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Genetic diversity in natural populations: a fundamental component of plant–microbe interactions

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Genetic diversity for plant defense against microbial pathogens has been studied either by analyzing sequences of defense genes or by testing phenotypic responses to pathogens under experimental conditions. These two approaches give different but complementary information but, till date, only rare attempts at their integration have been made. Here we discuss the advances made, because of the two approaches, in understanding plant–pathogen coevolution and propose ways of integrating the two.

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Introduction

Parasites are believed to be one of the main selective pressures on their hosts, and thus responsible for a never-ending struggle for escaping infection through genetic novelty/diversity, as illustrated by the Red Queen tale [1]. Indeed parasites, in adapting to the most common genotype of their hosts, indirectly favor rare host genotypes until they themselves reach a higher frequency and become new potential targets for the parasites. The hosts of parasites must constantly change their resistance strategies because parasites evolve rapidly. Thus for some time coevolution with parasites has been the hypothesis of choice to explain the maintenance of sex despite its short-term disadvantage over asexual reproduction, because sex generates new alleles and allelic combinations through recombination and segregation processes [2]. This coevolution process has been extensively investigated using theoretical models that show that host–parasite coevolution can generate 'arms races', that is, recurrent selective sweeps, each time favoring a novel resistance allele, but can also create conditions that maintain polymorphism at resistance loci (see [3] for a review). Indeed, systems of defense against parasites provide examples of high genetic variation, such as the major histocompatibility complex whose genes are among the most variable in vertebrates and include ancient allelic variants that have been maintained across speciation events [4]. Maintaining such high genetic diversity over such long evolutionary time scales presents a theoretical problem. To solve this problem many models require either cost of resistance and/or numerous complex additional factors like spatial structure or multilocus interactions [5,6]. Tellier and Brown [7••], however, have recently shown that indirect frequency-dependent selection on resistance and avirulence genes (i.e. the selection of a resistance gene depends on the frequency of the corresponding avirulence gene), cannot, in itself, promote stable polymorphism. Direct frequency-dependent selection (i.e. the selection of a resistance gene depends on its own frequency) is, however, a necessary prerequisite for the stability of resistance (and avirulence) polymorphism. Most above-mentioned factors invoked in the literature for generating stability represent special cases of this general solution, in that they create the conditions of direct frequency-dependent selection [7••]. On the contrary, multilocus interactions and costs of resistance or virulence are shown to generate only indirect frequency-dependent selection, and are thus not key factors for stable polymorphism [8].

In plant–pathogen systems there have been many studies on the diversity of genes involved in resistance to pathogens and associated phenotypic variation in both cultivated and natural species, and great variation can be found at every scale. In plant–pathogen interactions there has been much emphasis on the 'gene-for-gene' (GFG) mechanism of resistance [9]. This race-specific resistance that prevents the establishment of pathogen infection is mediated by host receptors that specifically recognize particular pathogen effectors. Often targeted against obligatory parasites, this resistance mechanism thus imposes a strong and intimate selection on specific couples of pathogens and their hosts. Theoretical models that have studied the evolution of such receptor loci and their matching effector loci in the parasites (avirulence loci) have shown that polymorphism of resistant and susceptible host alleles and virulent/avirulent parasite alleles can be maintained for long periods [7••].

So parasites are clearly important selective forces in nature and interactions between hosts and parasites are expected to favor novel and rare variants, thereby generating and maintaining genetic diversity for genes involved in the interaction. On the contrary, genetic variation is generated by random mutations and can be maintained transiently in

Glossary
Balancing selection: Evolutionary process that maintains genetic polymorphism within a population by frequency-dependent selection (advantage of rare alleles) or overdominance (heterozygote advantage).
Directional selection: Evolutionary process that favors a single and extreme phenotype in an environment.
Diversifying selection: Evolutionary process that favors divergent phenotypes simultaneously within the same environment.
F_{ST}: The proportion of heterozygote deficit in the entire sample that is because of differentiation in allele frequencies among populations.
Local adaptation: Higher performance of parasites (respectively hosts) when confronted with their local hosts (respectively parasites) compared to an antagonist from foreign populations.
Metapopulation: Group of spatially separated populations connected by gene flow and regularly experiencing local extinction and recolonization.
Neutral evolution: Evolutionary process that neither favors nor selects against new variants.
Paralogs: Genes that have diverged after a gene duplication event, in opposition to orthologs, that have diverged after a speciation event.
Positive selection: At the molecular level, evolutionary process that favors nonsynonymous substitutions that change the function of the protein.
Purifying selection (or stabilizing selection): At the molecular level, evolutionary process that acts against nonsynonymous substitutions.
Quantitative traits: Characters under polygenic control, often with continuous variation in populations.
RGA (RGC): Resistance gene analog (resistance gene candidate), a locus identified in whole genome analyses based on their structural homology (presence of NBS and LRR domains) with previously identified R-genes.

