

Independent deletions of a pathogen-resistance gene in *Brassica* and *Arabidopsis*

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ABSTRACT Plant disease resistance (*R*) genes confer race-specific resistance to pathogens and are genetically defined on the basis of intra-specific functional polymorphism. Little is known about the evolutionary mechanisms that generate this polymorphism. Most *R* loci examined to date contain alternate alleles and/or linked homologs even in disease-susceptible plant genotypes. In contrast, the resistance to *Pseudomonas syringae* pathovar *maculicola* (*RPM1*) bacterial resistance gene is completely absent (*rpm1-null*) in 5/5 *Arabidopsis thaliana* accessions that lack *RPM1* function. The *rpm1-null* locus contains a 98-bp segment of unknown origin in place of the *RPM1* gene. We undertook comparative mapping of *RPM1* and flanking genes in *Brassica napus* to determine the ancestral state of the *RPM1* locus. We cloned two *B. napus* *RPM1* homologs encoding hypothetical proteins with ≈81% amino acid identity to *Arabidopsis* *RPM1*. Colinearity of genes flanking *RPM1* is conserved between *B. napus* and *Arabidopsis*. Surprisingly, we found four additional *B. napus* loci in which the flanking marker synteny is maintained but *RPM1* is absent. These *B. napus* *rpm1-null* loci have no detectable nucleotide similarity to the *Arabidopsis* *rpm1-null* allele. We conclude that *RPM1* evolved before the divergence of the Brassicaceae and has been deleted independently in the *Brassica* and *Arabidopsis* lineages. These results suggest that functional polymorphism at *R* gene loci can arise from gene deletions.

The outcome of many plant–pathogen interactions is determined by disease-resistance (*R*) genes that enable plants to recognize invading pathogens and activate inducible defenses (1). A typical *R* gene allele encodes “race-specific” resistance to only one or a few strains of a single pathogen species (2). *R* gene loci are functionally polymorphic within a plant species and encode alternate alleles that either recognize different strains of the same pathogen or do not recognize any tested pathogen. Recent molecular studies have revealed that *R* genes often reside in complex loci consisting of the *R* gene and tightly linked homologs (3–8). These complexes can exist in both disease-resistant and disease-susceptible plant genotypes. The allelic diversity and functional specialization at *R* gene loci inspired H. H. Flor’s “gene-for-gene” hypothesis: An *R* gene specifies resistance only if the pathogen expresses a corresponding avirulence (*avr*) gene (9). If either component is nonfunctional, then the plant is unable to activate resistance responses. This model has successfully described a wide variety of plant–pathogen associations; however, we currently know little about the molecular basis and origin of *R* gene polymorphism.

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The most common molecular interpretation of the gene-for-gene hypothesis is that *R* genes encode specialized receptors that recognize the direct or indirect products (elicitors) of the corresponding *avr* genes (10). One implication of the receptor–elicitor model is that functional polymorphism at *R* loci could arise from gain-of-function mutations that enable the plant to recognize novel pathogen variants. Maintenance of multiple alleles or multiple linked genes with different recognition capabilities at an *R* locus would enable a host population to defend itself against the corresponding pathogens. *R* gene polymorphism could also, in principle, arise from loss-of-function mutations in *R* genes. Nonfunctional *R* gene alleles could be maintained if there is a cost of resistance in the absence of pathogen selection (11). Nonfunctional alleles could also serve as a repository of divergent sequences that could be contributed to related genes by recombination, thereby accelerating the evolution of novel *R* genes (12).

Recent cloning of *R* genes against diverse pathogens provides the tools for comparative analysis of *R* gene alleles within and between species, which will provide insight into the evolutionary history of *R* genes. We describe the structure of functional and nonfunctional alleles of the *RPM1* (resistance to *Pseudomonas syringae*, pathovar *maculicola*) bacterial resistance gene in *Arabidopsis thaliana* and a related crop species, *Brassica napus*. *RPM1* was initially identified in *A. thaliana* accession Col-0 through its ability to confer resistance to *P. syringae* isolates expressing either *avrRpm1* (13) or *avrB* (14). The *avrB* and *avrRpm1* avirulence genes are sequence unrelated, thus *RPM1* enables dual-specificity resistance. *RPM1* was isolated by map-based cloning and was shown to encode a protein with a putative amino-terminal leucine zipper, a consensus nucleotide binding site (NBS), and 14 C-terminal leucine-rich repeats (LRRs) (15). The *RPM1* protein is thus a member of the largest class of *R* proteins functionally characterized to date, the so-called NBS-LRR class (16). Previous analyses suggested that *RPM1* functional polymorphism in *Arabidopsis* arose from an intraspecific insertion or deletion of the *RPM1* gene (15). Here we provide molecular evidence that the evolution of *RPM1* predated the divergence of the Brassicaceae, and that independent deletions of *RPM1* have occurred in both *Arabidopsis* and *Brassica*.

