Evidence for a Cytoplasmically Transmissible Factor Affecting Recognition and Somato-sexual Differentiation in the Basidiomycete Stereum hirsutum

By DAVID COATES AND ALAN D. M. RAYNER*

School of Biological Sciences, University of Bath, Claverton Down, Bath BA2 7AY, UK

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A spontaneous change, possibly involving a mating-type switch, occurred during storage of a homokaryotic culture (F2.4) of the basidiomycete Stereum hirsutum and resulted in a dramatic transformation of its cultural and interactive properties. Subcultures grown on malt agar rapidly produced farinaceous hymenial surfaces if exposed to light, and were not receptive to nucleus migration when paired with compatible homokaryons. These properties were transmissible to other homokaryons by direct pairing, the recipients becoming transformed regardless of whether they were sexually or somatically compatible or incompatible with F2.4. With the incompatible sib F2.9, transformation apparently occurred without nuclear transfer.

Progeny monobasidiospore cultures from transformed F2.4 and F2.9 strains consistently belonged to two interaction groups, standard and reversed, the former reacting similarly to the corresponding untransformed strains against a range of sib homokaryons, the latter interacting in exactly the opposite fashion. Reversed-standard pairings resulted in all cases in an unusual 'quasi-compatible' reaction, characterized by development of an asymmetrically migrating farinaceous region.

Progeny from transformed F2.9 were dimorphic, being divisible into (a) slow-growing, dense and (b) fast-growing, effuse colony types, the former reverting spontaneously to the latter. In several cases mycelial 'mounds' developed on reverted slow, dense colonies. Basidiospore progeny and mycelial subcultures from mounds produced colonies with further mounds. These and further observations indicate pleiotropic effects initiated or mediated by a cytoplasmically transmissible, possibly insertional factor on recognition and developmental processes in S. hirsutum.

INTRODUCTION

Stereum hirsutum is a common member of a widespread group of wood-decaying basidiomycete species in the genera Stereum, Phanerochaete and Coniophora, which possess vegetative mycelia with multinucleate hyphal compartments and verticillate ('whorled') clamp-connections (Boidin, 1971). Occurrence of the latter on primary mycelia arising from single germinated basidiospores led to the belief that these fungi were homothallic (Boidin, 1971), but subsequent observations have shown that both outcrossing (heterothallic) and non-outcrossing (homothallic/amictic) populations occur (Coates et al., 1981; Boddy & Rayner, 1982; Rayner & Turton, 1982; Rayner et al., 1984).

Non-outcrossing populations have been found in Stereum sanguinolentum, S. rameale, S. subomentosum and Finnish collections of S. hirsutum. Their characteristic feature is the production, in the field, of homokaryotic basidiocarps giving rise to genetically homogeneous progeny sets exhibiting neither somatic nor mating incompatibility reactions. However, homokaryons of different genetic lineage are somatically incompatible.

Outcrossing populations are found in British collections of S. hirsutum, and in S. rugosum, S. gausapatum, Phanerochaete velutina and Coniophora puteana. All these fungi possess unifactorial homogenic incompatibility (mating) systems, allowing homokaryons with complementary
alleles at a single multiallelic compatibility locus (C-factor) to form a stable heterokaryon (secondary mycelium). Basidiospore progeny from heterokaryotic fruit bodies in these outcrossing species are genetically variable and consequently exhibit a variety of compatible and incompatible interaction patterns when interpaired.

As in other heterothallic basidiomycetes, somatic incompatibility, which usually takes the form of a pigmented or sparse demarcation zone between colonies, occurs between different heterokaryons and in certain homokaryon combinations. The genetic basis of somatic incompatibility is not certain. The reaction is often more intense between unrelated than related strains, and is probably due to recognition of self–non-self differences. Hence somatic compatibility, the intermingling without antagonistic interaction between mycelia, occurs in self-pairings and, increasingly, after cycles of inbreeding. Permanent expression of somatic incompatibility is only seen in the absence of a mating interaction, and this reciprocal relationship has given rise to the concept that mating compatibility overrides somatic incompatibility in self–non-self confrontations (Rayner & Todd, 1979; Todd & Rayner, 1980; Rayner et al., 1984).

