Hyphal and Mycelial Responses Associated with Genetic Exchange Within and Between Species of the Basidiomycete Genus Stereum

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Living hyphae of Stereum hirsutum behaved similarly prior to fusion on a cellophane membrane whether they were genetically the same or different. Fusions were generally initiated within 5-10 h of mycelial interdigitation. A period (10-35 min) of interfacial expansion usually preceded opening of the fusion pore, but in certain combinations involving an Australian homokaryon, MP16-15, refractile sheaths developed around hyphal penetration pegs. Between somatically incompatible heterokaryons, the cytoplasm in fusion compartments and some adjacent compartments progressively lysed and was replaced by refractile globules and wall deposits. Self fusions other than clamp connections resulted in little or unidirectional nuclear movement via the fusion pore followed by cycles of nuclear aggregation, division and septation. This sequence also followed fusion between mating-compatible pairs and ‘bow-tie’-forming pairs of sibling homokaryons for 2-3 h and 18-28 h respectively, before the onset of central or eccentric septal erosion and nuclear migration. Following migration, numerous intercalary septa were formed and there was no repair of eroded septa. An extended non-septate phase occurred within the Australian homokaryon, MP16-15, in which rapid (>10 μm s⁻¹), pulsed, unidirectional, long-range protoplasmic streaming occurred.

Certain combinations of homokaryons of different taxonomic species of Stereum, or of different breeding strategy or geographical origin, gave rise to unilaterally extensive degenerative reactions in plate culture, sometimes accompanied by production of free crystals of (+)-torreyol. Sequential septal erosion, nuclear migration and protoplasmic lysis were all observed in the partner which became degenerate. Constitutive septal erosion, nuclear migration and intercalary septal synthesis were observed in sectors of appressed mycelium in certain homokaryons after long-term storage.

INTRODUCTION

Gene flow in natural populations of eukaryotes is conditioned by the interplay between the opposing tendencies for the rejection of non-self, which maintains individual integrity, and access of non-self, which is a prerequisite of sexual outcrossing. Homobasidiomycete fungi provide a valuable model system for studying this interplay because of the reciprocal relationship which exists in them between rejection (heterogenic incompatibility) and access responses which follow fusion between sexually undifferentiated hyphae (Rayner et al., 1984). Stereum is one of several cosmopolitan basidiomycete genera with what has been termed ‘holocoenocytic nuclear behaviour’ (Boidin, 1971) and in which outcrossing is regulated by a unifactorial mating compatibility system (Ainsworth, 1987). Correspondingly, both homokaryons arising by germination of basidiospores and heterokaryons arising from matings between homokaryons with complementary alleles at a single mating factor have hyphae with multinucleate compartments and clamp connections at some septa. In this respect Stereum differs from other species in which self and non-self hyphal interactions have been extensively studied, e.g. Schizophyllum commune (Niederpruem, 1980a, b; Nguyen & Niederpruem, 1984; Todd & Aylmore, 1985) and Coriolus versicolor (Aylmore & Todd, 1984a),
where homokaryons are strictly monokaryotic, mating-type heterokaryons are dikaryotic with clamp connections and the mating system is bifactorial. On the other hand it resembles Phanerochaete velutina, whose hyphal interactions were described by Ainsworth & Rayner (1986) and Aylmore & Todd (1986a, b).

In this paper we report on cytological events during and after self and non-self hyphal fusions in Stereum, and relate these to interaction patterns between whole mycelia. We draw particular attention to the extensive degeneration of one of the mycelial partners, following its invasion by non-self nuclei, which can occur in combinations of strains of different taxonomic species, breeding strategy or geographical origin.

METHODS

Strains and pairings on plates. Fruit bodies of Stereum were collected and supplied to us from different parts of the world as shown in Table 1. Homokaryotic strains were obtained respectively from single basidiospores and excised tissue from these fruit bodies isolated onto 2% (w/v) malt extract agar (MA; 20 g Munton & Fison spray malt A, 20 g Lab M agar no. 2 per litre distilled water), usually containing 100 μg novobiocin ml⁻¹. Pairings within (controls) and between strains were usually made by placing mycelial disc inocula, 6 mm diameter, cut from the margin of actively growing colonies, approximately 1 cm apart in the centre of 9 cm diameter plastic non-vented Petri-dishes containing 15 ml 2% MA. The plates were incubated in darkness at 25 °C and examined weekly until the macroscopic appearance of the mycelia ceased to change rapidly (up to 4 weeks).

