protoplasts from pollen tetrads of Luffa cylindrica and Lycopersicon esculentum when treated with 5% cellulase, whereas within the same period about 70–80% isolated protoplasts was obtained in the case of Cajanus cajan and Zea mays. Young pollen tetrads were found to be most responsive to the enzyme treatment, with resulting release of protoplasts. Spherical, non-vacuolate protoplasts thus isolated were fairly uniform in size, having a large nucleus at the centre (figure 1). Pollen grains did not yield release of protoplasts, probably because of their exines being not responsive to either of the enzymes tested.

During the isolation process, less than 3% spontaneous fusion was observed. Even after following the method developed by Ito and Media[15] to cause spontaneous fusion, we did not observe more than 5% fusion. Agglutination or fusion was not induced through high or low temperature treatments.

Neither 0.45 M mannitol nor 0.56 M sucrose could induce fusion even after 4 h of treatment. But treatment with 0.4 M sodium nitrate for 4 h induced about 8–10% fusion. Over plasmolysis resulted when 1 M sodium nitrate was used. However, the most significant result was observed through treatment with Keller and Melchers's[13] high pH and high calcium fusion solution with the modification. Adherence of the naked protoplasts could be seen even after 10 min of treatment (figure 2). The rate of agglutination and fusion increases with the increase of time and after 30 min of treatment about 70–80% of agglutination was observed. Fusion of the adhered cells proceeds quickly to a dumb-bell shape structure (figure 3) followed by formation of a spherical shape (figure 4) with the mixing of the cytoplasm. In about 5% cases, nuclear fusion was observed after about 10 h of culturing. When several protoplasts were seen to fuse together, multilobed structures were evident, which after a lapse of time rounded off.

Work is in progress to find appropriate cultural conditions necessary for these haploid protoplasts for carrying out elaborate somatic genetics studies.

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Interspecific protoplast fusion and complementation in *Aspergilli*

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Summary. Protoplast fusion and nutritional complementation between auxotrophic mutants of *Aspergillus nidulans* and *Aspergillus fumigatus* has been achieved. It is concluded that the nutritional complementation may be due to interspecific aneuploidy.

We previously reported[2] that high-frequency intraspecific protoplast fusion and heterokaryon formation were obtained in *Aspergilli* and *Penicillia*. We now describe successful experiments to fuse protoplasts of auxotrophic mutants of taxonomically distant *A. nidulans* and *A. fumigatus*, resulting in nutritional complementation and interspecific aneuploidy of hyper-haploid type.

Material and methods. Stable mutants requiring lysine (lys) and adenine (ade) were produced by UV-irradiation from *A. nidulans* R21 (yellow conidia, and requiring *p*-aminobenzoic acid)[3,1] and *A. fumigatus* 5085 (wild-type)[1]. Back-mutation has never been observed with these mutants. The methods of protoplasts formation, fusion with polyethylene glycol (PEG) and regeneration were carried out under optimal conditions[2]. The complemented colonies were selected on minimal medium containing *p*-aminobenzoic acid (PABA). The complementation frequency is expressed as the number of colonies developing after PEG treatment in minimal medium compared to the number growing in yeast-extract medium. The method of staining conidial nuclei was based on that of PUHALLA[4].

Result and discussion. In PABA-containing minimal medium protoplasts were able to regenerate and develop into colonies in low frequency after interspecific protoplast fusion had been induced between *A. nidulans* lys and *A. fumigatus* ade or *A. nidulans* ade and *A. fumigatus* lys. We shall deal here merely with cases when only *A. nidulans* could be recovered from the interspecific fusion products; opposite cases, when only *A. fumigatus* could be regained, are also known and will be reported elsewhere. The main characteristics of these interspecific products are as follows.

In interspecific protoplast fusion, the complementation frequency was of the order of 10^{-5} whereas in intraspecific fusion as high as 40 to 60% can regularly be attained[2,5]. Complementation was never observed when mycelia of the two species were mixed and incubated in an attempt to achieve hyphal fusion, whereas intraspecifically complementation is common with these mutants and with others[6–9].

