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Phenotypic and Genotypic Variation in the Interaction between *Arabidopsis thaliana* and *Albugo candida*

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Two biotrophic parasites of the wild crucifer *Arabidopsis thaliana* (L.) Heynh. are being used to explore the molecular basis and evolution of genotype-specific recognition and host defense. Genes for recognition of *Peronospora parasitica* (downy mildew) are numerous in *A. thaliana* and located on four of the five chromosomes as described previously. Genes for recognition of the closely related parasite *Albugo candida* (white blister) are described here. In contrast to the former parasite, less than 15% of the host accessions tested were capable of recognizing either of two isolates of *A. candida*. The geographic regions represented by these accessions included countries in eastern and western Europe, Asia, North America and Africa. Extensive collections from England and Germany were required to identify examples of incompatible interactions. Phenotypic variation among incompatible interactions included reduced blister formation or complete lack of asexual reproduction by the parasite. Variation in the extent of the host response was also observed. Three host genes for recognition of *A. candida* (*RAC*), each associated with different interaction phenotypes, were identified through inheritance studies with three accessions. One of these genes at locus *RAC1* appeared to be completely dominant, whereas the other two genes were only partially dominant or recessive under certain conditions, possibly including the effect of genetic background. One of the latter two genes defined a second locus *RAC2*. *RAC1* was mapped to the top arm of chromosome 1 in the 1 cM interval between RFLP markers M254 and M253.

Additional keywords: incomplete dominance, Peronosporales, gene-for-gene.

"Two eyes are better than one." Enhanced perspective can be gained from having two points of reference; for this reason, we have chosen two naturally occurring biotrophic parasites of *Arabidopsis thaliana* (L.) Heynh. to explore the molecular basis and evolution of genotype-specific recognition in plant defense. The first parasite, *Peronospora parasitica* (Pers. ex Fr.) Fr. (downy mildew), was described previously with respect to its life cycle in *A. thaliana*, the characterization of interaction phenotypes, and a molecular genetic approach to confirming the existence of host loci involved in recognition of the parasite (Koch and Slusarenko 1990; Parker et al. 1993; Holub et al. 1994; Tör et al. 1994). The second closely related parasite, *Albugo candida* (Pers. ex Fr.) O. Kuntze (white blister), is described here in association with the same host species.

Downy mildew of *A. thaliana* has been shown to be a complex but nonetheless suitable parasitic symbiosis for molecular genetic investigation. Extensive reciprocal variation of interaction phenotypes has been observed following inoculation of 11 accessions of *A. thaliana* with seven isolates of *P. parasitica* (Holub et al. 1994). Phenotypic variation was characterized among the incompatible interactions with respect to asexual sporulation by the parasite and the symptoms of host response. The timing and degree of asexual reproduction by the parasite varied among the combinations and provided an indirect assessment of colonization. Necrosis of host cells ranged from minute flecks visible macroscopically 7 days after inoculation (dai) to more extensive pits clearly visible 3 dai and often expanding until the entire cotyledon was necrotic. By characterizing the phenotypic and genotypic variation observed in this symbiosis, a foundation was laid for investigating the process of genotype specific recognition (Holub et al. 1994). Twelve host loci associated with different phenotypes were postulated. Such evidence was used as the basis for efforts to map the fine-scale location of several host loci as a step towards eventual isolation of genes involved in host-parasite recognition (Parker et al. 1993; Tör et al. 1994).

A. candida also commonly occurs on *A. thaliana* in Europe (Lind, 1913; Jörstad 1964; Holub et al. 1991), and often in intimate association with *P. parasitica*. In southeast England, plants infected with *A. candida* were observed in more than 25% of *A. thaliana* populations examined during two consecutive springs (Holub et al. 1994); 9% of these host populations contained plants infected with both parasites. Among 31 host populations surveyed in northern England and southern Scotland in spring 1993, ten (33%) contained plants infected with *A. candida*, and five (17%) contained plants infected with both parasites.

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E. Holub and E. Brose contributed equally to the research and therefore are joint first authors.

Most crucifer species can be infected by *A. candida* but pathogenic variants of this parasite are in general highly specialized to a given host genus or species (Rostrup 1913; Hiura 1930; Napper 1933; Togashi and Shibasaki 1934; Pound and Williams 1963; Williams and Pound 1963; Jörstad 1964; Pidskalny and Rimmer 1985; Petrie 1988). This appears to be the case for *A. candida* collected from *A. thaliana*. In preliminary investigations with two isolates, *A. suecica* was the only other host species observed on which these isolates were capable of unrestricted asexual reproduction. These isolates were not parasitic on *Brassica* spp. or other wild crucifers commonly found growing alongside *A. thaliana* such as *Cardamine hirsuta* L. and *Capsella bursa-pastoris* (L.) Medic. However, in common with isolates obtained from other hosts, they were capable of producing minute blisters associated with necrotic flecks in white mustard (*Sinapis alba* L.).

Comparative studies of *P. parasitica* and *A. candida* in the common host *A. thaliana* will contribute to an understanding of the general features and important contrasts that may exist in parasitic symbioses of plants. Therefore, we have highlighted in this paper important aspects of the life cycle of *A. candida* in association with *A. thaliana*, describe the variation among interaction phenotypes observed following inoculation of a large collection of host accessions with two isolates of the parasite, and report the identification of three genes from at least two host loci associated with different interaction phenotypes.