natural populations by processes as mundane as drift and migration in a metapopulation context. Thus, it is important to ask how much of the observed variation in host populations has been selected by the interactions with parasites. Can we identify the role of parasite-mediated selection in generating and maintaining diversity over and above random processes and gene flow between populations that have diverged in isolation? If parasites are driving host evolution, what is the nature of the selective forces involved? Does parasite-driven adaptation shape host evolution predominantly through processes of balancing or positive selection?

Globally two types of information are available on the nature of diversity for resistance in plants and its evolutionary origins. Molecular population genetics studies have focused on particular genes involved at various levels of resistance mechanisms, investigating their polymorphism across worldwide samples of ecotypes in order to elucidate the selective forces that generated these polymorphisms [10••]. Typically such studies have been carried out on genes of major effect like the recognition genes of the GFG systems in the model plant *Arabidopsis thaliana* or in economically important crop species [6,11]. Ecological genetics studies, on the contrary, have investigated variation in global resistance phenotypes at the population and metapopulation scales, testing for local adaptation of parasites to their natural host populations (e.g. [12••]). These two approaches differ in their underlying questions and the information they give about the cause of resistance variation. Despite their potential complementarity [5,10••] there has been little communication between the two schools; a lack of communication strengthened by their differences in level of sampling and types of model systems. We propose here, however, that some crossinoculation of ideas between genetic and ecological genetic studies might help to solve some of the standing problems, such as whether selection has shaped the level of genetic diversity found in natural populations and how much of the detectable genetic variation is expressed. Integrating the knowledge of genotypic and phenotypic variation might enable the drawing of a more complete picture of parasite-driven selection on their hosts.

Here we discuss how some recent advances in molecular and ecological studies on plant–pathogen systems aid our comprehension of how host genetic diversity is shaped by coevolution with parasites and how this diversity in turn provides advantages against these enemies. Our goal is not to provide a detailed review of the works that have studied the selective histories of R-genes, already the subject of excellent reviews (e.g. [10••,13]). Instead, we illustrate the diversity of selective histories on genes involved in pathogen defense using R-genes that are instrumental in specific recognition of pathogen infection in plants and the diversity of selective responses in natural plant populations to current pathogen pressure. We explore the limitations of the two approaches and suggest how they could be integrated to develop more accurate hypotheses/explanations.

Molecular population genetics

The past few years have advanced our knowledge of the genes involved in plant resistance to pathogens. Most relevant for this paper, their identification, mapping, and sequencing have allowed elucidation of the selective forces acting on these resistance functions at the nucleotide level. Every nucleotide in the sequence is a window on past evolutionary events, revealing the nature of past selection responsible for current molecular patterns. Statistical methods can now discriminate among different evolutionary processes: neutral, positive, purifying, or balancing selection [14] (see glossary). These tests compare the frequency of allelic variants within (Tajima's *D*, Fu and Li's *D*) and among species (Hudson–Kreitman–Aguadé, HKA test), compare an observed rate of amino-acid replacements relative to silent mutations to neutral expectations (d*N*/d*S* test, K_a/K_s), or both (McDonald–Kreitman, MK test).

Rapid evolution in some R-genes

Among the many plant genes involved in pathogen resistance [15], R-genes that mediate the GFG-specific recognition of pathogens have received most attention. So far the majority of these genes belong to the nucleotide-binding

site (NBS)–leucine-rich repeats (LRR) type, coding for a class of cytoplasmic proteins with a NBS signaling domain and a LRR region, which is assumed in some cases to be where specificity to pathogen effectors or other intracellular targets is located. This LRR region can also be found in other R-genes coding for membrane receptors [13]. Some of these genes have been identified by the study of resistant/susceptible phenotypes of plants to pathogen strains, but many others have been localized in fully sequenced plant genomes based solely on their structural homologies to the first sequenced R-genes [16–18]. These RGC or RGA (see glossary) nonetheless give an idea of the extreme diversity of these gene families within species, for instance, in *A. thaliana* about 150 putative R-genes have been identified [17] and in rice about 480 [18].