The colonies resulting from interspecific fusion were thick, irregularly growing and differed markedly in appearance from intraspecific ones (Figure 1). The hyphae often exhibited deformations (Figure 2). Conidium-formation was infrequent, with one nucleus in each conidium. Diploidization has never been found. Nevertheless, conidia from interspecific colonies were able to germinate

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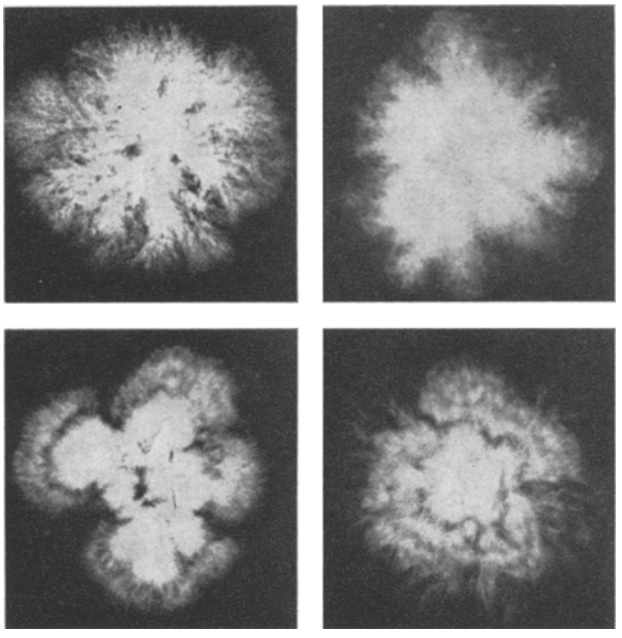

Fig. 1. Complemented colonies resulting from intraspecific and interspecific protoplast fusion on minimal medium. Top left: *A. nidulans* ade + *A. nidulans* lys. Top right: *A. fumigatus* ade + *A. fumigatus* lys. Bottom left: *A. nidulans* lys + *A. fumigatus* ade. Bottom right: *A. nidulans* ade + *A. fumigatus* lys.

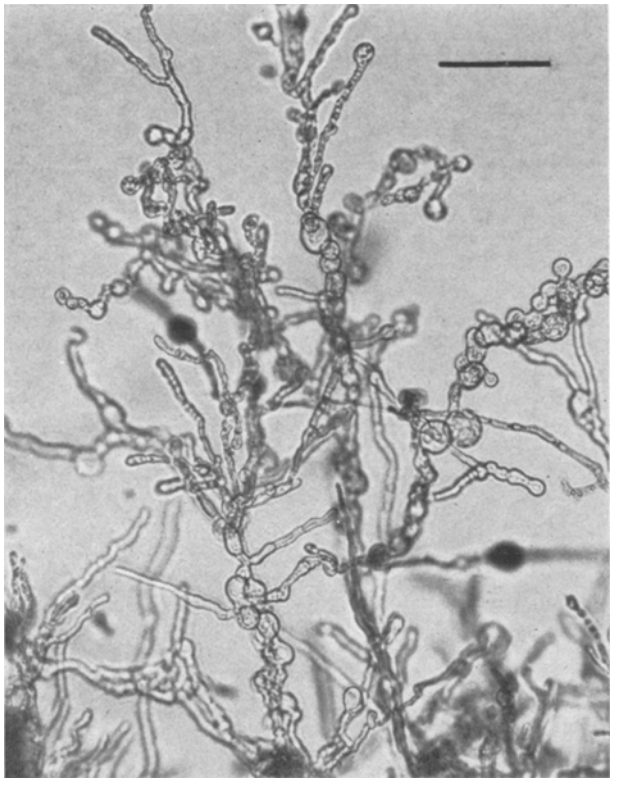

Fig. 2. Hyphae exhibiting characteristic deformations in the interspecific fusion product of *A. nidulans* lys + *A. fumigatus* ade. Marker represents 50 µm.

on minimal medium and develop into typical interspecific colonies. Haploid conidia in intraspecific colonies did not germinate on minimal medium, because of segregation into the parental auxotrophic mutants.

