

## GUEST EDITORIAL

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### **Fungal Vegetative Compatibility – Promises and Prospects**

In many filamentous fungi, physiologically distinct individuals of the same species can fuse asexually to form a heterokaryon. When the heterokaryon is stable, then the participants are said to be vegetatively compatible and to belong to the same vegetative compatibility group (VCG). Vegetative (heterokaryon) compatibility (VC) has a multilocus genetic basis that has been studied for over 40 years in model organisms such as *Neurospora*, *Aspergillus*, and *Podospora* (for recent reviews see references 2 and 5). In spite of this long period of study, however, the physiological mechanism(s) responsible for this trait has not been discerned. VCGs may differ from one another at one, some, or all

of the vegetative incompatibility (*vic*) loci that are dispersed throughout the genome and responsible for the VCG phenotype. It is nearly impossible to determine from phenotype alone the number of hetero-allelic *vic* loci responsible for an incompatible interaction. Within the last 10 years much of the basic theory and methodology that was developed for model organisms has been applied to plant pathogenic fungi. The primary use in pathogenic fungi has been for population studies in which VC serves as a polymorphic marker, but VC can be used also as a model for self-nonsel self recognition, as a tool in the construction of genetic maps and the rapid isogenization of strains; and, when the interaction mechanism is understood, as a novel target for antifungal agents. Both population analyses and mechanistic studies of VC will remain at the forefront of plant pathology research for the foreseeable future.

Much of the work with VC in pathogenic fungi has tested a model proposed by John Puhalla in 1985 (6). He suggested that VC could be used to subdivide populations into different VCGs and that these subdivisions were correlated with pathogenicity. This model assumes that the pathogens rarely, if ever, participate in recombination events that could lead to reassortment of the *vic* alleles to yield new VCG phenotypes. Under this model each VCG is essentially a clone, and VCG and pathogenicity are coincidentally correlated rather than related by a cause-and-effect association. This model has been applied most extensively to the different pathogenic forms of *Fusarium oxysporum*, and 10 years after the original proposal a few conclusions can be drawn. First, there is a strong correlation between pathogenicity and VCG in some pathogenic *formae speciales* of *F. oxysporum*, e.g. *apii*, *cubense*, *cyclaminis*, and *melonis*, with most members of the individual *forma specialis* confined to one or a few VCGs. The correlation is weak, or nonexistent, in others, e.g. *asparagi* and *lycopersici*, and the members of these *formae speciales* belong to a relatively large number of VCGs. Strains recovered from soil as saprophytes are almost always diverse with respect to VCG. Where these results are consistent with Puhalla's model, they confirm the belief that the pathogenic strains originated as clones, which may later evolve themselves, and suggest that it should be possible to develop VCG-based diagnostics. In cases not consistent with the model, we are forced to reconsider our view of *F. oxysporum*. No sexual stage is known for this species, and there is no obvious explanation for the presence of so many multigenic genotypes in the absence of an effective sexual genetic exchange mechanism.

VCGs are useful for the analysis of fungal populations in contexts other than those proposed by Puhalla. VCGs are a direct multi-genic assessment of a trait of adaptive importance within fungal populations. The laboratory analysis of VCGs with complementary nitrate-nonutilizing (*nit*) mutants, is technically simple and requires little more than basic microbiological materials (3). VCGs are well-suited for measuring genotypic diversity, e.g. the frequency of different genotypes within a population, and for determining if two strains are identical to one another.

However, the VCG technique is not a panacea for population analyses of pathogenic fungi. VCG analyses are not appropriate for determining if strains belong to different biological species or for assessing differences that occur above the species level. Although the VCG technique is useful for measuring genotypic diversity, it is not useful for assessing the levels of allele frequencies, e.g. the frequencies of alleles at different *vic* loci. Similarly, although the VCG technique is a powerful tool for determining clonality, it cannot be used for ascertaining the degree of relatedness, e.g. sibs, parents, cousins, etc. Finally,

the detection of heterokaryosis may be complicated by alleles that prevent formation of a heterokaryon, even if the component strains are vegetatively compatible, *e.g.* mutants at heterokaryon self-incompatibility (*hsi*) loci (4). When compared with some other multi-locus techniques, such as DNA fingerprint probes, VCGs require less technical equipment and laboratory sophistication, but may not sample as many loci and require more effort to interpret.

The basic biological mechanisms underlying VC are not well understood. Conceptually there are two classes of mechanisms that are important for heterokaryosis: those responsible for the establishment of the heterokaryon and those required for its maintenance, once it has been established. The genes required for establishment of a heterokaryon may have mechanical/structural roles, or as yet unidentified discriminatory roles. *hsi* mutants are one of the potentially large number of mutant types in this class.

Genes such as the *vic* loci play a crucial role in the maintenance of the heterokaryon. The killing mechanism, which results in the death of heterokaryotic cells that are vegetatively incompatible, is of primary interest. How do cells in different VCGs recognize each other and thus set off either mutual killing or acceptance? The interaction probably involves some cell membrane and periplasmic space components, since protoplasts behave differently from hyphal cells. It is likely that there are many ways in which recognition occurs and in which the killing process is initiated and/or mediated, since there are multiple *vic* loci and the alleles at these loci usually interact only with other alleles at the same locus. Consequently, it would not be surprising, for example, if understanding the mechanism by which one gene operates in *Neurospora* tells us very little about how other genes in *Neurospora* operate, much less how *vic* loci in pathogenic fungi function. I think it is likely that a relatively large sample of loci from a broad spectrum of fungi will have to be examined before general conclusions can be drawn about these loci as a class rather than as individuals. Conceivably, such an understanding might provide a method for inducing the physiological killing response, thus affording an environmentally friendly method for controlling fungal pathogens.

It will be necessary also to understand the regulatory network in which these loci function. If recent mutagenesis studies with *Neurospora* (1) are an indication, then these networks are very complex and will contain numerous interesting genes, probably with pleiotropic effects on other functions. To be successful, we must do more than simply clone these genes; to understand the role *vic* loci play in the life cycle is at least as important. These studies could lead to the development of 'universally compatible' strains. Such strains could be used as delivery vehicles for mycoviruses or other intracellular biocontrol agents into a population that was otherwise so subdivided by VCG as to make biological control by such transmission impossible.

For more direct pathogen control, agents that simulate an incompatible reaction might be used to induce the physiological killing response, providing a novel set of antifungal compounds. If the response is limited, it might be possible to eliminate pathogenic strains while leaving their nonpathogenic, and perhaps beneficial, relatives untouched. If the response mechanism is more general, then it might be possible to identify species-specific or a cluster-of-species-specific antifungal agent(s). In essence these compounds would trigger a 'suicide' reaction in the target, and could be environmentally friendly, quite specific in their action, and applicable in settings other than just plant protection, *e.g.* medical and veterinary uses. Studies of VC have greatly advanced our understanding

of populations of fungal pathogens, but progress resulting from an analysis of the mode of action of the *vic* loci will probably be responsible for even greater changes in plant pathology in the coming decade.

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