How to demonstrate barrage in Neurospora.

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Background

The standard barrage reaction. Barrage (French for *dam* or *barrier*) is the term used for a reaction that is seen when two strains are confronted that differ in alleles at a locus or loci governing the reaction (Esser and Meinhardt 1984, Rizet 1952). In *N. crassa*, strains of opposite mating type show vegetative (heterokaryon) incompatibility, failing to form stable heterokaryons. Aneuploid strains carrying both *mat A* and *mat a* genes in the same nucleus are grossly abnormal because of the *het*-incompatibility. When *mat A* and *mat a* mycelia of strains in OR background grow toward one another and come together on agar medium under suitable conditions, a barrage is formed between them, with two separate lines of perithecia separated by a 'clear' zone within which there is no or little surface growth (Griffiths 1979. See Fig. 37 in Perkins *et al.* 2001, Fig. 1B in Perkins and Raju 1986, Fig. 1F in Micali and Smith 2003). This has been called the Standard Mating Barrage.

The standard barrage reaction in *N. crassa* was first described by Griffiths (1979), who found it useful for teaching. Griffiths used *fluffy* strains, which conferred the advantage that no macroconidia are present to obscure the barrage area. One figure published by Dodge (1931) shows what appears to be a standard barrage. No barrage reaction occurs when standard *A* and *a* strains of *N. discreta* are confronted (Perkins and Raju 1986).

Opposite-mating-type *N. crassa* strains of genetic backgrounds other than OR do not necessarily give the standard mating reaction when confronted (Fig.1E in Micali and Smith 2003, and see below).
The barrage reaction between *mat A* and *mat a* is suppressed by the recessive gene *tol*, which suppresses mating-type-associated heterokaryon incompatibility (Newmeyer 1970). *N. tetrasperma* strains are naturally *tol*-minus (Jacobson 1992).

If one of the confronted *N. crassa* strains carries a *per* mutation, maternal origin of the perithecia can be distinguished on the basis of perithecial wall pigmentation (Figure 1). This made it possible to show that perithecia on opposite sides of the barrage originate from the mycelium on which they are formed and that fertilizing nuclei come from the strain on the opposite side of the clear zone (Perkins 1988a; Fig 37 in Perkins *et al.* 2001). This suggests (but does not prove) that trichogynes from one side extend across the barrage to the other side, where they pick up nuclei of the opposite mating type.

No barrage is seen when one of the *mat* genes of *N. crassa* is inactivated by mutation or when the heterokaryon incompatibility function is inactivated or suppressed by the recessive *tol* mutation. However, standard barrages were observed by F. J. Doe (personal communication) when strains differing in *het-c* alleles were confronted, even though *het*-incompatibility mediated by *mating type* or by genes at other *het* loci was absent (F. J. Doe, unpublished). These observations suggest that in some strains a direct relation exists between *het*-incompatibility and barrage formation of the standard type (Perkins 1988b, Perkins *et al.* 2001). However, further study is needed too determine how general this is in *N. crassa*. (In *Podospora anserina*, *het*-incompatibility is recognized routinely by barrage formation rather than by failure to complement in forced heterokaryons.)

Nonstandard barrage reactions. The simple standard reaction with two lines of perithecia is by no means universal, either in Neurospora or in other fungi (see Esser and Meinhardt 1984, Micali and Smith 2003). Nonstandard reactions are commonly seen when different strains are confronted, and barrage reactions are not limited to strains of opposite mating type. The interaction is sometimes asymmetric, with nuclei of one strain invading mycelia of the other while no invasion occurs in the opposite direction (Giffiths and Rieck 1981, Micali and Smith 2003). With nonstandard barrages, exceptions are found to the 1:1 relation that has been suggested to exist between *het*-incompatibility and the Standard Mating Barrage (Micali and Smith 2003).

Strains are listed below that are suitable for demonstrating the Standard Mating Barrage. Their usefulness is by no means limited to teaching. They should also be useful for further research into the mechanism, providing a reference to which experiments with nonstandard barrages can be compared. For strains and procedures used in examining nonstandard barrage reactions, see Micali and Smith (2003).