These molecular studies provide insights into the evolutionary history of this class of genes and raise new questions about the processes of parasite-driven selection on host resistance. First, detailed studies of individual R-genes or R-gene families have sometimes shown large excesses of nonsynonymous changes compared to neutral expectation ($K_a/K_s > 1$) particularly in the LRR region. Between paralogs of the same cluster/family, this pattern suggests diversifying selection, that is, adaptive divergence. This was the case in the R-gene families of *A. thaliana* (see [6] for a review), or in cereals [19]. On the contrary, an excess of nonsynonymous substitutions between allelic copies of the same gene suggests both positive selection acting to diversify alleles, and balancing selection maintaining polymorphism, as has been suggested for a fair number of R-genes. Some of them have maintained different resistant and susceptible alleles, for instance Cf-2 in *Solanum pimpinellifolium* [20], or even alleles with different recognition specificities as the RPP13 *Arabidopsis* gene whose alleles recognize different strains of the oomycete *Hyaloperonospora parasitica* [21]. Others harbor presence/absence polymorphisms [22]. Furthermore, some allelic polymorphisms are ancient, for example, with allelic polymorphisms shared among *Lycopersicon* species at the Pto gene [23••] and at type II RGC genes in several wild and cultivated *Lactuca* species [16]. In general, R-genes show stronger patterns of selection than sets of non-R-genes [23••,24], or random sequences in the genome [25••]. However, all resistance genes do not show the same clear traces of selection. The portrait of R-genes drawn by molecular genetics is thus one of a large class of genes with evolutionary histories ranging from highly to little selected [25••], with balancing selection maintaining polymorphism of divergent alleles within loci, diversifying selection driving the divergence of separate loci within gene families, and allelic polymorphism without detectable traces of selection despite phenotypic effects on resistance [26].

Molecular genetics also reveal a role of recombination in generating new alleles and genes, that is, the importance of sex in generating genetic variation involved in host–parasite interactions [27]. Indeed, R-genes are generally found within large families of highly related genes, or at least associated with a few paralogs. For instance, more than 20 genes are present at the Dm3 locus in lettuce [28]. Recombination would be central in the birth and death of these resistance genes, a hypothesis developed to explain the dynamics of R-gene families [29]. According to this scenario, rare events of unequal crossing-over would delete genes or give birth to new copies, which are then prone to recombination because of their high similarity to their neighbors. These new copies would, in turn, further favor other unequal crossing-overs and thus further losses and duplications. The new copies thereby generated would diverge in sequence through mutations or inter-allelic recombination, which would confer new specificity or knock them out.

Many loci or many alleles?

The results of these molecular studies have also raised new questions about the processes of resistance gene evolution and the organization of their diversity. One is the scale at which genetic diversity is maintained within plant genomes, and in particular the distinction between interlocus and intralocus variability. Some loci maintain high allelic polymorphisms while others show weak polymorphism but exist in multiple, divergent paralogous copies, the number of paralogs being sometimes even variable among individuals. Whether these two scales of diversity correspond to different strategies in the arms race against pathogens, are differently efficient, or whether they keep footprints of evolutionary events of different ages is still to be explored. Alternatively, this apparent discrepancy between many loci versus many alleles could be because of a sampling bias, intralocus analyses being more often performed on isolated genes rather than on large cluster families. Indeed, individuals may vary in paralog number and it is impossible to determine which allele is to be ascribed to which gene copy, rendering allelic analysis of large gene families impossible [10••]. Clearly, in the fight against ever changing parasites, it seems more advantageous for an individual to keep several genes with different specificities rather than at most two alleles at a single locus. On the contrary, maintaining multiple specificities in the same genotype can impose higher costs, if every gene adds its own cost [26,30] and/or increases the probability of autoimmune responses through epistatic interactions [31••]. Thus we are left with a number of open questions: Why are there so many resistance genes and what proportion is functional? Are ancient genes recycled for new resistance specificities or purged? Is this profusion of genes a by-product of their propensity to recombine?

Is sampling representative?