Pairings were initially made in all combinations amongst families of 15–20 sibs (from the same fruit body), allowing identification of which of the fruit bodies supplied had been produced by non-outcrossing or outcrossing mechanisms. In the former case, all sibs were morphologically identical, somatically compatible (i.e. they merged to form a uniform mycellial mat) and did not segregate into distinguishable mating-types. In the latter case, the sibs were variable morphologically and of two mating-types (criteria based on Coates et al., 1981).

Suitable tester strains representing each mating-type and nine non-outcrossing populations were selected and used to study the outcome of intraspecific non-sib pairings of S. hirsutum, and interspecific pairings between S. complicatum and S. hirsutum and between S. ochraceo-flavum and S. striatum. Examples of each interaction type were repeated one or more times and analysed further by subculturing from morphologically distinct zones and re-pairing with progenitors.

Cytological studies. Suitable strains were selected to study two or three examples of each of the following interactions typical of outcrossing, sympatric populations of S. hirsutum: self-fusions between heterokaryons; non-self fusions between mating-compatible and incompatible sibling homokaryons; self fusions, including clamp connection formation, in homokaryons and heterokaryons.

Additionally, non-sibling homokaryons were selected to study one or more examples of the following combinations of different taxonomic species, breeding strategy or geographical origin: MP16 × AF1 (one example); MP16 × EP1 (one); PB1 × EP1 (one); GP4 × F21 (two); EP3 × EP1 (one); FP3 × EP18 (one); K10 × EP1 (one); AF1 × JG2 (one); AF1 × JG1 (three); F2 × GP3 (one); EP1 × JG1 (one); EP18 × JG1 (one); JG3 × PB2 (three).

Preparatory to the cytological studies, mycelia of all these strains were grown on cellophane dialysis membranes (autoclaved separately in distilled water at 69 kPa for 20 min) overlying 0.02% MA. For all self fusions and pairings between heterokaryons, Australian and British homokaryons, and sibling homokaryons except those from Australia, a piece of cellophane (≤ 10 mm²) was then cut from the periphery of a single colony (self fusions) or to include growing margins from two adjacent colonies (non-self interactions) and loaded into a microculture chamber (for details, see Aylmore & Todd, 1984b). After overnight incubation at 25 °C in darkness, the chambers were transferred to a Wild M20 microscope with camera attachment and observed at 22–25 °C by phase-contrast optics over a period of up to 36 h. Photomicrographs were taken on Kodak Technical Pan 2415 film. A total of 20 cases of self fusion and five each of non-self fusion between heterokaryons, mating-compatible sibling homokaryons, incompatible sibling homokaryons and between Australian and British homokaryons were followed.

This protocol was altered for two pairs of Australian sibling homokaryons and the remaining non-sibling homokaryons. In these cases, similarly sized pieces of cellophane with overlying freshly interacting mycelia were transferred to sterile glass microscope slides coated with 0.02% MA and covered by a sterile coverslip. These interactions were allowed to recover for 1–2 h before 70 examples were observed and representatives photographed.

Sectors of appressed mycelium were also observed within five homokaryons from Britain and the Soviet Union after at least 2 years storage at about 5 °C under liquid paraffin. Representatives were photographed in situ in dishes of 2% MA.
Table 1. Origin of strains of Stereum used in pairing experiments

<table>
<thead>
<tr>
<th>Species and breeding strategy</th>
<th>Location</th>
<th>Substratum</th>
<th>Fruit body code</th>
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<td>Carpinus</td>
<td>EP1</td>
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**RESULTS**

Self and non-self responses prior to and at hyphal fusion

Few fusions occurred until 5–10 h after mycelial interdigitation. Before then apices repelled one another, oriented themselves parallel to lateral walls, ceased extension before or after lateral wall contact, and passed other hyphae without any apparent response. Thereafter, fusions occurred in rapid succession, frequently opening between appressed lateral walls (Fig. 1a) or establishing H-connections (Fig. 1b, c, e–j) between hyphae in close proximity. All compartments except old, extensively vacuolated ones could fuse. Some were observed with two open lateral wall fusion pores and a few had apices which simultaneously fused with a pair of compartments (Fig. 1c).

Curvature (homing) of apices to specific sites in lateral walls was rarely observed and only took place over a maximum distance of 40 μm. Such long-range fusions were usually tip-to-side (Fig. 1d, k) whereas H-connections almost invariably involved tip induction from a nearby lateral wall followed by tip-to-induced tip fusion (Fig. 1e–g) and occasionally short-range homing of the induced tip (Fig. 1h–j).