The interspecific mycelia could be cultivated and maintained indefinitely on minimal agar medium. The inoculated mycelia gave rise to central, thick, slow-growing colonies and rapidly-growing thin sectors fanning out from the central part. In both parts mycelia were complemented for lysine and adenine. Practically all conidia from both parts germinated on yeastextract medium or media containing the compound required by the *A. nidulans* mutant involved in the fusion. On minimal medium, almost all conidia taken from the central part germinated and developed into colonies, compared with only about 1% of the conidia from the rapidly-growing sectors.

If mycelia grown in minimal medium were transferred onto yeast extract medium, the above differences were even more pronounced. The slow-growing, thick, prototrophic central part became pigmented and produced many rapidly-growing sectors (Figure 3). Neither mycelial nor conidial inoculation from the sectors gave rise to colonies on minimal medium. In fact, the *A. nidulans* parental mutant was recovered in these cases. All these facts strongly recall the behaviour of intraspecific aneuploids of hyper-haploid type[10–12]. We explain our results by aneuploid formation between the two different species.

From a given colony we could not recover both parental mutants simultaneously or any kind of heterokaryons, despite the broad variations employed (general and selective media; mycelial and conidial inoculation; repeated protoplast formation from the fusion products and inoculation of different media with the protoplasts; 'transfusion' of *A. fumigatus* mitochondria into protoplasts of the fusion product; fusion of protoplasts derived from the interspecific fusion products of the two types).

With the parental pairs of the PABA-requiring *A. nidulans* lys and *A. fumigatus* ade, we obtained colonies which needed neither lysine nor PABA for growth; the complementation frequency was less than 10^{-8}, i.e. 3 orders of magnitude less than that with lysine alone.

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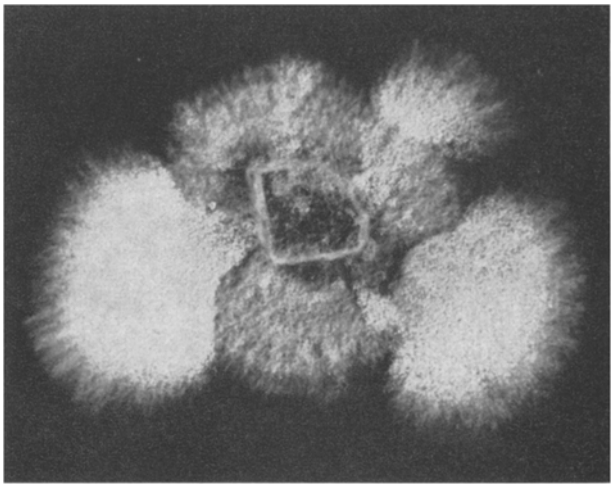

Fig. 3. Rapidly-growing sectors emerging from the slow-growing interspecific fusion product of *A. nidulans* lys + *A. fumigatus* ade on yeast-extract medium.

Consequently, no interdependency was observed between the losses of requirement for lysine and PABA. The same held for the reappearance of amino acid and PABA requirements. On a basic culture medium containing lysine but not PABA, rapidly-growing lysine-dependent sectors developed from transferred prototrophic colonies. Mycelia from these sectors became both lysine- and PABA-dependent after transfer onto media containing both compounds, and the original *A. nidulans* lys was recovered at this stage. The same result was obtained if the sequence of supplementing the vitamin-free culture medium was the reverse, with the exception that PABA-dependent sectors developed first. We assume that in these rare cases two chromosomes integrated from *A. fumigatus* into *A. nidulans*, and were then eliminated independently and rapidly when the selective pressure ceased.