MATERIALS AND METHODS

Genetic Mapping in *B. napus*. Clones from *A. thaliana* and *B. napus* were used to probe filters with genomic DNA from

This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: *avr*, avirulence; NBS, nucleotide binding site; LRR, leucine-rich repeat; *RPM1*, resistance to *Pseudomonas syringae*, pathovar *maculicola*; RFLP, restriction fragment length polymorphism. Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF10594–AF105106 and AF105139–AF105143).

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avirulent DC3000. Measurements of *in planta* bacterial growth confirmed that the *RPM1* transgene conferred levels of resistance that were comparable to wild-type Col-0 (not shown). We also determined that *RPM1* is sufficient for resistance in transgenic lines of the susceptible accessions Mt-0 and Fe-1 (not shown).

The Brassica *RPM1* Locus Is Syntenic with *RPM1* from Resistant *Arabidopsis*. The sequence comparisons of functional and null *RPM1* loci suggested that *RPM1* polymorphism resulted from either a single insertion or a single deletion of *RPM1* during *Arabidopsis* evolution. To distinguish between these competing hypotheses, we compared the organization of *RPM1* and flanking genes in *Arabidopsis* and a related crucifer, *B. napus* (oilseed rape). Diploid *Brassica* genomes are thought to have evolved from a hexaploid ancestor (21–23), and *B. napus* is a amphidiploid hybrid of *Brassica rapa* (A genome) and *Brassica oleracea* (C genome) (24). Thus, each single-copy *Arabidopsis* gene would be expected to map to six loci in *B. napus*, arranged in three homoeologous pairs.

We identified only two *RPM1* homologs in *B. napus* by hybridization with an *Arabidopsis* *RPM1* coding sequence probe. Only two *RPM1* RFLPs were detected in *B. napus* DNA digested with either of five restriction enzymes, and in each case the two RFLPs mapped to the same chromosomal loci (see below). Corresponding genomic clones (named 1A and 9N) were isolated, respectively, from libraries of spring (N-o-101) or winter (N-o-109) *B. napus* varieties. The hypothetical proteins encoded by 1A and 9N share 95% amino acid identity and each shares ≈81% identity with *Arabidopsis* *RPM1* (Fig. 2). Like *Arabidopsis* *RPM1*, the *B. napus* *RPM1* proteins contain a putative N-terminal leucine zipper, a consensus NBS, and 14 imperfect LRRs. Amino acid substitutions between *Arabidopsis* *RPM1*, 9N, and 1A are distributed relatively evenly over the protein. No clear evidence for positive diversifying selection (7) in the LRRs was found in comparisons of *RPM1* homologs (data not shown).

The 5' flanking regions of 1A and 9N are noticeably diverged from each other and contain several insertion/deletions, the largest of which is a 255-bp insertion in 9N, relative to 1A (not shown). Comparison of the 5' and 3' flanking regions of the *Arabidopsis* *RPM1* and 1A or 9N clones revealed little overall sequence conservation, with the exception of two potential 5' *cis* regulatory elements immediately proximal to the *RPM1* coding sequence. One motif is almost completely identical to a C/EBP binding site (25) (CCAAGT-TGCAAATGTGAAAGCAGT, beginning 218 bp upstream of the *RPM1* start codon).

9N and 1A were placed on an integrated genetic linkage map of *B. napus* (17) between RFLP markers pN148a & pO12e and pW108c & pO12c, on homoeologous chromosomes N1 (representing the A genome of *B. rapa*) and N11 (representing the C genome of *B. oleracea*), respectively (Fig. 3A). Surprisingly, each locus contains only one copy of *RPM1*.

To assess collinearity between the *B. napus* and *Arabidopsis* *RPM1* loci, we mapped two cDNA markers that closely flank *RPM1* in *Arabidopsis* (Fig. 1). The *GTP* probe was derived from an ORF located 700 bp centromeric to *Arabidopsis* *RPM1* that encodes a putative GTP-binding protein, and the *M4* probe was derived from an ORF ≈4 kb telomeric that encodes a putative UDP-glucosyltransferase. Both markers are single copy in *Arabidopsis* (not shown) and are present in *RPM1* and *rpm1-null Arabidopsis* accessions (Fig. 1). In *B. napus*, these probes mapped as cosegregating pairs to six loci, two of which correspond to the *B. napus* *RPM1* loci (Fig. 3A). The six loci map within large homoeologous segments of the *B. napus* genome (26). Sequencing and hybridization analysis of the *B. napus* *RPM1* phage clones confirmed synteny between the *RPM1* loci in *B. napus* and resistant *Arabidopsis* (Fig. 3B). The distance between the end of the *GTP* gene and the beginning of 1A and 9N is 293 and 562 bp, respectively, compared with