Mating compatibility in some species, including S. gausapatum, P. velutina and C. puteana, results in asymmetric development of the secondary mycelium preceded by unilateral or unequally bilateral nuclear migration and marked inhibitory or even lytic interactions. However, in British strains of S. hirsutum, mating compatibility allows homokaryons to donate nuclei to and accept nuclei from each other more or less equally, leading to production of a single stable heterokaryon, which is manifested by a gradual uniform change in colony morphology of both homokaryons. There is also a second, unlinked multiallelic locus (B-factor), heterozygosity at which typically causes a distinctive ‘bow-tie’ interaction which is only visible between mating-type (C-factor) incompatible homokaryons. This involves formation of a band of appressed mycelium with abnormal branching, widest at its edges, which is usually asymmetric and tends to advance preferentially into one homokaryon leading to partial or complete replacement of its nuclei by those of the other. As with mating, there is a reciprocal relationship between bow-tie formation and somatic incompatibility: in some pairings somatic incompatibility may be expressed rapidly, partially or wholly precluding bow-tie formation. In other pairings cessation of bow-tie migration is associated with development of a somatic incompatibility reaction, and in yet others somatic incompatibility is absent and bow-tie migration proceeds to completion.

Important questions arising from these observations concern the origin of the different patterns of somatic and mating incompatibility in these fungi, including the occurrence of non-outcrossing populations. Broader issues concern the regulatory mechanisms controlling expression of the functionally complementary systems of somatic and mating incompatibility, which respectively result in rejection and acceptance of non-self, together with the morphogenetic pathways culminating in mycelial differentiation and fruit body production. Here we report the discovery of a factor in a British strain of S. hirsutum, the behaviour of which appears to relate strongly to these issues. The factor has strong affinities with both the B- and C-factors of this species, but when acquired by homokaryons enhances somatic rejection, blocks nuclear migration, induces fruiting and conjugate association of nuclei, and is apparently cytoplasmically transmissible to other strains.

**METHODS**

*Strains and cultural procedures.* The homokaryotic strains of S. hirsutum used experimentally were derived from single basidiospores obtained from four different fruit bodies (D1, D2, F1, F2) collected from two field sites in south-west England as detailed by Coates et al. (1981). The culture medium was 2% (w/v) malt agar throughout. Experimental and confirmatory pairings were normally made by placing inocula cut from the margins of actively growing colonies, or subcultures from interaction plates, approx. 1 cm apart in 9 cm Petri plates. Incubation of plates was normally at 25 °C in dark or dark–light regimes. However, the exact cultural conditions were not critical in determining the interaction patterns. Monobasidiospore isolates were prepared from spore deposits produced by fruiting strains (see below), by making a suspension of the spores in sterile water, spreading this across agar plates, and then transferring single germlings to fresh medium with a sterile tungsten needle.
RESULTS

Origin of transformed strains

The present study resulted from the observation of a spontaneous change in the morphological and interactive properties in a stock culture of the homokaryon F2.4 which had been stored under mineral oil (liquid paraffin, sp.gr. 0.86-0.89; Fisons) at 5°C. This spontaneously changed strain, henceforth termed the 'source' strain, was apparently unable to receive nuclei from C-factor-compatible homokaryons, but on its own readily produced hymenial surfaces (see below) yielding deposits of viable basidiospores. None of our other British homokaryons of *S. hirsutum* has been observed to fruit in culture, and heterokaryons have only done so sporadically, after prolonged incubation. As detailed below, the fruiting and compatibility properties just described were transmissible, along with certain other characteristics, from the source strain to certain 'recipient' homokaryons – including both compatible and incompatible types – when these were paired against it. Henceforth the source strain, and the recipients changed by contact with it, will be described as 'transformed', this being indicated by the addition of '(t)' to the isolate codes. All the transformed strains were stable, retaining their properties even after repeated subculturing. The fruiting, other morphological characteristics and interactive properties of the transformed strains and their progeny (see below) provided the criteria for identifying transformation, and distinguished it from straightforward heterokaryotization of C-factor compatible strains. They also excluded the possibility that the source strain was simply a heterokaryon resulting from contamination of F2.4 by a compatible basidiospore.