A period (10–35 min) of interfacial expansion preceded pore opening, which then completely or incompletely (Fig. 1l, m) eroded this region. Hyphal contact involving the Australian homokaryon, MP16-15, either with its mating-compatible sibling MP16-1 or the British homokaryon AF1–6 often resulted in a phase during which one hypha developed a penetration peg. A sheathing deposit of refractile material underlying the lateral wall of the invaded hypha formed around the peg (Fig. 1n). Fusion ensued if this sheath was breached, but not if it remained intact.
Fig. 1. Responses prior to and during hyphal fusions in *S. hirsutum*. (a, b) Self fusions within a heterokaryon; arrows indicate (a) fusion pore between appressed lateral walls and (b) H-connections. (c, d) Self fusions within a homokaryon showing (c) an H-connection simultaneously involving one apical and two recipient intercalary compartments and (d) tip-to-side fusion following curvature of a main hyphal apex through 90°. (e–g) Sequence showing H-connection formation within a heterokaryon involving (e) hyphal tip induction (T) from a recipient site in the lateral wall, (f) tip-to-induced tip
Hyphal interactions in Stereum

Events at and after self fusions

In plate culture, self-pairings of all the strains resulted in the merging of both mycelia into a uniform mat. At the hyphal level, the sequence of events was similar in heterokaryons and homokaryons and an example of the latter is shown in Fig. 2(a–f). Opening of the fusion pore was marked by a bidirectional flow of cytoplasmic granules which was sometimes followed by an unpredictably unidirectional passage of nuclei, often interspersed with vacuoles. After between 16 min and 4 h, nuclei derived from one or both participant hyphae aggregated in the fusion compartment, but not in the pore itself, and entered a division cycle. Nuclear membrane dilation began over a 5 min period and then the nuclei concerned faded from view for 5–10 min before smaller, globular division products became visible. Cytoplasmic granules aggregated at the site of nuclear division, heralding septal synthesis, which became visible 8–14 min after the daughter nuclei. Fusion compartments could be further reduced in volume by a second cycle of nuclear aggregation and division within as little as 10 min, but could also increase in volume by further hyphal fusions.

A similarly timed sequence of nuclear aggregation and division, involving up to six nuclei and followed by septation, regularly occurred in extending apices and intercalary positions throughout the mycelium, even in the absence of nearby hyphal fusions. During the early phase of vigorous hyphal extension in the microculture chamber, apical septation was accompanied by clamp connection formation (Fig. 2g–j), with an interval of about 15–30 min between septation and hook cell outgrowth at the next site of nuclear division. Hook cells were frequently seen with two entrapped daughter nuclei, but one (Fig. 2k) or three (Fig. 2l) nuclei were sometimes present in hooks which successfully fused with the subtending hypha.

Events at and after non-self-fusions within sympatric populations

Fusions between heterokaryons. On plates, pairings between different heterokaryons resulted in somatic incompatibility reactions, mutual rejection leading to formation of a demarcation zone of sparse, typically pigmented mycelium, localized to the interaction interface as described by Coates et al. (1981).

Fusion pore opening between hyphae was followed by a bilateral lytic reaction (Fig. 3) in which nuclei only traversed the pore during an initial equilibration of intrahyphal pressure. Slowing of mitochondrial and cytoplasmic granular motion preceded progressive vacuolation with accompanying localized highly refractile wall accretion and production of similarly refractile globules. The latter were sometimes the only remaining intrahyphal structures in the fusion pore region within as little as 2.5 h. Nevertheless, apices of some fusion compartments maintained visible Spitzkenkörper with continued extension and septation during this time. During the next 12 h, vacuolation and lysis spread into neighbouring compartments past intact septa, including ones synthesized after pore opening. Localization of the lytic reactions resulted in fragmentation of the colony into living islets which could then sprout and found new growth centres.

