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Original article

Spatial pattern for resistance to a pathogen. Theoretical approach and empirical approach at the phenotypic and molecular levels

Claire NEEMA[a,*], Claire LAVIGNE[c], Juliette DE MEAUX[a,b], Isabelle CATTAN-TOUPANCE[b], Julio FRANCO DE OLIVEIRA[b], Alexandra DEVILLE[c], Thierry LANGIN[b]

[a] Laboratoire de pathologie végétale, INAPG, 16 rue Claude Bernard, 75231 Paris Cedex 05, France
[b] Laboratoire de phytopathologie moléculaire, IBP-Bât 630, Université Paris 11, 91405 Orsay, France
[c] Laboratoire d'Écologie, Systématique et Évolution, Bât. 362, UPRES-A 8079, Université Paris XI, 91405 Orsay, France

Abstract – A good understanding of the dynamics of host/pathogen interactions and of the factors that shape the spatial distribution of resistance genes is a prerequisite of metapopulation dynamic management of resistance genes. We studied the diversity and spatial structure of natural populations of common bean (*Phaseolus vulgaris*) for resistance to *Colletotrichum lindemuthianum*, the causal agent of anthracnose. This study was carried out in Mexico and Argentina both at the phenotypic level and at the molecular level for two families of resistance gene candidates (RGCs). Using a simulation model, we also investigated the effects of migration and selection on the spatial structure of resistance phenotypes in a metapopulation for two genetic determinisms of the interaction (gene-for-gene and matching allele). Our results showed a differentiation between the countries for all the markers and indicated that the RGC, polymorphic in both countries, do not behave as neutral markers. Comparison of the diversities for resistance to strains isolated from wild or cultivated plants suggested that, although there is local adaptation of *C. lindemuthianum* between the two countries, the coevolution process seems to occur at a very local scale with the maintenance of resistances to allopatric strains, a result consistent with simulations of the models.

fungal pathogen / model / resistance / population structure / wild populations / *Colletotrichum lindemuthianum* / *Phaseolus vulgaris*

* Correspondence and reprints
E-mail: neema@inapg.inra.fr

Résumé – Étude de la distribution spatiale des résistances à un agent pathogène, par des approches théorique et expérimentale aux niveaux phénotypique et moléculaire. Un préalable à une gestion dynamique en métapopulation des résistances aux agents pathogènes est une meilleure connaissance des interactions hôte/pathogène et des facteurs qui modifient la répartition spatiale des gènes impliqués dans l'interaction. Nous avons étudié, au Mexique et en Argentine, la diversité et la structuration de populations naturelles de haricot commun (*Phaseolus vulgaris*) pour la résistance à *Colletotrichum lindemuthianum*, responsable de l'anthracnose. Cette approche a été menée au niveau phénotypique, au niveau de marqueurs moléculaires neutres (RAPD) et au niveau de deux familles de gènes candidats pour la résistance (RGC). Nos résultats indiquent qu'il existe une différenciation pour tous les marqueurs entre les deux pays et que les RGC, polymorphes dans les deux pays, ne se comportent pas comme des marqueurs neutres. La comparaison des diversités pour les résistances à des souches sauvages ou isolées de cultivars suggère que, bien qu'il existe une adaptation de *C. lindemuthianum* à l'échelle des deux pays, la coévolution se ferait à une échelle très locale et maintiendrait des résistances à des souches allopatriques. Par ailleurs, nous avons simulé sur une métapopulation l'effet de la migration et de la sélection sur la répartition spatiale des phénotypes de résistance pour deux déterminismes génétiques de l'interaction (gène pour gène et « matching allele »). Pour les mêmes valeurs de paramètres, le niveau de compatibilité locale est moins élevé pour un déterminisme de type gène pour gène que « matching allele », l'asymétrie du système gène pour gène favorisant l'hôte lorsque de nombreux loci sont en jeu. Globalement, le niveau d'adaptation locale du parasite diminue lorsque la migration de l'hôte augmente. Une maladaptation locale peut même être observée, en particulier dans un système gène pour gène, si les pressions de sélection réciproques sont fortes. Dans le détail, le test d'une population d'agents pathogènes sur l'ensemble des populations hôtes indique que certaines populations hôtes possèdent des résistances à des populations pathogènes éloignées, et ce quel que soit le niveau d'adaptation locale. Ce résultat est cohérent avec ce qui est observé dans les données expérimentales.

Champignon phytopathogène / modèle / populations naturelles / résistance / structure populations / *Colletotrichum lindemuthianum* / *Phaseolus vulgaris*

1. INTRODUCTION

Genetic resources of crop plants and of their wild relatives have been kept in gene banks since the sixties. One major problem with these banks has been that the species no longer evolve following the selective pressure of the environment. The problem is particularly acute for the value of gene banks as a source of genes conferring resistances to pathogens or parasites. A common view of host-parasite coevolution is that parasites evolve in interaction with their host and exert a selection pressure on their hosts which results in the evolution of resistance in the host [2, 32]. If plants are kept in gene banks, populations of parasites could continue to evolve while plants would harbour resistance genes for avirulences that are no longer present in the pathogen populations. These

resistance genes would be inefficient if introduced in a crop. Some authors suggested that one solution to this problem would be the dynamic management of genetic resources of a plant species evolving in its natural environment [32]. The goal would be to maintain as much diversity as possible for all desirable traits, and in particular resistance genes, and allow the appearance of new genes or new gene combinations to resist new pathogenic strains. Such management has been performed on three isolated barley populations grown since 1974 near Cambridge, UK [33]. To maintain more diversity, dynamic management with metapopulations was later suggested, consisting of a number of populations of the crop species linked by gene flow and evolving under different pathogen pressures. However, the evolution of plant-pathogen interactions in metapopulations is still not well understood thus compromising the feasibility of such management.

The evolution of plant host-pathogen interactions in a metapopulation has been investigated both theoretically [25, 28, 29, 44, 58] and empirically [*e.g.* 3, 10, 34, 35, 36, 48] taking into account both the numerical and genetic dynamics. The evolution of resistance to powdery mildew has also been assessed [39, 46, 47] in an experimental metapopulation of winter wheat established in 1984 [32]. However, the number of plant host-pathogen interactions for which there is empirical evidence about the structure of resistance and virulence genes in natural or experimental (meta)populations is limited and little is known about the conditions (population sizes, pathogen pressure, migration rates...) that maintain high levels of diversity for resistance and/or durable resistance in these populations. The interactions between hosts and pathogens are, furthermore, governed by a number of different genetic systems (such as gene-for-gene or matching allele) that might result in a different evolution at the metapopulation level. The management of resistance genes in a metapopulation, therefore, still raises a large number of questions about number of populations, optimal population sizes, selective pressures to apply (introduced pathogens or not) and migration rates needed.