Morphological characteristics of transformed strains and their progeny

Both the source strain F2.4(t) and transformed incompatible sibs produced an unusually dense, tufted mycelium which, particularly at colony margins, after damage and/or exposure to light, rapidly acquired a superficial farinaceous covering of small, discrete hymenial surfaces (see Figs 3 and 4). These fruiting colonies also acquired an unusual pink coloration not seen in any of our many British field isolates of *S. hirsutum*. In contrast, transformed mating-type compatible strains were like normal secondary mycelia of *S. hirsutum* (see Coates et al., 1981) in having a crustose, often yellow-ochre pigmented morphology. They readily produced hymenia which, apart from their small size, were similar in form to field produced fruit bodies.

Monobasidiospore progeny from the source strain were rather variable, although much less so than is typically seen in progeny from field heterokaryons, in production of aerial mycelium and pigmentation; they were all rapid-growing and generally more effuse than the parental strain. A few readily produced farinaceous hymenial surfaces. By contrast 20 progeny from F2.9(t) (incompatible with F2.4 and apparently transformed without nucleus transfer –see below) fell into two types (Fig. 1a), with fast-growing, effuse morphology (11 isolates) and slow-growing, dense morphology (9 isolates), respectively. All the latter then reverted spontaneously to fast, effuse morphology (Fig. 1b), farinaceous hymenial surfaces subsequently developing in the reversion zone.

All F2.9(t) progeny eventually produced hymenia on their own, although less rapidly than the parental strain or when they were paired in quasi-compatible combinations with other homokaryons (see below). The characteristics of second generation progeny sets from these hymenia and also from fruiting source-strain progeny are given in Table 1. The pattern of occurrence of slow, dense and fast, effuse colony types from F2.9(t) shows a striking alternation between generations.

Ten progeny from a non-sib-related, mating-type compatible homokaryon (D1.9) transformed by the source strain were all fast, effuse, but otherwise highly variable in morphology, presumably reflecting the fact that the recipient strain had become heterokaryotic; that is, transformation was accompanied by passage of nuclei from the source strain. Two of the progeny isolates produced colonies with a 'ragged' morphology, producing marginal sectors at the colony margin which were morphologically indistinguishable from bow-tie regions (Fig. 1c).

Three reverted slow, dense first generation progeny strains from F2.9(t) produced tumour-like dense aggregations of hyphae (Fig. 2) somewhat similar to the 'mounds' described in *Schizo-
Fig. 1. Colony types in monobasidiospore progeny from transformed strains of Stereum hirsutum. (a) Slow, dense (left) and fast, effuse colony types from the recipient strain F2.9. (b) Spontaneous reversion of a slow, dense colony to fast, effuse growth. (c) 'Ragged' morphology due to apparent constitutive expression of bow-tie reactions in a control pairing (self x self) of a strain from the transformed non-sib D1.9(t).

Phyllum commune (Gaber & Leonard, 1981). Unlike S. commune, these mounds produced a superficial hymenial surface when exposed to light, and progeny monobasidiospore isolates from these, although fast, effuse types, themselves produced mounds, sometimes when overgrowing contaminant Penicillium colonies (Fig. 2a). By contrast, direct subcultures from mound tissue produced initially slow, dense colonies from which developed fast, effuse sectors, together with mounds in much larger numbers than on the original strains (Fig. 2b).