Fusions between mating-compatible and bow-tie forming sibling homokaryons. Mycelial interactions between sibling homokaryons from outcrossing populations were as described by Coates et al. (1981) and Coates & Rayner (1985). Compatible matings led to the bilateral emergence of a vigorous secondary mycelial phase, morphologically distinct from the progenitor contact and (g) fusion pore opening (arrowed). (h–j) Similar sequence which differed in that fusion followed induced hyphal tip curvature (T). A nucleus (arrowed) has migrated into the H-connection from the induced tip. (k) Pre-fusion tip-to-side contact within a heterokaryon following curvature of a hyphal apex towards a hook cell of a paired clamp connection. (l, m) Self fusion within a heterokaryon involving constriction (arrowed) of a previously globular organelle during (l) and persisting after (m) passage through a fusion pore which had been incompletely open for 48 min. (n) Refractile sheath (S) within an Australian homokaryon underlying a penetration peg (arrowed) extending from a British homokaryon. Bar markers represent 5 μm (n), 10 μm (a, c, e–m) and 50 μm (b, d). Time (min) is indicated in the upper right-hand corner of (e–g), (h–j) and (l, m).
Fig. 2. (a–f) Self fusion within a *S. hirsutum* homokaryon: (a) vacuole (V) traversing the fusion pore and entering the H-connection; (b–e) passage of a nucleus (N) across the H-connection; (f) septum (S) formed following and at the site of nuclear aggregation and division. (g–j) Clamp connection formation in a *S. hirsutum* heterokaryon: (g) hook cell initiation (H); (h–j) daughter nuclei (e.g. N), some of which are trapped in the hook cells. (k, l) Clamp connections in *S. hirsutum* homokaryons: (k) single nucleus (N) trapped in a non-septate hook cell adjacent to an aggregation of cytoplasmic granules (G) at a site of septal synthesis; (l) three nuclei (N) trapped in a septate hook cell following nearby septation in the subtending hypha. The bar marker represents 10 μm. Time (min) is indicated in the upper right-hand corner of (a–f) and (g–j).
Homokaryons. 'Bow-tie' interactions, controlled by a multiallelic locus expressed in certain mating-incompatible combinations, resulted in zones of appressed mycelium spreading bilaterally or unilaterally from the interaction interface. These zones were widest at their periphery and bordered by liquid droplet exudation.

Hyphal fusions between mating-compatible homokaryons resembled self fusions in that septation following nuclear division initially reduced fusion compartment volume. However, starting 2–3 h after fusion, this situation was temporarily reversed by septal erosion prior to nuclear migration (Fig. 4). Septal erosion affected both pre- and post-fusion septa, except when the latter were formed between pairs of already eroded septa. Interestingly, erosion occurred either centrally, resulting in the disappearance of the dolipore apparatus (Fig. 4c), or eccentrically, leaving the apparatus intact (cf. Fig. 6d, below). In both cases, erosion was often accompanied by an outward bulge at the junction of the septum and lateral wall (Fig. 4c). All septa in a fusion compartment could be eroded, after which nuclei migrated either towards or away from the recipient hyphal apices. Although nuclei were subsequently seen to migrate into fusion compartments, their origins could not be traced. Hyphae containing eroded septa (migration hyphae) often fused with hyphae originating from both mycelia, and their non-septate branches became vacuolated, lysed and delimited by septa near the branch point. An extended coenocytic phase, associated with rapid protoplasmic streaming, was detected in mating-compatible combinations of Australian homokaryons. However, such streaming was only studied in detail in the interaction between the Australian homokaryon MP16-15 and the British homokaryon AF1-6, described below.

Some 20 h after initial non-self fusion, aggregations of nuclei were observed adjacent to intact septa delimiting swollen, highly refractile compartments. In one such hypha containing three compartments whose lengths were approximately in the ratio 1:1:3, the time elapsing between consecutive septal erosions was 13, 32 and 68 min respectively. Erosion of each septum was marked by a violent, pulsed squirting of organelles through the pore (Fig. 4j–l), apparently damaging and deforming many nuclei, until there was an equilibration of refractility.

Fusions between bow-tie-forming sibling homokaryons only differed from corresponding mating-compatible responses in having a longer delay before the onset of septal erosion (Fig. 4m), which occurred 8–28 h after fusion pore opening. Migration hyphae with eroded septa were also observed within appressed sectors of individual homokaryons after storage.

Mycelial interactions between homokaryons of different species, breeding strategy and/or geographical origin

The results of these interactions are summarized in Table 2. Some interactions resulted in bilateral emergence of a vigorous, stable, secondary mycelium, as in fully compatible matings between sibling homokaryons. In other cases, a narrow, pigmented zone demarcated one strain from its opposing mycelium, and neither close examination nor subculturing revealed any
Fig. 4. Fusion between sibling homokaryons of *S. hirsutum*, nuclear migration and septation. *(a, b)* Passage of a nucleus (N) from a donor to a recipient hypha. *(c)* Site of the first eroded septum in the recipient hypha marked by an outward bulge (B) at its junction with the lateral wall. *(d)* Septa (S) formed after nuclear migration from the fusion compartment. *(e–h)* A nucleus (N) passing through an incompletely eroded septum and hyphal branch point (e–g from Ainsworth, 1987). *(i)* The same septum (S1) as in *(e–h)* after further erosion, a clamp connection with eroded septa and a newly synthesized septum (S2). *(j–l)* Nuclei (e.g. N) squirting through a newly eroded septum which delimited a swollen,
visible difference from its progenitor, implying that the strain retained its individual integrity. This feature was particularly characteristic of non-outcrossing strains.