The precondition of interspecific complementation in otherwise incompatible fungi may possibly be the disintegration of nuclei of one of the two species. Such a disintegration could be followed by the retention of one or a few chromosomes. This might be the reason for both the low frequency of complementation and our inability to recover both fusion partners simultaneously from a given colony.

Aneuploidy is a frequent consequence of interspecific fusion of animal cells[13,14]. Our results suggest that this might be valid on a broader scale in eukaryotes. Moreover, a directed and selective retention and propagation of foreign chromosomes might be achieved with fungi by using suitable mutants. This method may offer further possibilities in eukaryotic genetics.

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Crossing between nondiapausing and diapausing races of Sarcophaga peregrina

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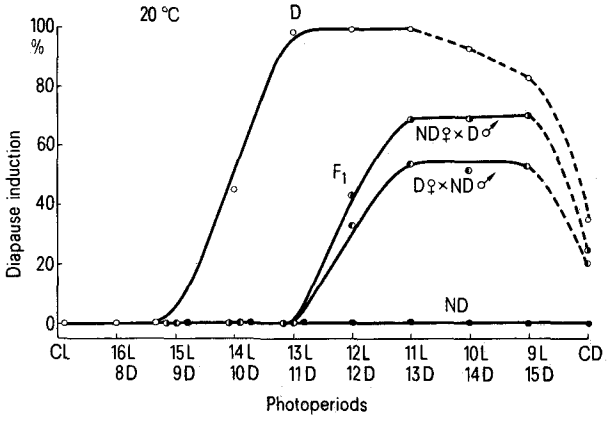
Summary. The temperate race (D) of *S. peregrina* undergoes pupal diapause in response to certain environmental conditions of photoperiod (13L:11D–11L:13D) and temperature (under 20 °C). The tropical race (ND) does not do so under the same circumstances. The tendency toward diapause was suppressed in 30–40% of the hybrids of crossings D♀ × ND♂ and ND♀ × D♂ even under such short day and low temperature conditions as 11L:13D–9L:15D, 20 °C. For entering diapause, the hybrids require a shorter day length (13L:11D) than that of (D) parents (15L:9D).

Sarcophaga peregrina Rob.-Desvoidy (Diptera: Sarcophagidae) is one of the synanthropic flesh flies which are commonly found in Japanese privies throughout Japan. Seven related species are distributed in and around the Indo-Australian area[2]. *S. peregrina* has the widest distribution, covering China, Japan, Volcano Is., Bonin Is., Mariana Is., Formosa, Hainan Is., India, Ceylon, Australia, New Guinea, New Britain, Samoa, Gilbert Is., and Hawaii. The northernmost boundary would appear to lie in Hokkaido[3]. This species is known to be a mechanical vector of aetiological agents of disease and its larvae are responsible for intestinal myiasis in these areas. Adults appear from May to September in Central Japan, and the facultatively diapausing pupa is known to be the overwintering form[4]. Under conditions of short day and low temperature, this flesh fly enters pupal diapause in Japan.



The induction of pupal diapause in Japanese diapause (D) and New Guinean nondiapause (ND) races of *Sarcophaga peregrina* and their hybrids (F_1) under different light regimes (at 20 °C). Each experiment was performed 3 times and the results averaged.

A New Guinean race whose colonies are derived from a single wild female collected in Wau (7.22 S, 146.40 E), alt. 1050–1250 m, Morobe District, in December 1973, was available for the present crossing experiment[5]. In a preliminary experiment it was found that the tropical race (ND) did not enter pupal diapause, even if parents and their offspring were bred under such light regimes and low temperature conditions as 14L:10D–12L:12D at 20 °C. A few of the offspring reared under the conditions: 20 °C, 11L:13D, however, developed into diapause pupae (2%). Nondiapausing colonies were selected and used for the crossing experiment. The 2 geographical races are morphologically distinguishable by the male genitalia, especially by the shape of the apical plate of aedeagus. Hybrid generations exhibited normal fertility of crossing. Eggs of each race are fertilized by sperms of the other, and at least some of the zygotes develop normally. Both

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