	1	50
<i>B. napus</i> <i>RPM1</i> -1An.....	
<i>B. napus</i> <i>RPM1</i> -9Ng.....	
<i>A. thal.</i> <i>RPM1</i>g.....	
Consensus	MASATVDVGI GLILSLLENE TLLLSGVHSE IEKMKKELLI IKSFLIEDTHK	
	Leucine Zipper	
	51	100
<i>B. napus</i> <i>RPM1</i> -1Atg.....	
<i>B. napus</i> <i>RPM1</i> -9Nw.....gt.....r.....s.....i.....	
<i>A. thal.</i> <i>RPM1</i>	hg.....	
Consensus	QDNGSTTTT T---TTTTQLF QTFVANTRDL AYQVEDIDE FTYHINGVRS	
	101	150
<i>B. napus</i> <i>RPM1</i> -1Am.....	
<i>B. napus</i> <i>RPM1</i> -9Nk.....	
<i>A. thal.</i> <i>RPM1</i>a.....i.....f.....r.....m.....	
Consensus	CTKLRRRAVHF P-YMWARHSI AQKLGAVNVM IRSISEMKR Y---QTYOGA	
	151	200
<i>B. napus</i> <i>RPM1</i> -1A	
<i>B. napus</i> <i>RPM1</i> -9N	
<i>A. thal.</i> <i>RPM1</i>	lppl.....d.....a.....n.....	
Consensus	SLVSHVDDGGG TKWVNHSISE SLFFSENSLV GIDAAKGLKI GWLLSPEPQR	
	201	250
<i>B. napus</i> <i>RPM1</i> -1A	
<i>B. napus</i> <i>RPM1</i> -9N	
<i>A. thal.</i> <i>RPM1</i>a.....	
Consensus	IVSVVVMGG SGRITLSANI FKSQTVRKHF ASYAWVTISK SVVIEDVFRF	
	P-loop	
	251	300
<i>B. napus</i> <i>RPM1</i> -1A	
<i>B. napus</i> <i>RPM1</i> -9N	
<i>A. thal.</i> <i>RPM1</i>d.....a.....g.....	
Consensus	MIKEFYKEAE TQIPGELYSL TYRELVEKLV EYLSKRYTF VLDVWNTGL	
	Kinase 2A	
	301	350
<i>B. napus</i> <i>RPM1</i> -1Av.....m.....	
<i>B. napus</i> <i>RPM1</i> -9Nn.....v.....l.....	
<i>A. thal.</i> <i>RPM1</i>y.....n.....d.....m.....p.....i.....t.....	
Consensus	WREISIALPD GISGRVYV-T TRRHWVASFS YGSGSRKHEI ELLKEDEAWV	
	Kinase 3A	
	351	400
<i>B. napus</i> <i>RPM1</i> -1Av.....	
<i>B. napus</i> <i>RPM1</i> -9Nl.....l.....	
<i>A. thal.</i> <i>RPM1</i>s.....p.....q.....t.....q.....p.....	
Consensus	LFCNKAFSGS LEECRFRNLE -IARKLVERC QGLPLATLGL GEMSTKRLKLE	
	Cons. Domain 2	
	401	450
<i>B. napus</i> <i>RPM1</i> -1Al.....	
<i>B. napus</i> <i>RPM1</i> -9Ns.....	
<i>A. thal.</i> <i>RPM1</i>k.....s.....t.....h.....i.....m.....f.....n.....	
Consensus	SEWKQVYNSL NWELNNLLEL KVVRSIL-LS FSDLPYPLKR CFLYCCLPFV	
	Cons. Domain 3	
	451	500
<i>B. napus</i> <i>RPM1</i> -1Ak.....	
<i>B. napus</i> <i>RPM1</i> -9N	
<i>A. thal.</i> <i>RPM1</i>i.....	
Consensus	NYRMKRKRLV RMVMAQRFVE PIRGVKAEV ADGLNELVY RRMILQVILWN	
	501	550
<i>B. napus</i> <i>RPM1</i> -1A	
<i>B. napus</i> <i>RPM1</i> -9N	
<i>A. thal.</i> <i>RPM1</i>a.....w.....v.....l.....yn.....s.....g.....a.....m.....ny.....s.....	
Consensus	PFGRPKVFKM HDVIREIALS ISKAERFCDV NGDDDDDD- AETAEDHTR	
	551	600
<i>B. napus</i> <i>RPM1</i> -1Al.....	
<i>B. napus</i> <i>RPM1</i> -9Nv.....	
<i>A. thal.</i> <i>RPM1</i>tp.....dsi.....a.....s.....saa.....k.....m.....l.....n.....d.....s.....	
Consensus	HLCIQEMRS GT-RRTNLET LLVCT--KHS IELPPSLKLL RALDLGSSGI	
	601	650
<i>B. napus</i> <i>RPM1</i> -1Af.....	
<i>B. napus</i> <i>RPM1</i> -9Nei.....	
<i>A. thal.</i> <i>RPM1</i>c.....m.....q.....kn.....k.....v.....k.....ie.....	
Consensus	SKLPD-LVTL FNLKYLNLGK TEVKELPRDF HRLINLETLM TRBSKVDLEP	
	651	700
<i>B. napus</i> <i>RPM1</i> -1A	
<i>B. napus</i> <i>RPM1</i> -9N	
<i>A. thal.</i> <i>RPM1</i>k.....r.....e.....r.....v.....k.....w.....	
Consensus	FGHWKLRKLR YLITFRNCYG HDSNWNVLVG TRVSPSICQL KGLQVMDCFN	
	701	750
<i>B. napus</i> <i>RPM1</i> -1Ak.....s.....	
<i>B. napus</i> <i>RPM1</i> -9Nt.....v.....i.....	
<i>A. thal.</i> <i>RPM1</i>c.....d.....n.....g.....d.....i.....d.....i.....n.....g.....	
Consensus	AEAEELTR-LG GMTQLTRISL VMIRREHGRD LCESLNKIKR LRFLSLTSDH	
	751	800
<i>B. napus</i> <i>RPM1</i> -1Ag.....	
<i>B. napus</i> <i>RPM1</i> -9Nr.....	
<i>A. thal.</i> <i>RPM1</i>d.....	
Consensus	EEEPLEID-L IATASIEKLF LAGKLERVPS WFSTLQNVTY LGLRGSQIQE	
	801	850
<i>B. napus</i> <i>RPM1</i> -1Ak.....	
<i>B. napus</i> <i>RPM1</i> -9Ns.....	
<i>A. thal.</i> <i>RPM1</i>lsi.....r.....p.....r.....q.....q.....e.....	
Consensus	NAIHYLQTLF KLVVLSFYNA YMGTLFCFAE GFENLKILDI VMGHLTEVV	
	851	900
<i>B. napus</i> <i>RPM1</i> -1Ae.....	
<i>B. napus</i> <i>RPM1</i> -9Nfel.....	
<i>A. thal.</i> <i>RPM1</i>d.....g.....y.....i.....n.....	
Consensus	IEDGAMVGIQ KLVVTRACVLE ESVPRGIENL VNLQELRLESH VSDQLVERIR	
	901	934
<i>B. napus</i> <i>RPM1</i> -1Aws.....*	
<i>B. napus</i> <i>RPM1</i> -9Nyt.....k.....*	
<i>A. thal.</i> <i>RPM1</i>gs.....r.....y.....dn.....*	
Consensus	GEEGVDRS-V KHIPAIKHHF RTEDGSPYVS LSS-	