Between these two extremes, many individual strains were subject to ingress of zones of degenerate morphology, which either persisted or were superseded by further mycelial development. These zones could occupy entire mycelia or only a localized band flanking the interaction interface. Mycelium within the zones contained collapsed aerial hyphae and exuded water droplets either throughout, if extensive, or at the boundary, if localized. Wherever degenerate mycelium encountered unoccupied medium, it had a lower extension rate than unaffected mycelium, was blotchy or mottled in appearance and contained encoiled hyphae with lysed compartments and irregularly spaced proliferations of attenuate branches.

In all cases where a degenerate morphology persisted, the effects were either unilateral, exclusive to one strain, or strongly asymmetric, more extensive in one strain than the other. Some examples are shown in Fig. 5.

Four extensive degenerative reactions of Australian homokaryons in combination with British or French strains were particularly striking, resulting in the proliferation of large numbers of aerial, filamentous crystals (Fig. 5a). These crystals have subsequently been identified as the sesquiterpene alcohol (+)-torreyol (A. M. Ainsworth and others, unpublished), and were consistently produced in over 500 replicates of the pairing MP16-15 × AF1-6.

The development of subcultures from degenerate mycelium varied. Commonly, the resulting mycelium lacked aerial hyphae, was strongly pigmented and had a slower extension rate than progenitor homokaryons. A strikingly lobate colony form, derived from an American strain in combination with a USSR homokaryon (Fig. 5b), is shown in Fig. 5(c). This colony form persisted through 10 serial subcultures. Pairings between such poorly-growing subcultures and their progenitors resulted in extensive degeneration of the homokaryon originally most affected.

Alternatively, subcultures from degenerate zones resulted, sometimes following sectoring, in development of vigorous mycelium, with extensive aerial growth. Such vigorous mycelium commonly resembled, or appeared identical to, the dominant (i.e. least affected) progenitor, even when sampled from well within the other strain. In interactions between S. complicatum and S. hirsutum, this situation was associated with the development of tufts of aerial mycelium in the degenerate S. hirsutum partner (Fig. 5d). In the case of S. striatum versus S. ochraceo-flavum, it was clearly associated with mycelial penetration by the former (Fig. 5e, f). Mycelial penetration was also common in non-outcrossing/outcrossing interactions, but here was not usually preceded by a degenerate reaction.

Subcultures from the torreyol-producing interaction, MP16-15 × AF1-6, yielded secondary mycelium from the British partner, and several disparate colony-types from the degenerate Australian mycelium.

**Fusions between homokaryons of different breeding strategy and/or producing unilaterally extensive degenerate mycelial interactions**

Fusion pores opened in all these interactions (e.g. Fig. 6a), and whilst no change was detected in non-outcrossing participants, migration hyphae developed in all outcrossing participants and within appressed sectors. These hyphae contained centrally and eccentrically eroded, clamped (Fig. 6b, c) and unclamped (Fig. 6c-e) septa, which were traversed by nuclei, as well as intact intercalary septa as occurred in mating-compatible and bow-tie-forming sibling combinations. Unlike these latter, however, most of the migration hyphae in degenerative combinations of homokaryons subsequently lysed with accompanying deposition of refractile material on walls and septa – as occurred near fusion compartments between somatically incompatible heterokaryons (see above).

In the Australian–British interactions (involving strains MP16-15 and AF1-6) lysis was observed ≥ 20 h after initial hyphal fusion and septal erosion produced an extended non-septate

highly refractile compartment (above). (m) Paired clamp connection with septa which have been eroded during a ‘bow-tie’ interaction. Bar marker represents 10 μm. Time (min) is shown in the upper right-hand corners.
Secondary mycelium is a vigorous, stable, mycelial phase morphologically distinct from progenitor homokaryons. ‘Degenerate morphology’ denotes zones of mycelium with collapsed aerial hyphae; exuded watery droplets; lysed, encoiled and abnormally branched hyphae. Isolate codes are as given in Table 1. All intraspecific, non-sibling combinations of outcrossers not specified led to bilateral emergence of secondary mycelium (160 cases). o, outcrossing; no, non-outcrossing breeding strategy.