Interactions of transformed strains

A summary of the interactions observed when the source strain was paired with a range of sib- and non-sib-related homokaryons is given in Table 2 and compared with those of F2.4 before transformation. In all cases a somatic incompatibility reaction developed along the junction line between F2.4(t) and any other strain. This was followed by a unilateral change in morphology
Recognition and differentiation in Stereum

Fig. 2. Mound formation: (a) mounds (arrowed) developing at sites of overgrowth of Penicillium contaminants on a plate multiply inoculated with fast, effuse basidiospore progeny from a mound formed previously on a reverted slow, dense colony; (b) mound-producing colony derived from a subculture from mound tissue.

and, sooner or later, production of hymenial surfaces in the recipient strain. When the recipient strain was not sib-related to (and hence was fully mating-type compatible with) the source strain, lines of apparent somatic incompatibility developed between adjacent sectors of the changed mycelium. Similar lines, or 'tracks', develop in matings between heterokaryons and homokaryons carrying unrelated nuclei in several basidiomycetes, where they delimit sectors of alternating genotype (Todd & Rayner, 1978; Rayner & Todd, 1979; Coates et al., 1981). However, in this case subcultures from different sectors intermingled with each other, indicating establishment of a stable secondary mycelium with somatic rejection only being expressed temporarily during passage of nuclei from the source strain.

By contrast, when recipient strains were sib-related and had previously produced bow-tie reactions with F2.4 (as with F2.15, Fig. 3a) the change in morphology followed rapidly behind a bow-tie-like front, the initial somatic incompatibility reaction tended to fade, and subcultures from the transformed zone were somatically compatible with the source strain. This was taken as evidence of complete replacement of the nuclei of the recipient strain by the source strain.

Finally, in pairings of F2.4(t) with a recipient strain (F2.9, Fig. 3b) with which F2.4 had been somatically incompatible, subcultures from the transformed zones were somatically compatible with and transformed F2.9 stocks, but were incompatible with the source strain. When paired against a range of sib-related homokaryons the subcultures produced somatic incompatibility reactions followed by transformation fronts in much the same pattern as had the source strain. Taken together with the morphological (see above) and interactive (see below) characteristics of progeny from F2.9(t) this strongly suggests that transformation had occurred without nuclear transfer, that is, by a cytoplasmically transmissible factor.
Table 1. Numbers of slow-dense and fast-effuse colony types in second generation progeny sets from source F2.4(t) and recipient F2.9(t) strains of Stereum hirsutum

<table>
<thead>
<tr>
<th>Origin and colony type of first generation progeny strain</th>
<th>Interaction type of first generation progeny strain*</th>
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<tr>
<td></td>
<td>Standard</td>
<td>Reversed†</td>
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<td></td>
<td>Fast-effuse</td>
<td>Slow-dense</td>
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<td>F2.9 (Slow-dense)</td>
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<td>F2.9 (Fast-effuse)</td>
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<td>F2.4 (Fast-effuse)</td>
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* See text and Tables 3 and 4 for explanation of interaction type.
† Viability of basidiospores from reversed progeny strains was very low compared with normal strains.

Table 2. Summary of interaction characteristics of strain F2.4 against other homokaryons of Stereum hirsutum before and after transformation

B-, C-factor relationship of homokaryon to F2.4

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<tr>
<td>F2.4 Fully compatible mating reaction</td>
<td>Bow-tie, not suppressed by somatic incompatibility, e.g. F2.15</td>
<td>Bow-tie suppressed by somatic incompatibility, e.g. F2.9</td>
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<tr>
<td>F2.4(t) Somatic incompatibility, unilateral heterokaryotization and transformation</td>
<td>Somatic incompatibility followed by replacement by F2.4(t)</td>
<td>Somatic incompatibility followed by transformation</td>
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* These results were not re-tested by subculturing and/or progeny analysis.
Recognition and differentiation in Stereum

Interactions of the source strain with mating-type incompatible sibs: (a) with strain F2.15 (right) showing unilateral migration of a bow-tie shaped front (small arrows), in which farinaceous source-strain mycelium is regenerating; (b) with strain F2.9 (right) showing migration of a transforming front (small arrows) from a somatic rejection line which initially developed between the colonies; f, farinaceous hymenial surfaces; si, somatic incompatibility reactions.