Fig. 2. Structural comparison of hypothetical *RPM1* proteins from *Arabidopsis* and *B. napus*. Putative functional motifs are emphasized in bold. The P-loop, kinase 2A, and kinase 3A motifs constitute a consensus NBS. Conserved domains 2 and 3 are present in all known NBS-LRR resistance genes, but their function is unknown. LRRs are designated by the highlighted XXLXLXX motif.

479 bp in *Arabidopsis*. The distance between M4 and 1A or 9N could not be determined precisely by restriction mapping but

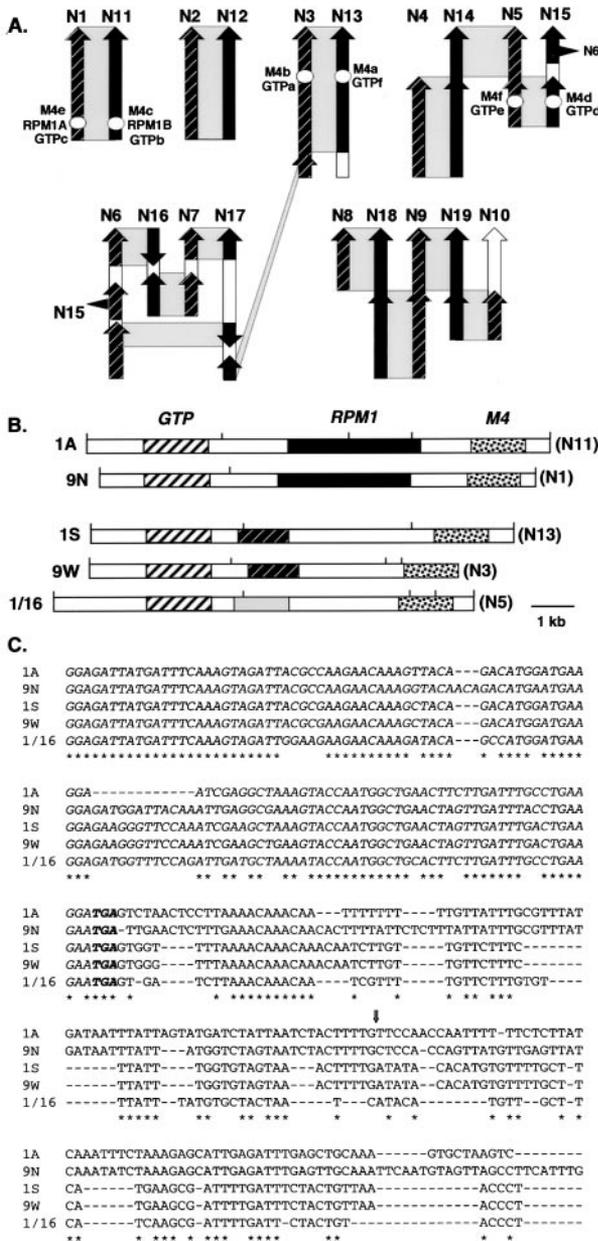


FIG. 3. Location and structure of functional and null *RPM1* loci in *B. napus*. (A) Schematic representation of the amphidiploid *B. napus* genome indicating the loci hybridizing to the *Arabidopsis* *RPM1*, *M4*, and *GTP* clones. Linkage groups N1 to N10 and N11 to N19 represent the A and C genomes, respectively. Hatched and filled areas of the arrows indicate regions of primary homeology between the A and C genomes. Unfilled areas represent regions for which primary homeology between the genomes has yet to be detected. (B) Schematic representation of *RPM1* and *rpm1-null* loci, based on restriction mapping of phage clones. The locus names are listed on the left and the chromosomes to which they map are listed on the right. 1A and 9N contain *RPM1*, and the other three phage are *rpm1-null*. Ticks indicate either *Xho*I or *Xba*I restriction enzyme sites. The *GTP*, *RPM1*, and *M4* genes are indicated above the figure. Equivalently shaded regions cross-hybridize. Unshaded regions were not tested. (C) Sequence alignment at the 5' breakpoint of sequence homology between *B. napus* *RPM1* and *rpm1-null*. The 3' ends of the *GTP* coding sequence are italicized, with stop codons emphasized in bold. Dashes represent gaps, and stars indicate sites that are identical among all five sequences. The arrow indicates the position at which similarity between the *RPM1* and *rpm1-null* loci breaks down completely.

is at least 3 kb in both cases. Thus, the order and approximate spacing of *GTP*, *RPM1*, and *M4* is conserved between 1A, 9N, and *Arabidopsis* *RPM1*. This synteny suggests that the func-

tional *RPM1* allele represents the ancestral state of the locus in *Arabidopsis*, and that the *Arabidopsis* *rpm1-null* allele was derived by deletion at some time after the divergence of *Arabidopsis* and *Brassica* lineages.

Southern hybridization suggested that the four other *GTP/M4* loci in *B. napus* (homologous chromosomes N3/N13 and N5/N15) did not contain any *RPM1*-hybridizing sequences (Fig. 3A). The absence of *RPM1* homolog was confirmed by analysis of corresponding genomic phage clones (Fig. 3B). These phage clones hybridized to the *GTP* and *M4* probes but not to *RPM1* coding sequence probes. Three *rpm1-null* loci were sequenced