Procedure

Optimal conditions for barrage formation using conidiating strains were described by Micali and Smith (2003): About 20 ml of synthetic cross medium (Westergaard and Mitchell 1947), with 1.5% sucrose and 1.5% agar , is used for each 8.5 cm diameter plate, giving an agar thickness of ~4 mm. Strains are confronted in pairs, each strain being inoculated twice on each plate, at spots 3 cm apart in alternate quadrants, using about 2 µl of a conidial suspension. Plates are incubated at 24°C in alternating dark and light. For nonconidiating strains, uniform sized pieces cut from a fresh mycelial lawn are used as inocula.

Standard *fluffy* testers are recommended for the standard reaction: *fl A* (FGSC 4317) and *fl a* (FGSC 4347), or *fl A* (FGSC 6682) and *fl a* (FGSC 6683) (Perkins *et al.* 1989). If a color marker is desired that will reveal the origin of the perithecial wall and identify which strain was the female parent, one of the following can be used: *fl; per-1 A* (FGSC 3311), *fl; per-1 a* (FGSC 3312), *fl; per-1 al-3 A* (FGSC 3960), or *fl; per-1 al-3 a* (FGSC 3120).

References

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DDP

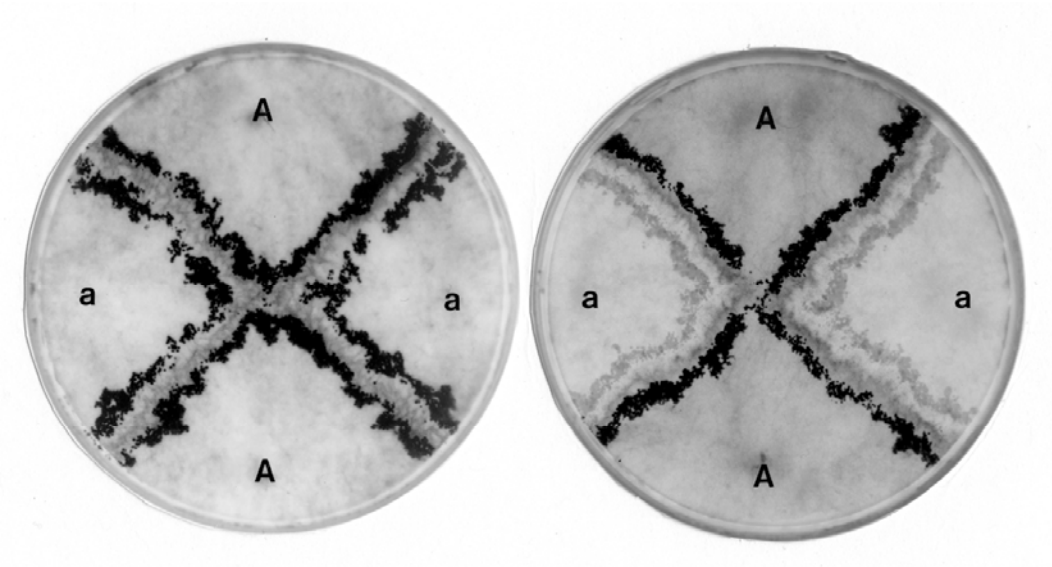

Fig 1. Barrage formation as a manifestation of vegetative (heterokaryon incompatibility. Strains of opposite mating type, *mat A* and *mat a*, were inoculated to crossing medium in alternate quadrants of the Petri dish at the positions marked. When unlike strains come together, a clear zone of inhibition is formed and lines of perithecia develop on each side of this barrage. Each strain acts as the maternal parent of perithecia on its own side of the barrage, and the strain on the opposite side acts as the fertilizing parent. This is shown in the plate to the right, where the *mat a* parent carried *per* allele PBJ1. The perithecial color mutant blocks formation of black pigment in the maternally-derived perithecial wall. The nonblack perithecia, which become orange as carotenoids develop, were fully as numerous as the black perithecia opposite them. Parents in these tests all carried a mutant *fluffy* allele, which improves visibility by eliminating macroconidia. Photo credit: D.D. Perkins.