal-like growth and appearance, are unrelated to true fungi. The somatic hyphae are coenocytic and the nuclei are diploid. Meiosis occurs just before mating in well-differentiated oogonia and antheridia. *P. infestans* is heterothallic with two mating types called A1 and A2 (Erwin and Ribeiro 1996). Since *P. infestans* does not display visual markers useful for genetic studies, molecular markers are needed for studies on inheritance. Current genetic studies are aimed at elucidating inheritance and cloning of mating-type genes (Judelson *et al.* 1995; Judelson 1996a) and genes responsible for fungicide resistance (Judelson and Roberts 1999) and race-specific avirulence (Carter *et al.* 1999; van der Lee *et al.* 2001a).

Generally, the sexual inheritance of molecular markers in *P. infestans* and related oomycetes appears to be Mendelian. So far, molecular markers have been exploited to construct genetic linkage maps from three oomycete species, *P. infestans* (van der Lee *et al.* 1997), *Phytophthora sojae* (May *et al.* 2002), and *Bremia lactucae* (Sicard *et al.* 2003). In *P. infestans*, however, deviations occasionally were found due to the presence of one or three alleles of a locus in the progeny, suggesting the occurrence of translocations, aneuploidy, and hemizygous regions (Judelson 1996a; Carter *et al.* 1999; Judelson and Roberts 1999; van der Lee *et al.* 2001b), and in *Phytophthora cinnamomi* similar abnormalities were reported (Dobrowolski *et al.* 2002). These studies were all based on multi-locus markers that did not span complete linkage groups.

To gain better insight into the genetics of *P. infestans*, we performed linkage analysis of markers in two F_1 progenies, integrated the two maps, and scanned the individual progenies for abnormalities. Previously, we constructed an amplified fragment length polymorphism (AFLP) linkage map based on an F_1 progeny obtained by crossing two Dutch field isolates and positioned the mating-type locus and six race-specific avirulence genes

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on this map (van der Lee *et al.* 1997, 2001a). We extended the linkage analysis using the same progeny and analyzed segregation in a second F_1 progeny obtained from two Mexican field isolates that are genetically unrelated to the Dutch field isolates. The maps are comparable; however, several cases were found where markers appear to be in a different order or not linked at all. This is particularly evident on linkage group III that contains the mating-type locus. The high-density maps also revealed trisomic progeny that originate from abnormalities during meiosis.

MATERIALS AND METHODS

***P. infestans* mapping populations:** Two F_1 populations were used for genetic analysis. One is derived from a cross between two Dutch *P. infestans* field isolates, 80029 (A1 mating type) and 88133 (A2 mating type). From this mapping population, called cross 71 and previously characterized by Drenth *et al.* (1995) and van der Lee *et al.* (1997), 76 progeny were analyzed. The other F_1 population is derived from a cross between two Mexican isolates, 580 (A1 mating type) and 618 (A2 mating type). This cross, called cross 68, was generated by L. J. Spielman at Cornell University (Ithaca, NY), and was first described and characterized by Goodwin *et al.* (1992). In this study 62 progeny of cross 68 were analyzed. For short periods of time isolates were subcultured on rye sucrose medium and for long-term storage isolates were kept in liquid nitrogen.

Marker generation: AFLP markers were generated as described by Vos *et al.* (1995), using the restriction enzyme combination of *Eco*RI-*Mse*I with two selective bases on each side. DNA isolation and template preparation were described before (van der Lee *et al.* 1997). Three types of markers were scored: A markers (genetic model Aa × aa), B markers (genetic model aa × Aa), and H markers (genetic model Aa × Aa). The same person scored all markers visually. Markers were named by the type of marker (A, B, or H) followed by the primer combination used to generate the marker and the position in the gel, either as a size estimate (indicated by the letter "s") or as a fragment number (indicated by an "f"). Markers that segregate in both crosses are called common markers and have an additional prefix C. Markers segregating in cross 71 are shown in uppercase (*e.g.*, CAE + AA/M + CAs201.9), while cross 68 markers are shown in lowercase (*e.g.*, cae + aa/m + cas 206.5). Fingerprinting of the two parental lines of cross 71 and 12 of its progeny was performed twice. Scoring accuracy was evaluated by the percentage of dissimilarity in the scorings between replicated individuals over the total number of scored markers using Microsoft Excel. For comparative studies precise length estimates of the AFLP markers are needed. Upon request the authors will provide more detailed information, including the visualization of the markers on gel. Apart from AFLP markers, some RFLP, RAPD, and allozyme markers were scored. These markers are also named by the probe, primer, or protein used to generate the marker followed by a fragment number or size. Some cross 68 markers were generated by A. Dyer and W. E. Fry from Cornell University and P. W. Tooley from the U.S. Department of Agriculture (Frederick, MD). For the RFLP, RAPD, and allozyme data, no quality assessment was performed. Allelic markers originating from the same RFLP or allozyme were treated as separate markers. The mating type was determined by checking for presence of oospores when co-cultured with tester strains of known mating type.

Linkage analysis: All individuals generated in the two crosses were analyzed for genetic distance using the Dice index within the NTSYS software package (Rohlf 1993). Isolates with a genetic similarity of 95% or more were considered genetically identical and only one of each set was used in the linkage analysis carried out in JoinMap 3.0 (http://www.kyazma.nl/join/jm_intro.htm). In the AFLP linkage analysis, progeny for which a large number of markers could not be scored reliably, as well as markers that could not be scored reliably on a large set of individuals, were set aside from the data set. Markers were assigned to linkage groups using LOD values ranging from 3.5 to 8.5. Only linkage groups with at least three markers were used for map calculation. To obtain a reliable grouping of the markers, we tested whether (i) the segregation ratio of the markers was in accordance with other markers in the group, (ii) the linkage phase of the markers could be determined relative to the other markers, (iii) the markers were linked to at least two distinct other loci given a LOD value of 1, and (iv) the markers showed clear polarity in the recombination events with other markers. Markers that did not meet these criteria were rejected. Finally, we analyzed whether the A and B markers separately had enough linkage data to support a single group, given a LOD threshold value of 3. To determine the order of markers in the linkage groups, we first calculated the order of markers originating from either the A1 parent (Aa × aa), resulting in an A map, or the A2 parent (aa × Aa), resulting in a B map. For the larger linkage groups with many A or B markers, A and B maps for each linkage phase (chromosome) also were calculated. The marker order was calculated using all linkage data with a LOD score of 1 or higher and a maximum distance of 40 cM with the Kosambi mapping function. A and B maps were used as a fixed framework for the integrated map containing H markers (Aa × Aa) as well as A and B markers. JoinMap 3.0 provides the opportunity to set aside markers that cause friction in the map, which is detected by a jump in the χ^2 value for the map upon addition of each marker. This χ^2 was set at the default value 5. When all markers were analyzed like this, JoinMap performed a second round attempting to position the markers that initially caused friction and were set aside, thereby using the map calculated in the first round as fixed order and applying the same χ^2 restriction. Maps were drawn with MapChart (Voorrips 2002).

Alignment of the maps of cross 71 and cross 68: The maps of cross 71 and cross 68 were aligned using markers that segregate in both crosses, the so-called common markers indicated with a C as prefix. Similar to the integration of the A and the B maps, which was done by markers present in both parents that segregated in the progeny, the alignment was based on markers that had an identical fragment size and an identical intensity and that segregated in both crosses. However, in contrast to markers generated within one cross, markers common in two crosses do not necessarily fit in the same genetic model in each of the crosses. For instance, an A marker in cross 71 (Aa × aa) may be an A (Aa × aa), B (aa × Aa), or H (Aa × Aa) marker in cross 68 and vice versa. The information for the comparison is more reliable when markers of the same type can be compared but more difficult if the comparison involves combinations of marker types. The alignment was done graphically and the position of the common markers was carefully examined using the calculations generated by JoinMap.

Graphical genotyping of the progeny: For the identification of trisomic or monosomic progeny, we made a graphical display of the markers of each linkage group for each of the progeny. Trisomic progeny were detected by the presence of all A markers or all B markers of a particular linkage group regardless of the linkage phase (chromosome) from which the markers originate (see Figure 3 as an example). Only linkage groups with five or more markers originating from the same parent with at least two changes of the linkage phase were

assayed. Graphical genotyping of the progeny was performed in Microsoft Excel and in GGT (van Berloo 1999).

RESULTS

Map construction in cross 71: In cross 71, 223 A markers, 241 B markers, and 165 H markers were scored, adding up to a total of 629 markers. Most H markers (Aa × Aa) were scored dominant for presence and absence but some AFLP markers allowed discrimination of the homozygous and heterozygous individuals in the progeny. All markers that passed the quality standard set for this map were AFLP markers. The AFLP markers were scored irrespective—and in many cases ignorant—of the number of replicates in the progeny. This provided us with a way to estimate the reliability of the data set. The reproducibility between replicate DNA samples ranged from 95.9 to 100%. Fingerprints derived from DNA isolated from different culture batches of the same individual after retrieval from liquid nitrogen appeared less consistent (95.9–99.6%) than fingerprints from DNA isolated from the same batch culture (96–100%). Of the 629 markers, 34 were scored on <50 progeny and these markers were excluded from further analysis. Similarly, 8 progeny had information on <520 markers and these progeny were also excluded from further analysis. Linkage analysis was thus performed with 595 markers on 68 progeny. The result of the linkage analysis is shown in Figure 1 and summarized in Table 1. Using a variable LOD value for grouping (ranging from 3.5 to 8.5), 548 of the 595 markers (92%) appeared to be linked to at least 2 other markers. For 534 of the 548 linked markers the criteria for reliable grouping (see materials and methods) could be met. Markers that did not meet the set criteria were largely H markers for which the linkage phase could not be determined and markers that did not show a clear polarity in the recombination events. In all cases the A and B markers separately had enough linkage data to support a single group at a LOD value of 3. In linkage groups containing regions with low marker density (LG IX, LG XI, LG XIII, LG A1-a; Table 1) only a single bridging link at a LOD value of 3 was found but at least two independent links could be established at a LOD value of 1. When the order of the markers in the A and B maps was calculated, only a limited number of markers were rejected on the basis of the χ^2 jump (7 and 4, respectively), indicating that there is not much friction in the data for marker ordering. Also, the comparison of linkage maps calculated for markers of a single linkage phase performed on linkage groups I, III, IV, V, and VIII did not reveal differences. The calculation of the integrated map (A, B, H) using the fixed order from the A and B map appeared to be slightly more difficult as judged by the χ^2 values and the compression of the genetic distance observed, for instance, at the top of LG IV (as shown in Figure 2C). Nevertheless, in most cases the relative position of the H markers in the map with the A and H markers is similar to the map with the B and H markers. An exception was LG III, which contains the mating-type locus. In this case the integration of the A and B map using the H markers caused great difficulties. Apart from the problems encountered in LG III (addressed below), most of the markers could be positioned on the integrated map—480 after the first and 508 after the second round. The 26 markers that could not be placed with this χ^2 restriction were not positioned on the map and are listed in the box in Figure 1. Most of the rejected markers fit best in dense regions where a single scoring error can lead to high χ^2 jumps. The added value of these markers is low.

