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Epistatic selection and coadaptation in the Prf resistance complex of wild tomato

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ABSTRACT

Natural selection imposed by pathogens is a strong and pervasive evolutionary force structuring genetic diversity within their hosts' genomes and populations. As a model system for understanding the genomic impact of host–parasite coevolution, we have been studying the evolutionary dynamics of disease resistance genes in wild relatives of the cultivated tomato species. In this study, we investigated the sequence variation and evolutionary history of three linked genes involved in pathogen resistance in populations of *Solanum peruvianum* (*Pto*, *Fen*, and *Prf*). These genes encode proteins, which form a multimeric complex and together activate defense responses. We used standard linkage disequilibrium, as well as partitioning of linkage disequilibrium components across populations and correlated substitution analysis to identify amino acid positions that are candidates for coevolving sites between Pto/Fen and Prf. These candidates were mapped onto known and predicted structures of Pto, Fen and Prf to visualize putative coevolving regions between proteins. We discuss the functional significance of these coevolving pairs in the context of what is known from previous structure–function studies of Pto, Fen and Prf.

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1. Introduction

Protein sequence variation at disease resistance loci in natural plant populations is a well-documented observation. The presence of this polymorphism, maintained by on-going coevolutionary interactions with pathogens, should allow for effective crop protection and has contributed to many successful breeding programs. However, due to the lengthy and costly process of generating cultivars with novel resistance phenotypes, great effort is placed on extending the lifetime of the plant cultivars currently available. To what degree this is possible and how this can be achieved is the focus of this special issue. At the heart of this challenge is to predict and/or identify which resistance genes pose an insurmountable hurdle to the pathogen and therefore will provide effective protection over multiple growing seasons. If such genes exist (which is perhaps unlikely due to the effectiveness of natural selection and evolutionary potential in nearly all pathogen populations), do these genes share common features that could act as targets in future breeding programs? A parallel line of investigation, which goes hand-in-hand with the first objective, is to determine the best deployment strategies to extend the lifetime of the resistance genes already available to breeders. Our work on this question emerges from prior observations about the genetic and phenotypic variation for disease resistance present in natural plant populations (Rose et al., 2004, 2005, 2007, 2011, 2012; Hoerger et al., 2012). Natural selection imposed by pathogens is exquisitely tuned to differences among plants in disease resistance and therefore, observations in natural populations may help to inspire more successful plant protection strategies.

In this paper, we describe the sequence variation and evolutionary history of three linked genes involved in pathogen resistance in wild tomato (*Pto*, *Fen*, and *Prf*). These genes encode proteins, which form a multimeric complex and together activate defense responses (Gutierrez et al., 2010; Ntoukakis et al., 2013). We are interested how the genetic linkage and the functional interaction combine to influence the sequence evolution of these genes. Since these genes reside in a small region of the tomato genome (60 kb), nearly all aspects of their evolutionary history are shared. A focus of this research is to determine to what degree these genes are coadapted to one another and how their roles in pathogen recognition mutually influence the evolution of neighboring and functionally linked genes. The presence and strength of epistatic selection in resistance gene complexes has broader implications because

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strong epistatic interactions can serve as a road-block to breeding programs, leading to genetic incompatibilities due to the disruption of R-gene functional units (reviewed in Bomblies, 2009).

The *Pto* resistance gene belongs to a small multigene family of five to six family members in the *Lycopersicon* clade (Martin et al., 1993), however functions have not been ascribed to all of these genes. The entire 60 kb region of chromosome 5 containing the *Pto* gene family has been sequenced from a susceptible *Solanum lycopersicum* cultivar and a resistant cultivar containing the *Pto* locus introgressed from the sister species *Solanum pimpinellifolium* (GenBank accessions AF220602 and AF220603). The two haplotypes share five orthologous, clustered genes (*Fen*, *Pth2*, *Pth3*, *Pth4* and *Pth5*).

Pto confers resistance to strains of *Pseudomonas syringae pv. tomato* expressing either AvrPto or AvrPtoB. It was the first race-specific R-gene to be isolated (Martin et al., 1993). This small gene without introns and the open reading frame (ORF) of 963 nucleotides encodes a functional serine/threonine kinase capable of autophosphorylation (Loh and Martin, 1995). The current model for Pto activation involves Pto binding to the pathogen ligand in the plant cell and a change in protein conformation, induced through this physical interaction. The stabilization of the Pto molecule in the proper conformation is dependent on Pto kinase activity. Next, the activated Pto protein transduces the signal, which is sensed by Prf to activate downstream plant immune responses. This includes the synthesis of antimicrobial compounds and results in programmed cell death at the site of infection (Rathjen et al., 1999; Sessa and Martin, 2000; Wu et al., 2004; Mucyn et al., 2006; Xing et al., 2007).

Fen, one of the *Pto* family members, is a functional serine/threonine kinase and confers sensitivity to the insecticide fenthion (Martin et al., 1994; Chang et al., 2002). The Fen protein shares 80% sequence identity with Pto, but does not confer AvrPto-dependent resistance (Scofield et al., 1996; Jia et al., 1997; Frederick et al., 1998). However, this paralog can recognize and activate defense responses to variants of AvrPtoB effector lacking E3 ubiquitin ligase activity (Rosebrock et al., 2007). Nonetheless, the wild type form of AvrPtoB ubiquitinates certain Fen alleles, which leads to their degradation in the plant cell. This suggests that the genes in the *Pto* cluster paralogs have experienced a complex history of host-pathogen coevolution.