to determine the 5' breakpoint: 1/16, 1S, and 9W, that correspond, respectively, to chromosomes N5, N13, and N3 (Fig. 3B). Sequence alignments confirmed that the *RPM1* gene was indeed absent from these loci. The breakdown of sequence similarity between the *Brassica* *RPM1* and *rpm1-null* loci is gradual, beginning with several small insertion/deletions just downstream of the *GTP* stop codon (Fig. 3C). By ≈ 90 bp downstream of the *GTP* stop codon (arrow in Fig. 3C), the *B. napus* *RPM1* and *rpm1-null* loci share no significant sequence identity. The breakpoints in the *rpm1-null* loci correspond to 210 bp and 475 bp, respectively, of 5' flanking sequence from 1A and 9N. No similarity was detected between the *Brassica* *rpm1-null* alleles and the 3' flanking regions of the *Brassica* *RPM1* gene, indicating that a significant region of the 3' flanking region is also absent from the *rpm1-null* loci. The 3' breakpoint was not mapped further.

The 1S and 9W *rpm1-null* loci are highly similar (>95%). In contrast, the sequence of the 1/16 null locus diverged significantly from 1S and 9W at 138 and 152 nucleotides downstream of the *GTP* termination codon, respectively. 1S and 9W, but not 1/16, share significant similarity in a region immediately 3' of *GTP* to a rice EST (RICS13430A), while 1/16 displays strong similarity to a valine tRNA and a small region of homology to *RPM1*.

There is extensive similarity between the *B. napus* *rpm1-null* alleles and the *Arabidopsis* *rpm1-null* alleles over the length of the *GTP* gene. Downstream of this gene, however, the *Arabidopsis* *rpm1-null* alleles share no significant similarity to any of the *Brassica* *rpm1-null* loci (data not shown). This indicates that the *Arabidopsis* and *Brassica* *rpm1-null* loci arose independently.

DISCUSSION

This study was designed to explore the molecular basis and origin of *RPM1* functional polymorphism in *Arabidopsis* and a related crop species. We examined *RPM1* structure in nine *Arabidopsis* laboratory accessions and found only two structures (haplotypes). The four disease-resistant accessions are almost identical to the reference Col-0 allele. In contrast, all five disease-susceptible accessions contain a null haplotype in which the entire *RPM1* gene is replaced by a short "filler" sequence. A Col-0 *RPM1* transgene restored resistance in three susceptible accessions. Thus, susceptibility in the examined accessions is caused by the complete absence of *RPM1*. The presence of only two haplotypes in the examined accessions suggests that *RPM1* polymorphism in *Arabidopsis* can be explained by a single molecular event that could be either (i) an insertion of *RPM1* in the progenitor of resistant accessions, or (ii) a deletion of *RPM1* in the progenitor of susceptible accessions.