On the map we marked the position of six avirulence genes (*AAvr1*, *BAvr2*, *AAvr3*, *BAvr4*, *AAvr10*, and *AAvr11*), the mating-type locus (*A-MAT*), and one PCR marker (AS1-LOC) representing the S1 locus linked to the mating-type locus (Judelson 1996b). The mapping information available for these loci did not meet the quality criteria set for construction of the AFLP linkage map in this study (see materials and methods) but their map position is relevant for comparison with other genetic studies performed in *P. infestans*. Therefore these loci were placed manually on the basis of direct distance to the closest markers. In Figure 1 they are italicized to indicate the lower confidence level. The lower confidence level is also applicable to one manually placed AFLP marker (CAE + GA/M + CGs172 on LG VIII) that was informative for aligning the maps of two different crosses (see below). Finally, we analyzed six RFLP markers, five generated by probe RG57 (ARG57.16, BRG57.18, BRG57.7, BRG57.3, RG57.8; Goodwin *et al.* 1992) and one by cDNA probe APPI122.2. Also, for these markers the mapping information did not meet the quality criteria set for mapping in this study but they were all assigned to a linkage group (see box in Figure 1).

The integrated linkage map of cross 71 comprises 23 linkage groups (Figure 1) of which 13 contain markers from both parents (A, B, and H markers). The latter are referred to as major linkage groups and are labeled with roman numbers. The remaining 10 minor linkage groups are composed of markers from a single parent and are named by their parental origin, A1 or A2, followed by a letter. Except for LG A1-a (81.6 cM) and A2-a (52.8 cM), the minor linkage groups are relatively small (3–32 cM). LG III-a, XII, A1-d, and A1-e showed strong aberrations in the segregation ratios (listed in Table 1). LG A1-d and LG A1-e contain only A markers of a single linkage phase; LG VIII and LG IX also contain a large number of markers of only a single linkage phase. The major linkage groups have a high marker density with an average interval of 2.1 cM. However, some linkage groups, such as LG XII, or regions, such as the top of LG XI or the lower region of LG IX, are low in marker density, particularly for markers derived from one of the parents or from a specific linkage phase.

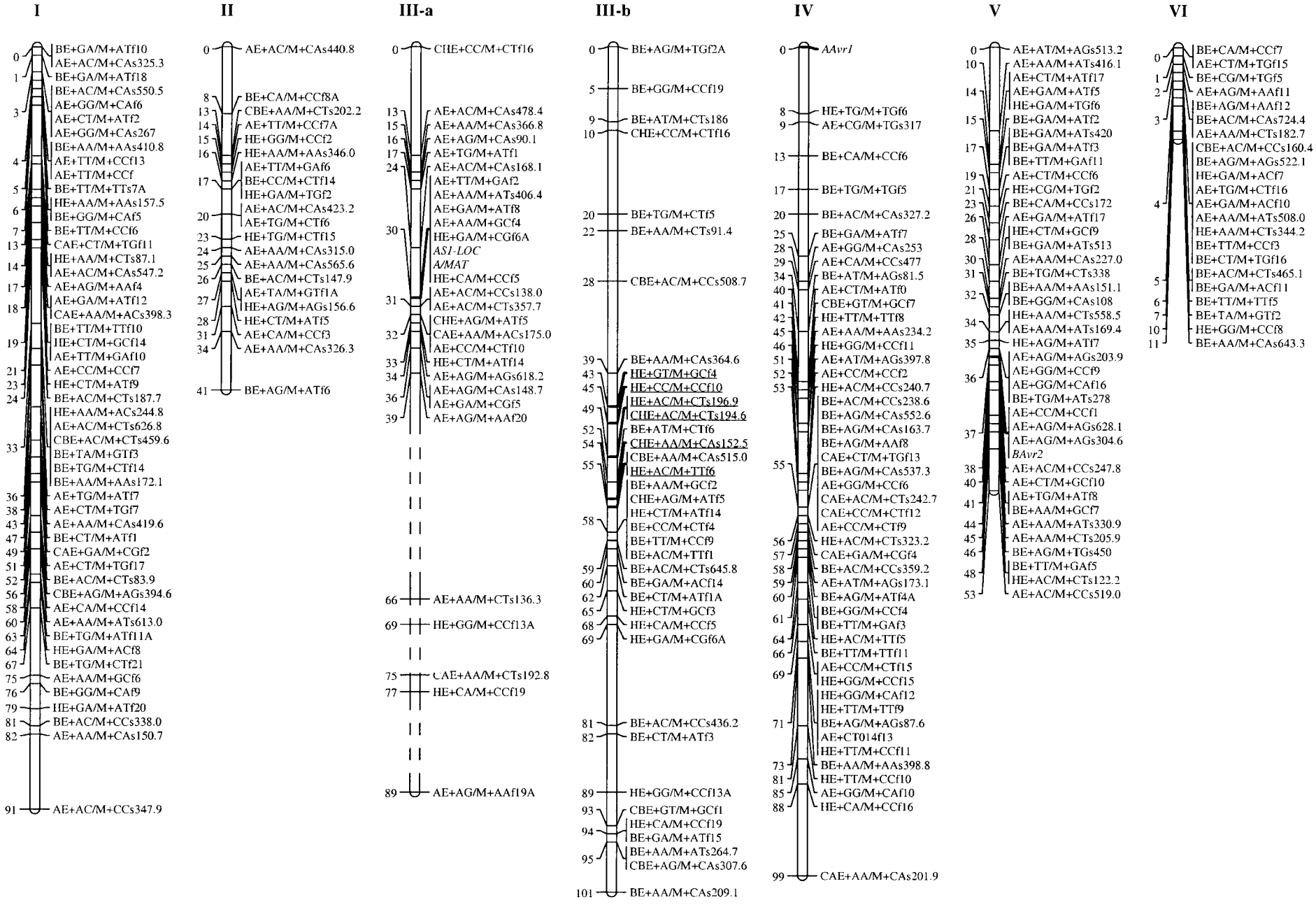
Some linkage groups contain clusters of markers in coupling phase of a single parent, originating from a single chromosome. The length of the linkage groups ranges from 3 to 101 cM. Particularly short for the number of markers it contains is LG VI with 22 markers on 10.7 cM; in this case it is even unclear if the map length in centimorgans extends beyond the experimental noise generated by the integration of the A and B map.

The integration of the A and B map using the H markers caused difficulties in LG III, the LG containing the mating-type locus. Closer examination revealed a cluster of six H markers (underlined in Figure 1) strongly linked to the B markers in LG III but not linked at all to any of the A markers in this linkage group. This is remarkable since in the corresponding segment A markers are present and should have been linked if the H markers on the III-a map were in the same position. This indicates that the A and B map were dissimilar for LG III. Consequently, we did not construct an integrated map of LG III but instead constructed separate A and B maps called III-a and III-b. In the two maps the position of the H markers was calculated by adding each and every H marker separately to avoid interference by other H markers. We analyzed whether the six H markers from III-b, which had no linkage to the A markers in III-a, were linked to A markers in other linkage groups but we could not identify even weak linkage (LOD <1). Three other H markers had low LOD values as compared to the A markers. The H marker on top of III-b (CHE + CC/M + CTf16) had a LOD value of 1 with some A markers of III-a, and two H markers at the bottom of III-b (HE + GG/M + CCf13A and HE + CA/M + CCf19) were linked to three A markers that also linked together (AE + AA/M + CTs136.3, CAE + AA/M + CTs192.8, AE + AG/M + AAf19A). Although the LOD values were low, the data supported the fusion of these three A markers with LG III-a. Segregation ratios were similar and the direct distance between the A markers as well as between the H markers matched with the calculated distances and the distance of these markers on the III-b map. Therefore we added these markers to LG III-a despite the fact that the significance for linkage is lower (as indicated by the dashed line in LG III-a in Figure 1).

Map construction in cross 68: From cross 68, 62 F_1 progeny were available. In these progeny 465 AFLP, 17 RFLP, 31 RAPD, and two allozyme markers were scored as well as the mating type. The majority of the markers were AFLP markers and the reliability of this data set, as judged by the scoring of replicated samples, ranged from 99.8 to 99.9%. Fifteen progeny had information on <443 markers and these progeny were excluded from further analysis. Also DNA markers that were scored on <32 progeny were excluded from further analysis, as were markers that segregated with an aberrant segregation ratio ($\chi^2 > 5$). Linkage analysis was performed with the remaining 425 markers on 47 progeny. Using variable LOD values ranging from 3.5 to 8.5, 392 markers could be grouped into 24 linkage groups (Figure S1 and Table S1 at http://www.genetics.org/supplemental/): 8 major linkage groups containing A, B, and H markers and 16 minor linkage groups, 8 with markers originating from the A1 parent and 8 with markers from the A2 parent. The order of the markers was calculated but in some of the linkage groups the relatively low number of H markers and the limited number of progeny made integration of the A and B maps more difficult. For some linkage groups, integration was supported by multi-allelic markers which, when treated as independent markers, mapped within 4–10 cM in the same linkage group. Overall, the map generated from the linkage analysis in cross 68 is more fragmented than the map of cross 71.

Alignment of the maps of cross 71 and cross 68: For alignment of the maps we used markers segregating in both crosses. These common markers were identified by fingerprinting the four parental lines and a number of their progeny side by side, followed by careful inspection of the fingerprints for bands with identical length and intensity. We started the alignment of the two maps by grouping the common markers per linkage group. Groups of two or more common markers that were mapped on one linkage group in cross 71 also mapped on one linkage group in cross 68 (Table 2) but there were clear exceptions, for instance, in LG I and LG III. We retested the grouping of these markers, and all groupings appeared reliable. The numbering of the linkage groups in cross 68 follows the numbering of the linkage groups in cross 71 as much as possible. As a consequence, linkage groups in cross 68 indicated by roman numbers are not necessarily major linkage groups and the A1 and A2 linkage groups are not necessarily minor linkage groups. Subsequently, we tested if linkage groups could be merged on the basis of linkage information obtained from the other cross. For example, the exchange of common markers from LG XIII

Figure 1.—Genetic linkage map of *P. infestans*. The map is based on the segregation of markers in cross 71 and is composed of 13 major linkage groups with A, B, and H markers (I–XIII) and 11 minor linkage groups with either A markers (A1-a–A1-f) or B markers (A2-a–A2-d) and with or without H markers. Markers are indicated on the right, cumulative distances (in centimorgans) on the left. All markers have prefixes A, B, or H according to their origin. Markers with the prefix C, so-called common markers, also segregate in cross 68. Nomenclature of the markers is further explained in the text. Markers and loci shown in italics were placed manually. Underlined markers in LG III-b are H markers that are linked to B markers but not to any of the A markers in LG III. Markers that were rejected by the χ^2 jump restriction are listed in the box.