Diversity for resistance in natural populations has been reported in different host-pathogen interactions [7, 19, 41, 48]. In most of the interactions studied, resistance polymorphism was observed on different geographical scales, even on very local ones, and population differentiation has often been shown. For example, frequencies of resistance to eight races of *Melampsora lini* were very different in 10 populations of *Linum marginale* in Australia and resistance differentiation seemed to occur at the metapopulation level [34]. In some cases, differentiation for resistance was correlated with environmental conditions; like in populations of *Avena barbata*, *A. fatua* and *A. ludoviciana* where resistances to *Puccinia coronata* were more frequent in a region favourable to pathogen development [13]. Population structure for resistance was also correlated with pathogen population structure in the *Amphicarpaea bracteata*–*Synchitrium decipiens* interaction where resistances were more frequent for allopatric strains [48]. There is therefore evidence that the resistance structure of

Table Ia. Pattern of compatible (= infection) and incompatible (= no infection) interactions in a gene-for-gene model with two loci. The host is diploid, the pathogen haploid. Resistance is dominant. I: incompatible, C: compatible.

Pathogen genotypes	Host genotype			
	R1-R2-	R1-r2r2	r1r1R2-	r1r1r2r2
Avr1Avr2	I	I	I	C
Avr1vir2	I	I	C	C
vir1Avr2	I	C	I	C
vir1vir2	C	C	C	C

a plant species may occur on different spatial scales; little is known, however, of its evolution over time. Burdon and Thompson [11] studied temporal changes in resistance to *M. lini* in one population of *L. marginale*. They detected a marked change in the resistance structure of this population over a 6-year period; but the local pathotypes were not obviously responsible for the changes in resistance frequencies.

Most studies at the population level are based on phenotypic data, and the genetics of most interactions is still subject to debate. There exist different models describing the genetics of the interaction between a host and a parasite. The two extreme cases are the gene-for-gene model, first described by Flor [23] and later by Burdon [6,9] and widely used in plant breeding, and the matching allele model [26], mainly used in theoretical modelling. The biological model behind the gene-for-gene (GFG) model is the interaction between an elicitor encoded by the avirulence gene of the parasite and a receptor encoded by the resistance gene of the host [17]. The interaction, governed by a large number of bi-allelic loci, is incompatible if the host expresses an *R* gene at any locus, which enables it to recognise the product of an *Avr* gene at the corresponding locus (Tab. Ia). The matching allele model (MAM) assumes one locus with a large number of alleles. The host is able to resist the parasite if both partners possess the matching alleles at that locus (Tab. Ib). Empirical evidence in favour of the gene-for-gene model has been reviewed in Thompson and Burdon [57]. A number of interactions, however, clearly do not follow the gene-for-gene model. More than two alleles for example can be observed at one locus as in the well-studied flax/*Melampsora lini* interaction for which at least 13 alleles have been described at the L locus [52]. Two loci can also interact to confer resistance on a single *Avr* product [14, 18]. Frank [26] argues that data are not sufficient to distinguish between a gene-for-gene interaction and a matching allele interaction in natural systems. As an answer, a review of the evidence in favour of gene-for-gene systems was provided by Parker [49].

In an attempt to improve our knowledge on the evolution of resistance patterns in a metapopulation, we adopted a two-pronged approach. First, we investigated the possibility of a direct estimation of the genetic diversity for

Table Ib. Pattern of compatible and incompatible interactions in a matching allele model with two alleles. The host is diploid, the pathogen haploid.
I: incompatible, C: compatible.

Pathogen genotypes	Host genotype		
	A1A1	A1A2	A2A2
A1	I	I	C
A2	C	I	I

resistance in natural populations of wild common bean (*Phaseolus vulgaris*) to *Colletotrichum lindemuthianum*, the causal agent of anthracnose. Population structures of wild common bean in Mexico and Argentina were thus compared for different markers: neutral markers, RGC markers and resistance phenotypes. Second, we investigated, in metapopulation models, possible differences in the outcome of gene-for-gene and matching allele interactions. In particular, we looked at whether increasing migration among populations or the selective pressures involved in the interaction would have the same effect on the pattern of host resistance in both models.

2. MATERIALS AND METHODS

2.1. Diversity of wild populations of common bean

2.1.1. The experimental model

The model studied is the interaction between the common bean, *Phaseolus vulgaris*, and the anthracnose agent, *Colletotrichum lindemuthianum*. *C. lindemuthianum* is a haploid fungus with no known sexual stage. It is dispersed over short distances (< 1 m) by splashing and possibly over longer distances in seeds. Wild common bean populations are found in the centres of diversity of the species, in Latin America, from Northern Mexico to North-Western Argentina [5]. The wild populations studied were geographically isolated from cultivated populations by large distances. The interaction between common bean and *C. lindemuthianum* is thought to follow a gene-for-gene relationship and several major resistance genes have been characterized in cultivars [38,51].

2.1.2. Sampling

The wild common bean is a predominantly selfing, annual, climbing plant. In natural populations, plants are grouped in patches of sizes ranging between 10 and 25 plants. Seeds were collected along 500 km transects in Argentina in May 1992, and in Mexico in October 1994 and January 1995 when pods were mature and anthracnose was present. Argentina and Mexico are situated respectively

in the Andean and in the Mesoamerican centre of diversity of the common bean. For this study, the progeny of 50 plants from 15 sites in Argentina and of 27 plants from 11 sites from Mexico were analysed. Seeds were multiplied in the CIAT (Centro Internacional de Agricultura Tropical, Cali, Columbia).

2.1.3. Molecular analyses

2.1.3.1. DNA extraction

The extraction was carried out as already described [16].

2.1.3.2. RAPD PCR procedure

Amplification procedures were performed as described by Cattan-Toupance *et al.* [16]. Two primers (Operon Technology) were used for amplification: primer E12 (TTATCGCCCC) and primer F10 (GGAAGCTTGG). Most RAPD markers have been shown to have a Mendelian inheritance in common bean [1]. We thus considered amplified fragments as single locus for resampling procedures.