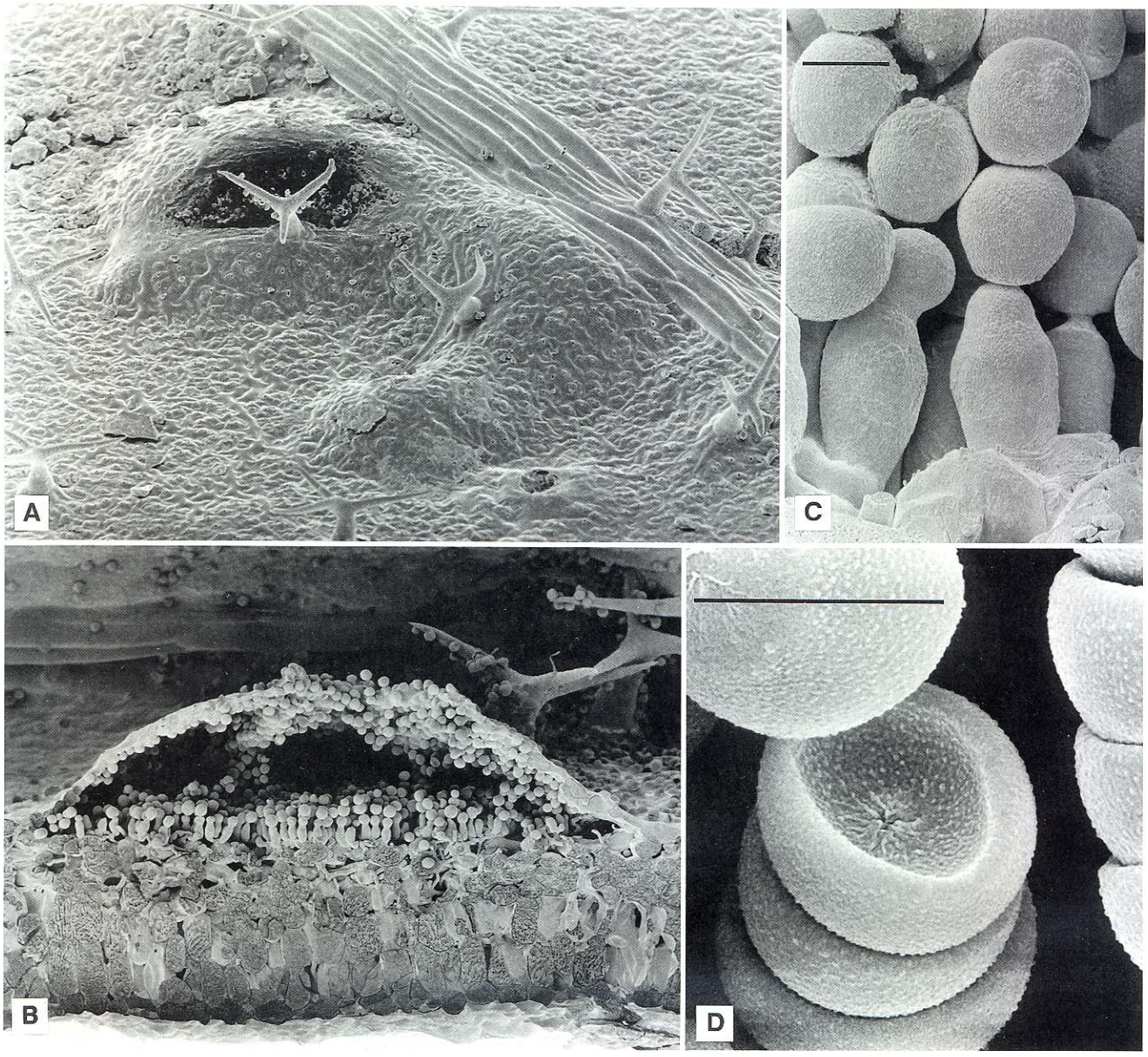

Fig. 1. Scanning electron photomicrographs of asexual reproduction by *Albugo candida* in *Arabidopsis thaliana*: **A,** Surface view of a sorus; **B,** freeze-fracture cross section through a leaf blade and a sorus; **C,** zoosporangia formed in chains from basal mother cells; **D,** dehydrated zoosporangia. Bars represent 10 microns.

RESULTS

Life cycle of *A. candida* in *A. thaliana*.

The most distinguishing attribute of *A. candida* is the production of white sori or blisters in host tissue (Figs. 1A, B and 2A). These blisters result from the subepidermal growth of unbranched, clavate sporangiophores or mother cells (Fig. 1C) which each bear a chain of deciduous zoosporangia (approx. 12 μm diameter). The protuberance of blisters is most likely caused by the outward force of the growing mass of zoosporangia. The zoosporangia have a catenulate outerwall, and are collapsed from dehydration late in development

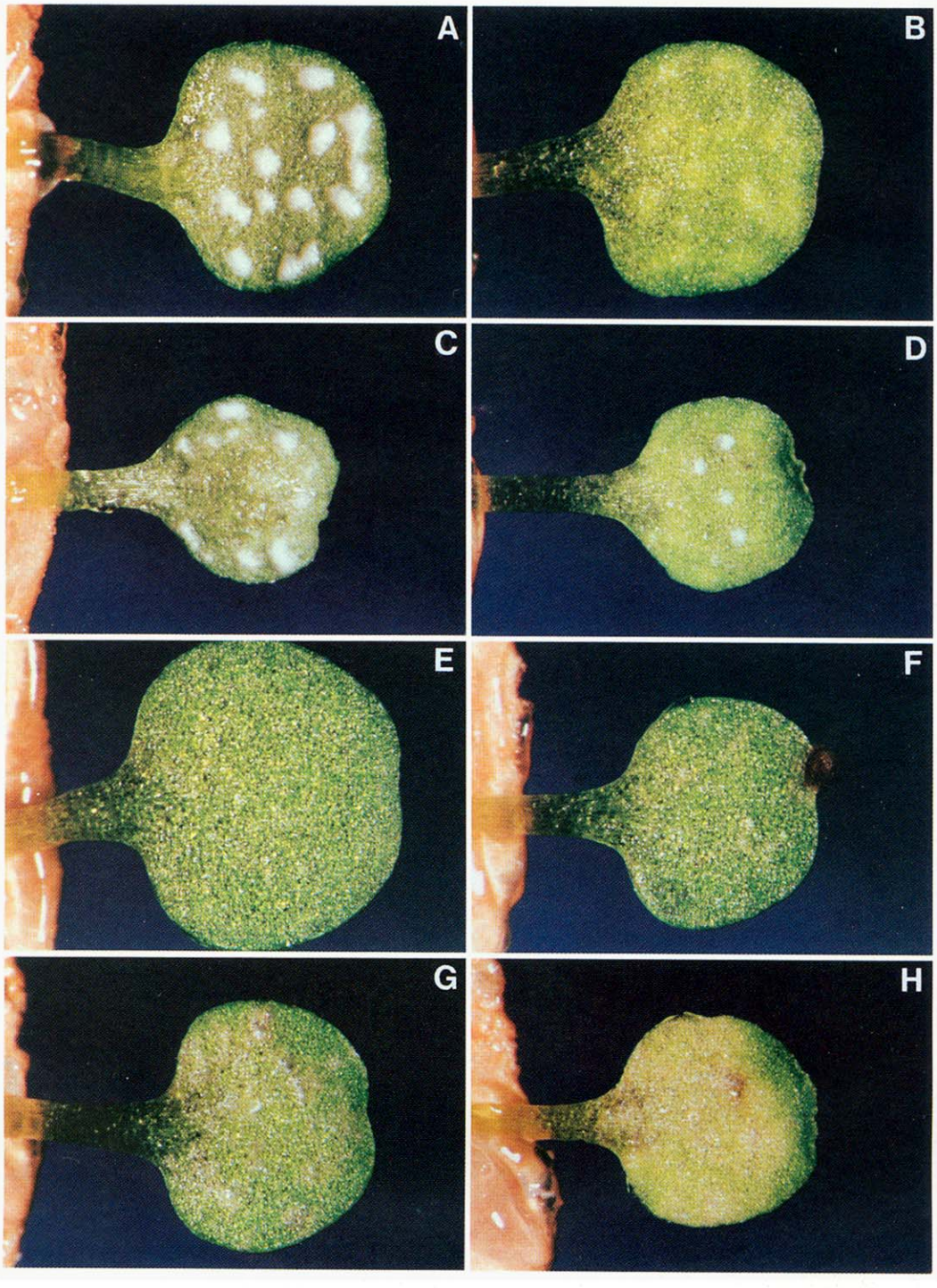

Fig. 2. Variation of interaction phenotype of *Arabidopsis thaliana* cotyledons 7 days after inoculation with *Albugo candida*: **A, B,** Upper and lower surface exhibiting green host tissue (no apparent response) and heavy production of blisters (fully compatible); **C, D,** upper and lower surface exhibiting necrotic flecks in center and surrounded by low production of blisters; **E,** noninoculated; **F,** necrotic flecks without blisters; **G,** necrotic cavities without blisters; **H,** necrotic flecks and a brown patch with bright yellowing of tissue surrounding the lesions.

(Fig. 1D). It is at this stage that zoosporangia are dispersed by air currents or rain droplets when the host epidermis of the sorus has been ruptured.

Water is essential for the germination of zoosporangia. They rehydrate rapidly, returning to a spheroid shape (Fig. 3B). The cytoplasm completes a process of differentiation into four to six biflagellate zoospores (approx. 5 μm diameter). The zoospores do not have a cell wall and are thus essentially motile protoplasts. They are released together through a preformed operculum of the sporangium (Fig. 3C, D) as soon as 1 h after rehydration, so it is likely that most differentiation of cytoplasm into zoospores takes place while the zoosporangia are maturing in the sorus. The process of zoospore release can, however, be delayed or inhibited entirely by environmental factors such as high temperature, solute concentration or host physiology such as leaf turgor during development of sori (Melhus 1911; Napper 1933; Edie and Ho 1970). Upon release, the zoospores immediately disentangle and become motile (Fig. 3D, E).

On the surface of *A. thaliana* zoospores are attracted to stomatal pores where they encyst (Fig. 3A) by a process of flagella loss, becoming spheroid and producing a cell wall. Encystment usually occurs at the center of a stomatal pore (Fig. 3F). Using scanning electron microscopy, 75 to 95% of stomatal pores on the upper surface of *A. thaliana* cotyledons were observed to be plugged with an encysted zoospore using our standard inoculum concentration and conditions for incubation. Fewer than 5% of the pores were plugged by more than one spore. Environmental cues such as chemical attractants or physical topography of the leaf surface may guide the zoospores of oomycetes to stomatal pores (Royle and Thomas 1973; Deacon and Donaldson 1993). Interestingly, when we compared inoculated cotyledons which had been incubated either in the light or in the dark, no clear difference was observed in the ability of *A. candida* zoospores to locate stomatal pores of *A. thaliana*: 75% of pores were plugged after incubation in the light and 78% were plugged after incubation in the dark.