Interactions of progeny from transformed strains

Interactions within monobasidiospore progeny families from the source strain and from the recipient strain F2.9(t) are given in Table 3(a and b, respectively). In both cases the progeny fell exactly into two 'quasi-compatibility' groups, members of different groups interacting to produce asymmetrically migrating bow-tie-shaped regions superseded by the production of aerial mycelial tufts and numerous farinaceous hymenia (Fig. 4). This suggests that quasi-compatibility was due to allelic difference at a single locus. Non-quasi-compatible combinations of source strain progeny were generally somatically incompatible or/and produced bow-tie reactions, which, together with the cultural variability of these progeny (see above) indicates substantial heterozygosity in F2.4(t). By complete contrast, non-quasi-compatible combinations of F2.9(t) progeny were all somatically compatible and appeared morphologically alike (the slow, dense–fast, effuse dimorphism was independent of quasi-compatibility grouping, but by the time the interactions were tested all slow, dense forms had reverted). This indicates substantial genetic identity between F2.9(t) progeny and hence makes it very unlikely that F2.9(t) itself contained any nuclei from F2.4(t); this would be consistent with transformation of F2.9 by a cytoplasmically transmissible factor, originating by some mechanism eliciting a substantial genetic change in the source strain.

When the progeny strains from F2.4(t) and F2.9(t) were paired against a range of sib homokaryons (Table 4a) they were seen to fall into two classes: members of one group, henceforth termed standard, gave reactions identical to the corresponding F2.4 and F2.9 strains before transformation; members of the other interaction group, henceforth termed reversed, were quasi-compatible against previously mating-type incompatible (common C-factor) strains. This suggests that basidia of transformed strains contain one nucleus conferring standard and one conferring reversed characteristics. This was confirmed by the consistent interactions in source–recipient strain progeny combinations (Table 4b), although the interestingly weak interactions in standard–standard pairings suggest loss of somatic incompatibility function between these strains. Both reversed and standard strains were fully compatible with non-sib-related homokaryons (Table 4c), although in all cases the interactions had some quasi-compatible characteristics (asymmetric migration fronts, production of mycelial knots and aerial tufts).
Fig. 4. 'Quasi-compatible' reaction between standard and reversed progeny (see text) from the source strain, showing asymmetric development of a bow-tie-shaped region (limits indicated by arrows) with tufts and farinaceous hymenial surfaces (f); i, inoculation sites.

Table 3. Interactions within progeny sets from transformed isolates of Stereum hirsutum

Bs, strong bow-tie reaction (liquid exudation); Bw, weak bow-tie reaction (no liquid exudation); . . . indicates subsequent development of strong mutual antagonism reaction; As, strong mutual antagonism (somatic incompatibility); Aw, weak mutual antagonism; Q, quasi-compatible reaction; I, intermingling.

(a) F2.4(t) progeny

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(b) F2.9(t) progeny

| 20 Bw | Aw | As | Bw | As | Bw | Aw | Bw | Bw | I | I | I | I | Q | Q | Q | Q | Q | Q | 6 |
| 19 Bw | Aw | Aw | Bw | As | Bw | Bw | Bw | Bw | Bw | I | I | I | I | Q | Q | Q | Q | Q | Q | 7 |
| 18 Aw | Aw | Bw | As | As | Bw | Bw | Bw | Bw | Bw | 1 | I | I | I | I | Q | Q | Q | Q | Q | 8 |
| 17 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | 1 | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 9 |
| 16 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 10 |
| 15 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 11 |
| 14 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 12 |
| 13 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 13 |
| 12 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 14 |
| 11 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 15 |
| 10 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 16 |
| 9 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 17 |
| 8 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 18 |
| 7 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 19 |
| 6 Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | Bw | I | I | I | I | I | I | I | I | I | I | I | I | I | I | Q | Q | Q | Q | Q | Q | 20 |