I
0 BE+GA/M+ATf10
0 AE+AC/M+CAs325.3
1 BE+GA/M+ATf18
BE+AC/M+CAs550.5
3 AE+GG/M+CAf6
AE+CT/M+ATf2
AE+GG/M+CAs267
BE+AA/M+AAs410.8
4 AE+TT/M+CCf13
AE+TT/M+CCf
5 BE+TT/M+TTs7A
6 HE+AA/M+AAs157.5
BE+GG/M+CAf5
7 BE+TT/M+CCf6
13 CAE+CT/M+TGf11
14 HE+AA/M+CTs87.1
AE+AC/M+CAs547.2
17 AE+AG/M+AAf4
18 AE+GA/M+ATf12
CAE+AA/M+ACs398.3
19 BE+TT/M+TTf10
HE+CT/M+GCf14
AE+TT/M+GAf10
21 AE+CC/M+CCf7
23 HE+CT/M+ATf9
24 BE+AC/M+CTs187.7
HE+AA/M+ACs244.8
AE+AC/M+CTs626.8
33 CBE+AC/M+CTs459.6
BE+TA/M+GTf3
BE+TG/M+CTf14
BE+AA/M+AAs172.1
36 AE+TG/M+ATf7
38 AE+CT/M+TGf7
43 AE+AA/M+CAs419.6
47 BE+CT/M+ATf1
49 CAE+GA/M+CGf2
51 AE+CT/M+TGf17
52 BE+AC/M+CTs83.9
56 CBE+AG/M+AGs394.6
58 AE+CA/M+CCf14
60 AE+AA/M+ATs613.0
63 BE+TG/M+ATf11A
64 HE+GA/M+ACf8
67 BE+TG/M+CTf21
75 AE+AA/M+GCf6
76 BE+GG/M+CAf9
79 HE+GA/M+ATf20
81 BE+AC/M+CCs338.0
82 AE+AA/M+CAs150.7
91 AE+AC/M+CCs347.9
II
0 AE+AC/M+CAs440.8
8 BE+CA/M+CCf8A
13 CBE+AA/M+CTs202.2
14 AE+TT/M+CCf7A
15 HE+GG/M+CCf2
16 HE+AA/M+AAs346.0
AE+TT/M+GAf6
17 BE+CC/M+CTf14
HE+GA/M+TGf2
20 AE+AC/M+CAs423.2
AE+TG/M+CTf6
23 HE+TG/M+CTf15
24 AE+AA/M+CAs315.0
25 AE+AA/M+CAs565.6
26 BE+AC/M+CTs147.9
AE+TA/M+GTf1A
27 HE+AG/M+AGs156.6
28 HE+CT/M+ATf5
31 AE+CA/M+CCf3
34 AE+AA/M+CAs326.3
41 BE+AG/M+ATf6
III-a
0 CHE+CC/M+CTf16
13 AE+AC/M+CAs478.4
15 AE+AA/M+CAs366.8
16 AE+AG/M+CAs90.1
17 AE+TG/M+ATf1
24 AE+AC/M+CAs168.1
AE+TT/M+GAf2
AE+AA/M+ATs406.4
AE+GA/M+ATf8
30 AE+AA/M+GCf4
HE+GA/M+CGf6A
AS1-LOC
A/MAT
HE+CA/M+CCf5
31 AE+AC/M+CCs138.0
AE+AC/M+CTs357.7
CHE+AG/M+ATf5
32 CAE+AA/M+ACs175.0
AE+CC/M+CTf10
33 HE+CT/M+ATf14
34 AE+AG/M+AGs618.2
36 AE+AG/M+CAs148.7
AE+GA/M+CGf5
39 AE+AG/M+AAf20
66 AE+AA/M+CTs136.3
69 HE+GG/M+CCf13A
75 CAE+AA/M+CTs192.8
77 HE+CA/M+CCf19
89 AE+AG/M+AAf19A
III-b
0 BE+AG/M+TGf2A
5 BE+GG/M+CCf19
9 BE+AT/M+CTs186
10 CHE+CC/M+CTf16
20 BE+TG/M+CTf5
22 BE+AA/M+CTs91.4
28 CBE+AC/M+CCs508.7
39 BE+AA/M+CAs364.6
43 HE+GT/M+GCf4
45 HE+CC/M+CCf10
HE+AC/M+CTs196.9
49 CHE+AC/M+CTs194.6
52 BE+AT/M+CTf6
54 CHE+AA/M+CAs152.5
55 CBE+AA/M+CAs515.0
HE+AC/M+TTf6
BE+AA/M+GCf2
CHE+AG/M+ATf5
58 HE+CT/M+ATf14
BE+CC/M+CTf4
BE+TT/M+CCf9
BE+AC/M+TTf1
59 BE+AC/M+CTs645.8
60 BE+GA/M+ACf14
62 BE+CT/M+ATf1A
65 HE+CT/M+GCf3
68 HE+CA/M+CCf5
69 HE+GA/M+CGf6A
81 BE+AC/M+CCs436.2
82 BE+CT/M+ATf3
89 HE+GG/M+CCf13A
93 CBE+GT/M+GCf1
HE+CA/M+CCf19
94 BE+GA/M+ATf15
BE+AA/M+ATs264.7
95 CBE+AG/M+CAs307.6
101 BE+AA/M+CAs209.1
IV
0 AAvr1
8 HE+TG/M+TGf6
9 AE+CG/M+TGs317
13 BE+CA/M+CCf6
17 BE+TG/M+TGf5
20 BE+AC/M+CAs327.2
25 BE+GA/M+ATf7
28 AE+GG/M+CAs253
29 AE+CA/M+CCs477
34 BE+AT/M+AGs81.5
40 AE+CT/M+ATf0
41 CBE+GT/M+GCf7
42 HE+TT/M+TTf8
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BE+AC/M+CCs238.6
BE+AG/M+CAs552.6
BE+AG/M+CAs163.7
BE+AG/M+AAf8
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BE+AG/M+CAs537.3
AE+GG/M+CCf6
CAE+AC/M+CTs242.7
CAE+CC/M+CTf12
AE+CC/M+CTf9
56 HE+AC/M+CTs323.2
57 CAE+GA/M+CGf4
58 BE+AC/M+CCs359.2
59 AE+AT/M+AGs173.1
60 BE+AG/M+ATf4A
61 BE+GG/M+CCf4
BE+TT/M+GAf3
64 HE+AC/M+TTf5
66 BE+TT/M+TTf11
69 AE+CC/M+CTf15
HE+GG/M+CCf15
HE+GG/M+CAf12
HE+TT/M+TTf9
71 BE+AG/M+AGs87.6
AE+CT014f13
HE+TT/M+CCf11
73 BE+AA/M+AAs398.8
81 HE+TT/M+CCf10
85 AE+GG/M+CAf10
88 HE+CA/M+CCf16
99 CAE+AA/M+CAs201.9
V
0 AE+AT/M+AGs513.2
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AE+CT/M+ATf17
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HE+GA/M+TGf6
15 BE+GA/M+ATf2
BE+GA/M+ATs420
17 BE+GA/M+ATf3
BE+TT/M+GAf11
19 AE+CT/M+CCf6
21 HE+CG/M+TGf2
23 BE+CA/M+CCs172
26 AE+GA/M+ATf17
HE+CT/M+GCf9
28 BE+GA/M+ATs513
30 AE+AA/M+CAs227.0
31 BE+TG/M+CTs338
BE+AA/M+AAs151.1
32 BE+GG/M+CAs108
HE+AA/M+CTs558.5
AE+AA/M+ATs169.4
35 HE+AG/M+ATf7
AE+AG/M+AGs203.9
36 AE+GG/M+CCf9
AE+GG/M+CAf16
BE+TG/M+ATs278
AE+CC/M+CCf1
37 AE+AG/M+AGs628.1
AE+AG/M+AGs304.6
BAvr2
38 AE+AC/M+CCs247.8
40 AE+CT/M+GCf10
41 AE+TG/M+ATf8
BE+AA/M+GCf7
44 AE+AA/M+ATs330.9
45 AE+AA/M+CTs205.9
46 BE+AG/M+TGs450
48 BE+TT/M+GAf5
HE+AC/M+CTs122.2
53 AE+AC/M+CCs519.0
VI
0 BE+CA/M+CCf7
AE+CT/M+TGf15
1 BE+CG/M+TGf5
2 AE+AG/M+AAf11
BE+AG/M+AAf12
3 BE+AC/M+CAs724.4
AE+AA/M+CTs182.7
CBE+AC/M+CCs160.4
BE+AG/M+AGs522.1
HE+GA/M+ACf7
AE+TG/M+CTf16
4 AE+GA/M+ACf10
AE+AA/M+ATs508.0
HE+AA/M+CTs344.2
BE+TT/M+CCf3
BE+CT/M+TGf16
5 BE+AC/M+CTs465.1
6 BE+GA/M+ACf11
7 BE+TT/M+TTf5
10 BE+TA/M+GTf2
11 HE+GG/M+CCf8
BE+AA/M+CAs643.3