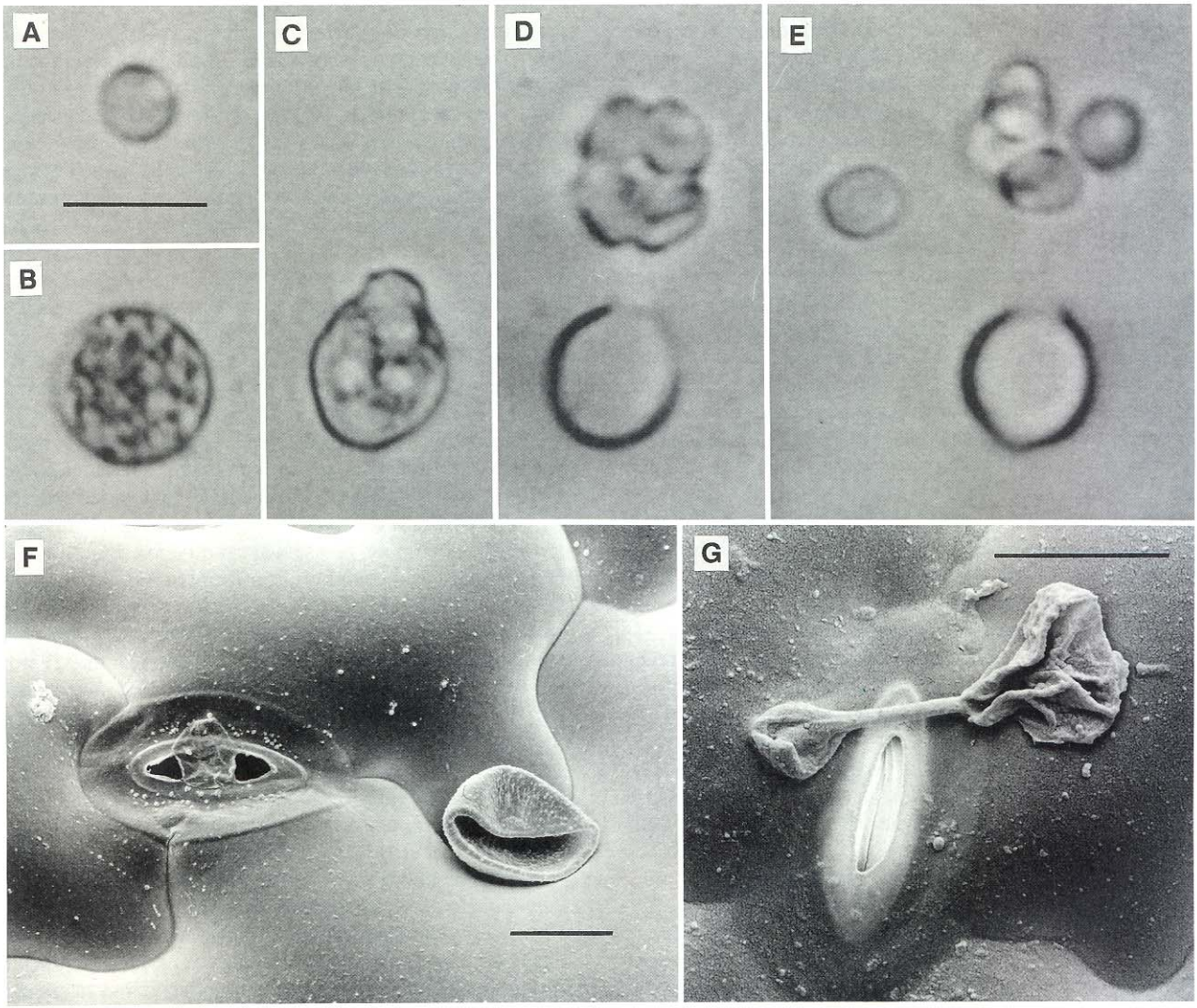

Fig. 3. The zoosporic stage of *Albugo candida*: **A,** Encysted zoospore; **B,** zoosporangium prior to germination; **C-D,** germinating zoosporangium and release of zoospores via the operculum; scanning electron photomicrograph of a zoospore cell wall above the opening of a stomata (left) and an empty zoosporangium (right); **F,** SEM showing growth of germ tube from condiosporangium of *Peronospora parasitica* and direct penetration between anticlinal walls of a stomatal guard cell and the adjacent epidermal cell. Bars represent 10 microns.

The zoospores germinate by means of a short germ tube that, in the case of one that has come to rest on a stomatal pore, will extend into the substomatal chamber. If environmental factors such as temperature or water quality has caused the zoospore to encyst prematurely before successfully locating a pore, then the spore will attach to the leaf surface where it came to rest, and the germ tube will attempt to reach a pore. Once the germ tube has entered the stomatal chamber, it produces an appressorium, penetrates between the host cells, and produces a single primary vesicle (the first haustorial structure) in one of the adjacent mesophyll cells. If the combination of host and parasite is compatible, the parasite produces branched hyphae that ramify through the mesophyll producing globose haustoria within host cells at regular intervals. Much of the colonization of host cotyledons will occur within 48 h after penetration. Small bumps that will eventually become sori begin to appear on the lower epidermis of cotyledons three days after penetration. The developing sori can be detected visually from the upper cotyledon surface, through the thin layer of host cells, as lighter green patches (Fig. 2B). The formation of sori appears to be affected less by variable environmental conditions than asexual sporulation by *P. parasitica* that usually does not occur unless there is suitable temperature and high humidity during several hours of darkness. The asexual inoculum of *A. candida* may also be more resilient in the dehydrated state than the comparable conidiosporangia of *P. parasitica* that must remain hydrated. For both parasites, asexual reproduction provides a means for rapid development of an epidemic.

A. candida also has a sexual stage that provides an opportunity for genetic recombination and a means of prolonged dormancy. As soon as 4 days after inoculation (dai), *A. candida* produces male and female gametangia called antheridia and oogonia, respectively. These are globose-like organs produced at the tips of hyphae. The antheridium grows towards and attaches to the surface of an oogonium. After fertilization, the oogonium develops into an oospore (about 25 μm diameter) which has a thick, verrucose or tuberculate cell wall and easily distinguished from oospores of *P. parasitica* (photograph in Holub et al. 1993). The oospores are typically golden brown when fully mature, as soon as 7 dai. Both isolates of *A. candida* that we collected from *A. thaliana* appear to be self-fertile (homothallic).

When the host tissue disintegrates into the soil, the oospores are liberated and will remain dormant until conditions are suitable for germination. Some oospores may be capable of germinating immediately, while others may require further aging. Oospores of some pathotypes of *A. candida* have been observed to germinate and release zoospores, but they can also germinate by producing a germ tube directly (Petrie and Verma 1974; Verma and Petri 1975).

Characterization of interaction phenotypes.

As for our previously reported investigation of *P. parasitica* (Holub et al. 1994), a cotyledon assay conducted under controlled-environment conditions was used to minimize nongenetic variation. Infection of seedlings may be most biologically relevant as the stage at which *A. candida* or *P. parasitica* impose the greatest selection for host incompatibility. The fecundity of another wild crucifer, *C. bursa-pastoris,* was more reduced after seedling infection by either biotrophic parasite than when infection occurred at older growth stages of the host (Alexander and Burdon 1984). Additionally, we focused on describing the interaction phenotype (i.e., combining observations of both host response and parasite reproduction) that was visible macroscopically, thereby enabling us to examine large numbers of seedlings.

A fully susceptible or compatible combination of host accession and parasite isolate typically exhibited profuse formation of white blisters first becoming evident 5 days after inoculation (dai) in the absence of any macroscopically visible host response (Fig. 2A,B). The blisters always formed in profusion on the lower surface of cotyledons regardless of whether inoculum was placed on the upper or lower surface. The blisters were sufficiently mature to release viable zoosporangia 7 to 10 dai. This interaction phenotype is referred to as GB, an abbreviation for green cotyledons (i.e., no host response) with the formation of blisters by the parasite. Copious oospore production was also evident 7 dai in the GB phenotype.