To infer the ancestral state of *RPM1* in *Arabidopsis* and thereby distinguish between the above hypotheses, we examined *RPM1* structure in *B. napus*. *B. napus* contains the *Brassica* A and C genomes, each of which contains three equivalents of an hypothesized *Arabidopsis*-like progenitor genome. Thus, if hypothesis i is correct, then we would expect *B. napus* to contain six copies of *RPM1* between the *GTP* and *M4* flanking markers. We indeed found two *B. napus* loci that

are collinear with the *GTP/RPM1/M4* gene organization found in the resistant *Arabidopsis* accessions. Sequencing of three null loci revealed two distinct molecular structures, on N3/N13 and N5, that were divergent from each other and from the *Arabidopsis rpm1-null* haplotype. Thus, we propose the model outlined in Fig. 4. *RPM1* existed as a single copy gene between *GTP* and *M4* before the divergence of *Arabidopsis* and *Brassica* progenitor species. The *Arabidopsis rpm1-null* haplotype arose from a subsequent deletion of *RPM1* in the *Arabidopsis* lineage. The alternative model is that *RPM1* was independently inserted between *GTP* and *M4* in the *Brassica* and *Arabidopsis* genomes, which seems extremely unlikely. In *Brassica*, the *GTP/RPM1/M4* locus was triplicated, and *RPM1* was subsequently deleted from two loci (Fig. 4). The structural similarity and homoeologous positions of the 1S and 9W null loci suggest that these two null loci were derived from the same progenitor, whereas the divergent structure of 1/16 relative to 1S and 9W suggests that 1/16 was derived from a different deletion event.

The simple organization of the *RPM1* locus differs from most *R* loci characterized to date, which contain clustered *R* gene families and other repeated sequences. Several lines of evidence suggest that this repetitive organization can promote structural divergence of *R* genes by unequal crossovers and gene conversion (7, 27, 28). This mode of evolution likely accelerates the evolution of novel *R* genes in response to coevolving pathogens. In contrast, *RPM1* exists as a single-copy gene in *Arabidopsis*, and the collinearity between the *Arabidopsis* and *Brassica RPM1* loci suggests that this single-copy organization has persisted over a relatively broad evolutionary time span, based on an estimated divergence of *Arabidopsis* and *Brassica* lineages approximately 10 million years ago (29). Thus it seems unlikely that recombination has significantly influenced *RPM1* evolution. One explanation for this conservation of single-copy gene organization is that *RPM1* is a versatile resistance gene with multiple specificities and is therefore not subject to strong pressure for rapid structural divergence. This idea is consistent with the dual specificity of *RPM1* for two unrelated avirulence gene products. In addition, *avrRpm1* can function as a pathogen virulence factor on *Arabidopsis* (30). This target of *RPM1* is therefore likely to be important for some pathogens and not easily discarded. Unpublished experiments (N. Gunn and E. Holub, personal communication) have demonstrated that 20 of 50 Col-incompatible *P. s. maculicola* isolates from *Brassica* are moderately or fully compatible with a Col-*rpm1* mutant. These data suggest that *RPM1* is an effective resistance gene against a variety of pathogenic bacterial isolates. It is tempting to speculate that complex *R* gene loci represent active battle-

grounds in arms races, while simple loci like *RPM1* reflect a relatively stable outcome.

We do not know if *RPM1* function is conserved in *Brassica*; however, the *B. napus* cultivars from which the 1A and 9N genes were cloned are not resistant to *Pst* DC3000 with *avrRpm1* or *avrB* (M. Grant and J. Taylor, personal communications). Preliminary experiments (not shown) indicate that neither 1A nor 9N can substitute functionally for *Arabidopsis RPM1* in transgenic *rpm1-null Arabidopsis* accessions. These data suggest that 1A and 9N have diverged from *Arabidopsis RPM1* in their ability to recognize the *avrRpm1* and *avrB*-dependent elicitors and/or in their ability to interact with other components of the resistance response in *Arabidopsis*. Perhaps *Brassica* species contain multiple alleles with different recognition capabilities at *RPM1*, reminiscent of the *L* locus in flax (12). It is currently unknown whether different *B. napus* lines, or other *Brassica* species, have different *RPM1* copy numbers.