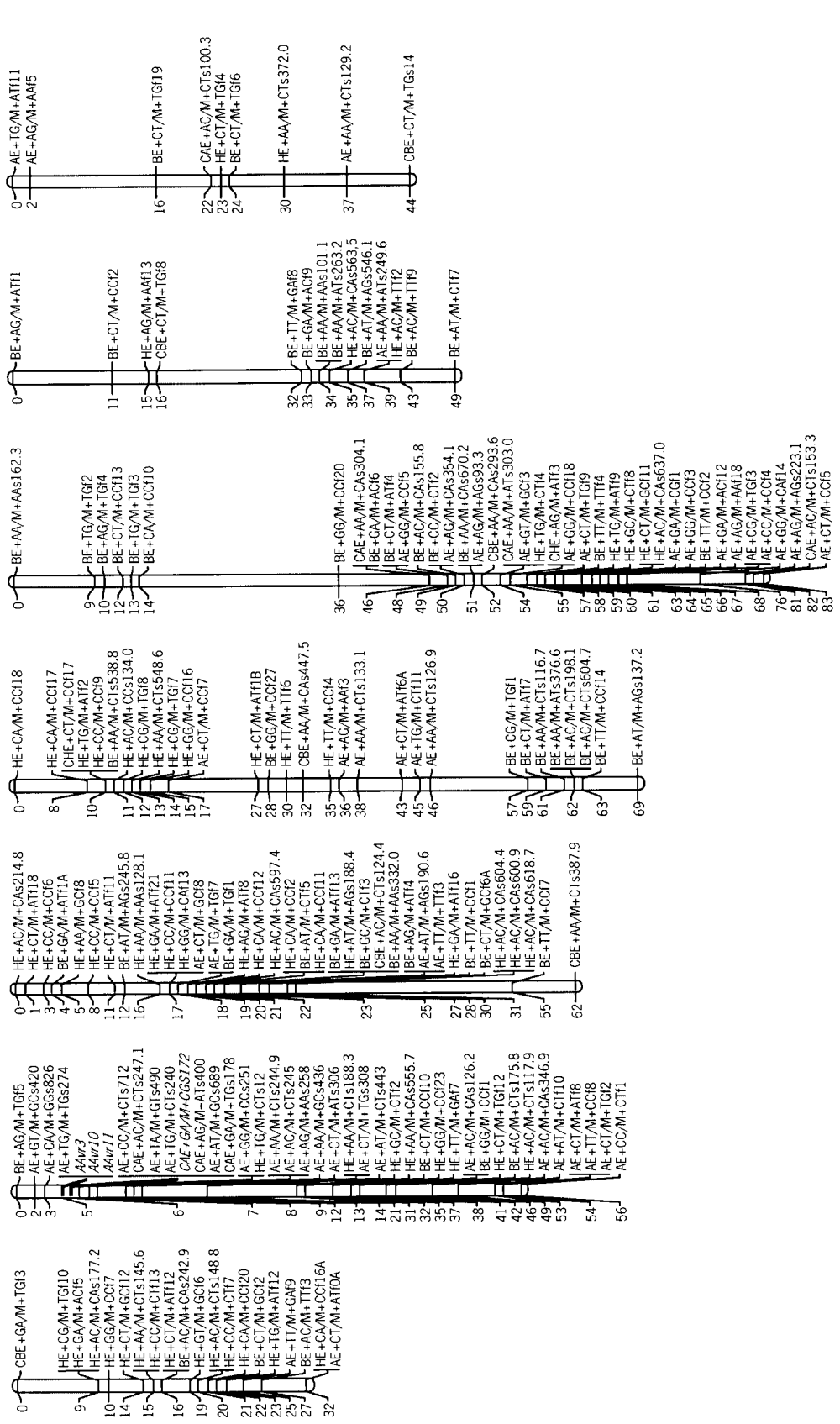


FIGURE 1.—*Continued.*

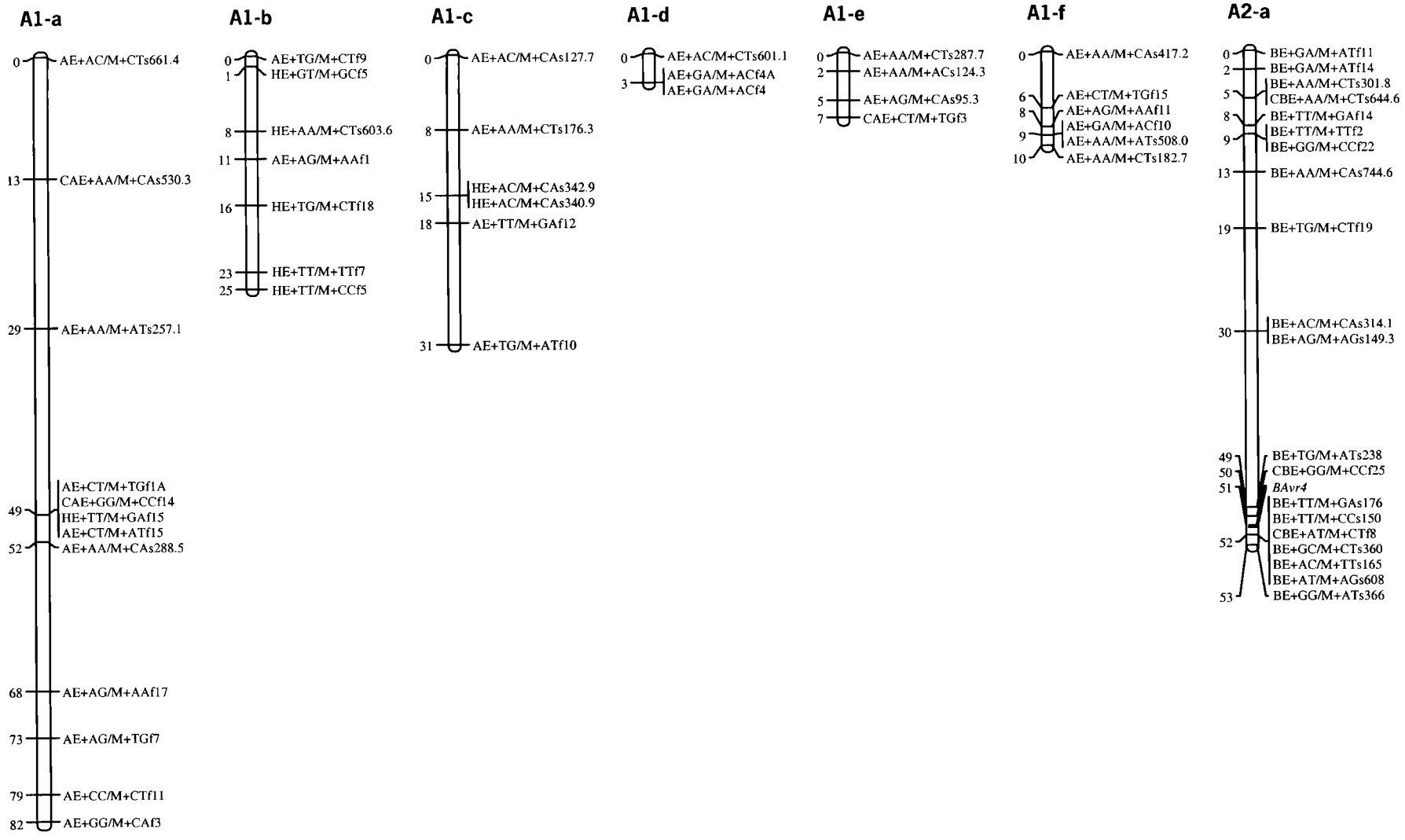


FIGURE 1.—*Continued.*

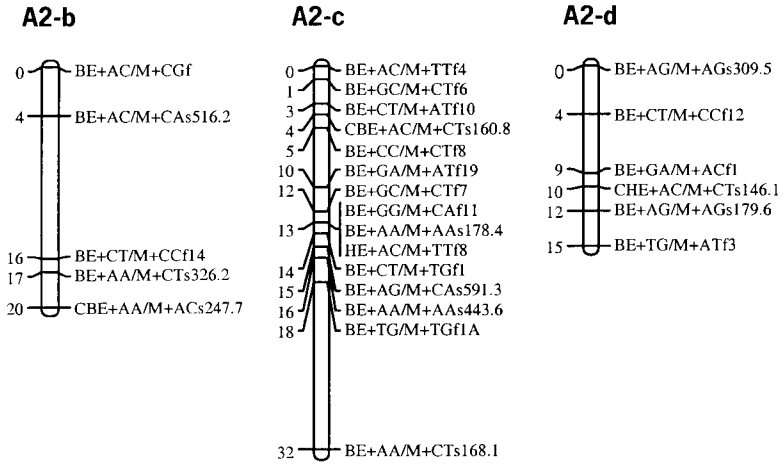


FIGURE 1.—*Continued.*

Markers that could be grouped but not positioned	
LG I	HE+GA/M+ATf9
	BE+AC/M+CCs328.3
	AE+AG/M+TGf8
	BE+AA/M+ACs97.5
	BE+AG/M+CAs223.1
	BE+TT/M+CCf12
	BRG57.18
	ARG57.16
LG II	AE+CA/M+CCf3
	HE+AA/M+AAs346.0
	BE+AG/M+Atf6
LG IV	BE+TT/M+CCf16
	BE+GA/M+ACf2
	AE+AA/M+GTs88.1
	AE+TT/M+GAf4
	AE+CC/M+CCf3a
	AE+CC/M+CCf3
	AE+AG/M+AAs118
	BRG57.7
	BRG57.3
	APPI122.2
LG V	AE+CT/M+TGf10
	BE+GA/M+ACs113
	HE+CG/M+TGs6
LG VIII	HE+AA/M+CTs124.7
	BE+GC/M+CTf1
	HE+GG/M+Caf7
LG IX	AE+CG/M+TGf9
	CBE+TA/M+GTf4
LG XI	BE+TG/M+TGf4
	HE+CC/M+CCf8
LG A2-a	BRG57.8

TABLE 1

Linkage groups of *P. infestans* and statistics on the map construction

Linkage group		No. of markers					LOD group[b]			Positioned[c] A + B + H		Rejected[d]				No. of linkage phases			Segregation ratio[e]	
Current	Previous	Total	A	B	H	C[a]	A + B + H	A	B	1	2	Quality	A + B + H	A	B	A	B	H	A	B
I	I	57	25	24	8	5	5	>3	>3	48	51	0	6	1	0	2	2	2		
II	II (part)	24	11	6	7	1	5.5	>3	>3	19	21	0	3	1	0	2	2	2		
III-a	III	20 + 7[f]	20	0	+7[f]	2 + 2[f]	>5[g]	>3[g]	>3[g]	20 + 7[f]	20 + 7[f]	0	0	0	\	2	NA	2	****	
III-b	III	40	0	26	14	8	5	>3	>3	32	37	3	0	\	0	NA	2	2		
IV	IV	57	23	21	13	6	5	>3	>3	42	48	2	7	3	4	2	2	2		
V	V	42	21	14	7	0	4.5	>3	>3	32	39	0	3	0	0	2	2	2		
VI	VI (part)	25	7	14	4	1	3.5	>3	>3	20	22	3	0	0	0	2	2	2		***
VII	VII	21	2	4	15	1	4	>3	>3	17	20	1	0	0	0	2	2	2		
VIII	VIII	42	27	5	10	4	4	>3	>3	37	37	2	3	1	0	1	1	1		
IX	IX	39	5	14	20	3	4.5	>3	1	37	37	0	2	0	0	2	1	1		
X	X + A2-e	32	6	13	13	2	4	>3	>3	30	30	2	0	0	0	2	2	1		***
XI	A1-b + A2-b + V (part)	41	18	16	7	5	5	>3	>2.7	31	39	0	2	1	0	2	2	2		
XII	II (part)	14	1	10	3	1	4	NA	>3	14	14	0	0	0	0	2	2	2	*****	
XIII	VII (part)	9	4	3	2	2	4	1	>3	9	9	0	0	0	0	2	2	1		
A1-a	A1-a	12	11	0	1	2	5.5	1	NA	12	12	0	0	0	\	2	NA	1		NA
A1-b	—	7	2	0	5	0	4	>3	NA	7	7	0	0	0	\	1	NA	2	***	NA
A1-c	—	6	4	0	2	0	6.5	>3	NA	6	6	0	0	\	0	2	NA	1		NA
A1-d	—	3	3	0	0	0	5.5	>3	NA	3	3	0	0	0	\	1	NA	NA	*******	NA
A1-e	II (part)	4	4	0	0	1	4.5	>3	NA	4	4	0	0	0	\	1	NA	NA	*******	NA
A1-f	VI (part)	6	6	0	0	0	10	>3	NA	6	6	0	0	0	\	1	NA	NA		NA
A2-a	A2-a	21	0	21	0	3	6	NA	>3	20	20	1	0	\	0	NA	2	NA	NA	
A2-b	—	5	0	5	0	1	8.5	NA	>3	5	5	0	0	\	0	NA	2	NA	NA	
A2-c	A2-c	15	0	14	1	1	4	NA	>3	15	15	0	0	\	0	NA	2	1	NA	
A2-d	A2-d	6	0	5	1	1	6.5	NA	>3	6	6	0	0	\	0	NA	2	1	NA	
Total		548	200	215	133	50	NA	NA	NA	480	508	14	26	7	4	NA	NA	NA	NA	NA

For each LG, the code in the current map, the code in the previous map (VAN DER LEE *et al.* 1997), the number of markers, the LOD value for grouping of the markers, the number of markers positioned/rejected, the number of linkage phases of the markers, and the maximal deviation from the expected segregation ratio are given. If informative, the markers are split according to their parental origin (A, B, or H). See MATERIALS AND METHODS for nomenclature. NA, not applicable.