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<th>Identity and origin of ‘participant A’</th>
<th>Identity and origin of ‘participant B’</th>
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<th>Persistent degenerate morphology only in B</th>
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<tr>
<td>S. hirsutum, no, Scandinavia, USSR, S. hirsutum, o, UK (AF1, F2), Romania (K6–12, EP2, 3, 11, JS1, 2, 4, N1)</td>
<td>S. hirsutum, o, USA (PB1, 3, 4)</td>
<td>36</td>
<td>0</td>
<td>36 (R)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Australia (MP5, 13, 16–19)</td>
<td>54</td>
<td>0</td>
<td>54 (R)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>USSR (EP1, 18)</td>
<td>108</td>
<td>0</td>
<td>108 (R)</td>
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<tr>
<td></td>
<td>USA (PB1, 3, 4)</td>
<td>36</td>
<td>0</td>
<td>36 (R)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* These interactions involved either temporary degenerate morphology superseded by regeneration of the progenitor (D), or an unchanged morphology associated with a narrow pigmented, demarcation zone (R).
† Following temporary degenerate morphology.
phase, predominantly in the Australian strain. Extensive, sustained (up to $\geq 30$ min within an individual hypha), rapid ($\geq 10 \mu m \ s^{-1}$), pulsed, unidirectional protoplasmic streaming occurred within this phase, mostly away from the British strain towards peripheral Australian hyphal apices. Between these pulses, mitochondria and nuclei resumed their normal, independent movements, but during the pulses they were carried with the flow. The process of streaming, which we have recorded on videotape, resulted in some migration hyphal compartments coming to contain very few organelles, whilst others became densely packed, congealed and highly refractile. By $36$ h after initial hyphal fusion, new growth centres were initiated in the Australian strain from isolated compartments which had been unaffected by protoplasmic lysis.

**DISCUSSION**

Our observations imply a complex interplay between mechanisms maintaining or overriding the genetic integrity of individual *Stereum* mycelia, which perhaps can most easily be visualized in the form of a flow diagram. That depicted in Fig. 7 illustrates a hierarchical sequence of recognition response options, each of which is in turn preconditioned by selection of a particular previous option: it is based both upon the current study, and on work with basidiomycetes other than *Stereum*.

The primary option concerns whether or not a mechanism can be implemented which brings potential fusion sites into contact. If not, then the potential for genetic interchange through fused protoplasts is obviated, as occurs, for example, between different, reproductively isolated populations ('anastomosis groups') of *Rhizoctonia solani* (= *Thanatephorus cucumeris*) (e.g. Anderson, 1984). The operation of this mechanism is probably specific to closely related populations or taxa – and perhaps to mycoparasites, which home in on signals from their hosts (cf. Kemp, 1977; Rayner, 1986). The similarity of the precontact responses in all the interactions presently studied probably reflects affinity between the strains. By contrast, hyphal contacts, not leading to fusion, probably occur by chance or do not occur at all (due to long-range inhibition) in more unrelated combinations such as *S. hirsutum* x *S. gausapatum* and *S. rameale* or *S. ochraceo-flavum* x *S. subtomentosum* respectively. Mycelial interactions in both the latter combinations result in neither participant invading the other and rapid cell death occurring in confronting hyphae (unpublished observations).

Mechanisms leading to contact may be telemorphotic (induction of hyphal tip formation at a distance) or tropic (homing). In *Phanerochaete velutina*, long-range homing over up to $250 \mu m$, leading to tip-to-side fusions, was common, as was curvature of longer 'donor' to shorter 'recipient' branches during the formation of H-bridges (Ainsworth & Rayner, 1986). Consequently we proposed that incipient branch initiation sites in the lateral wall of recipient hyphae instigated and directed the process. However, in *Stereum*, long-range homing leading to tip-to-side fusion was much rarer and occurred over shorter distances than in *P. velutina*, and it was the shorter rather than the longer hypha which exhibited curvature during H-bridge formation. This suggests that the instigative roles of branch apices and initiation sites may vary in different fungi.