Seedling vigor appeared to be unaffected by an infection resulting in the GB phenotype. The expansion of cotyledons with this phenotype was similar to that of noninoculated cotyledons, and growth during the first 4 to 6 weeks of a rosette appeared similar to that of noninoculated seedlings. However, subsequent growth of such seedlings was often affected by systemic infection. This typically culminated with the profuse eruption of blisters on lower and upper surfaces of rosette leaves, petioles, stems, stem leaves, and occasionally siliques. In a severe infection, the bolting stem was crippled, producing little or no seed. True leaves bearing heavy sporulation were often turned upward thus exposing blisters on the lower surface to wind currents. This may effectively improve the long-distance dispersal of the parasite, but could also improve short-distance dispersal by funneling zoosporangia into puddles of rain water at the base of rosettes.

Incompatible phenotypes were classified using the GB phenotype as a benchmark for comparing cotyledon symptomology, and a notation equivalent to that described for *P. parasitica* (Holub et al. 1994). Differences could not be detected in the timing of asexual sporulation by *A. candida*. However, reduced or light blister formation (L) was observed in some interactions. The blisters were smaller in size; and in some cases (e.g., Edi-0 inoculated with *Acem1*), they consistently appeared on the upper surface of cotyledons (U) around the periphery of a host lesion (Fig. 4C,D). The major types of host response observed were minute necrotic flecks (F) and the more visible formation of necrotic cavities (C) (Fig. 4F,G). The cavities formed as macroscopic depressions in the cotyledon surface that terminated expansion before 7 dai. The more prominent, expansive lesions described as necrotic pits following inoculation with *P. parasitica* was not observed following inoculations of any *A. thaliana* accessions with *A. candida*. However, the incompatible interaction between Ksk-1 and *Acem1* was accentuated by distinct yellowing (Y) that extended beyond the necrotic lesions (Fig. 4H). The necrosis in this case was usually confined to flecks, but occasionally formed larger lesions such as the brown patch visible in Figure 4H. High temperature (>20 C) is one environmental condition that appears to enhance the expansion of necrotic lesions (E. Brose, unpublished).

Identifying sources of incompatibility among accessions of *A. thaliana*.

The search for phenotypic variation among interactions between *A. candida* and *A. thaliana* began with inoculation of host accessions which had been collected from distant localities world wide. These accessions included Nd-1, Col-5, Ler-1 from Germany and all of the accessions at the end of the list beginning with Ag-0 (Table 1). The geographic regions represented by these accessions included countries in eastern and western Europe, Asia, North America, and Africa. They all exhibited a compatible GB phenotype following inoculations with *A. candida* isolates *Acem1* and *Acks1*. This was in sharp contrast to the wide variation of incompatible phenotypes observed following inoculation of the same accessions with isolates of *P. parasitica* (Holub et al. 1993).

The search for phenotypic variation was continued using a more extensive collection of *A. thaliana* from Germany and the UK. Most of the interactions observed with these additional accessions were compatible following inoculations with both *A. candida* isolates. However, examples of incompatible phenotypes were found such as the FYN phenotype following inoculation of Ksk-1 with *Acem1* (Table 1). Incompatibility with *Acem1* (12 out of 92) appeared to be more common than to *Acks1* (8 out of 93). With *Acem1*, incompatibility was more common among accessions from the UK (9 out of 27) than among accessions from Germany (2 out of 44). Those accessions that exhibited incompatibility following inoculation with *Acem1* frequently exhibited a GB phenotype following inoculation with *Acks1*. There was no confirmed example of a reciprocal interaction; however, it may be possible to select such a variant from the heterogeneous populations of Bur-0 and Kin-0.

Identifying *A. thaliana* genes associated with specific recognition of *A. candida*.

Three accessions of *A. thaliana* were chosen to examine the inheritance of variation for interaction phenotype following inoculation with isolate *Acem1*: Ksk-1 which exhibited necrotic flecks surrounded by yellowing and no blisters (FYN)(Fig. 2H); Ksk-2 which exhibited necrotic flecks surrounded by green tissue and no blisters (FGN)(Fig. 2F); and Cnt-1 that exhibited necrotic cavities and the restricted or low production of blisters (CL). Each of these accessions was crossed with the fully susceptible accession Col-5 (Table 2). The F_2 progeny from each cross segregated in a manner suggesting that the FYN, FGN and CL phenotypes were simply inherited (Table 2).

In the cross between Col-5 and Ksk-1, the progeny segregated 38:12 for incompatible interactions (FYN and FGN) versus full susceptibility (GB). Thus, Ksk-1 appears to carry a single dominant gene (for 3:1, $\chi^2 = 0.03$, $P = 0.90$) for the ability to recognize isolate *Acem1*. F_3 families derived from self-pollination of the F_2 individuals were inoculated with *Acem1* (Table 3). Among the 28 families derived from F_2 individuals exhibiting the FYN or FN phenotype, 12 were uniform for the Ksk-1 phenotype and the remaining 26 segregated. The 12 F_3 families derived from F_2 individuals exhibiting the GB phenotype were all uniform for the Col-5 (GB) phenotype. In addition, F_1 progeny from the cross between Col-5 and Ksk-1 exhibited the same phenotype as Ksk-1. On the basis of this further evidence, Ksk-1 appears to carry a single, completely dominant gene for recognizing *Acem1*. The locus for this gene was named *RAC1* (abbreviation for "recognition of *Albugo candida*") and was mapped to the top arm of chromosome one (described below).

Arabidopsis thaliana Seed Stock	Chromosome 1 Locus				
	M201	M254	*RAC1*	M253	M299
Wei-1	W	W	W	W	W
Ksk-1	K	K	K	K	K
2168	W	W	K	K	K
2160	W	W	W	K	K
2250*	W/K	W/K	W	W	W
2348, 2473	K	K	K	W	W
2069, 2074, 2121, 2127, 2144, 2338, 2367	W	W	W	W	K
2065, 2082, 2087, 2104, 2270, 2273, 2337	K	W	W	W	W
2094, 2103, 2240*, 2246*, 2307*, 2272*	W/K	W	W	W	W
2051, 2285, 2330	K	K	K	K	W
2012*, 2057*, 2248*, 2325	K	K	K	K	W/K
2079, 2155, 2320, 2469	W	K	K	K	K
2140, 2264*	W/K	K	K	K	K
2380, 2096	W/K	W/K	W/K	W/K	K
2344	W/K	W	W	W	W/K
2309	W/K	W	W	W	K
2099, 2169	K	W	W	W	K
2015	K	W/K	W/K	W/K	K

* F_3 Wei-1 x Ksk-1, all others are the F_6 generation

Fig. 4. Segregation of loci among F_3 and F_6 families of the cross between Wei-1 and Ksk-1 that were critical in the mapping of *RAC1*.

In the cross between Col-5 and Ksk-2, the data suggest that the ability of Ksk-2 to recognize isolate *Acem1* is most likely a recessive trait. The F_2 progeny segregated 85:148 for incompatible interactions (FGN and FB) versus full susceptibility (GB) and all of the F_1 individuals exhibited a Col-5 phenotype (GB). Recognition may not be completely recessive judging from the chi-squared test of the F_2 segregation (for 1:3, χ^2 = 16.38, P = <0.01). When F_3 families derived from 60 F_2 individuals were inoculated with *Acem1*, 15 were uniform for the Ksk-2 phenotype and 12 were uniform for the Col-5 phenotype (Table 3). A majority of the 33 segregating families were derived from F_2 plants that exhibited the GB phenotype (for 1:2:1, χ^2 = 1.02, P = 0.61). The locus for this gene from Ksk-2 was named *RAC2*, on the basis of recombination with the locus *RAC1* (described below).