[a] Number of common markers (C) that also segregate in cross 68; for furthers details see Table 2.

[b] LOD value that groups markers in the LG in the integrated map (A + B + H) and the maps of the A and B markers separately.

[c] Number of markers positioned in the first (1) and second (2) round of JoinMap3.0 (further explanation in MATERIALS AND METHODS).

[d] Number of rejected markers using a restriction of a χ^2 jump of 5 in the integrated map (A + B + H) and the maps of the A and B markers separately.

[e] Maximum deviation from the expected segregation ratio. ***$P < 0.01$, ****$P < 0.005$, *****$P < 0.001$, ******$P < 0.0005$, *******$P < 0.0001$.

[f] LG III is split and the H markers in LG III-a are also present in LG III-b; to prevent counting these markers twice, these markers are not added to the total.

[g] For core region, see RESULTS.

and A2-a suggested that these two groups compose one linkage group. Markers on LG XIII[71] and LG A2-a[71], however, were not linked and could not be merged in one linkage group despite the fact that enough relevant marker data were available.

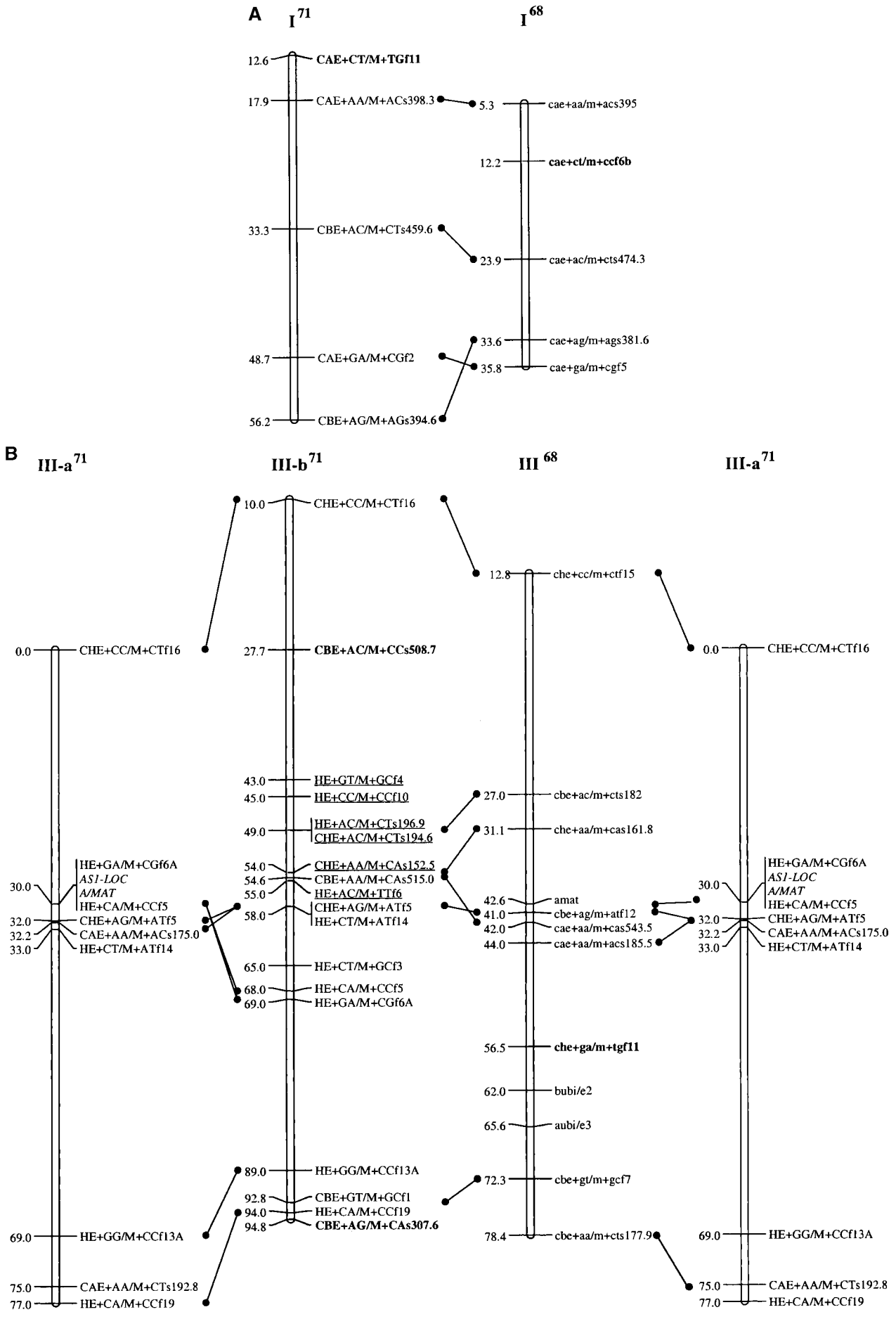
The different groupings already suggested that an overall integration of the two maps on the basis of common markers was not possible. We then compared the order of the markers within the five linkage groups for which four or more common markers were available (*i.e.*, LG I, LG III, LG IV, LG VIII, and LG XI). In LG VIII and LG XI, alignment of the maps from cross 68 and cross 71 showed minor differences in the order of the markers that remained within the mapping resolution and also the distances between the markers were similar (Figure 2, D and E). In the comparison of LG IV (Figure 2C), the order and the distance between the markers was similar for four marker pairs but not for marker pair CAE + AA/M + CAs201.9-cae + aa/m + cas206.5. In cross 71, marker CAE + AA/M + CAs201.9 was mapped distal from markers HE + CA/M + CCf16 and AE + GG/M + CAf10 (Figure 1) and also the position of marker cae + aa/m + cas206.5 in cross 68 was clear from the relative position to markers cae + ac/m + cts228.3 and cae + ct/m + tgf17 (Figure S1 at http://www.genetics.org/supplemental/). In these cases all markers originated from the same parent and therefore provided maximum mapping resolution. Also, the fact that marker CAE + AA/M + CAs201.9 was quite distant from the other common markers in cross 71 whereas cae + aa/m + cas206.5 was close to the common markers in cross 68 made an identical position unlikely.

In cross 71 the A and B map of LG III could not be integrated (Figure 1) but there was no problem with the integration of LG III in cross 68. Of the six markers that distinguished III-a[71] from III-b[71] (HE + GT/M + GCf4, HE + CC/M + CCf10, HE + AC/M + CTs196.9, CHE + AC/M + CTs194.6, CHE + AA/M + CAs152.5, and HE + AC/M + TTf6), two (CHE + AA/M + CAs152.5 and CHE + AC/M + CTs194.6) had a corresponding marker in cross 68 (che + aa/m + cas161.8 and cbe + ac/m + cts182). Both these markers were positioned on LG III[68] (Figure 2B). Furthermore, marker che11m15s161.8 was linked to both A and B markers in LG III[68], demonstrating that the two parents of cross 68 are not dissimilar in this region. Further comparison revealed that the integrated map of LG III[68] is dissimilar to both the III-a[71] and the III-b[71] map. The corresponding markers for markers CBE + AC/M + CCs508.7 and CBE + AG/M + CAs307.6 (cbe + ac/m + ccs588.6 and cae + ag/m + cas284.9, respectively) were not positioned on LG III[68]. Instead, cae + ag/m + cas284.9 was linked (LOD value of 5.9) to marker PEPI in cross 68 on a LG with no other common markers and marker cbe + ac/m + ccs588.6 was linked (LOD value of 6.5) to RG57/1H on LG I(part)[68] and, conversely, marker CBE + GA/M + TGf3 corresponding to marker che + ga/m + tgf11 positioned on LG III[68] was not mapped on LG III in cross 71 but on LG VII[71]. Nevertheless, seven other markers and the mating-type locus were linked and their relative positions were similar.

In LG I, the differences in the order and the distance of four of the six common markers was within the mapping resolution (Figure 2A). In cross 68, marker cbe + ct/m + tgf13a, corresponding to marker CAE + CT/M + TGf11 in cross 71, was not linked to any of the markers from LG I[68], but this can be due to the absence of B and H markers in the corresponding part of the map in cross 68. However, marker cae + ct/m + ccf6b was located on LG I[68] but the corresponding marker in cross 71 (CHE + CT/M + CCF17) was mapped on LG X[71].

Part of the progeny is trisomic for one or more chromosomes: While constructing a linkage map, the inheritance of markers in each individual progeny can be visualized in the order of the map by graphical genotyping. This procedure is generally used to identify errors in marker scoring. Such errors often result in apparent double crossing-over events, but these are unlikely to occur. In this study we used graphical genotyping to identify aberrant progeny. In both cross 68 and cross 71, part of the progeny contained all markers from a particular chromosomal pair from one of the parents. One example of trisomy of LG IV is shown in Figure 3. One of the progeny of cross 71, D12-17, contained all LG IV markers from the A1 parent regardless of whether the markers were in coupling phase (same chromosome) or in repulsion phase (homologous chromosome). In

Figure 2.—Alignment of the maps of LG I (A), LG III (B), LG IV (C), LG VIII (D), and LG XI (E) generated for cross 71 and cross 68. For clarity only relevant parts of the linkage groups and relevant markers are shown. The lines connect the map positions of the aligned markers. Common markers for which the corresponding marker in the other cross is on another linkage group or is not grouped are indicated by boldface type. Note that the marker codes of the common markers can be different due to the parental origin and/or the size estimate or fragment number. In B, the calculated maps of LG III-a and LG III-b from cross 71 are aligned with the integrated map of LG III from cross 68. LG III-a[71] is shown twice to facilitate the comparison to both LG III-b[71] and the integrated LG III map of cross 68 (III[68]). "A/MAT" on III-a[71] and "amat" on III[68] represent the mating-type locus. Underlined markers in LG III-b[71] are H markers that are linked to B markers but not to any of the A markers in LG III-a[71]. In C, A and B maps (IV-a[71], IV-a[68], IV-b[71], and IV-b[68], respectively) and integrated maps (IV[71] and IV[68]) are shown. Note the compression of the H markers in the IV-b[71] map.