2.1.3.3. RFLP markers

Total DNA was digested with the two restriction enzymes *Hin*dIII and *Hae*III. Southern blot and hybridisation experiments were carried out as already described [30]. Two probes corresponding to two Resistance Gene Candidates (RGCs) were used. These two clones were obtained by a candidate gene approach with two degenerate primers designed from two domains which are conserved among the NBS-containing resistance genes [31]. These two clones (PRLJ1 and B2) were characterised by Ferrier-Cana [22] and Geffroy *et al.* [31], and grouped in two classes according to their hybridisation pattern. One of them (PRLJ1) displayed the complex pattern characteristic of a multigene family and was localised on the linkage group B4 on the common bean integrated linkage map [27].

2.1.3.4. Phenotypic studies

Plants were tested for their resistance or susceptibility to three sets of strains of *C. lindemuthianum*. The first set consisted of six strains isolated from cultivated beans of different origins and characterised for their avirulence spectra against cultivars possessing known resistance genes. The second set consisted of 10 strains isolated from wild common bean collected in Mexico and 10 strains isolated from wild common bean collected in Argentina. Strains from wild and cultivated plants present different patterns for RAPD markers [54]. Plants were also tested against seven strains for which resistance specificities were mapped on the linkage group B4 [31].

Five seeds of each plant were sown in vermiculite for a week and then spray-inoculated with a 5×10^6 spores·ml^{-1} suspension. Spore suspensions were prepared by flooding 10-day-old fungus cultures with distilled water. Conidia were dislodged by scraping the culture surface with a spatula. Concentration was adjusted to 5×10^6 spores·ml^{-1} by counting with a hematocytometer. The inoculated plants were incubated in a growth chamber at 19 °C with saturated humidity. Disease symptoms were scored 7 days after inoculation and reactions classified as resistance (R) or susceptibility (S).

2.1.4. Data analysis

Within-country diversity was determined for molecular markers and resistance phenotypes by Nei's unbiased diversity index [45]. Genetic differentiation between countries and random association among pairs of different markers within countries were tested using Fisher's exact test provided by the GENEPOP software [53].

2.2. The metapopulation models

The model is adapted from the METAPOP program initially intended to describe a metapopulation of one species. It is an individual-based model, where the genotype of each individual is encoded.

The two simulated species are a parasite and its host plant. The parasite is asexual and haploid while the host is diploid and reproduces sexually (all individuals being hermaphrodites). Both species are assumed to have discrete non-overlapping generations with no age structure and generation times are the same. The host and the parasite can potentially exist on 100 discrete sites.

2.2.1. Within-population processes

Within-population dynamics follow a Lotka-Volterra model, adapted from Frank [24] to a host-parasite system. Because of sexual reproduction, independent strains of hosts and parasites could not be described as in Franck [24] or Gandon *et al.* [28]. The variation between generations of the numbers of hosts ΔH_t and of parasites ΔP_t are given by

$$\Delta H_t = H_t \left[r_h \left(1 - \frac{H_t}{K_h} \right) - d\, c_t\, P_t \right]$$
$$\Delta P_t = P_t \left(r_p\, c_t\, H_t - s \right).$$

H_t and P_t are the population sizes of hosts and parasites at generation t, r_h and r_p are their intrinsic growth rates and K_h is the carrying capacity of a site for the host. The proportion of compatible interactions in the population at

generation t, *i.e.* the measured probability that a parasite can infect a host in that population, is c_t. The pathogenicity of the parasite on the host, *i.e.* the decrease in population growth due to a single parasite virulent on all hosts, is d. As a consequence, the decrease in host population growth due to the local parasite population depends on d, on the number of parasites and on the probability that a parasite is virulent on a host in that population. The independent mortality rate of the parasite is s. Whereas H_t, P_t and c_t were allowed to change during the simulations, the other parameters were fixed at $K_h = 200$, $d = 0.01$, $s = 0.6$, $r_h = 0.5$ and $r_p = 0.07$. Gandon *et al.* [28] found that in such a system, the populations are locally stable when $K_h\, r_p - s < 1$ which is not the case here. These values, however, allowed stable coexistence of the host and the parasite at the metapopulation level for all simulations presented here.

The equations above provide the number of offspring that will build the following generation both for the host and the parasite. To determine the relative contribution of each genotype to these offspring, it was necessary to describe the selection pressure to which each individual host and parasite was submitted at any generation t. The relative survival of a given individual parasite offspring i to the following generation was given by $(1 - wp)ci + wp$ where ci is the proportion of hosts that the individual i can infect in the population, and wp is a fixed parameter describing the relative survival of individual i if it cannot infect any host ($wp < 1$). Results will be presented for two values of wp: $wp = 0.9$, and $wp = 0.5$ respectively meaning little and much selection against avirulent genotypes. Symmetrically, the relative survival of a given individual host offspring i to the following generation was given by $(1 - wh)(1 - Ci) + wh$ where Ci is the proportion of parasites that are virulent on individual i, and wh is a fixed parameter describing the relative survival of individual i if it is infected by a parasite ($wh < 1$). Results will be presented for two values of wh: $wh = 0.9$, and $wh = 0.5$ respectively meaning little and much selection for resistant genotypes.

2.2.2. Metapopulation processes

The metapopulation contains 100 sites distributed along a 10×10 grid. Each site can potentially host a parasite and a host population. Migration occurs through seeds and pollen for the host. Migration of the parasite occurs by asexual migrants. The migration rate is defined as the proportion of seeds/pollen/individuals of a population that originates from adjacent populations. For simplicity the same migration rate m_h was assumed for both seeds and pollen. For the parasite, the migration rate is m_p. Migration only occurs between adjacent sites.

2.2.3. *Genetics of the interaction*

Two different genetic systems were simulated for the control of the interaction between the parasite and its host. A gene-for-gene system was simulated for ten bi-allelic loci. In the parasite, at locus i the two alleles are virulent (vir_i) and avirulent (Avr_i) while in the host the two alleles at each corresponding locus are susceptible (s_i) and resistant (R_i) with R_i dominant over s_i. In such a system, the relationship between the host and the parasite is compatible (*i.e.* the parasite can damage the host) if, at none of the 10 loci, the host has one R_i allele while the parasite is Avr_i. A matching allele system [24, 26] was simulated for one locus with 10 alleles. The interaction between the host and the parasite is incompatible if their alleles match.

The mutation rate from one allele to another was set at 0.0001.

2.2.4. *Conditions of a simulation*

All simulations begin with small populations of ten hosts and ten parasites. In the gene-for-gene model, all hosts are susceptible and all parasites are avirulent at all loci. This quickly selects for resistance genes in the host. For the matching allele model, the initial frequencies of all alleles are equal on average, making mutation ineffective at creating new variability.