To date, molecular analyses include several biases that complicate the extension of their results to more ecologi-

cal questions of host adaptation to parasites. In the first place, with few exceptions [23••,32••,33] molecular analyses on resistance genes have been performed on few individuals (ecotypes) of 'species-wide' samples. Therefore the variation detected may not represent variation at geographical scales relevant for host–parasite coevolution and it is impossible to detect the scale at which different selection histories have been operating, that is, differentiation among isolated populations or divergent selection within populations. Furthermore, the need for good knowledge of the studied genome has restricted most molecular studies to economically important crops (e.g. tomato, potato, rice, and lettuce), where identifying resistant varieties was a major motivation, or to *A. thaliana*, already a model in genetics. The genetic diversity of resistance genes found in such species that have probably undergone drastic bottlenecks during domestication, or with particular reproductive strategies such as *A. thaliana*, may not be representative of the more general natural plant–parasite interactions. This kind of bias causes problems because evolutionary models used in most statistical tests of molecular population genetics are based on assumptions that may not be met for populations subjected to large demographic fluctuations or for nonrandom mating systems. Taking into account demographic effects may, for instance, require more intensive sampling at the population scale [34,35]. More generally, molecular studies on genes of interest should always be compared with the existing neutral diversity, which keeps a trace of nonselective events that have shaped the genome.

Despite these biases and limitations molecular genetics has yielded promising results. As a whole, many genes involved in plant resistance to pathogens show patterns of diversifying and balancing selection, and recombination has played an important role in generating this diversity. Hence, so far these findings are in accordance with the Red Queen hypothesis, that is, that hosts are continually evolving in response to selective pressure imposed by their parasites. How then, in real populations, do hosts respond to this selection on their phenotypes?

Ecological genetics

Variation in resistance phenotypes

Phenotypic variation for resistance to parasites in natural host populations occurs at all scales. However, when observing variation in disease levels in nature and even in disease resistance under experimental conditions, care must be taken to separate genetic from nongenetic effects. The expression of disease resistance can be modified by environmental conditions ([36], but see [37]), so an experimental approach is needed to verify that variation in disease resistance is genetic. Indeed, only the genetic component of phenotypic variation in disease resistance can respond to selection, so understanding the genetic basis of phenotypic variation is crucial to understanding the evolutionary responses of hosts to pathogens.

Does it have a genetic basis and, if so, is the variation adaptive?

Resistance may either be governed by genes with major effects that generate race-specific resistance via a GFG relationship with their pathogens or be of quantitative nature, presumably governed by many genes with small effects, though there may be a possible common genetic basis for quantitative and qualitative resistance (discussed in [37]). Since many genes for resistance are introduced to crops from wild relatives it is clear that wild populations harbor such genes [38]. Experimental crosses (e.g. [39,40]), assessment of similarity among relatives [41], and differences among host inbred lines [42,43] indicate that phenotypic differences have a genetic basis. Studies of natural populations have revealed major-gene resistance in some natural systems [39,40] and quantitative variation in resistance in others (e.g. [36]). It is, however, important to know at what scale this genetic variation is found because different evolutionary rules apply to variation maintained within versus among populations.

In general, variation within populations may be neutral and in the process of being lost by drift, may result from mixing of populations that are subjected to different selection pressures within a coevolutionary mosaic [44] and represents the balance between local selection, migration and drift, or may be actively maintained by local balancing selection, either direct frequency-dependence or a balance between conflicting selection pressures. Variation among populations may be adaptive, being the response to local selection pressures by differentiated pathogen populations, or may result from metapopulation or regional stochastic processes such as founder events.

Most pathogens greatly reduce the fitness of their hosts ([45], but see [42,43,46]) and should select for resistance. Indeed, as discussed above, some resistance genes show strong evidence of a long history of positive and diversifying selection in some cases and balancing selection in others. Still it is unclear how much of the genetic variation among and within populations that is commonly found for resistance phenotypes [40,47,48] results from local versus regional processes for the former, and heterogeneous or balancing selection in the local context for the latter. Patterns of among and within population genetic variation for resistance to pathogens may be generated by (nonselective) processes operating beyond the scope of the local population.

Two case studies: do pathogens select for resistance?

