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Mating Behaviour in *Onychium siliculosum* (Desv.) C. Chr.

I P SINGH, A R S NIRANJAN and S K ROY

Cytogenetic Laboratory of Vascular Plants, Department of Botany, Banaras Hindu University, Varanasi 221005

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Onychium siliculosum of Central Himalayas was found highly adapted for inter-gametophytic selfing and crossing. Intra-gametophytic selfing was less in the species indicating the presence of significant degree of genetic load in the populations. Such high degree of genetic load can restrict the distribution which is reflected by sporadic or disjunct occurrence of the species in the investigated areas.

Key Words: Fern gametophyte, Mating system, Intra- and Inter-gametophytic selfing

Introduction

The reproductive mechanisms, which determine the distributional patterns of a fern taxon can be profitably studied in their uniquely free-living, autotrophic gametophytes. A number of morphological features such as the sequence of gametangial ontogeny, their location on the gametophyte and the frequency of unisexual gametophytes have been found to influence the determination of intra-gametophytic selfing or inter-gametophytic crossing within a given species. Inter-gametophytic crossing results in various levels of heterozygosity depending on the origin of gametes (Klekowski & LLoyd 1968). The extent of genetic load in the sporophyte of a fern is dependent upon the genetic constitution and mating system of the fern in question. If the availability of the fusing partners is unconditional, the distribution of the taxon would be expected to be even; otherwise, in the ecological niche, it would be limited. The mating system of the golden fern, *Onychium siliculosum* was examined from this point of view.

Materials and Methods

The spores of diploid (n-29) sexual species without any clonal multiplication system were collected from individuals growing sparsely in patches on ferruginous red gravel soil at lower altitudes (*ca* 1645 m) in Pithoragarh hills (Central Himalayas). These were stored in desiccator, surface sterilized with 2% sodium hypochlorite solution and uniformely sown on 25 ml of autoclave-sterilized (15lb/in^2) inorganic

medium (Klekowski 1969) at *p*H 5.3 in Petri-dishes. The plates were maintained at 24±2°C under continuous white fluorescent illumination at an intensity of 250–300 ft—c in a culture room.

Prior to the initiation of gametangia, the gametophytes were randomly isolated into four sets of populations, viz., (*i*) 25 singly isolated gametophytes, (*ii*) 25 cross cultures (each plate containing two different gametophytes originated from two different sporophytes), (*iii*) 25 paired cultures (each plate containing two gametophytes of the same parental sporophyte) and (*iv*) 25 composite cultures (each plate having 12 gametophytes of different origin). After the formation of sex organs in these populations, the plates were flooded with sterilized double distilled water twice a week to facilitate fertilization. The frequency of sporophyte production was later estimated. While one set of stock culture was watered, the other set was left unwatered to serve as an apogamous control.

Results

The spores germinated within 5 days showing 92% germinability. The adult prothallus was large, two-winged with a midrib, wing cells remaining uniformly thin and the margin undulating outwards. Sex organs remain restricted to the midrib and its adjacent parts. Antheridia started initiating after 30 days of germination on the posterior end adjacent to the rhizoids and the prothalli became bisexual within 6 days. The archegonial necks were oriented towards antheridia and rhizoids.

The production of male and female gametangia on the same gametophyte makes it morphologically hermaphroditic but functionally they always remain unisexual because by the time the archegonia mature, most antheridia on the same gametophyte are spent up. Table 1 shows that maximum antheridiate prothalli formation occurred after 40 days of germination. At the same time archegoniate prothalli were less in number and hermaphroditic prothalli were still lesser. This made intra-gametophytic selfing less probable and encouraged inter-gametophytic selfing and crossing. After 70 days one antheridial prothallus, 15 archegoniate prothalli and one bisexual prothallus were observed in a population. This type of gametangial sequence favoured intergametophytic selfing and crossing having capacity in time for intragametophytic selfing. Composite cultures yielded 81.3% sporophytes, pair cultures 70%, cross cultures 78% and isolates 40% sporophytes (table 2). This sequence and frequency of sporophyte production indicate less capacity for intra-gametophytic selfing. Crossing possible only in favourable spots permits patchy distribution of the species.