Alleles are attributed at random to individuals to constitute the initial populations. The populations are then allowed to evolve for 200 generations and statistics are collected from the 200th to the 500th generation. This makes the collected statistics largely independent of the initial conditions.

One main problem with the gene-for-gene model is that after a large number of generations, all parasites carry the *vir* alleles but there is polymorphism for the R-alleles in the host. The host is then fully susceptible to the parasite, which leads to host population crashes. This unrealistic situation is due to the reduced number of loci used. Obviously if more loci were introduced in the model, this phenomenon would be delayed. To overcome this problem there were two possibilities. The first was to introduce a fitness cost associated with virulence alleles, but evidence for this type of cost is scarce and it would be difficult to parameterise, while its effect on the outcome of the interaction is obvious. We decided that a locus would be reset to its initial state both in the host and the parasite once all the metapopulation became fixed for the *vir* allele at that locus. This is similar to assuming that a new resistance gene appears in the host producing a receptor that is able to recognise a new molecule in the parasite. Five simulations were performed for each set of parameters.

2.2.5. *Measured statistics*

At each generation each individual parasite is tested for compatibility against all the individual hosts at the same site. The proportion of local compatible interactions is averaged over individual parasites for each site and afterwards

averaged over all sites. The resulting proportion is the local compatibility at the studied generation *loct*. To produce a synthetic parameter *loc*, the local compatibility during a simulation is averaged from generation 200 to 500.

To measure the local adaptation of the parasites to their hosts, all individual parasites are also tested for compatibility against all hosts of the other sites at each generation. As above, by averaging over parasites and sites, this provides an "exotic" compatibility for each generation *exott*. Local adaptation at generation t is measured as $adapt = (loct - exott)/loct$. The synthetic parameter *adapt* is obtained by averaging *adapt* from generation 200 to 500.

At the 500th generation, the compatibility of each parasite population on all host populations is determined.

3. RESULTS

3.1. Diversity of wild bean populations

3.1.1. Molecular polymorphism

Twelve polymorphic RAPD products were obtained with the two primers used, among the plants tested in Mexico and Argentina. Polymorphism was greater in Mexico with ten polymorphic markers and only two polymorphic in Argentina.

RFLP experiments were carried out with four enzyme-probe combinations (*cf.* 2.1.3.3.). Hybridisation with PRLJ1 always resulted in multi-band patterns, while probe B2 gave more simple hybridisation patterns. Hybridisation of probe PRLJ1 to DNA digested with *Hae*III revealed six to twelve DNA fragments in each plant. A total of 13 different DNA fragments, ranging between 6 and 1.6 kb, were obtained for this enzyme-probe combination. Ten of these fragments were polymorphic and used for further analysis. The enzyme-probe combination *Hin*dIII-PRLJ1 gave the same type of hybridisation results.

Hybridisation of probe B2 to DNA digested with either *Hae*III or *Hin*dIII gave one to three DNA fragments in each plant. Further analysis was carried out on five polymorphic DNA fragments hybridised with the *Hae*III-B2 combination.

3.1.2. Phenotypic polymorphism

Plants from Argentina and Mexico were first tested against six strains isolated from cultivated common bean. Resistance phenotypes observed in the two countries ranged from susceptibility to the six strains to resistance to the six strains. However, most of the interactions tested were incompatible, which means that wild plants were generally resistant to these strains isolated from

Table II. Diversity of wild common bean in Mexico and Argentina for molecular and phenotypic markers, estimated with the Nei unbiased diversity index.

Region	Sampling size	RAPD	RFLP PRLJ1 probe *Hae*IIIenzyme	RFLP B2 probe *Hae*IIIenzyme	Resistance Set of strains from cultivars	Resistance Set of wild strains
Argentina	50	0.31	0.19	0.20	0.30	0.36
Mexico	27	0.34	0.37	0.17	0.37	0.45

common bean cultivars. If we assume that resistance to each strain is an independent factor, all the resistance factors detected were polymorphic in the two countries.

Plants were also tested against 10 strains isolated from wild plants of each country. Most of the interactions between plants and strains from the same country were compatible, while incompatible interactions were the most frequent when plants were inoculated with strains isolated in the other country. However, resistance to each strain tested, was observed in each country, and all the resistance factors were polymorphic in the two countries.

3.1.3. Population diversity

Nei's unbiased diversity index was used to estimate the diversity in each country for molecular and resistance phenotypic markers. Diversity for RAPD and resistance markers was almost similar in Argentina and Mexico (Tab. II). Less diversity was observed in the two countries for polymorphic bands revealed with RFLP using *Hae*III-B2 probe, while probe PRLJ1 revealed a greater diversity in Mexico.

3.1.4. Population structure

Differentiation between Mexico and Argentina was estimated by the Wright parameter F_{st}. The results showed a differentiation between the two countries for all the markers studied (Tab. III). Differentiation was higher for RAPD markers than for molecular and phenotypic markers involved in pathogen resistance. Differentiation for resistance to wild strains was higher than resistance to strains from cultivars. No difference is observed in the differentiation index given by the two RFLP probes. The F_{st} parameter for the PRLJ1 probe, however, is similar to that observed for resistance to the strains isolated from cultivars.

Table III. Differentiation parameters between Mexico and Argentina for molecular and phenotypic markers, estimated by F_{st}. 95% confidence intervals are given in brackets.

RAPD	RFLP PRLJ1 probe *Hae*IIIenzyme	RFLP B2 probe *Hae*IIIenzyme	Resistance to strains from cultivars	Resistance to wild strains
0.85	0.35	0.44	0.30	0.57
[0.69; 0.94]	[0.195; 0.48]	[0.32; 0.65]	[0.173; 0.43]	[0.47; 0.66]

3.1.5. Correlation between markers

Statistical associations were tested between RAPD, RFLP and resistance markers. Only 1% of the associations tested were significant at the 5% level. Most of the combinations therefore were in random association.

In particular, no significant correlation was found between RFLP patterns obtained with the PRLJ1 probe and resistance phenotypes to seven fungus strains whose resistance specificities have been mapped to the same linkage group of that PRLJ1 clone.

3.2. Metapopulation modelling

3.2.1. Local compatibility

All results are presented as means and standard errors over five simulations. For the same parameter sets, the level of local compatibility was much higher in the matching allele model than in the gene-for-gene model (Fig. 1). The most consistent result between models is that increased host migration decreased the local level of compatibility. Increased parasite migration raised the level of local compatibility except when selection was weak in the gene-for-gene model. For the matching allele model, this effect was more pronounced when host migration was high ($mh = 0.10$). The effect of the intensity of selection on both partners also differed between the two models: more intense selection ($wp = wh = 0.5$ *versus* $wp = wh = 0.9$) resulted in less local compatibility in the gene-for-gene model while it resulted in similar local compatibility in the matching allele model.