The null organization of the *rpm1* alleles was unanticipated because other *R* loci, both simple and complex, contain allelic or closely linked homologous genes even in disease-susceptible plant genotypes (4, 6, 7, 12). The existence of highly related homologs in *R* gene clusters implies that *R* gene duplication and divergence can lead to gains-of-resistance function that generate intraspecific functional polymorphism at *R* gene loci. Our results demonstrate that intraspecific polymorphism can also arise from gene deletions. Intraspecific *R* gene copy number variation has been observed in several other studies (3–8). For example, recent comparative mapping of *R* gene homologs in grass species revealed several loci in which *R* gene homologs were present at a given locus in one species but undetectable at the syntenic position in other grass species (31). The authors suggested that lack of synteny at *R* loci could be explained by *R* gene translocation and/or rapid sequence divergence of *R* genes after speciation. *R* gene deletion is a third potential explanation for lack of synteny at *R* loci. Based on the assumption that different *R* loci are subject to different modes of selection, it seems likely that both expansion and contraction occur at *R* loci. These events could be driven, respectively, by selection for novel *R* genes and selection against superfluous or costly *R* genes (see below).

The sequences of the null loci provided no clues as to the mechanism of *RPM1* loss. It seems most likely that the *Arabidopsis* locus was spontaneously deleted and that the 98-bp segment represents filler DNA inserted in place of *RPM1*. The imperfect 9-bp direct repeat in Col-0 at the exact 3' junction of the deletion suggests the insertion/excision of an Ac-like transposable element, but no other sequence evidence for transposons was observed in resistant or susceptible alleles. Filler DNA has been associated with spontaneous deletions and transposon excisions at other loci and in most cases is derived from sequences close to the deletion (32, 33). The donor of the 98-bp filler is unknown but may be revealed as more *Arabidopsis* sequences become available.

At first glance it seems counterintuitive that a gene like *RPM1* with obvious selective advantages would be deleted. The deletions of *RPM1* may be stochastic events with no selective consequences in the absence of pathogen pressure. Alternatively, *RPM1* may be selectively disadvantageous in the absence of pathogen pressure. This "cost of resistance" hypothesis is supported by the presence of almost identical null alleles in *Arabidopsis* accessions from Germany, Switzerland, and North Africa, which implies a rapid proliferation of the *rpm1-null* allele. Furthermore, a forthcoming study will present evidence that the *rpm1-null* haplotype is relatively ancient and that *RPM1* and *rpm1-null* haplotypes are present in natural *Arabidopsis* populations from Europe, Asia, Africa, and North America (E. Stahl and J. Bergelson, personal communication). The widespread distribution and apparent conservation of both haplotypes suggests that each haplotype is selectively

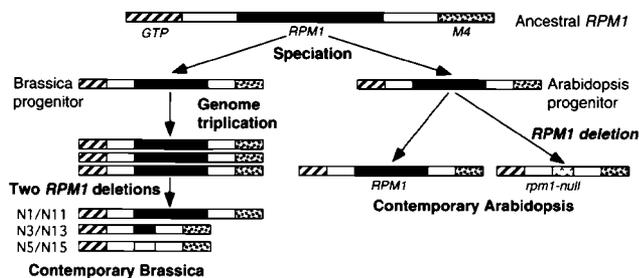


FIG. 4. Model for the evolution of *RPM1* in the Brassicaceae. The ancestral state of *RPM1* is inferred from structural conservation between *Arabidopsis* and *Brassica* loci. Subsequent deletion events are hypothesized to have occurred independently in *Arabidopsis* and *Brassica* because of structural dissimilarities between the *Arabidopsis* and *Brassica* null loci. The structural dissimilarity between the N5 and N3/N13 loci further suggests that *RPM1* has been deleted twice in *Brassica* after the genome triplication.

advantageous under different environmental conditions and that both haplotypes have been maintained by balancing selection.

Direct evidence for an *RPM1*-associated fitness penalty may be provided by carefully designed fitness comparisons of isogenic lines that differ only by the presence or absence of *RPM1*. Extending such comparisons to different types of *rpm1* mutant alleles may illuminate the molecular basis of the penalty. Perhaps *RPM1* predisposes the plant to inappropriate defense induction by spurious stimuli. Because induced resistance involves significant metabolic reprogramming (34) and localized cell death (35), even a low level of inappropriate defense induction could impose significant fitness costs. A homolog of the *Pto* resistance gene (*Fen*) triggers a resistance-like reaction in response to herbicide application (36). Disease lesion mimic mutants, which likely arose from recombination between linked *R* genes, have been identified at the maize *RPI* resistance gene complex (37). These examples suggest that the same mechanisms that accelerate the evolution of novel resistance genes may also generate disadvantageous alleles. It is worth noting that deletion alleles are unable to revert, recombine, or evolve a new inappropriate specificity and could thus be less costly than missense alleles. Perhaps frequent recombination within *R* gene clusters serves a dual purpose by facilitating both the creation of useful *R* genes and the removal of costly or superfluous *R* genes. Further examination of *RPM1* and other pathogen-resistance loci will reveal the prevalence and selective importance of gene deletions.

