

## MATING SYSTEM IN *PHYMATODES* *SCOLOPENDRIA* (BURM) CHING

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THE gametophyte represents the sexual phase of the life cycle of a fern. It is a free-living, autotrophic, integrated multicellular system, yet small enough to be cultured in numbers *in vitro* and examined under the microscope. Gametangial differentiation in them is sequential which largely determines their mating system. Thus, observations on the growth and differentiation of the gametophyte from spore germination to emergence of sporophyte have been made to find the predominant mating system in *Phymatodes scolopendria*, a sexual diploid ( $n = 36$ ) member of the family Polypodiaceae. The spores were obtained from single and different sporophytes from Singapore, surface-sterilized with 2% sodium hypochlorite solution, washed and sown uniformly on autoclave sterilized (15 lb/in<sup>2</sup>) inorganic nutrient media of Dyer<sup>1</sup> (pH 5.8) maintained at  $22 \pm 2^\circ\text{C}$  under continuous white fluorescent light of 250–300 ft-c intensity in a culture room. Prior to the initiation of gametangia 22 days after sowing, cordate gametophytes were randomly isolated into three sets of populations, viz (a) 15 single isolated gametophytes in different petriplates, (b) 20 pairs of gametophytes, each petriplate containing one pair and (c) mixed culture of several petriplates, each containing 20 gametophytes. These gametophytes were allowed to grow until they attained sexual maturity by about 36 days; Thereafter fertilization was ensured by flooding them with a film of water more than once. Appropriate tests indicated the absence of apogamy in the population.

The spores germinated in 4 days, showing 98%

germinability. Antheridia started to initiate after 24 days of germination followed by archegonia in another five days (table 1). By and large the prothalli are protandrous so that when the archegonia are mature most of antheridia are spent up and the prothalli are functionally unisexual, earlier male and later female. However, the intervention of a brief hermaphroditic phase cannot be ruled out altogether, which raises some possibility of intragametophytic selfing in an otherwise overwhelming preponderance of intergametophytic mating. The emergence of first sporophytic leaf started in mixed culture after 52 days of germination followed by those in pairs and lastly in isolate cultures. The sporophyte production was highest viz 95.7% in mixed, medium 52.5% in pairs and lowest 6.6% in isolates, which indicated that *P. scolopendria* took to cross-breeding due to the accumulation of recessive sporophytic lethals in its genepool in the absence of incompatibility barrier<sup>2, 3</sup>. And in doing so, it has exploited the opportunities of genetic evolution population-wise by way of selection from the hybrid swarms. The results of the breeding experiment also indicated the chances of intragametophytic selfing, however small in the gametangial sequence of this species oriented towards cross breeding. It would mean that single spores blown by strong wind currents on to distant islands in the Sembilan Strait would theoretically retain the capacity of colonization by producing homozygous sporophytes. But surely, a sporophyte of this kind of genotype would be weak in a habitual cross-breeder, and therefore, the practicability of such colonizations by single spore invasions is likely to be low. For its extension, therefore, *P. scolopendria* has to depend largely on multispore invasion process, which in other words, renders the species as a bad colonizer. A survey of its distribution in and around Singapore corroborated this theoretical deduction by the revelation of its poor occurrence in

Table 1 Sex ratio in composite culture of *P. scolopendria*

Days after sowing	Sample size	Sterile	Male	Female	Hermaphrodite
25	40	40	—	—	—
28	40	38	2	—	—
30	40	36	4	—	—
33	40	5	34	1	—
35	40	—	34	4	2
38	40	—	8	28	4
40	40	—	—	32	8
45	40	—	—	35	5
48	40	—	—	38	2

**Table 2** Breeding test for hybridization of *P. scolopendria*, sporophyte formation in isolate, pair and mixed culture

Sex expression	Population	No. of gametophyte studied	No. of gametophyte produced sporophyte	%Gametophyte producing sporophyte
Male to hermaphrodite and female	Isolate (15 gametophytes)	15	1	6.6%
	Pairs (40 gametophytes)	40	5 × 2 = 10 11 × 1 = 11	52.5%
	two gametophytes per petriplate Mixed (20 gametophytes per petriplate) 20 petriplates	400	383	95.7%

these places. The 50% of sporophyte formation in pairs of gametophytes in the present series of experiments is probably not significant because there are equal chances of the members constituting the pairs to be sibs or nonsibs as no particular care was taken to keep them apart in this way. This is perhaps evident from the result of the percentage of emerging sporophytes from such cultures, which deviated significantly from those of the composite cultures (table 2).

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## EFFECT OF ALTERED SURFACE TENSION ON HYPHAL MORPHOGENESIS IN SOME FUNGI

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FUNGAL hyphae, generally form branches, some distance behind the apex, alternately on the two sides. These laterals may branch further in a similar manner. This system of branching has been found to be optimal

for absorbing nutrients from substratum<sup>1</sup>. Nutritional<sup>2, 3</sup>, chemical<sup>4-6</sup> as well as physical factors such as temperature<sup>7</sup>, osmotic shock<sup>8</sup> and the physical nature of the surface<sup>9</sup> are known to influence hyphal branching. However, the influence of an important physical factor viz surface tension on hyphal differentiation is not worked out so far. In the present paper an attempt has been made to find out the influence of surface tension on hyphal differentiation of fungi belonging to different taxa.

Single spore isolates of *Bipolaris sorokiniana* (Sacc in Sorok) Shoemaker, *Botryodiplodia theobromae* Pat., *Polyporus versicolor* L ex Fries and *Syncephalastrum racemosum* Cohn and a single hyphal tip culture of *Rhizoctonia solani* Kuhn were used. A plug of mycelium cut from the margin of an actively growing colony was placed on Czapek-Dox agar medium amended with Tween 80 (polyoxyethylene sorbitan monooleate-Sigma). This is a non-toxic neutral detergent and was added to the medium to give a final concentration of 0.5, 0.8 or 1.0%. Growth was observed after 48 hr of incubation at 30 ± 1°C. The number of hyphal tips formed was counted and the results were analyzed using student's *t* test.

Addition of surface tension depressant to the medium increased significantly the number of branches formed in both aseptate and septate fungi (table 1). The branching potential of *B. theobromae*, *R. solani* and *S. racemosum* was increased 6, 5 and 4 folds respectively when the surface tension was reduced. However, the magnitude of hyphal branching due to reduced surface tension in *B. sorokiniana* and *P. versicolor* was less than that of the other fungi studied.

Although reduced surface tension alters the growth characteristics of prokaryotes<sup>10, 11</sup>, the mechanism of