3.2.2. Local adaptation of the parasite

Positive values of local adaptation mean that the parasite has a higher probability of being able to infect hosts at its local site than at other sites, while negative values mean that the parasite is better at attacking hosts of other sites. The parasite is then considered maladapted. Both situations were observed during the simulations but the level of local adaptation was globally

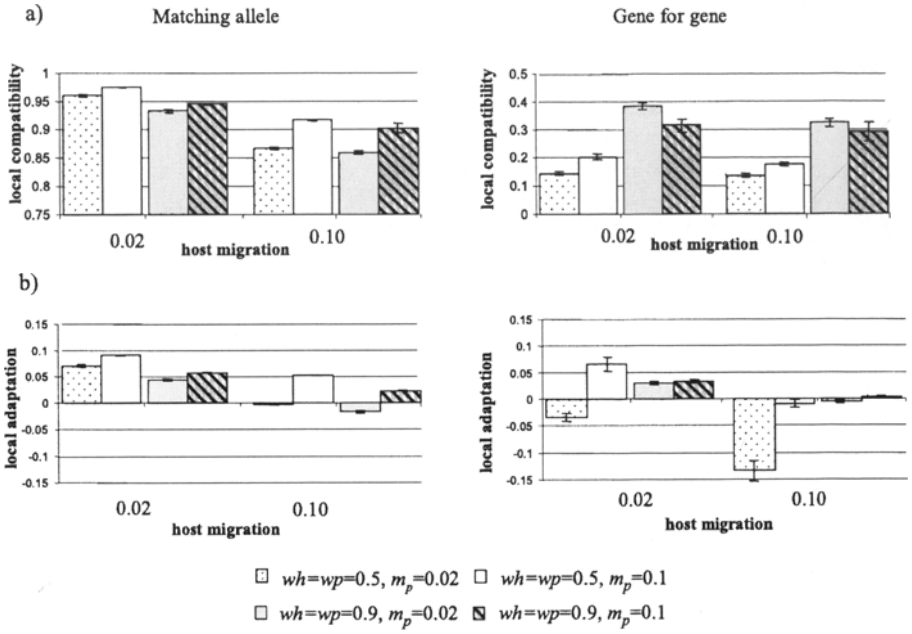

Figure 1. Local compatibility (a) and local adaptation (b) in a gene-for-gene and a matching allele model as a function of host migration, parasite migration and the intensity of selection. Bars in white indicate strong selection ($wh = wp = 0.5$) and bars in grey weak selection ($wh = wp = 0.9$). Dotted areas indicate a parasite migration rate of 0.02 and plain or hatched areas a parasite migration rate of 0.1. Note that the scales are different for the two models in figure a.

higher in the matching allele model than in the gene-for-gene model. High levels of maladaptation were only observed in the gene-for-gene model. There are two results consistent between the two models. For a given set of parameters, increased host migration decreased the level of local adaptation or increased maladaptation while increased parasite migration had the inverse effects. These effects, however, were of a large magnitude only if the selective pressure was strong ($wp = wh = 0.5$).

4. DISCUSSION

As pointed out by Thompson [56] with the geographic mosaic theory of coevolution, the coevolutionary process in plant-pathogen associations cannot be understood without taking into account the different spatial scales on which it occurs. The importance of considering the spatial context to understand the evolution of host-parasite interactions is now generally recognized [59]. We consider the dynamic management of plant resistance genes on a metapopulation

scale. Theoretical models have shown that the evolution of resistance gene frequencies in a metapopulation depends on the life-histories of both partners [28] and on the dynamics of the interaction (epidemic *versus* endemic) [25, 44]. Empirical data about the genetic structure of natural populations is limited and would be necessary to validate the models. Even less is known, to our knowledge, about the influence of the genetic determinism of the interaction on the spatial structure of resistance, although it appears essential to know how the choice of the genetic model used will influence management recommendations. Here we investigated the effects of two genetic systems and of life history traits by varying the selection coefficients and migration rates of both partners. Strong *versus* weak selection in the pathogen differentiates obligate *versus* facultative pathogens while strong *versus* weak selection in the host differentiates killer *versus* debilitator pathogens [8].

4.1. Local compatibility

The two genetic systems of the interaction that we modelled are those commonly described as the two extremes, with most natural systems probably being a combination of both. When all diploid host genotypes (with dominant resistance) are confronted with all haploid parasite genotypes, the symmetry of the relationship differs between models. In the matching allele model, the proportion of incompatible interactions is $2/(n+1)$ where n is the number of alleles considered. In a gene-for-gene model, this proportion becomes $1-(2/3)n$ where n is the number of bi-allelic loci. With ten alleles in the MAM and ten loci in the GFG model, the proportions of incompatible relationships were respectively 0.18 and 0.98. This large difference in symmetry between models likely explains the lower local compatibility levels observed in the GFG model. A higher number of alleles has, indeed, been shown to decrease the global level of compatibility by increasing the asymmetry between the two partners in a matching allele model [28]. Geffroy *et al.* [31] observed a level of compatibility of 0.5 to 0.8 when strains of *C. lindemuthianum* from Mexico, Argentina and Ecuador were tested on plants from the same country. We feel that the level of local compatibility cannot, however, be used as a criterion to determine which genetic model drives these interactions in natural populations. In fact, local compatibility would also depend on numbers of loci/alleles and pathogen population sizes. It suposedly also depends on the generation time of the pathogen [36], although halving the generation time of a parasite compared to its hosts did not modify the pattern of local compatibility in a simulation model with a matching allele model with two alleles. In our model, the level of local compatibility also depended, to a lesser extent, on other life-history parameters. In the MAM, the local level of compatibility tended to decrease when host migration increased and increase when the parasite migration rate increased. A possible explanation is that migration is a source of new resistance or virulence alleles [28, 29]. The effect of migration of both partners was less

obvious in a GFG model. More parasite migration tended to increase the level of compatibility, but only when selection was strong.

4.2. Local adaptation: population structure at the phenotypic level

Non-zero levels of local adaptation indicate a metapopulation structure with population differentiation for resistance and virulence, a result predicted by Burdon and Thrall [12] if migration rates are low enough. The dynamics in the different populations are out of phase, with different genotypic combinations being selected in different populations. As can be seen in Figure 2, testing single pathogen populations on many host populations does not give a clear view of the effect of geographic distance on that structure. In particular, high levels of compatibility can be observed between populations close or far apart. In the well-studied interaction between *Linum marginale* and *Melampsora lini* in Southern Australia, Burdon and Thrall [12] for example report differences in the resistance structure of host populations situated a few hundred metres apart and differences in pathotype structures of pathogen populations situated 300 m apart.