After contact, protoplasmic continuity depends on opening of the fusion pore. If the pore does not open, further progress may involve, apart from renewed growth away from the contacted hypha, either penetration or encoiling of the latter, responses which are more commonly associated with mycoparasitism (e.g. Barnett & Binder, 1973). The formation of penetration pegs in the Australian strain, MP16-15, by both sibling and British interaction partners suggests that this is due to intrinsic properties of MP16-15, perhaps associated with delay in fusion pore opening and (+)-torreyol production. Similar penetration pegs have been observed between certain strains in anastomosis group 2-2 of *Rhizoctonia solani* (Yokoyama & Ogoshi, 1986). Encoiling occurred in a wide variety of interactions, including the bow-tie, which has been shown to be under allelic control (Coates & Rayner, 1985). Certain abnormal 'self-parasitic' strains have been detected in *S. hirsutum*, which both encoil their own hyphae and are unilaterally encoiled in interactions with normal outcrossing homokaryons: production of these
Fig. 5. Unilaterally degenerative mycelial interactions between homokaryons of *Stereum*. (a) British-Australian interaction in which the lower half of the dish (British inoculum) contained secondary mycelium whilst the upper half (Australian) developed a persistently degenerate morphology beneath an abundance of filamentous (+)-torreyol crystals (arrowed). (b) USSR-USA interaction, showing degeneration of the USA homokaryon (upper inoculum). (c) Subculture from a degenerate USA mycelium, similar to that in (b), showing lobate morphology. (d) *S. complicatum*- *S. hirsutum* interaction, showing degeneration and emergence of aerial mycelial tufts (arrowed) within *S. hirsutum* (upper inoculum). (e) Interaction between *S. striatum* (upper) and *S. ochraceo-flavum*, showing bow-tie-shaped degenerate zone lying between pigmented rejection zones at the interaction interface (P) and the border (B) of remnant *S. ochraceo-flavum*. There is some vigorous mycelium spreading into the
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Fig. 6. (a–e) Hyphae involved in interactions between homokaryons of *S. hirsutum* and *Stereum complicatum*: (a) non-self fusion pore (P); (b) clamp connection with eroded septa in *S. hirsutum*; (c) intact (I), centrally (C) and eccentrically (E) eroded septa in *S. complicatum*; (d) eccentrically eroded septum with apparently intact dolipore apparatus and lateral wall bulge (B) in *S. complicatum*; (e) centrally eroded septum with lateral wall bulge (B) in *S. hirsutum*. Bar markers represent 5 μm (b, d, e) and 10 μm (a, c).

abnormal strains appears to be under simple genetic control (Rayner & Coates, 1987; A. M. Ainsworth, D. Coates & A. D. M. Rayner, unpublished).

Events following protoplasmic continuity depend on whether or not there is recognition of non-self. Self fusions are characterized by the absence of protoplasmic lysis or septal erosion and nuclear migration. However, previous studies indicated that a fundamental distinction existed in the behaviour of the holocoenocytic species *P. velutina*, and species such as *Chondrostereum purpureum*, *Coriolus versicolor* and *Schizophyllum commune* with strictly monokaryotic and dikaryotic hyphal compartments. Whilst self fusions in the latter species were associated with a 'nuclear replacement' reaction involving the destruction of resident nuclei in recipient compartments, those in *P. velutina* involved initiation of a division cycle and septation (Ainsworth & Rayner, 1986). In this respect the holocoenocytic *Stereum* species presently studied resembled *P. velutina*, although they differed from the latter in that septa did not form directly across the fusion pore following nuclear aggregation and division.

Recognition of non-self can result in two very different responses: acceptance and rejection. The balance between these responses varies, and is important in understanding gene flow and competition within basidiomycete populations. Rejection, which involves initiation of a programme of cell death obviating parasitic or genomic takeover (see below), is typically localized, as observed between different heterokaryons in the present study. However, it may also have contributed to the lysis in extensive degenerative reactions between homokaryons which occurred too late to prevent nuclear invasion through the sexually unspecialized hyphae.

degenerate zone from *S. striatum*. (f) Subculture (centre) from a degenerate zone between *S. striatum* and *S. ochraceo-flavum* paired with the progenitor strains. The *S. ochraceo-flavum* (lower) strain became degenerate near the confrontation zone, whilst a *S. striatum* mycelial front (arrowed) penetrated into the degenerate zone.
As implied above, similar cell death responses occur in interspecific mycelial interactions either on contact in 'hyphal interference' reactions (Ikediugwu & Webster, 1970) – as between S. hirsutum and S. gausapatum – or at long-range – as between S. rameale or S. ochraceo-flavum and S. subtomentosum. The requirement for hyphal fusion to initiate this response within species but not between species presumably reflects the degree of genetic difference between the interactants.