In the cross between Col-5 and Cnt-1, the data suggest that the ability of Cnt-1 to recognize isolate *Acem1* exhibits partial dominance. Although the F_1 plants exhibited a Cnt-1 phenotype (CL) following inoculation, the F_2 progeny segregated 240:101 for incompatible (CL and CN) versus full susceptibility (GB) and therefore suggested that a completely dominant gene is unlikely (for 3:1, χ^2= 3.88, P = 0.05). F_3 families

Table 1. Interaction phenotypes between accessions of *Arabidopsis thaliana* and two isolates of *Albugo candida*

A. thaliana		*A. candida* isolate		*A. thaliana*		*A. candida* isolate	
Accession	Geographic Source	*Acem1*	*Acks1*	Accession	Geographic Source	*Acem1*	*Acks1*
Bea-1	Bearsted, Kent, UK	GB[a]	GB	La-0	Landsberg, Germany	GB	GB
Bid-1	Biddenden, Kent, UK	GB	GB	Ler-1	Landsberg, Germany	GB	GB
Cal-0	Calver, Derbyshire, UK	GB	GB	Li-1	Limburg, Germany	GB	GB
Chr-1	Chartham, Kent, UK	GB	GB	Ma-0	Marburg, Germany	GB	GB
Cnt-1	Canterbury, Kent, UK	CL	CN	Mnz-0	Mainz, Germany	GB	GB
Ci-0	Crief, Tayside, UK	GB	GB	Nd-1	Niederzenz, Germany	GB	GB
Crl-1	Carlisle, Cumbria, UK	GB	GB	No-0	Nossen, Germany	GB	GB
Edi-0	Edinburgh, Lothian, UK	FLU	FL	Ob-0	Oberursel, Germany	GB	GB
Ema-1	East Malling, Kent, UK	GB	GB	Old-0	Oldenburg, Germany	GB	GB
Frd-1	Fordwich, Kent, UK	FL	GB	Ove-0	Ovelgönne, Germany	GB	GB
Igt-1	Igtham, Kent, UK	GB	GB	Po-0	Poppelsdorf, Germany	GB	GB
Kil-0	Killearn, Central Scotland, UK	GB	GB	Pr-0	Praunheim, Germany	GB	GB
Ksk-1	Keswick, Cumbria, UK	FYN	GB	Pt-0	Pötrau, Germany	GB	GB
Ksk-2	Keswick, Cumbria, UK	FN	GB	Rd-0	Rodenbach, Germany	GB	GB
Kyl-1	Kyle of Lochalsh, Highland, UK	GB	GB	Sg-1	St. Georgen, Germany	GB	GB
Lan-0	Lanark, Strathclyde, UK	FN	FN	Sh-0	Schwiegershausen, Germany	GB	GB
Lha-1	L. Harbledown, Kent, UK	GB	GB	Si-0	Siegen, Germany	GB	GB
Mai-1	Maidstone, Kent, UK	FN	GB	Sp-0	Spandau, Germany	FL	GB
Mc-0	Mickles Fell, Cumbria, UK	GB	GB	Vi-0	Vilbel, Germany	nt	FN
Sco-1	Scotney Castle, Kent, UK	FN	GB	Wü-0	Würzburg, Germany	GB	GB
Set-0	Settle, Yorkshire, UK	GB	GB	Wl-0	Wildbad, Germany	GB	GB
Sis-1	Sissinghurst Garden, Kent, UK	FN	FN	Wt-1	Wietze, Germany	GB	GB
Su-0	Southport, Merseyside, UK	GB	GB	Xxx-0	Unknown	GB	GB
Sy-0	Isle of Skye, Highland Scotland	GB	GB	Ag-0	Argentat, France	GB	GB
Wis-1	Wisley Garden, Surrey, UK	GB	GB	Ms-0	Moscow, Russia	GB	GB
Wma-1	West Malling, Kent, UK	GB	GB	Oy-1	Oystese, Norway	GB	GB
Bur-0	Burren, Ireland	GB	GB&FN[b]	Te-0	Tenela, Finland	GB	GB
Bd-1	Berlin, Germany	GB	GB	Hi-0	Hilversum, Netherlands	GB	GB
Aa-0	Aua, Germany	GB	GB	Pi-0	Pitztal, Austria	GB	GB
Bay-0	Bayreuth, Germany	GB	GB	Mh-0	Mülhen, Poland	GB	GB
Bch-1	Büchen, Germany	GB	GB	Wei-1	Weiningen, Switzerland	GB	GB
Bsch-0	Buchschlag, Germany	GB	GB	Di-0	Dijon, France	GB	GB
C24	Landsberg, Germany	GB	GB	Pla-0	Playa de Aro, Spain	GB	GB
Col-5	Landsberg, Germany	GB	GB	Sue-0	Granada, Spain	GB	GB
Da-0	Darmstadt, Germany	GB	FN	Per-1	Perm, (USSR)	GB	GB
Dr-0	Dresden, Germany	GB	GB	Rld-2	Rzhev, Russia	GB	GB
Ei-7	Eifel, Germany	FN	FN	Ws-1	Wassilewskija, Byelorussia	GB	GB
Eil-0	Eilenburg, Germany	GB	GB	Kas-1	Kashmir	GB	GB
Er-0	Erlangen, Germany	GB	GB	Mt-0	Martuba, Libya	GB	GB
Est-0	Estland, Germany	GB	GB	Can-0	Canary Islands	GB	GB
Fe-1	Freiburg, Germany	GB	GB	Cvi-0	Cape Verde Island	GB	GB
Fi-0	Frickhofen, Germany	GB	GB	Tsu-0	Tsu, Japa n	GB	GB
Fr-2	Frankfurt, Germany	GB	GB	Kin-0	Kindalville, MI, USA	GB & FL[b]	FL & FN[b]
Gie-0	Gießen, Germany	GB	GB	Yo-0	Yosemite, CA, USA	GB	GB
Gö-0	Göttingen, Germany	GB	GB	*A. suecica*	Helsinki, Finland	GB	GB
Ha-0	Hannover, Germany	GB	GB	*A. suecica*	Brüssel, Belgium	GB	GB
Je-0	Jena, Germany	FN	GB	*A. suecica*	Göttingen, Germany	GB	GB
Kb-0	Kronberg, Germany	GB	GB	*A. griffithiana*	Unknown (AIS)	FN	FN
Kl-0	Köln, Germany	GB	GB	*A. wallichii*	Unknown (AIS)	FN	FN

[a] Interaction phenotypes were characterized as follows: host response was recorded as necrotic flecks (F), necrotic cavities (C), yellowing which surrounds necrotic lesions (Y), or green, apparently non-responding (G); asexual reproduction of *A. candida* was recorded as blisters produced (B), blisters produced but noticeably low in number and small in size (L), or no blisters produced (N). nt = not tested.

[b] Population was apparently heterogeneous.

were tested from 48 F_2 plants (Table 3) and a ratio very close to 1:2:1 was observed ($\chi^2 = 0.21$, P = 0.90). Hence, a single partially dominant gene from Cnt-1 appeared to be associated with recognition of *Acem1*. Interestingly, there was a high frequency of nonparental class of F_2 individuals that exhibited no blister formation. This might indicate overdominance of the gene when in the heterozygous condition (i.e., the incompatibility observed is apparently greater than that observed for seedlings of selfed Cnt-1) or else indicate a genetic contribution from the compatible parent that affects resistance. The hypothesis of overdominance was not substantiated by data from the F_1 individuals because they uniformly permitted formation of blisters. In addition, data from the F_3 families demonstrated that a majority of the heterozygous F_2 individuals were in class CB and all of the homozygous incompatible F_2 individuals were in class CN (Table 3). The same F_3 families were inoculated with the second parasite isolate, *Acks1*, which is also recognized by Cnt-1. There was complete correspondence with the data obtained using *Acem1* so it appears that the same gene from Cnt-1 is responsible for recognition of both isolates.

To test whether a compatible parent can influence the expression of disease resistance in progeny, both Ksk-2 and Cnt-1 were crossed with five accessions that each exhibited a GB phenotype when inoculated with *Acem1* (Ler-1, Nd-1, Oy-1, Ws-3, and Wei-1). Among the crosses with Ksk-2, inheritance of incompatibility appeared to vary in dominance. The cross between Wei-1 and Ksk-2 was an extreme example (8:51). Differences between the parents in seed germinability is unlikely to explain this variation because the incompatible parent Ksk-2 requires a period of seed maturation after harvest and cold treatment before germination, whereas the susceptible parents typically do not require pretreatment for germination. Variation was also observed among the crosses with Cnt-1, although less pronounced as with Ksk-2.

Mapping *RAC1*.