Interactions between progeny from the non-sib-related recipient strain D1.9(t) are given in Table 5. No directly somatically incompatible interactions occurred (these would normally be expected between at least some progeny from a non-sib-composed secondary mycelium) and the progeny belonged to two groups which were fully mating-type compatible with each other. However, a proportion of the fully compatible interactions had some quasi-compatible characteristics. Remarkably, all non-mating-type compatible combinations at least initially gave bow-tie reactions, although some of these were followed by unusual patterns of mycelial regeneration, suggesting quasi-compatibility.
Table 4. Interactions of F2.4(t) and F2.9(t) progeny against sib and non-sib homokaryons and each other

Symbols as for Table 3, others as follows: S, homokaryons with standard interaction behaviour; R, homokaryons with reversed behaviour; SD, slow-dense morphology; FE, fast-effuse morphology; C, fully compatible; *, interactions with quasi-compatible characteristics; f, farinaceous hymenial surfaces. (C1, C2, B1, B2) refer to the compatibility (C) and bow-tie (B) allele designations of each isolate. F2.4(t) and F2.9(t) refer to progeny of transformed F2.4 and F2.9 isolates.

(a) Against sibs

F2 untransformed sibling homokaryons

<table>
<thead>
<tr>
<th>Homokaryon genotype</th>
<th>C2B2</th>
<th>C2B1</th>
<th>C1B1</th>
<th>C1B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homokaryon code no.</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Interaction type</td>
<td>3</td>
<td>16</td>
<td>15</td>
<td>9</td>
</tr>
</tbody>
</table>

- F2.4(t) progeny
  - (genotype C1B1)
    - R: 6 As Bw As Bs Q Q Q Q
    - S: 8 As Bw As As Q Q Q Q
    - 10 As Bs Bw Bs Q Q Q Q
    - 1 C C C C Aw As Bs As
    - 3 C C C C Aw As Bw As
    - 7 C C C C Bw Bw Bs As
    - 5 As Aw Aw As Q Q Q Q
    - 14 Bs Aw Bs As Q Q Q Q

- F2.9(t) progeny
  - (genotype C1B1)
    - R: 4 C C C C As Bs Aw I
    - 6 C C C C As Bs As I
    - 18 C C C C As Bs As I
    - 19 C C C C As Bs As I

(b) Against each other

F2.9(t) progeny (genotype C1B2)

<table>
<thead>
<tr>
<th>Interaction type</th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony type</td>
<td>SD</td>
<td>FE</td>
</tr>
<tr>
<td>Progeny code no.</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

- F2.4(t) progeny
  - (genotype C1B1)
    - S: 1 Bw Bw Bw Bw Bw Q Q Q Q
    - 2 Bw Bw Bw Bw Bw Q Q Q Q
    - 6 Q Q Q Q Q Q Bs Bs Bs Bs
    - 8 Q Q Q Q Q Q Bs Bs Bs Bs

(c) Against non-sibs

<table>
<thead>
<tr>
<th>Homokaryons</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

- F2.9(t) progeny
  - R: 5 Co Co Co Co Co Co Co Co
  - S: 14 Co Co Co Co Co Co Co Co
  - 18 Co Co Co Co Co Co Co Co
  - 4 Co Co Co Co Co Co Co Co

DISCUSSION

Although they depend on the natural markers provided by recognition factors rather than conventional nuclear markers (which are not presently available), the present results strongly suggest that transformation of the S. hirsutum source strain is associated with production of a
cytoplasmically transmissible mobile factor. This putative factor has the property, unlike many cytoplasmic determinants in higher fungi (e.g. Caten, 1972; Anagnostakis, 1982; Brasier, 1983, 1984) of readily gaining access across a somatic incompatibility barrier. It apparently directly or indirectly affects not only the mechanism determining acceptance or rejection of non-self in mating and/or somatic interactions, but also reproductive differentiation, slow, dense–fast, effuse mycelial transitions and the production of mycelial tumours or mounds. Thus it may offer a new insight into the mechanisms underlying these phenomena and their evolutionary significance.