Here we will summarize the results from two particularly well-studied systems and see what encouraging or sobering messages emerge. In particular we are interested in whether patterns of selection by parasites on hosts and

patterns of host response are consistent with what the genes are telling us. The genotypic evidence clearly demonstrates that molecular variation for host resistance genes can be shaped by both balancing and directional selection. It is tempting to suppose that the selective agents were the parasites themselves, but how can we determine this? A first approach is to see whether phenotypic responses to current parasite-mediated selection are consistent with the footprints of selection in genomes. Footprints of positive selection, for example, would be consistent with the rapid repeated invasions of new resistance types, balancing selection with negative frequency dependence that maintains intrapopulation variation for multiple resistance types. If selection is strong, the spread of a new resistance type will be transient and rapid, limiting our ability to witness it. On the contrary, though parasites are omnipresent, they may be at such low prevalence that most susceptible individuals escape their attention entirely, generating very weak selection differentials that are hence difficult to detect. Occasional epidemics, however, should offer an opportunity to observe selection in action.

1: Linum marginale–Melampsora lini

The *Linum marginale–Melampsora lini* interaction, native to southern Australia, is one of the best-characterized natural plant–pathogen interactions. Genetic variation for host and parasite traits relevant to their interaction has been studied at a number of spatial and temporal scales. Natural pathogen populations vary for the occurrence and frequency of different pathotypes, as determined by detailed inoculation studies on a set of host lines carrying different resistance genes. More relevant for our purpose here, host populations show heterogeneity for resistance structure at all scales investigated to date — from clustering of resistance types within populations to differences in frequency of particular race-specific resistances among populations, between adjacent metapopulations, and between eastern and western Australia (see [49] for a summary). In the Kiandra Plain of southern Australia *L. marginale* shows (weak) isolation by distance for race-specific resistance to its rust *M. lini*, though there is no corresponding geographic pattern for pathotypes in the pathogen populations [48]. Nonetheless, over the same geographic scale, pathogen populations are strongly differentiated for their infection profiles and show strong local adaptation, with higher performance of the pathogen in sympatric than allopatric combinations [50••]. Parasites must be exerting strong selection pressure for increased resistance because they are locally adapted to their hosts. However, at the scale of the 10 km between the northern and southernmost populations studied, local selection by the pathogen was not sufficient to eradicate the traces of migration even on this highly selected aspect of the phenotype. Parasite-mediated selection is operating, but gene flow among host populations dampens the ability of local populations to respond to this selection.

The within-population dynamics of flax resistance variation against flax rust, however, provides some rather puzzling results. A parasite epidemic should select for increased resistance, particularly against the local parasites responsible for the epidemic. However, though a rust epidemic that eliminated almost 80% of the flax population in a long-term demographic plot led to a change in frequency of the various resistance phenotypes in the population, this change was not in any predictable direction. Those phenotypes most resistant to the local pathogen strains actually decreased in frequency while the most susceptible ones increased [51••]. Therefore, predicted changes in resistance, in response to even strong parasite-mediated selection, may be swamped by genetic effects such as linkage with genes under even stronger selection or demographic effects such as age structure. On the contrary, change in the frequency of the various resistance phenotypes in unpredictable directions following an epidemic may imply balancing selection that could maintain polymorphisms for multiple resistance types over long periods.

2: Plantago lanceolata–Podosphaera plantaginis

Plantago lanceolata, host of the powdery mildew *Podosphaera plantaginis* in the Åland archipelago in Finland shows genetic variation for resistance within and among populations and metapopulations, but similarity in resistance phenotypes among populations does not increase with geographic proximity either among [47] or within [52••] populations. The former suggests little gene flow among host populations, in particular because the pathogen populations show higher infectivity on plants from their local population than those from even other nearby populations [53], which should give an advantage to immigrant resistance types. Differentiation for resistance among host populations appears then to be non-adaptive, resulting from stochastic processes such as the founder effect and also to be little affected by current genetic exchange among host populations, unlike in the flax example. In both systems, then, parasites are exerting selection for increased resistance but the response of flax hosts is hindered by the influx of inappropriate resistance types whereas plantain hosts are constrained by the set of resistance types that initially colonized each host population.

The lack of clustering of similar resistance phenotypes within populations implies highly localized gene dispersal together with strong local selection by different sets of pathogens. Within populations, over a scale of tens of meters, hosts from areas that had high pathogen pressure over the previous four years are more resistant than those from patches with low or no history of infection during the same period, implying that pathogen-mediated selection is locally strong enough to eliminate susceptible phenotypes [52••]. These very localized differences, however, do not translate into a more general population response,

because populations with a history of disease are not more resistant than those free from disease [47]. Thus local selection increases resistance at the small scale within populations but is insufficient to render host populations as a whole more resistant to their local pathogens, possibly because prevalences are too low or pathogen populations are too transient [47]. These findings present convincing evidence of parasite-mediated selection leading to the evolution of resistance, with increase in particular resistance phenotypes over the short term. The transient nature of parasite epidemics causes temporal heterogeneity in the strength and possibly the direction of selection, because resistance is pathogen race-specific [47], so parasite-mediated selection and the host response is consistent with both positive and balancing selection on resistance genes.