The DNA from 26 homozygous susceptible (GB) and 14 homozygous resistant (FYN) F_3 families were used in the initial phase of mapping *RAC1*. Families that were thought to be segregating were excluded. Additional homozygous material was produced from 122 random F_2 individuals that were carried through single seed descent until the F_6 generation. Among these F_6 inbred lines, 41% exhibited a uniform FYN phenotype characteristic of Ksk-1. RFLP markers from each chromosome were used systematically to sample regions of the genome until cosegregating markers were detected. Recombination of less than 5% was found only on chromosome 1 (Table 4) and *RAC1* was eventually located to a 1.0 cM interval approximately half-way between M254 and M253 (Table 4, Fig. 5).

Linkage relationship between *RAC* genes.

The F_2 progeny from the cross between Ksk-2 and Ksk-1 segregated 421:5 for incompatible and fully susceptible (GB) phenotypes following inoculation with *Acem1* (Table 2). Assuming that a single recessive and a single completely dominant gene were segregating in this cross, it is unlikely that the

Table 2. Distribution of interaction phenotypes among F_1 and F_2 progeny of crosses between accessions of *Arabidopsis thaliana* inoculated with *Albugo candida* isolate *Acem1*

A. thaliana cross (female × male)			Interaction phenotypes[a]						non-GB:GB ratio[b]			
Phenotype[a]	Accession	Generation	CN	CB	FYN	FGN	FGB	GB	Observed	Predicted	χ^2	P
GB × FYN	Col-5 × Ksk-1	F_1	0	0	6	0	0	0	6:0			
	Col-5 × Ksk-1	F_2	0	0	37	1	0	12	38:12	3:1	0.03	0.90
GB × FGN	Col-5 × Ksk-2	F_1	0	0	0	0	0	7	0:7			
	Col-5 × Ksk-2	F_2	0	0	0	76	9	148	85:148	1:3	16.38	<0.01
	Ws-3 × Ksk-2	F_2	0	0	0	15	0	83	15:83	1:3	4.91	0.03
	Wei-1 × Ksk-2	F_2	0	0	0	2	6	51	8:51	1:3	4.12	0.05
	Ler-1 × Ksk-2	F_2	0	0	0	9	11	65	20:65	1:3	0.10	0.76
FGN × GB	Ksk-2 × Oy-1	F_2	0	0	0	18	18	71	36:71	1:3	4.26	0.05
	Ksk-2 × Nd-1	F_2	0	0	0	12	7	67	19:67	1:3	0.39	0.61
GB × CL	Col-5 × Cnt-1	F_1	0	5	0	0	0	0	5:0			
	Col-5 × Cnt-1	F_2	128	112	0	0	0	101	240:101	3:1	3.88	0.05
	Ws-3 × Cnt-1	F_2	43	19	0	3	2	24	67:24	3:1	0.09	0.76
	Wei-1 × Cnt-1	F_2	46	18	0	3	0	17	67:17	3:1	1.02	0.35
	Ler-1 × Cnt-1	F_2	28	13	0	0	2	17	43:17	3:1	0.36	0.60
	Oy-1 × Cnt-1	F_2	34	6	0	0	7	14	47:14	3:1	0.14	0.70
	Nd-1 × Cnt-1	F_2	32	16	0	0	1	25	49:25	3:1	3.05	0.08
FGN × FYN	Ksk-2 × Ksk-1	F_1	0	0	3	2	0	0	5:0			
	Ksk-2 × Ksk-1	F_2	18	5	307	90	1	5	421:5	13:3	86.39	<0.01
FGN × CL	Ksk-2 × Cnt-1	F_1	2	2	0	0	0	0	4:0			
	Ksk-2 × Cnt-1	F_2	114	46	0	363	5	22	528:22	13:3	78.55	<0.01
GB	Col-5	S_4	0	0	0	0	0	10	0:10			
FYN	Ksk-1	S_4	0	0	10	0	0	0	10:0			
FGN	Ksk-2	S_4	0	0	1	8	0	0	9:0			
CL	Cnt-1	S_4	1	8	0	0	0	0	9:0			

[a] Interaction phenotypes were characterized as follows: Host response was recorded as necrotic flecks (F), necrotic cavities (C), yellowing which surrounds necrotic lesions (Y), or green, apparently non-responding (G); asexual reproduction of *A. candida* was recorded as blisters produced (B), blisters produced but noticeably low in number and small in size (L), or no blisters produced (N).

[b] For use in chi-squared test of selected models for predicting the number of host genes associated with incompatibility. Ratios are drawn from the observed behavior of selfed-progeny of parental accessions and F_1 progeny of crosses: Ksk-1 and Cnt-1 each predicted to carry a single, dominant allele for recognition of the parasite; Ksk-2 predicted to carry a single, completely recessive recognition allele.

two genes are independent (for 13:3, $\chi^2 = 86.39$, $P = <0.01$). Two completely dominant, independently segregating genes is also unlikely (for 15:1, $\chi^2 = 18.73$, $P = <0.01$). Similarly, F_2 progeny from the cross between Ksk-2 and Cnt-1 segregated 528:22 suggesting that the two predicted genes were not segregating independently (for 13:3, $\chi^2 = 78.55$, $P = <0.01$; for 15:1, $\chi^2 = 4.75$, $P = 0.04$). This evidence may suggest that host genes for recognition of *Acem1* from three sources are located on the same arm of chromosome 1. However, the cross between Ksk-1 and Cnt-1 remains to be tested.

DISCUSSION

The three examples of genotype-specific recognition examined were each associated with characteristic phenotypic expression. In the case of accession Ksk-1 following inoculation with isolate *Acem1*, the host resistance was inherited as a dominant trait. A single Ksk-1 allele at locus *RAC1* was associated with the expression of incompatibility and, because the phenotype was unambiguous, the allele is well suited for fine-scale mapping and molecular isolation. The *RAC1* allele is similar in this respect to the Nd-1 allele at *RPP1* identified following inoculation with *P. parasitica* isolate *Emoy2* (Holub et al. 1994). The Ksk-1 allele at *RAC1* is a major genetic component of the ability to recognize *Acem1*. If, as it appears, that genes identified from other accessions are closely linked to *RAC1*, then the Ksk-1 allele should provide a useful anchor to investigate further an important region of chromosome 1. Genes involved in recognition of other pathogens may also reside in this region of the genome. An allele from Wei-1 at the locus *RPP9* was previously reported to cosegregate with *RAC1* (Holub et al. 1994).

Table 3. Progeny tests of F_3 families from the crosses between three *Arabidopsis thaliana* accessions and Col-5 following inoculation with *Albugo candida* isolates *Acem1*

Cross	F_2 Phenotype[a]	Number of F_3 families: Uniform, No Blisters	Uniform, Blisters	Segregating
Col-5 (GB) × Ksk-1 (FYN)	FYN	12	0	25
	FN	0	0	1
	GB	0	12	0
Col-5 (GB) × Ksk-2 (FN)	FN	15	0	0
	FB	0	0	2
	GB	0	12	31
Col-5 (GB) × Cnt-1 (CB)[b]	CN	11	0	7
	CB	0	0	18
	GB	0	12	0

[a] Interaction phenotypes were characterized using an assessment of host and parasite attributes: host response was recorded as necrotic flecks (F), necrotic cavities (C), yellowing which surrounds necrotic lesions (Y), or green, apparently nonresponding (G); asexual reproduction of *A. candida* was recorded as blisters produced (B), blisters produced but noticeably low in number and size (L), or no blisters produced (N).

[b] The F_3 families behaved identically following inoculations with *Acem1* and *Acks1* suggesting that the same recognition allele from Cnt-1 recognizes both isolates.

In the case of accession Ksk-2, resistance to *Acem1* was inherited as a completely recessive trait. A second locus, *RAC2* was postulated for the Ksk-2 allele because recombination was observed with the allele at *RAC1* from Ksk-1. No analogous example of recessive resistance has yet been reported for recognition of *P. parasitica*.

In the third example of resistance to *A. candida*, a single allele was postulated to be associated with the incompatible phenotype expressed by Cnt-1. The cavity response associated with this allele was readily visible whether the gene was in the homozygous or heterozygous condition. However, restricted formation of blisters was typical, as indicated by the phenotypes for selfed Cnt-1 and F_1 progeny derived from the cross with Col-5. The phenotype expressed by Cnt-1 is analogous in this respect to the FDL phenotype associated with expression of the *RPP4* allele of Col-0 for recognition of *P. parasitica* (Holub et al. 1994). The cross between Ksk-1 and Cnt-1 was not available, so it is unknown whether the Cnt-1 allele for *Acem1* recognition resides at *RAC1*. However, recombination with *RAC2* was observed.