A
I 71
I 68
12.6 CAE+CT/M+TGf11
17.9 CAE+AA/M+ACs398.3
5.3 cae+aa/m+acs395
12.2 cae+ct/m+ccf6b
33.3 CBE+AC/M+CTs459.6
23.9 cae+ac/m+cts474.3
33.6 cae+ag/m+ags381.6
48.7 CAE+GA/M+CGf2
35.8 cae+ga/m+cgf5
56.2 CBE+AG/M+AGs394.6
B
III-a 71
III-b 71
III 68
III-a 71
10.0 CHE+CC/M+CTf16
12.8 che+cc/m+ctf15
0.0 CHE+CC/M+CTf16
27.7 CBE+AC/M+CCs508.7
43.0 HE+GT/M+GCf4
45.0 HE+CC/M+CCf10
27.0 cbe+ac/m+cts182
49.0 HE+AC/M+CTs196.9
CHE+AC/M+CTs194.6
31.1 che+aa/m+cas161.8
54.0 CHE+AA/M+CAs152.5
54.6 CBE+AA/M+CAs515.0
55.0 HE+AC/M+TTf6
30.0 HE+GA/M+CGf6A
AS1-LOC
A/MAT
HE+CA/M+CCf5
42.6 amat
41.0 cbe+ag/m+atf12
42.0 cae+aa/m+cas543.5
44.0 cae+aa/m+acs185.5
32.0 CHE+AG/M+ATf5
32.2 CAE+AA/M+ACs175.0
33.0 HE+CT/M+ATf14
58.0 CHE+AG/M+ATf5
HE+CT/M+ATf14
65.0 HE+CT/M+GCf3
68.0 HE+CA/M+CCf5
69.0 HE+GA/M+CGf6A
56.5 che+ga/m+tgf11
62.0 bubi/e2
65.6 aubi/e3
89.0 HE+GG/M+CCf13A
72.3 cbe+gt/m+gcf7
92.8 CBE+GT/M+GCf1
94.0 HE+CA/M+CCf19
94.8 CBE+AG/M+CAs307.6
69.0 HE+GG/M+CCf13A
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75.0 CAE+AA/M+CTs192.8
77.0 HE+CA/M+CCf19

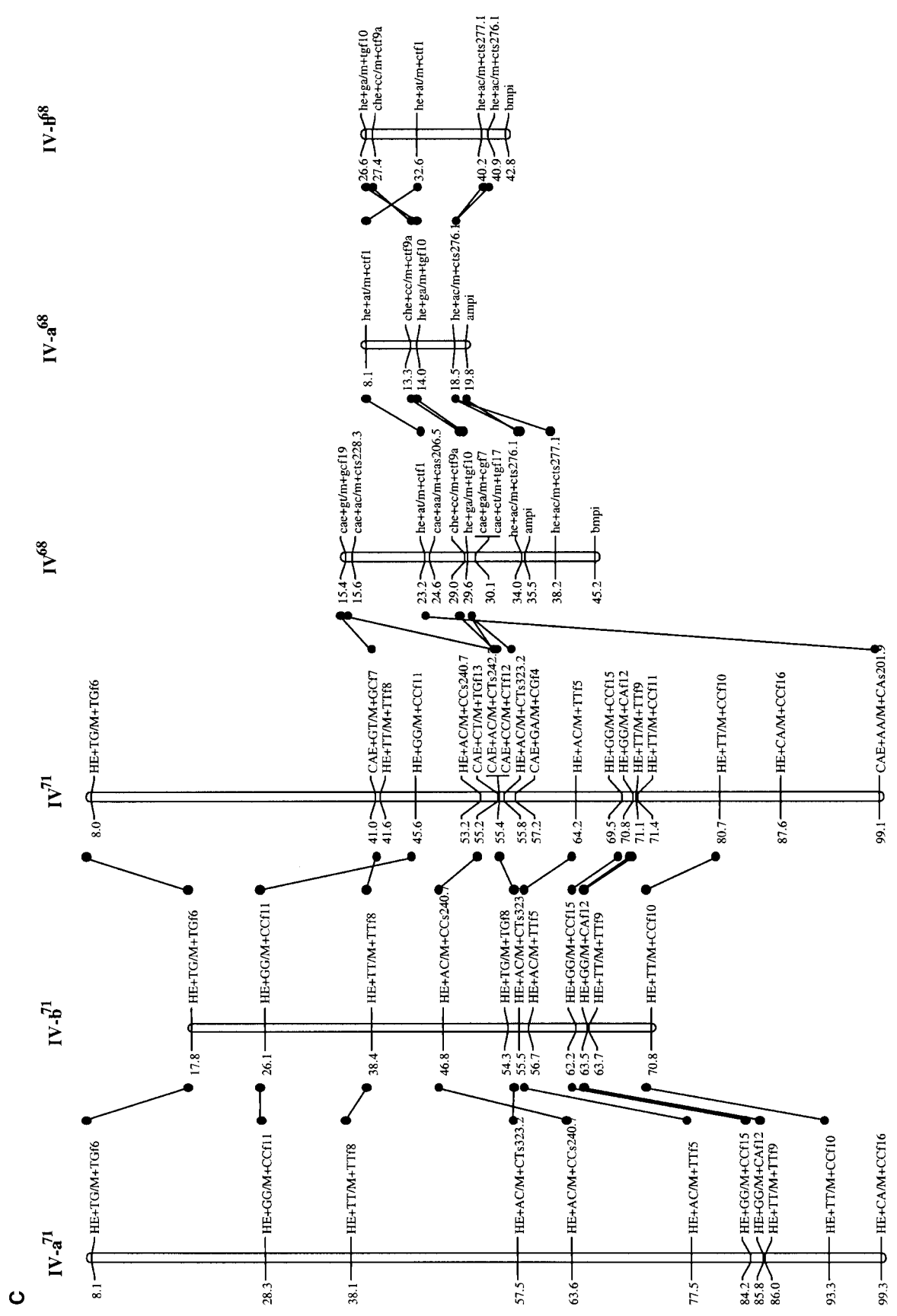

FIGURE 2.—*Continued.*

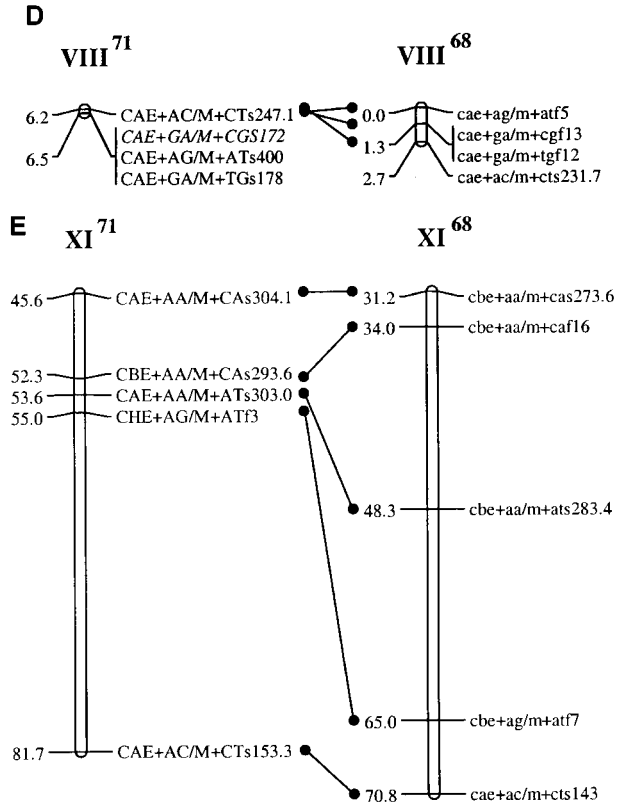

FIGURE 2.—*Continued.*

addition, D12-17 received a third chromosome represented by LG IV markers derived from the A2 parent. D12-17 thus received both homologous chromosomes of a specific pair from one parent and one recombinant chromosome from the other parent.

The exact number of trisomic progeny is difficult to give since some linkage groups contained only markers of a single linkage phase or the number of phase transitions was too low. As was mentioned above and illustrated in Figure 4, there was a strong tendency for tightly linked markers to occur in coupling phase. Still, by analyzing all linkage groups with two or more phase transitions we could obtain an estimate using these criteria: 8 cross 71 progeny were trisomic on the basis of the A map while 5 progeny were trisomic on the basis of the B map. When the information on the A and B map was combined, 11 progeny (16.1%) were trisomic: 8 for one linkage group and 1 for two, 1 for three, and 1 for six linkage groups (Table 3 and Table S2 at http://www.genetics.org/supplemental/). For some linkage groups no trisomy was found and consequently none of the progeny appeared to be triploid. Furthermore, in cross 71 we did not find progeny that were monosomic, as would be detected by the absence of all markers from one of the parents for a particular linkage group. Using the same criteria for cross 68, 10 progeny were trisomic on the basis of the B map while 1 was trisomic on the basis of the A map. In total, 11 progeny were trisomic and also one putative monosomic genotype was identified. If multi-allelic markers were available, the trisomic isolates always correlated with the identification of three alleles of RFLP or isoenzyme markers. However, the presence of a single or three RFLP or isoenzyme alleles frequently did not correlate to monosomy or trisomy of the linkage group.

DISCUSSION

Construction of a genetic linkage map is instrumental for inheritance studies in various ways: it generates markers for phenotypic traits, it is imperative for positional cloning, and it allows detection of aberrations from Mendelian inheritance. Previous studies in *P. infestans* suggested non-Mendelian inheritance in particular at the mating-type locus (JUDELSON *et al* 1995; VAN DER LEE *et al.* 1997). With the genome-wide analysis of *P. infestans* presented here we aim to put these findings in perspective.

