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X International Fungal Biology Conference
Ensenada, Mexico
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MSA Meeting
University of Kentucky
Lexington, KY, USA
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9th International Mycological Congress
Edinburgh, UK

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A simple and rapid method to determine vegetative compatibility groups in fungi

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Vegetative compatibility in fungi reflects phenotypic differences (or similarity) among individuals representing the population of a species (Leslie 1993). Thus, individuals (genotypes) of a fungal species having the same heterokaryon (*het*) or vegetative incompatibility (*vic*) loci can fuse to form a heterokaryon (Glass et al 2000). Fungal isolates that form stable heterokaryons are then considered to belong to same vegetative compatible group (VCG). In contrast, isolates that are different at one or some or more of these loci will not anastomose. Rather, programmed cell death or apoptosis occurs in the mycelial cells that are in contact with an isolate representing a different VCG (Anagnostakis 1987, Leslie 1993). In the case of fungi which have coloured or dark mycelium in culture (such as most *Botryosphaeriaceae and Cryphonectriaceae*) failure to anastomose is observed as a thick barrage line between the two different isolates. For such species, tests in Petri dishes make it relatively easy to determine the VCG's for a population of isolates and this provides a robust view of population diversity (Burgess et al 2001, van Heerden and Wingfield 2001). For fungi with light coloured mycelium, for example species of *Fusarium*, barrage zones between isolates having different VCG's are difficult to discern. In such cases, it is necessary to produce *nit* mutants to define the individuals in culture (Klittich and Leslie 1988, Swift 2002).

Where it is possible to define the VCG's of individuals in the population of fungi, such tests are relatively simple and inexpensive. This approach also provides a means of understanding the genetic diversity in a fungal population where molecular markers and the concomitant sophisticated facilities may not be readily available. A disadvantage of determining VCG's in culture is that it is necessary to challenge every strain with every other strain in a population. This can be extremely time consuming and laborious. For example, where only two isolates are tested per Petri dish and there are 10 isolates in the collection to be studied, a total of 45 comparisons will be needed. An obvi-

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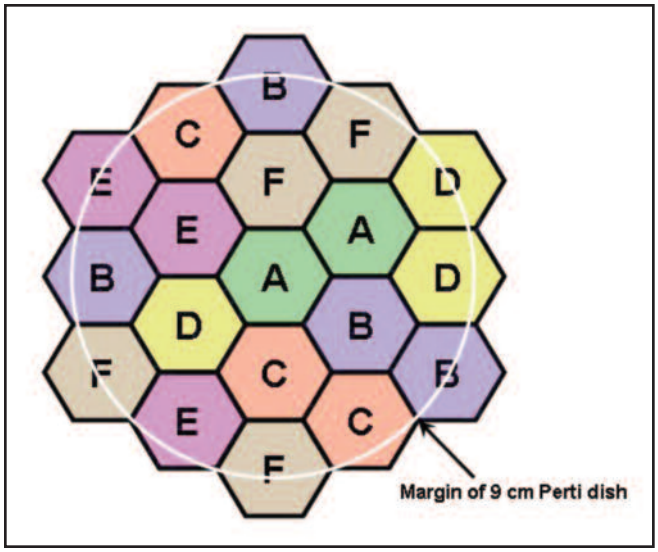

Figure 1. Template in which six isolates (A-F) can be compared on a single plate including control pairings where each isolate is compared with itself. A small mycelium plug (1 mm^2)) is placed in the centre of each hexagon

ous solution to the problem is to compare as many isolates as possible on a single Petri dish.

In order to increase the efficiency of VCG tests on Petri dishes, we have designed a template (Fig 1) where six isolates can be paired in all possible combinations on a single 9 cm Petri dish. This template has been used extensively (Smith et al 1996, Smith et al 2000, van Heerden and Wingfield 2001). A pairing schedule was then developed using this template to show how 31 isolates can be compared in all possible combinations in only 31 Petri dishes (Table 1) (Burgess et al 2001).

The pairing schedule described here to optimise VCG tests in culture, was originally derived empirically. Interestingly, however, it actually represents a specific block design known as a Steiner system. The design also represents a projective plane since every two plates have exactly one isolate in common (van Lint and Wilson, 2001). Thirty-one isolates appears to be an ideal number, and if any additional isolates are added to a study, the number of plates required for the tests would increase dramatically. For larger populations we suggest first comparing isolates in groups of 31 and thereafter, once duplicate VCG's are noted and removed, to compare the full population. For smaller populations we suggest duplicating some of the isolates to make up the set of 31. This approach will improve the speed, efficiency and reproducibility of vegetative compatibility tests.

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Table 1. The pairing schedule for 31 isolates in which each isolate is paired with itself and all other isolates in 31 Petri dishes.[1]

Plate no.	Position on template					
	A	B	C	D	E	F
1	1	2	3	4	5	6
2	1	7	8	9	10	11
3	1	12	13	14	15	16
4	1	17	18	19	20	21
5	1	22	23	24	25	26
6	1	27	28	29	30	31
7	2	7	12	17	22	27
8	2	8	13	18	23	28
9	2	9	14	19	24	29
10	2	10	15	20	25	30
11	2	11	16	21	26	31
12	3	7	13	19	25	31
13	3	8	14	20	26	27
14	3	9	15	21	22	28
15	3	10	16	17	23	29
16	3	11	12	18	24	30
17	4	7	14	21	23	30
18	4	8	15	17	24	31
19	4	9	16	18	25	27
20	4	10	12	19	26	28
21	4	11	13	20	22	29
22	5	7	15	18	26	29
23	5	8	16	19	22	30
24	5	9	12	20	23	31
25	5	10	13	21	24	27
26	5	11	14	17	25	28
27	6	7	16	20	24	28
28	6	8	12	21	25	29
29	6	9	13	17	26	30
30	6	10	14	18	22	31
31	6	11	15	19	23	27

[1]This design allows for cross checking (*i.e.,* if isolate 1 has same VCG as isolate 11 and isolate 25 then isolate 11 and 25 should also be compatible).