First sporophytic leaf initiation started in composite cultures after 78 days of germination followed by pair cultures and lastly in isolate cultures. At the termination of the experiment gametophytes which were unable to show the emergence of sporophytes were observed to contain dead embryos and had become bisexual at different stages of abortion, showing that fertilization had indeed occurred repeatedly but growth of sporophytic tissue was terminated early. This shows that one or more recessive lethal genes on being homozygous in the young sporophyte caused embryo abortion. The unwatered control never produced sporophytes until flooded with sterile water showing the absence of apogamy. Vegetative Propagation was also absent in this species.

Discussion

The pattern of gametangial ontogeny conferred a high probability of inter-gametophytic selfing and crossing in populations. Intra-gametophytic selfing as evident from less sporophytes in isolate cultures was impaired due to genetic-incompatibility between the

Table 1 *Chronological changes in sex ratio of a stock culture of* O. siliculosum

Days after germination	Sample size	Neuter	Male	Female	Herma-phrodite
30	25	24	1	—	—
33	25	20	5	—	—
36	25	11	12	1	1
40	25	—	18	4	3
50	25	—	10	9	6
60	25	—	4	15	6
70	25	—	1	15	9

Table 2 *Breeding test for* O. siliculosum *populations : Percentage of gametophyte not producing sporophyte in isolate, cross, pair and composite cultures*

Sex expression	Population	No. of gameto-phyte studied	No. of gameto-phyte producing sporophyte	Percentage of gameto-phyte not producing sporophyte	% of sporophyte Production	Observed lethal
Male	Isolate	25	10	60	40%	Nil
to	Pair	50	35	30	70%	+
herma-	Cross	50	39	22	78%	Nil
phrodite	Composite	300	244	187	81.3%	Nil

gametes of the same genetic constitution. It is possible that the gametophytes of isolate cultures were functionally unisexual, i.e., having either archegonia or antheridia at a given time which made fertilization impossible or, homozygosity of lethal genes resulting from intra-gametophytic selfing prevented the emergence of living sporophytes.

The frequency of sporophyte production in *O. siliculosum* was maximum in composite cultures and lowest in isolate cultures. Klekowski (1972) observed in *Pteridium aquilinum* that as the number of self-incompatibility loci decreases, the occurrence of sporophytes in pair population of gametophytes increases. Sporophyte production was 70% in pair cultures and 40% in isolates in the present taxon showing a self-compatibility system at work. Isolate cultures yielded sporophyte in *O. siliculosum*. Inter-gametophytic selfing occurred at a high frequency to eliminate all genetic variants which form lethal homozygous combinations (genetic load). This indicated that the native areas were initially colonized by homozygous sporophytes developing from self-compatible gametophytes. The possible explanation for the absence of sporophyte in isolate cultures was the genetic constitution of the zygotic genotype. The lethals were being collected as long as they remain in a heterozygous state, but would express their lethality in the zygote at any stage soon after they became homozygous (Crumpacker 1967).

According to Klekowski (1969), LLoyd (1974) and Singh and Roy (1977), the pattern

of gametangial ontogeny from male to hermaphrodite conferred a high probability of inter gametophytic selfing and crossing followed by less possibility of intra-gametophytic selfing. The results of the present study on *O. siliculosum* corroborate this view. Khare and Kaur (1979) ascribed the same gametangial ontogeny in *Cyclosorus parasiticus* to favour inbreeding system. *O. siliculosum* is initially adapted for inter-gametophytic selfing and possesses the capacity for intra-gametophytic selfing during development, thus leading one to believe that populations of this species possess less genetic diversity as reported in *Osmunda regalis* (Klekowski 1973, LLoyd 1974). Intra-gametophytic selfing predominates during the early stages of colonization of a new habitat. Thus it could be concluded that *O. siliculosum* could be a good colonizer of barren land but its mating system seems to be of little value in so far as the improvement of this character is concerned. Genetically, *O. siliculosum* could be identified as a highly heterozygous species.

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