Though plantain and powdery mildew appears a convincing example of parasite-mediated microevolutionary change in resistance it is not completely clear that resistance phenotypes measured under controlled greenhouse conditions represent resistance phenotypes perceived by pathogen populations in nature. Plants grown from seeds from the same set of populations were hand inoculated with pathogen strains isolated from naturally infected populations or were set out in the field within those same populations. Parasites show local adaptation when inoculated in the greenhouse but not for natural field transmission and plants from the different source populations vary in the form of infection to which they were more resistant [54••]. Hence increased resistance in host patches with a long history of selection by the powdery mildew parasite should be verified under more natural inoculation conditions to ensure that the apparent response to selection is solid.

To summarize, selection by pathogens for increasing host resistance occurs in natural populations but selection pressures are heterogeneous in time and space and only highly local responses to this selection are detectable. Host resistance may change as a result of parasite epidemics, but not always in predictable or apparently adaptive ways. Nonetheless, molecular genetics studies reveal strong footprints of diversifying and balancing selection for some resistance genes, implying that the selection, though too weak or transient to cause clear shifts in phenotypes, leaves undeniable traces in host genomes. What kinds of studies, then, could combine these types of evidence into a coherent picture?

Integrating phenotypic and molecular approaches

Choosing the right species and scales

A first step to integrate molecular and ecological information would be to use comparable model species and sampling scales. Interesting insights about wild *Lactuca*, *Lycopersicon*, and *Flax* species can be gained from the molecular studies of their cultivated relatives [55] and it is encouraging that some recent molecular studies have adopted a more exhaustive sampling approach, examining variation within natural populations of wild relatives of cultivated species for which resistance genes have been identified. At the gene family level Sicard *et al.* [33] investigated the diversity of putative resistance genes (RGC2) in *Lactuca* for both cultivated and wild species, and included within population as well as among population comparisons of haplotypes. A similar approach was taken with wild populations of common bean *Phaseolus vulgaris* [32••,56]. The allelic polymorphism of individual genes was also studied at the interpopulation and intrapopulation level for three recognition genes of *A. thaliana*, RPS2, RPP8 and RPP13 [57], and for the Pto gene in wild *Lycopersicon* species [23••]. In all these cases high haplotype or allelic diversity could be found even within populations, demonstrating that genetic diversity for resistance exists at small scales and can hence respond to the action of selection by pathogens within populations.

Quantitative resistance — finding QTLs

It is important to note that major-gene resistance does not explain all variation in resistance phenotypes. Hence we need more complete information on what generates the resistance phenotype and this necessitates correlating molecular variation (for both functional genes and neutral markers) with (quantitative) phenotypic variation.

Quantitative resistance, exhibiting continuous variation in populations and usually under polygenic control, exists and may be widespread (e.g. [58,59]). It can also take various forms (e.g. constitutive resistance versus induced resistance) and involve diverse pathways (e.g. salicylic acid induction versus jasmonic acid induction). The loci contributing to this phenotypic variation are called quantitative trait loci (QTLs). They can be identified, for instance, by analyzing the selfed or backcrossed progeny of an F1 cross between two individuals that are highly differentiated for both the trait of interest and molecular markers, but several other designs can also be used [60]. Associations are then sought between alleles of the markers and status for the trait, using refined statistical analyses [60], which allow determination of how many genes control the trait and their respective importance. QTLs for resistance to pathogens have been identified for several crops (e.g. [61–63]) but also *A. thaliana* [37]. QTL analyses require large numbers of individuals, which, though labor-intensive, grants high statistical power. Therefore, even though some RGA colocalize with QTLs [64], QTL analyses can detect genes with small effects as well as genes that do not belong to already familiar gene families, including regulatory genes or regions. QTLs can also be used to investigate whether loci involved in resistance have pleiotropic effects [60], such as costs on other important functions. The selective history of QTLs

themselves or of their closely linked markers [65] can be identified, to determine whether genes involved in quantitative and qualitative resistance have similar kinds of evolutionary histories.