From this genotypic evidence, we have attributed the observed incompatibility to the expression of single host genes and corresponding, at present hypothetical, parasite genes. These are referred to as recognition genes because we postulate that they are involved in the earliest stages of incom-

Table 4. Recombination in F_3 and F_6 Wei-1 × Ksk-1 families between the *RAC1* locus and molecular markers from the five chromosomes of *Arabidopsis thaliana*

Chromosome	Locus[a]	F_3 n	F_3 r	F_3 % r[b]	F_6 n	F_6 r	F_6 % r[b]
1	M201	80	12	15	93	15	17
	M335	80	3	4	122	12	11
	M402	80	1	1	85	7	8
	M254	80	1	1	100	3	3
	M253	80	0	0	125	7	6
	M299	80	1	1	115	14	13
2	M216	74	43	58	64	33	52
	M336	76	42	55	34	20	59
3	M243	0	0	NT	69	36	52
	M433	76	46	61	0	0	NT
	M457	80	37	46	31	17	55
4	M506	76	36	47	0	0	NT
	M557	80	36	45	71	36	51
	RPP2	80	37	46	92	43	47
5	M447	80	43	54	92	50	54
	M331	60	31	52	43	30	70

[a] Loci designated by "M" were previously identified by segregation of restriction fragment length polymorphisms (Chang et al. 1988). *RPP2* was defined as a locus associated with recognition of *Peronospora parasitica* (Tör et al. 1994).

[b] Percentage recombination was estimated as the number of changes (R) between a pair of loci divided by the total number of cases examined (n). For F_3 lines, r was estimated on the basis of chromosomes (e.g., homozygous Wei-1 at locus 1 and homozygous Ksk-1 at locus 2 was counted as two changes; a heterozygous and homozygous combination was counted as one change), and n was twice the number of lines examined. For F_6 lines, r was calculated on a per lineage basis (e.g., homozygous Wei-1 at locus 1 and homozygous Ksk-1 at locus 2 was counted as one change; assumes that the individual lineage inherited two copies of the same change via selfing).

patibility; it remains to be seen; however, what role they actually play in disease resistance. In some cases, such as Cnt-1, the transduction of non-self recognition to host response and ultimately to an expression of resistance is deficient. This accession exhibits a genetically dominant host response, evident as necrotic cavities, but only expresses weak resistance because the parasite is capable of restricted asexual reproduction even in plants homozygous for the Cnt-1 allele. A high proportion of F_2 progeny from the cross between Cnt-1 and Col-5 exhibited no blisters, in contrast to selfed Cnt-1 or F_1 progeny. This is clearly not an example of over-dominant expression of the Cnt-1 allele. It will therefore be important to consider that another gene (or genes) plays a role in modifying the interaction phenotype, even one inherited from the susceptible parent (Col-5). The influence of at least another gene was suggested by differences in segregation ratios at F_2 among crosses of Ksk-2 with several susceptible accessions and to a lesser extent among similar crosses using Cnt-1 (Table 2).

Parasite genotype was also observed to influence the expression of Cnt-1 resistance. In contrast to the blisters that formed following inoculation of Cnt-1 with *Acem1*, no blisters have been observed following inoculation with isolate *Acks1*. This is most readily explained by a genetic difference between the parasite isolates because analyses of F_3 Col-5 × Cnt-1 families suggested that *Acem1* and *Acks1* are recognized by the same Cnt-1 allele.

Advanced inbred lines will enable further dissection of disease resistance to determine whether more than one gene is involved in a given interaction phenotype. Critical analyses of F_6 inbreds from the cross between Wei-1 and Ksk-1 unexpectedly revealed a quarter that exhibited a phenotype similar to Ksk-2 (FN without yellowing) following inoculation with *Acem1* and incubation at 15°C (E. Brose, unpublished). The same inbreds had exhibited a GB phenotype in previous experiments that were incubated at the standard temperature of 20°C and were therefore thought to carry the Col-5 allele at *RAC1*. An attempt is now being made, using the cooler environment, to determine whether Ksk-1 carries a second, temperature-sensitive gene and to locate it in the genome. It would be difficult to locate such a gene using material from the F_2 or F_3 generations if it is associated with a recessive phenotype and hypostatic to the Ksk-1 allele at *RAC1*. The influence of temperature and physiological age of host tissue has been reported for phylogenetically-related symbioses (Liu and Rimmer 1990; Judelson and Michelmore 1992).

Genes for specific recognition of *A. candida* appear to be much less frequent in *A. thaliana* than those for recognition of *P. parasitica*. In the case of the later parasite, over half of the host accessions were capable of recognizing any given isolate of the parasite (Holub et al. 1994), whereas fewer than 10% of the accessions recognized the two isolates of *A. candida*. Over 20 isolates of *P. parasitica* have been examined thus far and none were compatible on more than 50% of *A. thaliana* accessions (E. Holub, unpublished). Additional *A. candida* isolates should be tested to determine whether variants exist in natural populations that can be recognized by a wider range of accessions. It will also be worthwhile continuing genetic analyses of additional germplasm with the current isolates to define other regions of the *A. thaliana* genome that carry *RAC* genes. The numerous recessive phenotypic markers already available in the accession Landsberg *erecta* should be especially useful for mapping new *RAC* loci since this accession is fully susceptible to both isolates of *A. candida*.

There is no obvious explanation for why *RAC* genes should occur less frequently in *A. thaliana* than *RPP* genes involved in the recognition of *P. parasitica*. Only UK isolates have been examined thus far, so a more recent immigration of *A. candida* to the UK might explain the difference between the parasites. Otherwise, they share similar opportunities for evolving on a common host. Both are commonly observed as mutual travellers having similar geographic range, and often coming into intimate contact (Sansome and Sansome 1974; Chaurasia et al. 1982; Alexander and Burdon 1984; Bains and Jhooty 1985; Holub et al. 1993). And, synchrony of their life cycles with that of *A. thaliana* is very similar so it is unlikely that one is more opportunistic by imposing selection pressure at a vulnerable growth stage of the host. Another hypothesis is that *A. candida* has evolved an effective means of evading recognition by the host. It may have the ability to "switch off" host defenses before being recognized or else to actively induce susceptibility of the host to further colonization. This has been suggested from the observation that prior infection of *A. thaliana* by *A. candida* can allow an otherwise incompatible isolate of *P. parasitica* to colonize the same host tissue (Holub et al. 1993).

The variation in genetic dominance relationships and in phenotypic expression of incompatibility is worth emphasizing because it demonstrates that evolutionary refinement of plant defense is possible without necessarily altering the specificity of recognition. The mechanistic basis of such phenomena is unresolved and awaits a greater understanding about the interaction between host and parasite gene products. For instance, it is unknown whether less than complete dominance results from an affect of gene dosage (partial dominance of the resistance allele), or from expression of the alternative "susceptibility" allele that interferes with disease resistance (codominance of two alleles). Dominance is not exclusively an attribute of alleles at a single locus because the interaction phenotype may depend on the host genetic background, the genotype of the parasite and nongenetic environment (Barrett 1983; Person and Mayo 1974; Roelfs 1988). The ability to characterize disease resistance is also greatly dependent on technique; dominant resistance observed macroscopically may appear less dominant if reevaluated at a microscopic level.

These phenomena are common features of crop-pathogen interactions. For instance, there have been increasing reports suggesting that partial dominance of disease resistance is perhaps the rule rather than the exception and that completely recessive inheritance is also common (Crute 1985; Crute and

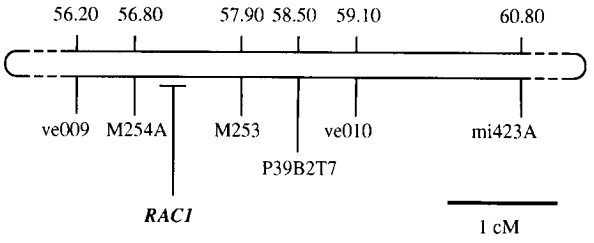

Fig. 5. Location of *RAC1* on the upper arm of chromosome 1 in *Arabidopsis thaliana* mapped relative to loci defined by restriction fragment length polymorphisms.