In the model, the degree of local adaptation or maladaptation was larger when selection pressures on both partners were larger, independently of the genetics of the interaction. This is because stronger local selection tends to increase the divergence between population dynamics. On the contrary, more migration decreased the differentiation among populations. Generally, the model predicted local adaptation in most cases. As expected for an MAM, local adaptation was more positive when migration rates were low and the parasite migrated more than its host, while maladaptation was only possible when the host migrated more than the parasite [28]. The pattern appears similar although more marked in a GFG model. In particular, when selection was strong, much larger levels of maladaptation were observed. This could again be because the asymmetry in a GFG model favours the host, so that when migration introduces new resistance genes they are at a large selective advantage. In fact, many studies have shown a local adaptation of the parasite to its host [36] with interactions as different as between host plants and fungi [48], microsporidians and Daphnia [21] or snails and trematodes [42,43] where parasites migrate more than their hosts. A similar result was observed both at the scales of the centres of diversity [31] and at a more local scale for the interaction between *P. vulgaris* and *C. lindemuthianum* [15]. A case of maladaptation with hosts that migrate more than the pathogens [20] is reported by Kaltz *et al.* [37]. The generality of the effect of migration rates on local adaptation therefore seems robust although only if large reciprocal selective pressures are involved in the interaction.

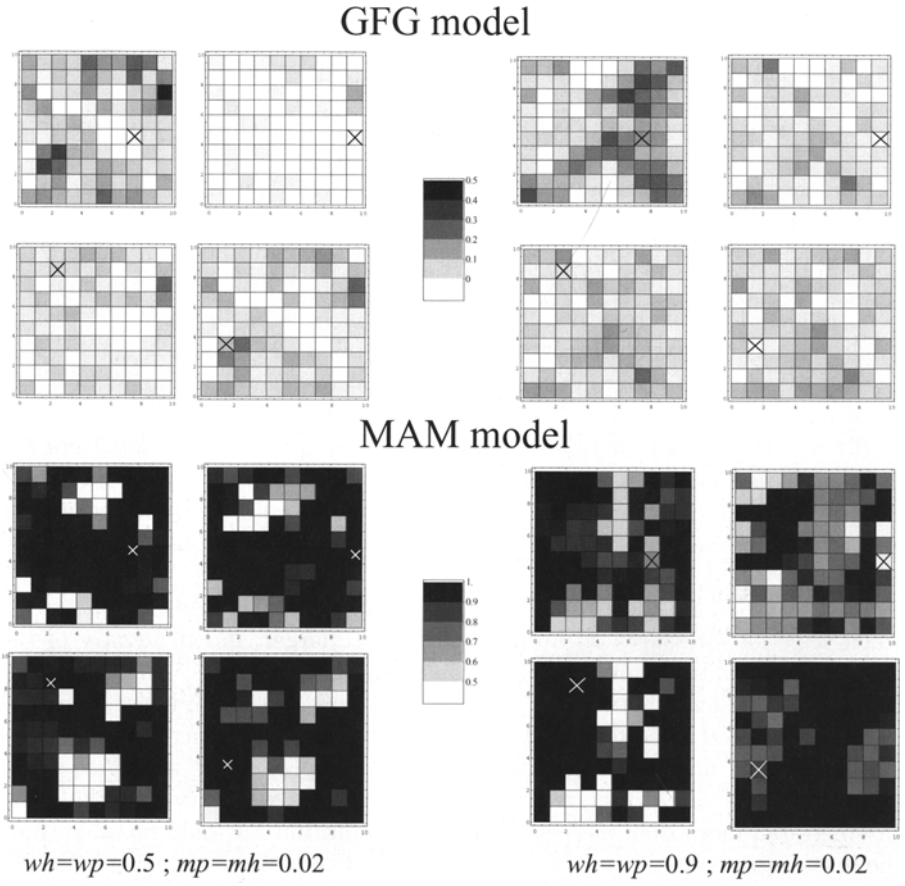

Figure 2. Examples of the mean compatibility of the pathogen population marked with a cross on all host populations of the metapopulation. For the GFG, white indicates no compatibility and black 50% compatibility. For the MAM, the scale is moved upward, white indicates less than 50% compatibility and black 100% compatibility. $wh = wp = 0.5$ indicates strong selection and $wh = wp = 0.9$ weak selection. m_p and m_h are the migration rates of the parasite and the host, respectively.

4.3. Population structure at the genetic level

As expected because of the low genetic exchange between wild populations of *P. vulgaris* from Mexico and Argentina, the differentiation observed for neutral markers is very strong [4]. This is consistent with results on a larger sample of 216 plants (128 plants from Mexico and 88 plants from Argentina, data not shown). The differentiation of the two diversity centres is also strong but significantly weaker for the other markers (RGC and resistance phenotypes), suggesting that other evolutive forces and not drift alone act upon these parts

of the genome. The second largest differentiation index is for the resistance pattern against wild strains of the pathogen. A general local adaptation of the pathogen to its host populations has been demonstrated by Geffroy *et al.* [31] at the scale of the two studied countries and, therefore, a significant differentiation was expected. Why this differentiation is less than for RAPD markers is more difficult to explain. The metapopulation theory indicates that, for a given level of migration, similar selection in different populations reduces the differentiation among populations at the selected loci. However, in this study, among the wild strains, most were allopatric to the plants tested, even within the country, and it is unlikely that they exerted a direct selection on the host plants. Similarly, it is extremely unlikely that direct selection explains the lowest F_{st} found for resistances to strains isolated from cultivars that evolved in totally different environments. A lower F_{st} value for resistances to strains from wild plants than from cultivated plants was also obtained with the largest sample of 216 plants. A possible explanation could be found in the organisation of resistance genes. It has been observed in different species that genes for resistance to pathogens are clustered in complex loci on the host genome [40, 50, 52, 55]. The probe RPLJ1 and the resistance to anthracnose strains were mapped at such a cluster in a cross between two cultivars [31]. Our results could be explained by the fact that selection by local strains maintains a diversity for resistance at these clusters through selection for genes that also confer resistance on other strains or other pathogens, or through hitchhiking effects on the rest of the cluster. Similar selection in both countries would be applied to the diversity thus maintained, which could explain the lower F_{st} values than for RAPD and would constitute a "reservoir" of resistance genes. Only a study at a more local geographic scale would allow us to test this hypothesis.