An important step in developing such insight will be identification of the molecular nature of the factor and mechanisms leading to its production, and this will obviously require much further analysis than has been possible so far. However, its effect on recognition, passage across somatic rejection zones and patterns of migration of transformation fronts and quasi-compatible reactions strongly suggest molecular affinities, if not some form of identity with the *S. hirsutum* B- and C-factors.

In trying to understand the evolution of mating systems in Basidiomycotina, and the ways in which these interact with somatic incompatibility systems, it is important to examine mycelial interactions in species with diverse mating and nuclear behaviour as well as in intensively studied species such as *Schizophyllum commune* and *Coprinus cinereus* which both have tetrapolar incompatibility and clamp connections only on the dikaryon. Following their own investigations of species of *Stereum*, *Phanerochaete* and *Coniophora*, Rayner et al. (1984) proposed three fundamental processes underlying secondary mycelium formation. Access migration allows ingress of donor nuclei into an acceptor mycelium at a rate dependent on the genetic relationship between donor and acceptor so that asymmetric or unilateral migration patterns are commonly observed. Acting alone, it results in abnormal branching and inhibition of colony extension and donor nuclei may migrate predominantly via lateral fusions between hyphae, resulting in bow-tie phenomena. Acceptor migration, which is dependent on an initial access step, occurs via pre-existing hyphae at a rate determined by the acceptor only so that different donor nuclei migrate at equal rates. Stabilization allows production of a stable secondary mycelium in which there is maintenance of a 1:1 nuclear ratio in dikaryotic or multikaryotic hyphal compartments. Of these three processes, access is the one during which the critical interaction with somatic rejection is believed to occur.

The occurrence of these three processes in various permutations goes some way towards explaining the varying incompatibility patterns in different species and populations of *Stereum*, *Phanerochaete* and *Coniophora* (see Introduction). The present results are consistent with this idea, and in particular show how one pattern of behaviour can be switched to an entirely different one within a population. In normal British strains of *S. hirsutum* the C-factor results, in stabilized acceptor migration, with the B-factor an unlinked, apparently redundant access factor whose operation does not normally result in formation of a stable heterokaryon. By contrast, the quasi-compatibility between standard and reversed progeny of transformed strains can be
Recognition and differentiation in Stereum

Fig. 5. Summary of mating interactions of strains F2.4 and F2.9 before transformation together with those of their progeny monobasidiospore isolates produced following transformation. C1 and C2 represent the two mating-type compatibility factors occurring in field strain progeny. The scheme shows how various strain interactions can be explained if the C-factor is subdivided into A and B components controlling acceptor migration (and stabilization?) and access migration, respectively. Identical B components prevent access resulting in incompatibility; difference only at B confers quasi-compatibility; difference at both A and B components confers full compatibility. Production of reversed progeny is dependent on a switch in the B component to its counterpart from the field heterokaryon.

Interpreted as being due to stabilized access migration, acceptor migration not occurring. Enhanced fruiting in transformed strains and their homokaryotic progeny indicate how a further switch to non-outcrossing could be effected, and it seems significant that homokaryons of non-outcrossing Finnish strains strongly resemble transformed British strains in their fruiting and other cultural properties (A. M. Ainsworth, personal communication).