A high-density genetic linkage map for *P. infestans* using cross 71: The markers analyzed consist largely of AFLP markers and reliability of this data set as judged by replicate samples of the same isolate was high (nearly 99%). All segregating markers, even those that showed strong deviation of the expected segregation ratios, were scored and analyzed. Over 90% of the high-quality markers are linked in 23 linkage groups and >85% of these markers could be positioned. Markers that could not be mapped are largely dominantly scored H markers, which intrinsically are less informative and therefore more difficult to group and position. The robustness of the grouping was tested by building the maps with the two independent marker types, which could be done for all linkage groups except for LG III. The integration of the A and the B maps resulted in a map with 508 AFLP markers distributed over 13 major and 10 minor linkage groups. The major linkage groups are dense in markers with an average marker spacing of ~2.0 cM. Nevertheless, some regions are low in marker density, particularly for markers originating from one of the parents or from one linkage phase, indicating that the map is far from saturated. Also, the number of linkage groups is remarkably high for the expected 8–10 chromosomes (SANSOME and BRASIER 1973). It seems that although >90% of the markers are linked, some parts of the genome remain uncovered, resulting in gaps in the linkage map. This problem may be caused by low polymorphism between the parental isolates for the homologous chromosomes in some regions. The alternative perspective would be that most markers originate from hypervariable regions and the tight clustering of markers in linkage phase seems to point in this direction. Hemizygous regions were identified before (JUDELSON 1996b; VAN DER LEE *et al.* 2001b) and may explain this observation.

Comparison with the previous maps of cross 71: In general, the grouping in the new map fits well with that of the first genetic linkage map of *P. infestans* and is identical to the partial maps presented before (VAN DER LEE

TABLE 2

Alignment of the maps of cross 68 and cross 71

		Linkage groups of cross 68																	
Linkage groups of cross 71	MT[a]	I A, H	II A, B, H	III A, B, H	IV A, B, H	VI A	VIII A, H	IX A, B, H	X B	XI A, B, H	XIII A, H	A1-e B, H	A2-a A, B, H	A2-b B, H	A2-c A, B, H	A2-d A	I(part) B, H	68-1 A, B, H	NG68[b]
I	A, B, H	**4**															1		
II	A, B, H		**1**																
III[c]	A, B, H			**8**													1		1
IV	A, B, H				**6**														
VI	A, B, H					**1**													
VII	A, B, H			1															
VIII	A, B, H						**4**												
IX	A, B, H							**2**											1
X	A, B, H	1							1										
XI	A, B, H									**5**									
XII	A, B, H																		1
XIII	A, B, H										**1**		1						
A1-a	A, H																		2
A1-e	A											**1**							
A2-a	B, H					1					1		**1**						
A2-b	B													**1**					
A2-c	B, H														**1**				
A2-d	B, H															**1**			
NG71[d]															1			1	3[e]

Numbers represent the numbers of common markers in each linkage group. Those in boldface type are on linkage groups with the same numbering in the two crosses. Linkage groups that do not contain common markers are not listed: five LGs of cross 71 (LG V, A1-b, A1-c, A1-d, and A1-f) and seven of cross 68 (un68-2–un68-8). Common markers and linkage groups shown in Figure 2 are underlined.

[a] MT: marker types present on the relevant linkage groups. In cross 71, major LGs (I–XIII) contain marker types A, B, and H and minor LGs (A1-a–f and A2-a–d) marker types A (and H) or B (and H), respectively (see Figure 1). The numbering of the LGs in cross 68 follows the numbering of the LGs in cross 71 and, as a consequence, major and minor LGs do not necessarily have roman numbers or A1 and A2 prefixes, respectively. For the map of cross 68 and numbers and marker types in each linkage group, see supplemental data at http://www.genetics.org/supplemental/.

[b] NG68: common markers not grouped in cross 68 but positioned on linkage groups in cross 71.

[c] Comprises LG III-a and LG III-b.

[d] NG71: common markers not grouped in cross 71 but positioned on linkage groups in cross 68.

[e] Common markers not grouped in cross 68 or cross 71.

TABLE 3

F_1 progeny from cross 71 with trisomic linkage groups

	Linkage groups of cross 71[a]																																				
	I		II		III		IV		V		VI		VII		VIII		IX		X		XI		XII		XIII												
F_1 progeny	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A1-a	A1-b	A1-c	A1-d	A1-e	A1-f	A2-a	A2-b	A2-c	A2-d	Total[b]
T15-3			—	X							X		—	X	—	—	—	—			—		—		—	—		—	—	—	—	—					3
T15-5			—						X				—		—	—	—	—			—		—		—	—		—	—	—	—	—					1
T20-4			—						X				—		—	—	—	—			—		—		—	—		—	—	—	—	—					1
T30-3			—				X						—		—	—	—	—			—		—		—	—		—	—	—	—	—					1
D12-16			—	X									—		—	—	—	—			—		—		—	—		—	—	—	—	—					1
D12-17			—				X						—		—	—	—	—			—		—		—	—		—	—	—	—	—					1
D12-22			—										—		—	—	—	—			—		—		—	—		—	—	—	—	—				X	1
E12-6			—	X						X		X	—		—	—	—	—	X		—		—	X	—	—		—	—	—	—	—			X		6
E12-7			—										—		—	—	—	—			—		—		—	—	X	—	—	—	—	—					1
E12-11			—										—		—	—	—	—	X		—		—		—	—		—	—	—	—	—					1
E12-18			—									X	—		—	—	—	—			—		—	X	—	—		—	—	—	—	—					2

X, two copies of that particular linkage group originating from either the A1 (A) or A2 (B) parent. —, trisomy could not be determined because the linkage group shows only one or no phase transition.

[a] The major linkage groups, containing markers from both parents, are split in A and B to indicate the parental origin of the trisomy. A, A1 parent 80029; B, A2 parent 88133.

[b] The number of trisomic linkage groups that could be detected in each of the F_1 strains.

et al. 1997; 2001a). Compared to the previous map, LG I, IV, V, VIII, IX, A1-a, A2-a, A2-c, and A2-d remain within the same boundaries upon the addition of the new markers (Table 1). The higher marker density revealed dissimilarity between the III-a and III-b map that was not noted previously. Some minor linkage groups could be integrated in a major linkage group: LG A2-e was integrated into LG X, and A1-b, A2-b, and part of LG V were fused to the new LG XI. The most important differences from the previous map are found in LG II, VI, and VII, all of which are now split into two or more linkage groups (Table 1). In the case of LG II, markers with distorted segregation ratios erroneously merged three linkage groups and hence LG II is now split in LG II, LG XII, and LG A1-e. In the case of LG VI and LG VII, the number of bridging H markers in the previous map appeared to be too low and LG VI is now split in LG VI and LG A1-f, while LG VII is split in LG VII and LG XIII.

The current AFLP map was constructed with more markers using more stringent LOD values and stricter criteria. With the exception of three markers that were forced to the end of their linkage group by the JoinMap algorithm, the order and distance between the markers in the current map and the previous map is similar, at least within the mapping resolution. Occasionally markers from different parents "slide" over each other, which is quite understandable, given the mapping resolution of the H markers. The software program JoinMap3.0 is much more sophisticated than the JoinMap1.4 version used for the first map. JoinMap3.0 allows easy identification of markers with aberrant segregation ratios and markers that cause friction in the map are set aside temporarily. The Windows-based user-interface makes it easy to analyze effects of different settings and contributions of individual markers or individual progeny to the map. Nevertheless, construction of integrated maps based solely on dominant markers remains difficult and since there is no direct linkage information between A and B markers, integrated maps should be handled with caution. Reassessment of the present grouping will be needed when more markers are added. We anticipate that with more markers, groups will merge, generating a number of linkage groups that is closer to the predicted number of 8–10 chromosomes (Sansome and Brasier 1973).

Translocations and other aberrations at LG III: In cross 71, the A and the B map of LG III are dissimilar in the region close to the mating-type locus. A group of six H markers, in the middle of LG III-b, are not linked to markers positioned on LG III-a. This does not involve a deletion, since by nature the H markers (Aa × Aa) are present in both parents. This absence of linkage therefore is reminiscent of a translocation. However, we could not identify the repositioning of this region to any of the other linkage groups. The translocation does not seem to correlate to the mating type as such,

since the parental isolates of cross 68 do not appear to differ in this region. Judelson *et al.* (1995) found only two of the four expected combinations of gametes in the progeny of some crosses, suggesting balanced lethals and possibly generated by balanced translocations. In cross 71 the translocation does not seem to be balanced: all four allelic combinations were found, but then again segregation ratios for the A1 and A2 mating-type locus and markers from the A1 parent on the same chromosome are distorted (van der Lee *et al.* 1997). The progeny mapped in this study were generated from oospores obtained by infecting potato leaves with the parental strains and were recovered from sporulating lesions formed on leaves floating on water mixed with soil containing the oospores (*in vivo*; Drenth *et al.* 1995). Remarkably, when progeny of the same parental strains were generated from oospores obtained by co-cultivation on rye medium and germination of these oospores on water agar (*in vitro*), the progeny showed no distorted segregation ratios for the mating-type locus or for markers linked to the mating type (T. van der Lee and F. Govers, unpublished results). The fact that the distorted segregation ratios were found only in the *in vivo* progeny suggests that progeny with the A1 mating type have an advantage during *in vivo* development, survival, and/or growth. In this respect it may be significant that the A1 mating type was distributed all over the world while the A2 mating type was restricted to some areas (Fry *et al.* 1992). Even now, in populations where sexual reproduction occurs, the ratio of A1 to A2 is biased for the A1 mating type, particularly after prolonged periods that favor vegetative growth (Zwankhuizen *et al.* 1998, 2000). Another remarkable feature is that in all studies reported, including this study, the A1 mating type is dominant, whereas A2 isolates of *P. infestans* can be self-fertile (Smart *et al.* 2000). Obviously, the mating-type locus is one of the most challenging and intriguing areas for genetic studies in *P. infestans*.

Alignment of two maps reveals more translocations: The map of cross 68 with 363 markers positioned on 24 linkage groups is less dense and more fragmented than the map of cross 71. Following the successful integration of A and B maps on the basis of common H markers, we used comigrating AFLP fragments as common markers for integration of the maps of cross 71 and cross 68. We anticipated that the map of cross 71 could serve as a backbone for the map of cross 68 and that areas with low marker density, caused by low polymorphism between parental isolates of one cross, would benefit from markers obtained in the same region in the other cross. In general the grouping and order of the common markers in the two crosses matched, but we also found several dissimilarities. This points toward differences in grouping and/or ordering of markers in the parental isolates of cross 68 and cross 71 and is reminiscent of translocations (Table 2, Figure 2). It is true that comigrating AFLP fragments do not necessarily represent the same fragment, but since H markers could readily be used to integrate A and B maps there is no reason why C markers could not be used for the same purpose. These findings suggest that translocations are not rare in Phytophthora and other studies support this. In *P. sojae* rearrangements were found within a linkage group (MacGregor *et al.* 2002), whereas in *P. infestans* a marker linked to the mating-type locus appeared to have translocated to another linkage group (Randall *et al.* 2003). Translocations create the possibility of obtaining one, two, or three copies of a locus or region, which may result in balanced lethals (Judelson 1996a) or in high frequencies of nonviable oospores, as observed in many crosses (Knapova *et al.* 2002). On the other hand, the flexibility to have one, two, or three copies of a genomic region can be advantageous for adaptation.