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1. Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. & Jones, J. D. G. (1995) *Science* **268**, 661–667.
2. Crute, I. R. (1985) in *Mechanisms of Resistance to Plant Diseases*, ed. Fraser, R. S. S. (Kluwer Academic Press, Dordrecht, Holland), pp. 80–143.
3. Anderson, P. A., Lawrence, G. J., Morrish, B. C., Ayliffe, M. A., Finnegan, E. J. & Ellis, J. G. (1997) *Plant Cell* **9**, 641–651.
4. Jia, Y., Loh, Y.-T., Zhou, J. & Martin, G. B. (1997) *Plant Cell* **9**, 61–73.
5. Ori, N., Eshed, Y., Paran, I., Presting, G., Aviv, D., Tanksley, S., Zamir, D. & Fluhr, R. (1997) *Plant Cell* **9**, 521–532.
6. Parker, J. E., Coleman, M. J., Szabo, V., Frost, L. N., Schmidt, R., van der Biezen, E., Moores, T., Dean, C., Daniels, M. J. & Jones, J. D. G. (1997) *Plant Cell* **9**, 879–894.
7. Parniske, M., Hammond-Kosack, K. E., Golstein, C., Thomas, C. M., Jones, D. A., Harrison, K., Wulff, B. B. H. & Jones, J. D. G. (1997) *Cell* **91**, 821–832.
8. Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C. & Baker, B. (1994) *Cell* **78**, 1101–1115.
9. Flor, H. H. (1956) *Adv. Genet.* **8**, 29–54.
10. Keen, N. T. (1990) *Annu. Rev. Genet.* **24**, 447–463.
11. Simms, E. L. (1996) *Bioscience* **46**, 136–145.
12. Ellis, J. G., Lawrence, G. J., Finnegan, E. J. & Anderson, P. A. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 4185–4188.
13. Debener, T., Lehnackers, H., Arnold, M. & Dangl, J. L. (1991) *Plant J.* **1**, 289–302.
14. Bisgrove, S. R., Simonich, M. T., Smith, N. M., Sattler, N. M. & Innes, R. W. (1994) *Plant Cell* **6**, 927–933.
15. Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W. & Dangl, J. L. (1995) *Science* **269**, 843–846.
16. Jones, D. A. & Jones, J. D. G. (1996) *Adv. Bot. Res. Adv. Plant Pathol.* **24**, 90–167.
17. Sharpe, A. G., Parkin, I. A. P., Keith, D. J. & Lydiat, D. J. (1995) *Genome* **38**, 1112–1121.
18. Robert, L. S., Robson, F., Sharpe, A., Lydiat, D. J. & Coupland, G. (1998) *Plant Mol. Biol.* **377**, 763–772.
19. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987) *Current Protocols in Molecular Biology* (Wiley, New York).
20. Innes, R. W., Bisgrove, S. R., Smith, N. M., Bent, A. F., Staskawicz, B. J. & Liu, Y.-C. (1993) *Plant J.* **4**, 813–820.
21. U, N. (1935) *Jpn. J. Bot.* **7**, 389–452.
22. Lagercrantz, U. & Lydiat, D. J. (1996) *Genetics* **144**, 1903–1910.
23. Cavell, A., Lydiat, D. J., Parkin, I., Dean, C. & Trick, M. (1998) *Genome* **41**, 62–69.
24. Parkin, I. A. P., Sharpe, A. G., Keith, D. J. & Lydiat, D. J. (1995) *Genome* **38**, 1122–1131.
25. Wedel, A. & Ziegler-Heitbrock, H. W. (1995) *Immunobiology* **193**, 171–185.
26. Scheffler, J. A., Sharpe, A. G., Schmidt, H., Sperling, P., Parkin, I. A. P., Luhs, W., Lydiat, D. J. & Heinz, E. (1997) *Theor. Appl. Genet.* **94**, 583–591.
27. Hulbert, S. H. (1997) *Annu. Rev. Phytopathol.* **35**, 293–310.
28. Ellis, J. & Jones, D. (1998) *Curr. Opin. Plant Biol.* **1**, 288–293.
29. Muller, J. (1981) *Bot. Rev.* **47**, 1–42.
30. Ritter, C. & Dangl, J. L. (1995) *Mol. Plant–Microbe Interact.* **8**, 444–453.
31. Leister, D., Kurth, J., Laurie, D. A., Yano, M., Sasaki, T., Devos, K., Graner, A. & Schulze-Lefert, P. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 370–375.
32. Doseff, A., Martienssen, R. & Sundaesan, V. (1991) *Nucleic Acids Res.* **19**, 579–584.
33. Wessler, S., Tarpley, A., Purugganan, M., Spell, M. & Okagaki, R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8731–8735.
34. Somssich, I. E. & Hahlbrock, K. (1998) *Trends Plant Sci.* **3**, 86–90.
35. Dangl, J. L., Dietrich, R. A. & Richberg, M. H. (1996) *Plant Cell* **8**, 1793–1807.
36. Martin, G. B., Frary, A., Wu, T., Brommonschenkel, S., Chunwongse, J., Earle, E. D. & Tanksley, S. D. (1994) *Plant Cell* **6**, 1543–1552.
37. Hu, G., Richter, T. E., Hulbert, S. H. & Pryor, T. (1996) *Plant Cell* **8**, 1367–1376.