Norwood 1986; Hammond-Kosack and Jones 1994; Holub et al. 1994; Hooker 1967; Kesseli et al. 1993; Kolmer and Dyck 1994; Lawrence et al. 1994; Mindrinos et al. 1994).

The findings reported here demonstrate that these important phenomena can also be observed in the parasitic symbiosis of a wild plant species where there is tremendous potential for resolving the molecular mechanism of plant defense. It should therefore be possible to rigorously test hypotheses like the ones suggested above for explaining variation in dominance. Although such hypotheses may be mutually exclusive for a given case study, none of them may yield a universal explanation. Therefore, comparative biology both within and between pathosystems will be increasingly important to achieve a satisfactory understanding of the diversity and evolution of plant defense.

MATERIALS AND METHODS

Isolates of *A. candida*.

The methods used to collect and maintain cultures and store frozen inoculum of *A. candida* have been published elsewhere (Dangl et al. 1992). Refinements of these methods are described below.

Both isolates of the parasite were obtained from natural populations of *A. thaliana* found in the UK: *Acem1* was collected from a host population found growing between polythene tunnels at Horticulture Research International, East Malling, Kent; *Acks1* was collected from a small private rose garden in Keswick, Cumbria. Cultures of both isolates were initiated from a mixture of zoosporangia removed from a single rosette leaf. However, to reduce possible heterogeneity in the inoculum, they were each subsequently regenerated for three generations from single blisters.

A. thaliana germplasm.

The *A. thaliana* accessions used in this study were obtained from the following sources: Col-5, Cnt-1, Ema-1, Ksk-1, Ler-1, Nd-1, Oy-1, Rld-2, Tsu-1, Wei-1, and Ws-3 were as described for a previous investigation (Holub et al. 1994); Bea-1, Bid-1, Chr-1, Crl-1, Frd-1, Igt-1, Ksk-2, Kyl-1, Lha-1, Mai-1, Sco-1, Sis-1, Wis-1, and Wma-1 were second-generation, single-seed decent accessions produced from plants collected by the first author from natural populations in the UK; Kin-1 was obtained from the Crucifer Genetic Cooperative (Madison, WI); Aa-0, Ag-0, C24, Est-0, and Ms-0 were obtained from the Nottingham Stock Centre; Can-0, Cvi-0, Kas-1, Mh-0, Mt-0, Per-0, Pi-0, Pla-0, Sue-0, and Te-0 were obtained from the collection of A. R. Kranz and B. Kirchheim via J. Dangl (Köln, Germany); and the remainder were obtained directly from the Kranz collection.

Growing plants.

Seed was sown on the surface of a soil mix consisting of six parts commercial peat-based seedling compost containing macro-nutrients (Levington F2 mix), one part vermiculite and one part fine sand. The ingredients were mixed and sieved (5 mm mesh). After sowing, the soil was moistened from below by placing the pots in a pan of water. Pots were drained, placed in a tray, and covered with a clear polystyrene cloche. Pots remained covered for the duration of an experiment to maintain humidity suitable for uniform seedling emergence and parasite infection. The cloche also reduced the risk of cross-contamination between isolates of *A. candida* and *P. parasitica*. Pots were stored for 5 to 14 days in the dark at 8°C before placing in the growth room. This served to vernalize sown seed, but also provided a convenient stock of plant material. Pots were transferred to walk-in growth rooms for germination at 18 to 20°C, under a 10-h photoperiod at a photon flux density of 150 to 200 $\mu E\ m^{-2}\ s^{-1}$.

Plants used for inoculum production were transplanted into 7.5-cm pots. A single rosette of 10 or more leaves was grown in each pot before the plants were inoculated with numerous drops of zoospore inoculum. Occasionally, plants that had been inoculated as seedlings were also transplanted for later use in inoculum production. These plants often became systemically infected and were eventually covered extensively with blisters. The heaviest infection was achieved by opening vents in the top of the cloche to reduce humidity. Although plants grown in this way could provide a source for inoculum for several weeks, a continuous turnover of stock plants was produced because the older blisters were readily contaminated by asexual sporulation of *P. parasitica* and were often preferentially consumed by fungus gnat larvae or foliar mites.

For experimentation, plants were grown in packs of 20 2 × 2 × 3 cm pots. A row of four pots was reserved for sowing control accessions of *A. thaliana* (usually fully susceptible accessions such as Oy-1, Col-5, and Wei-1). Test material was sown in the remaining 16 pots, arranged in a row of four pots for each of four seed lines. Eight to ten seeds were sown per pot.

Inoculation.

Sporangia of *A. candida* were germinated as previously described for the production of zoospore inoculum (Dangl et al. 1992). It was technically difficult to estimate the effective concentration of *A. candida* zoospores because germination of sporangia was asynchronous and the percentage germination between batches of sporangia was highly variable. Nevertheless, the inoculum was routinely adjusted to a concentration of ca. 2×10^4 sporangia per ml immediately after rehydration (maximum zoospore concentration would have been about 8×10^4). The suspension was incubated at 12 to 15°C for 2 h. By this time, zoospores began to be released, and the suspension was refrigerated (8°C) or placed in a container of ice slush. Refrigeration was essential for extending the duration of zoospore motility, especially for experiments conducted during summer months.

For experimentation, 7-day-old seedlings were inoculated by placing 2 µl of inoculum on each cotyledon (about 40 to 160 zoospores). After inoculation, the plants were placed in a clear plastic box to retain sufficient soil moisture and relative humidity for infection. Growth chamber conditions were the same as described above. *A. candida* grown on a fully susceptible accession typically began to produce blisters by 7 dai. Most characteristics of phenotypes could be seen with the naked eye, thereby making it practical to examine large numbers of inoculated seedlings. A dissecting microscope at low magnification (20×) was routinely used for confirming the phenotype identity.

Cryo-scanning electron microscopy.

Samples were viewed in a frozen, hydrated condition using a Hexland CT1000 Cryotrans System that was attached to a

Cambridge Instruments S200 scanning electron microscope (SEM). Cotyledons were mounted to the sample holder using Leit-C conductive carbon cement (Neubauer, Münster, Germany), then frozen either directly on the stage of the cryo-preparation chamber (–180° C) or by plunging into nitrogen slush at –210°C and transferred into the preparation chamber using the vacuum transfer device (VTD). Freeze fracturing was achieved by striking a vertically-mounted cotyledon with a cooled knife assembly in the cryo-preparation chamber. Loose fragments of tissue were removed by inverting the sample holder.

Samples were transferred to the SEM cold stage using the VTD and viewed uncoated using an accelerating voltage of 5 kV. Surface water was sublimated for removal by temporarily setting the cold stage at –80° C. Samples were sputter coated with gold in the cryo-preparation chamber for about 5 min at 1.5 kV and 1.5 to 2.0 mA. The sample was then returned to the SEM cold stage using the VTD. Samples were examined and photographed at –180° C using an accelerating voltage of 4 to 14 kV in the presence of an anticontaminator at –190° C.

Mapping the *RAC1* locus using RFLP analysis.

Plant DNA was prepared from F_3 and F_6 families from the cross between Wei-1 and Ksk-1. At least 20, 6-week-old plants were grown in a greenhouse for each F_3 family, and at least 50 3-week-old plants were grown for each F_6 family. The plants were placed in the dark for two days before DNA was isolated by the method of Dellaporta et al. (1983).

Restriction digests, Southern blotting, and hybridization of labeled probes to isolated plant genomic DNA was carried out by using standard techniques (Ausubel et al. 1990). RFLP probes were lambda genomic clones (Chang et al. 1988: designated by "M"). Probes were labeled using the Pharmacia Oligolabelling system according to the manufacturer's instructions.

RFLP markers were mapped relative to the resistance locus *RAC1* using the computer program MAPMAKER v1.0 Macintosh (Lander et al. 1987). It was not possible to estimate the relative distances between loci from the combined data of the F_6 and nonrandom F_3 lines. The distances shown in Figure 5 were therefore obtained from the F_8 Ler-0 × Col-4 inbred lines (Lister and Dean 1993) as reported in AAtDB release 3.1 (Cherry et al. 1992).

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