The patterns of differentiation presented by the resistance gene candidates (RGCs) families are closer to those of the resistances than the RAPD markers, suggesting that they are not selectively neutral, but are linked to or involved in resistance. Since no statistical relationship was observed between the resistance phenotypes and RFLP patterns, our results suggest that the polymorphism observed for RFLP markers is indicative of the diversity of the putative "reservoirs" of resistance genes rather than a direct reflection of a local coevolution. Whether this polymorphism is also indicative of the differentiation for resistances to other pathogens remains to be tested.

More generally, a similar picture emerges from the model and the data. The models tend to show that within a metapopulation, when migration is low, the probability of finding a plant resistant to a given strain is similar in every host population except in the exact population of origin of the strain. The data provide essentially the same picture with mainly similar patterns of resistance to strains isolated from wild or cultivated plants. This suggests that the coevolution between host and pathogen would be very local. More data at a local scale are needed to understand whether this coevolution directly influences the patterns of resistance to other strains of the same pathogen, or

even to other pathogens. This would be crucial to know, before more modelling is done on the effect of different managements of genetic resources.

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REFERENCES

[1] Adam-Blondon A.F., Sévignac M., Dron M., A genetic map of common bean to localize specific resistance genes against anthracnose, Genome 37 (1994) 915–924.

[2] Allard R.W., The genetics of host-pathogen coevolution: Implications for genetic resource conservation, J. Hered. 81 (1990) 1–6.

[3] Antonovics J., Thrall P.H., Jarosz A.M., Genetics and the spatial ecology of species interactions: the *Silene-Ustilago* system, in: Tilman D., Kareiva P. (Eds.), Spatial ecology: the role of space in population dynamics and interspecific interactions., Princeton University Press, Princeton, 1997, pp. 146–170.

[4] Beccera Velasquez L., Gepts P., RFLP diversity of common bean (*Phaseolus vulgaris*) in its centers of origin, Genome 37 (1994) 256–263.

[5] Berglund Brücher O., Brücher H., The South American wild bean (*Phaseolus aborigineus* BurK.) as ancestor of the common bean, Econ. Bot. 30 (1976) 257–272.

[6] Burdon J.J., Diseases and plant population biology, Cambridge University Press, 1987.

[7] Burdon J.J., Phenotypic and genetic patterns of resistance to the pathogen *Phakopsora pachyrhizi* in populations of *Glycine canecens*, Oecologia 73 (1987b) 257–267.

[8] Burdon J.J., The structure of pathogen populations in natural plant communities, Ann. Rev. Phytopathol. 31 (1993) 305–323.

[9] Burdon J.J., The distribution and origin of genes for race-specific resistance to *Melampsora lini* in *Linum marginale*, Evolution 48 (1994) 1564–1575.

[10] Burdon J.J., Jarosz A.M., Temporal variation in the racial structure of flax rust (*Melampsora lini*) populations growing on natural stands of wild flax (*Linum marginale*): local *versus* metapopulation dynamics, Plant Pathol. 41 (1991) 165–179.

[11] Burdon J.J., Thompson J.N., Changed patterns of resistance in a population of Linum marginale attacked by the rust pathogen *Melampsora lini*, J. Ecol. 83 (1995) 199–206.

[12] Burdon J.J., Thrall P.H., Spatial and temporal patterns in coevolving plants and pathogen associations, Amer. Nat. 153 (1999) S15–S33.

[13] Burdon J.J., Oats J.D., Marshall D.R., Interactions between *Avena* and *Puccinia species.* I The wild hosts: *Avena barbata* Pott. ex Link, *A. fatua* L., *A. ludoviciana* Durieu, J. Appl. Ecol. 20 (1983) 571–584.

[14] Caicedo A.L., Schaal B.A., Kunkel B.N., Diversity and molecular evolution of the RPS2 resistance gene in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. USA 96 (1999) 302–306.

[15] Cattan-Toupance I., Diversité et répartition de la résistance à *Colletotrichum lindemuthianum* dans des populations naturelles de *Phaseolus vulgaris* LM., Thèse de l'Institut national agronomique Paris-Grignon, 1997, 110 p.

[16] Cattan-Toupance I., Michalakis Y., Neema C., Genetic structure of wild bean populations in their south Andean center of origin, Theor. Appl. Genet. 96 (1998) 844–851.

[17] Cheng J., Smith-Becker J., Keen N.T., Genetics of plant-pathogen interactions, Curr. Opin. Biotechnol. 9 (1998) 202–207.

[18] Christ B.J., Person C.O., Pope D.D., The genetic determination of variation in pathogenicity, in: Wolfe M.S., Caten C.E. (Eds.), Populations of plant pathogens: their dynamics and genetics, Blackwell Scientific Publications, Oxford, 1987, pp. 7–19.

[19] Clarke D.D., Bevan J.R., Crute I.R., Genetic interactions between wild plants and their parasites, in: Day P.R., Ellis G.J. (Eds), Genetic and plant pathogenesis, Blackwell Scientific Publications, Oxford, 1987, pp. 195–206.

[20] Delmotte F., Bucheli E., Shykoff J.A., Host and parasite populations structure in a natural plant-pathosystem, Heredity 82 (1999) 300–308.

[21] Ebert D., Virulence and local adaptation of a horizontally transmitted parasite, Science 265 (1994) 1084–1086.

[22] Ferrier-Cana E., Caractérisation d'analogues de gènes de résistance chez le haricot commun *Phaseolus vulgaris*, regroupés à des locus contenant des spécificités de résistance vis-à-vis du champignon phytopathogène *Colletotrichum lindemuthianum*. Thèse de Doctorat Université Paris 11, 2000, 196 p.

[23] Flor H.H., Host-parasite interaction in flax rust. Its genetics and other implications, Phytopathol. 45 (1955) 680–685.

[24] Frank S.A., Ecological and genetic models of host-pathogen coevolution, Heredity 67 (1991) 73–83.

[25] Frank S.A., Coevolutionary genetics of plants and pathogens, Evol. Ecol. 7 (1993) 45–75.

[26] Frank S.A., Recognition and polymorphism in host-parasite genetics, Phil. Trans. Roy. Soc. London B 346 (1994) 283–293.

[27] Freyre R., Skroch P., Geffroy V., Adam-Blondon A.F., Shirmohamadali A., Johnson W.C., Llca V., Nodari R.O., Pereira P.A., Tsai S.M., Tohme J., Dron M., Nienhuis J., Vallejos C.E., Gepts P., Towards an integrated linkage map of common bean 4. Development of a core linkage map and alignment of RFLP maps, Theor. Appl. Genet. 97 (1998) 847–856.