Both Pto and Fen proteins do not act alone, but require a second protein, Prf, for the activation of disease resistance. *Prf* is a large gene embedded within the *Pto* gene cluster, although it is phylogenetically unrelated to *Pto* and its paralogs. In *S. pimpinellifolium* Rio Grande 76R, the 3′ end of this gene is located about 500 bp from the ORF of *Fen* and 24 kb from the ORF of *Pto*. The complete transcribed region of *Prf* is almost 11 kb and contains five introns. The protein coding region is 5.5 kb long. The resultant Prf protein is a large molecule (209.7 kDa) and contains NBS-LRR motifs, common to many other plant R-proteins (Salmeron et al., 1996).

Both of the kinases, Pto and Fen, functionally interact with the same N-terminal portion of Prf (Mucyn et al., 2006, 2009; Ntoukakis et al., 2009). Silencing of Prf prevents signaling by Fen or Pto, indicating that Prf acts epistatically to Fen and Pto. Recognition of *avrPtoB* by alleles of Pto from tomato expressed in *Nicotiana benthamiana* was only possible if the tomato allele of Prf was also co-expressed. The *N. benthamiana* allele of Prf could not complement this phenotype. This indicates that specific pairs of interacting partners are required for the full range of resistance (Balmuth and Rathjen, 2007; Mucyn et al., 2009). Thus, not only are *Pto* and *Fen* physically linked with *Prf*, which may indirectly affect their evolutionary history, but the functional protein interaction may require coadaptation between these molecules.

The molecular set up of this resistance cluster is analogous to a socket-wrench set, with Prf serving as the wrench handle or drive and Pto and its paralogs representing the individual sockets, adapted to recognizing different pathogen ligands. The physical linkage of Prf and the Pto cluster means that the entire set can be bred as a unit. This has advantages because the coadapted unit is not disrupted during the breeding process; e.g. the functional components do not become separated. As demonstrated in different studies, disrupting an R-gene functional unit can lead to unregulated cell death and hybrid incompatibilities (reviewed in Bomblies, 2009). As such, this small cluster of genes resembles the genetic cassettes constructed by modern breeders. The main difference is that the combination of specificities in a single plant genotype is guided by natural evolutionary history – including non-selective influences such as drift and demography – rather than by breeders and molecular geneticists. As a consequence, the number and breadth of specificities (and hence the range of pathogen protection) may be more limited than what is desired from a breeder's perspective, but the advantage of this "evolutionary genetic cassette" is that the individual components are guaranteed to function with one another.

What is the likelihood that these three genes represent a coadapted gene complex? Previous studies have shown that activity of tomato Fen is suppressed when combined with the allele of Prf from *N. benthamiana* (Mucyn et al., 2009). Likewise, *Pto* is subject to a mixture of balancing and purifying selection (Rose et al., 2007, 2011). This suggests that some types of *Pto* and *Fen* may function best with certain types of *Prf*. In this study we addressed these following questions: (1) Do the tightly linked genes *Pto*, *Fen* and *Prf* evolve in a correlated fashion? (2) Does epistatic selection operate in the Pto signaling pathway? (3) Does the maintenance of allelic variation at the *Pto* and *Fen* genes lead to the maintenance of allelic diversity at the *Prf* locus? We identified amino acid positions that are candidates for coevolving sites between Pto/Fen and Prf using standard linkage disequilibrium, as well as partitioning of linkage disequilibrium components across populations and correlated substitution analysis. These candidates were mapped onto known and predicted structures of Pto, Fen and Prf to visualize putative coevolving regions between proteins. Functional significance of these coevolving pairs is discussed in the context of what is known from previous structure–function studies of Pto, Fen and Prf.

2. Materials and methods

2.1. Plant materials

For this study, we collected multiple individuals of *Solanum peruvianum*, a species closely related to the cultivated tomato and endemic to the western coast of South America. This species is widespread and often occurs in large stands in central and southern Peru and northern Chile (reviewed in Chetelat et al., 2009). Individuals of *S. peruvianum* are diploid, obligate outcrossing, short-lived perennials and census population sizes range from a few plants to several hundred (tgrc.ucdavis.edu).

We sampled this species from three different geographical locations: (1) Canta (Central Peru, 11°31′S, 76°41′W; 2050 m altitude) (2) Nazca (Southern Peru, 14°51′S, 74°44′W; 2130 m altitude), and (3) Tarapaca (Northern Chile, 18°33′S, 70°09′W: 400 m altitude). The samples from Nazca and Canta, gathered in May 2004 by T. Staedler and T. Marczewski, are described in Staedler et al. (2008). Six plants were collected per population. DNA was extracted from leaf material using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). Seeds from the Tarapaca population were collected by C. Rick in April 1986 and stored at the Tomato Genetics Resource Center (TGRC) at the University of California, Davis (tgrc.ucdavis.edu; accession LA2744). This accession,

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