Quantitative resistance — selection versus drift?

Quantitative resistance revealed by QTL analysis, as all quantitative traits, will vary among populations and only some of this variation is adaptive. One can determine how much of this differentiation results from the action of selection (i.e. is adaptive) and how much from nonselective processes such as migration or genetic drift by comparing neutral molecular marker variation (F_{ST}) and variation in phenotypes (Q_{ST}). Q_{ST} partitions quantitative genetic variation in a way analogous to F_{ST} into within-population and between-population components [66]. If the observed differentiation of a quantitative trait is significantly higher than that of neutral molecular markers ($Q_{ST} > F_{ST}$), the trait has most probably undergone diversifying selection, and local adaptation could occur despite gene flow. By contrast, significantly smaller values of Q_{ST} than F_{ST} suggest the action of uniform selection ($Q_{ST} < F_{ST}$). Similar values of Q_{ST} and F_{ST} indicate no detectable effect of selection: selection may act, but is swamped by gene flow. Indeed, this is the population analogy to comparing the degree of genetic divergence of resistance genes with that of neutral genes within the same genome, which enables one to differentiate their respective histories. In the case of Q_{ST} versus F_{ST} we differentiate the history of particular traits of interest from the history of neutral genes within the same populations. Q_{ST} analysis assumes that the genetic basis for phenotypic variation is purely additive. Even quantitative resistance phenotypes, however, may involve some genes with nonadditive effects [37], in particular when they interact with parasite genotypes in a race-specific manner [67]. Accurate Q_{st}/F_{st} comparisons for resistance traits would thus require appropriate experimental protocols that limit as much as possible such nonadditive effects. For instance, separate analyses should be conducted for resistance against different pathogen genotypes.

Conclusion: linking genotypes and phenotypes

Although we find some undeniable footprints of positive selection for resistance genes, there are few clear examples of natural selection by parasites generating a positive response for currently functional resistance types in natural populations. One important challenge, then, is to understand how selection, that appears to generate only highly localized and diffuse responses, sometimes in the wrong direction, filters its way through the phenotypic noise to leave such distinct traces at the genome level. Obviously these processes occur at very different time scales. Genomic footprints of selection are the summation of a long history of selection that may have been more or less diffuse. Current selection pressures are operating on phenotypes that are, themselves, the product of past selective episodes. Detecting a response to selection in real time will require strong, consistent selective pressures. We propose that human activities, such as biocontrol and species introductions, offer just such opportunities. Some selection pressures on resistance in such populations can be inferred and quantified, for example in weeds subjected to biocontrol efforts, or for emerging diseases, and rapid evolutionary response can be observed [68].

Few studies have analyzed variation in natural populations, and even fewer have associated phenotypic and molecular analyses (but see [32,33,69]). The few studies using such an integrative approach have shown that the patterns of interpopulation differentiation found for putative R-genes and neutral molecular markers do not correspond to the variation in resistance phenotype [32••,56], suggesting that other factors affect resistance, or that the particular putative R-genes were not involved in this resistance phenotype. In addition to such detailed studies of small-scale variation at R-genes and their analogs in natural populations, we require information on variation in other loci that influence the resistance phenotype such as those revealed by QTL analyses and more importantly the pattern of variation (Q_{ST}) in the focal trait itself — the resistance phenotype. Rapid advances in sequencing techniques now render such population studies possible, so we look forward to studies that link genetic variation with the small-scale differences in resistance phenotypes available to the action of local selection by pathogens.

Parasite's side of the mirror

In parallel to plant resistance, pathogens also show a large diversity of pathogenicity phenotypes and their adaptation to local hosts has been demonstrated in many systems [12••]. Much progress has been made in the past few years in understanding the evolutionary history of parasite genes involved in infection [70]. The avirulence molecules triggering receptors of known resistance genes have now been identified from well-known bacterial and filamentous pathogens and some of them have undergone strong selective pressures (e.g. [71]). Linking the selective patterns acting on parasite pathogenicity to those acting on host resistance will be an exciting challenge in the future. But as for host resistance, an integration of molecular and ecological data, and in particular better knowledge of the genetic structure of parasite populations [72,73], is also desirable for the investigation of parasites' genetic diversity of traits involved in pathogenicity.

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