Non-self acceptance is specific to outcrossing homokaryons, and involves initiation of nuclear migration pathways. There have been numerous studies of the rates of nuclear migration and genetic control of its initiation by the B-mating-type factor in fungi such as Schizophyllum commune and Coprinus cinereus with bifactorial mating compatibility (e.g. Raper, 1983). However, the patterns and pathways of migration, how they are related to secondary mycelium
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emergence or degenerative reactions, and their occurrence in species with unifactorial compatibility systems have been relatively neglected. Moreover, the mechanisms of nuclear migration are still uncertain, although septal erosion by R-glucanase, protoplasmic streaming and microtubule-assisted processes have all been implicated (Raper, 1983).

With respect to septal erosion, a significant observation in the present study was the occurrence of both central and eccentric dissolution of the septal wall, the latter process leaving the dolipore apparatus apparently intact. Since only the septal swelling is composed of pure R-glucan, most authors have emphasized the removal of the dolipore apparatus by R-glucanase as the primary determinant of migration (e.g. Giesy & Day, 1965; Mayfield, 1974; Marchant & Wessels, 1974; Casselton, 1978). However, the occurrence of eccentric erosion, which has also been recorded by Jersild et al. (1967) and Bistis (1970) adds another dimension to the process.

With respect to nuclear migration per se, although the involvement of both independent migration and protoplasmic flow has been reported and acknowledged (e.g. Niederpruem, 1980a; Raper, 1983) the former was regarded as the dominant process and the latter as at most partial and limited, with a rate not more than 10-20-fold that of mycelial extension. Whilst both mechanisms were observed during the present study, the sustained and extensive streaming seen in coenocytic hyphae in interactions involving the Australian strain MP16-15 represents a significant departure from previous observations. Its rate, at about 100-fold greater than that of mycelial extension, is more in keeping with the very rapid nuclear migration rate reported by Ross (1976) in Coprinus congregatus.

With regard to genetic control and pattern of nuclear migration, the occurrence of the process in the bow-tie interaction strengthens the proposal that the multiallelic factor responsible is homologous with the B-mating-type factor in bifactorial fungi (Coates & Rayner, 1985). Moreover, it may, after duplication and transposition from the compatibility factor to another chromosome, represent an intermediate stage between unifactorial and bifactorial control. The delay before onset of migration compared with mating-compatible interactions may relate to the fact that migration into hyphae existing prior to non-self fusion is restricted, associated with expression of a rejection reaction (Coates & Rayner, 1985; P. Scard, personal communication).

Given that nuclear migration occurs, the next issue concerns whether or not the invading nuclei form a stable relationship with resident nuclei and cytoplasm. If they do, then a stable secondary mycelial phase (mating-type heterokaryon) will emerge. If they do not, a variety of possibilities opens up, all or some of which may contribute to degenerative interactions and their sequels: (1) post-migrational cell death responses and other adverse effects may result in weak growth of the heterokaryon; (2) varying proportions of nuclei may occur in different parts of the same mycelium, resulting in different growth patterns; (3) one nuclear type may gain a competitive advantage over the other type by genomic suppression, superior division rates or active destruction; (4) fusions between nuclei and internuclear genetic transfer may generate recombinant types. Some of these possibilities, which have far-reaching implications in evolution and speciation (Rayner, 1988), are illustrated by phenomena which have been detected in organisms other than basidiomycetes.

One of these phenomena involves what has been called ‘post-fusion somatic incompatibility’ between plasmodia of the myxomycete Physarum polycephalum. This phenomenon involves invasion of the protoplasm by nuclei from dominant ‘killer’ phenotypes, associated with the destruction of resident nuclei of ‘sensitive’ strains. The latter nuclei are enclosed in vacuoles and expelled from the cytoplasm but also frequently undergo fusion with the invasive nuclei (Lane & Carlile, 1979).

Another relevant phenomenon has been reported in a heterokaryon of the ascomycete Neurospora crassa containing leucine-dependent and -independent nuclei. Displacement of the independent by the dependent nuclei led to self-extinction on minimal medium (Ryan & Lederberg, 1946). Also in certain N. crassa heterokaryons, nuclei carrying a ‘weakly dominant’ allele I are able to inhibit multiplication of i nuclei, hence producing an I homokaryon, if the proportion of the I nuclei is ≤ 70% (Pittenger & Brawner, 1961).

The latter case involves purely nuclear interactions within a common cytoplasm. However, an important role for cytoplasmic factors in many unilateral degenerative reactions (e.g. British-
Australian) is evident from the fact, that these can follow reciprocal nuclear exchange, and hence the association of the same nuclei in different cytoplasms. This finding has now been substantiated by studies of restriction fragment length polymorphism in nuclear and mitochondrial DNA (unpublished).

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