Trisomic progeny: Previously, the occurrence of trisomic progeny in two Phytophthora species, *P. infestans* and *P. cinnamoni*, was postulated on the basis of the presence of three alleles of multi-locus markers (Carter *et al.* 1999; Dobrowolski *et al.* 2002). In those studies the number of markers was limited and the presence of three alleles could not be assessed for a complete linkage group. In this study we used a different approach to identify trisomic progeny and demonstrated that trisomy extends over the whole chromosome. For each linkage group with at least 5 markers from one of the parents and two linkage-phase transitions, we tested whether we could detect the presence of two copies of a chromosomal set of one of the parents using the linkage phase of the markers. If all markers from a particular parent are present in the progeny regardless of the linkage phase, then both parental chromosomes are transmitted to its offspring. As summarized in Table 3, we found significant numbers of trisomic progeny for specific chromosomes. Obviously, with this method trisomy of linkage groups with a few markers or only one or no phase transition remains undetected whereas more markers and more phase transitions result in more solid data. For LG IV, *e.g.*, with 21 markers originating from parent 80029 and six phase transitions we could demonstrate that trisomy extends over at least 50 cM of a total of 99 cM (Figure 3). We also identified progeny that had only a part of a linkage group in triplicate (Table S2, at http://www.genetics.org/supplemental/). In cross 68, some of the multi-allelic markers suggested the presence of one or three copies of a chromosome pair but this was not confirmed by other markers on the same linkage group, whereas other linkage groups were clearly trisomic. This indicates that, apart from trisomy, other aberrations, possibly translocations or postfusion instability, do occur. Remarkably, in three progeny of cross 71 the intensity of a group of markers linked to the avirulence gene cluster *Avr3-Avr10-Avr11* was significantly reduced to ~50% (data not shown), also pointing at postfusion deletions or mitotic gene conversions (as described for

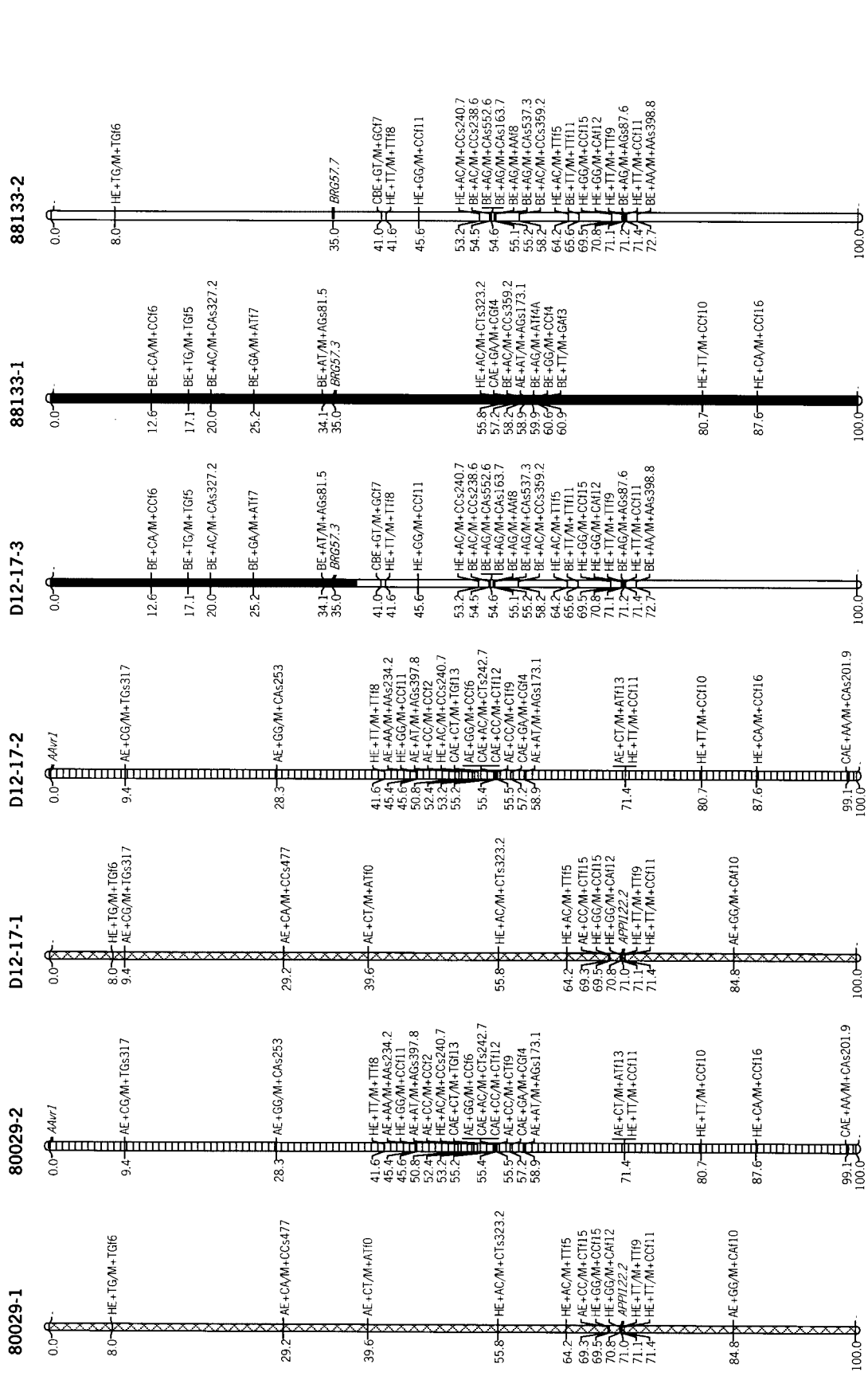

FIGURE 3.—Graphical representation of LG IV of the parental isolates of cross 71 and F_1 individual D12-17 trisomic for LG IV. Only the markers present in a particular isolate are indicated. Calculation of the linkage phase was done in JoinMap. Filling patterns of the bars indicate parental origin. Note the recombination breakpoint in one of the three homologous chromosomes in D12-17.

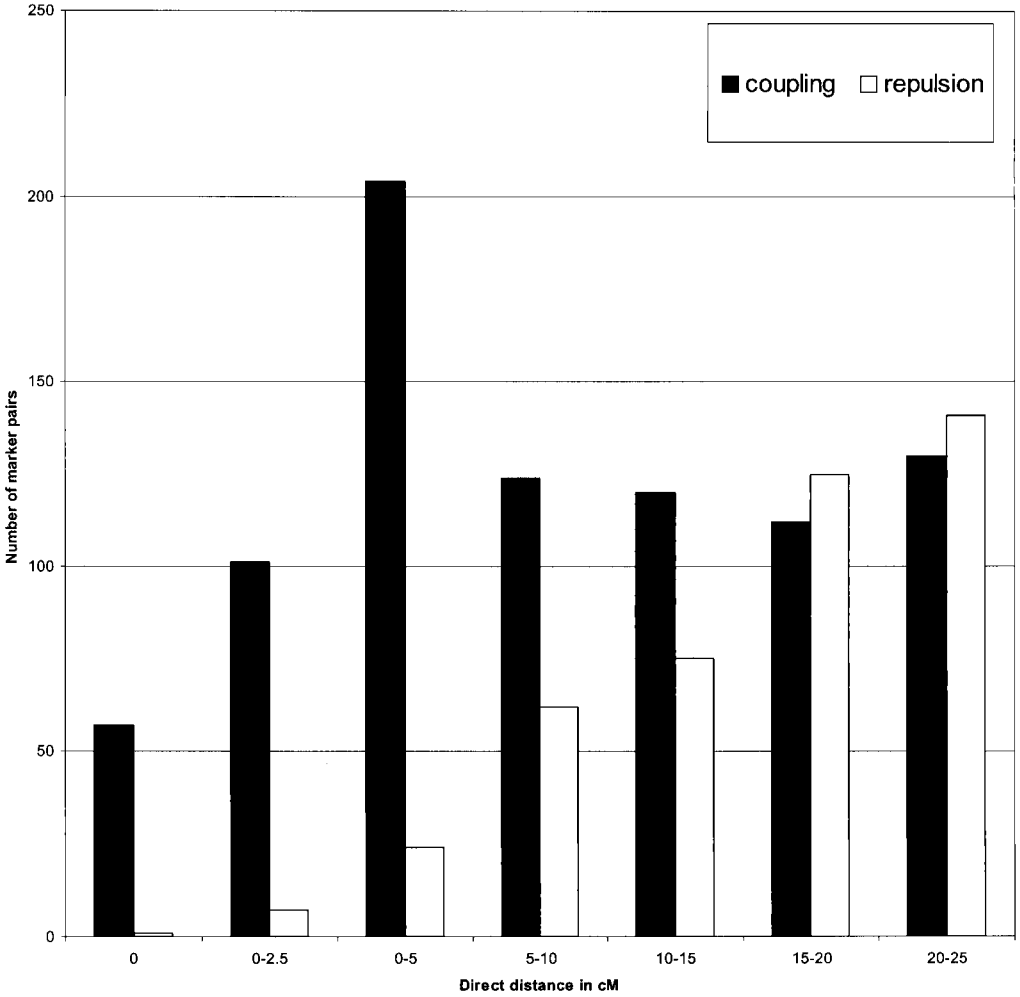

FIGURE 4.—Strongly linked markers are mostly in coupling phase. For 1282 A marker pairs (Aa × aa; Aa × aa) in cross 71 with a LOD value of 3 or higher, the linkage phase was determined. Markers are grouped in classes according to their pairwise genetic distance (*x*-axis) and the number of marker pairs in each class (*y*-axis). Note that the two bars representing markers within a genetic distance of 0–5 cM are an accumulation of markers represented by the first two sets of two bars (genetic distance 0 and 0–2.5 cM, respectively) plus markers with a genetic distance of 2.5–5.0 cM.

P. sojae by CHAMNANPUNT *et al.* 2001). Because we can detect trisomy only in linkage groups with sufficient markers and phase transitions, assessment of the precise percentage of trisomic progeny is impossible but definitely 10–16% of the progeny from both crosses have one or more trisomic linkage groups. In contrast, we encountered only a single individual that was possibly monosomic for one linkage group and no progeny that were triploid.

The parental isolates of cross 71 are both field isolates and their genetic diversity is in line with the diversity found in the Dutch field population. No crossing barriers appear to exist between the parental isolates, as the progeny were no more heterozygous than the parental isolates (data not shown). The parental isolates were also used for mixed inoculations in field trails and numerous oospores were found (DRENTH *et al.* 1995). The cross 71 progeny, including the trisomic progeny, are pathogenic on potato. We therefore think that trisomy in *P. infestans* is not an artifact and that it occurs in a natural environment. Oomycetes have a multinucleate coenocytic mycelium and variable chromosome numbers may be less problematic. We hypothesize that trisomy and subsequent instability of chromosomal regions, or loss of complete chromosomes, contribute significantly to the notorious genetic flexibility of *P. infestans*.

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