The behaviour of the transmissible factor here reported thus seems to be closely associated with access function. The production of standard and reversed progeny by strains transformed without nuclear transfer, together with their other characteristics imply that the transformed strains behave as functional heterokaryons with nuclei associating conjugately as though of complementary mating-type. The quasi-compatibility of reversed strains with standard strains and untransformed sibs carrying the same C-alleles as F2.4 would be explained if the C-factor in reversed strains is changed to a different allelic configuration. Furthermore, the incompatibility of reversed strains with sibs carrying different C-alleles from untransformed F2.4 but full compatibility with non-sibs (Fig. 5), indicates that the changed configuration is not entirely novel, since with the C-factor being multiallelic, this would lead to full compatibility in both cases. The only explanation, perhaps analogous to the cassette system in Saccharomyces cerevisiae (e.g. Herskowitz & Oshima, 1981), seems to be the cytoplasmic carriage of non-expressed complementary mating alleles into the untransformed source homokaryon, expression of these being associated with nuclear insertion and transformation. However, to account for the quasi-compatible reaction, it cannot be the entire C-factor which is switched (this would give normal compatibility reactions between standard and reversed strains). We suggest instead that the C-factor is complex, conferring access, acceptor and stabilization functions, and that only that part conferring access (and stabilization?) is switched. As indicated in Fig. 5 this would account for the full range of reactions of reversed and standard strains. It may also provide a basis for understanding the large number, and occasional
constitutive expression, of bow-tie interactions followed by apparent quasi-compatibility amongst progeny from the transformed non-sib D1.9(t).

A major problem in interpretation concerns the heterogeneity of F2.4(t) progeny compared with the homogeneity of F2.9(t) progeny. This might be because in the source strain the factor must have been produced endogenously (it cannot have been acquired) and that this has somehow necessitated major disruption of the genome perhaps leading to chromosomal alteration which becomes manifest following recombination at meiosis. By contrast, the acquisition of the factor by a recipient strain would not necessitate major genomic disruption in this way.

The effects of transformation on fruiting are both remarkable and of practical utility in facilitating genetical analysis and manipulation. An important feature was that although greatly enhanced in them, fruiting was not confined to the functionally heterokaryotic transformed strains. It also occurred (particularly following damage, slow, dense-fast, effuse transitions, mound formation, interactions with other colonies, etc.) in their homokaryotic progeny. This indicates that the factor acts as a key unlocking the morphogenetic pathways leading to fruiting. Currently, understanding of the transition to fruiting in Basidiomycotina is difficult due to the wide range of factors, both exogenous (including light, damage, interactions with other microorganisms) and endogenous (cyclic AMP, specific inducer genes) which have been implicated (e.g. Manachere, 1980; Esser et al., 1977; Leslie & Leonard, 1979; Uno et al., 1981), together with the discrepancies between observation of abundant homokaryotic fruiting in some species (Esser et al., 1977) and the enhancement of fruiting in the dikaryotic state in others, including Schizophyllum commune (De Vries & Wessels, 1984). The pleiotropic effects of the factor we describe impinge on all these areas, and hence may provide a clue as to how the diverse mechanisms underlying fruiting may be integrated.

Finally, the effects on slow, dense-fast, effuse dimorphism and production of mycelial mounds in reverted slow, dense progeny strains is further indication of the profound disturbance of differentiation pathways brought about by the transforming factor. Again, the mechanisms require clarification, but it seems very significant that mounds in dikaryotic strains show similar characteristics and production of mycelial mounds in reverted slow, dense progeny strains is further indication of the profound disturbance of differentiation pathways brought about by the transforming factor. Again, the mechanisms require clarification, but it seems very significant that mounds in dikaryotic strains of Schizophyllum commune were produced in response to unilateral transfer between conjugate nuclei of a transposable gene on the A mating-type chromosome (Gaber & Leonard, 1981). Such transfer also involves loss of certain genes, and it may be that the reduced somatic incompatibility expression in standard source-receiver strain progeny interactions (Table 4b) could result from such loss, which could in turn provide a mechanism enabling stable association between nuclei of different genotype.

Note added in proof. Reciprocal transformation of F2.4 by F2.9(t) has recently been achieved, and resulting basidiospore progeny analysed; these showed similar characteristics and interactions to progeny of F2.9(t).

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REFERENCES