[28] Gandon S., Capowiez Y., Dubois Y., Michalakis Y., Olivieri I., Local adaptation and gene for gene coevolution in a metapopulation model, Proc. Roy. Soc. London B. 263 (1996) 1003–1009.

[29] Gandon S., Ebert D., Olivieri I., Michalakis Y., Differential adaptation in spacially heterogeneous environments and host-parasite coevolution, in: Mopper S., Strauss S.Y. (Eds.), Genetic structure and local adaptation in natural insect populations, Chapman & Hall, New York, 1998, pp. 325–342.

[30] Geffroy V., Creusot F., Falquet J., Sévignac M., Adam-Blondon A.F., Bannerot H., Gepts P., Dron M., A family of LRR sequences in the vicinity of the $Co-2$

locus for anthracnose resistance in *Phaseolus vulgaris* and its potential use in marker assisted selection, Theor. Appl. Genet. 96 (1998) 494–502.

[31] Geffroy V., Sicard D., de Oliveira J.C.F., Sévignac M., Cohen S., Gepts P., Neema C., Langin T., Dron M., Identification of an ancestral gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*, Mol. Plant-Microbe Interact. 12 (1999) 774–784.

[32] Henry J.P., Pontis C., David J.L., Gouyon P.H., An experiment on dynamic conservation of genetic resources with metapopulations, in: Seitz A., Loeschcke V. (Eds.), Species conservation: A populational-biological approach, Birkhaüser Verlag, Basel, 1991.

[33] Ibrahim K.M., Barret J.A., Evolution of mildew resistance in a hybrid bulk population of barley, Heredity 67 (1991) 247–256.

[34] Jarosz A.M., Burdon J.J., Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*: II. Local and regional variation in patterns of resistance and racial structure, Evolution 45 (1991) 1618–1627.

[35] Jarosz A.M., Burdon J.J., Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. III Influence of pathogen epidemics on host survivorship and flower production, Oecologia 89 (1992) 53–61.

[36] Kaltz O., Shykoff J.A., Local adaptation in host-parasite systems, Heredity 81 (1998) 361–370.

[37] Kaltz O., Gandon S., Michalakis Y., Shykoff J.A., Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: evidence from a cross-inoculation experiment, Evolution 53 (1999) 395–407.

[38] Kelly J.D., Young R.A., Proposed symbols for anthracnose resistance genes, Annu. Rep. Bean Coop. 39 (1996) 20–24.

[39] Le Boulc'h V., David J.L., Brabant P., de Vallavieille-Pope C., Dynamic conservation of variability: responses of wheat populations to different selective forces including powdery mildew, Genet. Sel. Evol. 26 (1994) 221s–240s.

[40] Leister D., Kurth J., Laurie D.A., Yano M., Sasaki T., Devos K., Graner A., Schulze-Lefert P., Rapid reorganization of resistance gene homologues in cereal genomes, Proc. Natl. Acad. Sci. USA 95 (1998) 370–375.

[41] Lenné J.M., Variation in reaction to anthracnose within native *Stylosanthes capitata* populations in Minas Gerais, Brazil, Phytopathol. 78 (1988) 131–134.

[42] Lively C.M., Jokela J., Clinal variation for local adaptation in a host-parasite interaction, Proc. Roy. Soc. London B 263 (1996) 891–897.

[43] Manning S.D., Woolhouse M.E.J., Ndamba J., Geographic compatibility of the freshwater snail *Bulinus globosus* and schistosomes for the Zimbabwe Highveld, Int. J. Parasit. (1995) 37–42.

[44] Morand S., Manning S.D., Woolhouse M.E.J., Parasite-host coevolution and geographic patterns of parasite infectivity and host susceptibility, Proc. Roy. Soc. London B 263 (1996) 119–128.

[45] Nei M., Estimation of average heterozygocity and genetic distance from a small number of individuals, Genetics 89 (1978) 583–590.

[46] Paillard S., Goldringer I., Enjalbert J., Doussinault G., de Vallavieille-Pope C., Brabant P., Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. I- Is specific seedling resistance selected?, Theor. Appl. Genet. 101 (2000) 449–456.

[47] Paillard S., Goldringer I., Enjalbert J., Trottet M., David J., de Vallavieille-Pope C., Brabant P., Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. II- Adult plant resistance, Theor. Appl. Genet. 101 (2000) 457–462.

[48] Parker M.A., Local population differentiation for compatibility in an annual legume and its host-specific fungal pathogen, Evolution 39 (1985) 713–723.

[49] Parker M.A., The nature of plant-parasite specificity, Evol. Ecol. 10 (1996) 319–322.

[50] Parniske M., Hammond Kosack K.E., Golstein C., Thomas C.M., Jones D.A., Harrison K., Wulff B.B., Jones J.D.G. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the $Cf-4/9$ locus of tomato, Cell 91 (1997) 821–832.

[51] Pastor-Corrales M.A., Tu J.C., Anthracnose, in: Schwartz H.F., Pastor-Corrales M.A. (Eds.), Bean production problems in the tropics, Columbia, CIAT, 1989, pp. 77–105.

[52] Pryor T., Ellis J., The genetic complexity of fungal resistance genes in plants, Adv. Plant Pathol. 10 (1993) 281–305.

[53] Raymond M., Rousset F., A population genetics software for exact tests and ecumenicism, J. Hered. 86 (1995) 248–249.

[54] Sicard D., Buchet S., Michalakis Y., Neema C. , Genetic variability of *Colletotrichum lindemuthianum* in wild populations of common bean, Plant Pathol. 46 (1997) 355–365.

[55] Song W.Y., Pi L.Y., Wang G.L., Gardner J., Holsten T., Ronald P.C. Evolution of the rice $Xa21$ disease resistance gene family, Plant Cell 9 (1997) 1279–1287.

[56] Thompson J.N., The coevolutionary process, University of Chicago Press, Chicago, 1994.

[57] Thompson J.N., Burdon J.J., Gene-for-gene coevolution between plants and parasites, Nature 360 (1992) 121–125.

[58] Thrall P.H., Antonovics J., Theoretical and empirical studies of metapopulations: population and genetic dynamics of the *Silene-Ustilago* system., Can. J. Bot. 73 (1995) S1249–S1258.

[59] Thrall P.H., Burdon J.J., Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial, J. Ecol. 85 (1997) 743–753.