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## RESEARCH ARTICLE

### EXPERIMENTAL STUDIES ON GAMETOPHYTIC GENERATION OF *DRYOPTERIS* SPECIES

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#### ABSTRACT

A fern gametophyte is a small, photosynthetic organism capable of normal development in culture. It has usually both the sex organs leading to the belief that self-fertilization is probably the rule. But this would lead to great deal of homozygosity and consequently little morphological variation. But it is generally found that ferns exhibit a lot of morphological and cytological variation which is due to cross fertilization. The present study is an attempt to investigate the reasons for these variations by studying the gametophytic generation in *Dryopteris caroli-hopei* Fraser.-Jenk., *D. chrysocoma* (Christ) and *D. cochleata* (Buch.-Ham. ex D.Don). Ripe spores of these species were raised on ½ Knop's nutrient medium and their growth was studied. It was noticed that in a population of gametophytes some were fast growing and some were slow growing. The slow growing gametophytes usually remain only male throughout the life.

## INTRODUCTION

Fern gametophyte is a small structure with usually both the sex organs. It is the simplest photosynthetic organism which is capable of normal development in culture. Each gametophyte can be examined intact under the microscope. Since it usually bears both the sex organs so the general tendency is to believe that self-fertilization is probably the rule. But this suggests that due to great deal of homozygosity there should be very little morphological variation. However, the general experience of the fern workers is that the ferns exhibit a lot of morphological and cytological variations which is obviously due to cross fertilization. To analyse this hypothesis various workers have done a great deal of work like Klekowski and Lloyd (1968), Lovis (1977), Walker (1979), Cousen (1979), Verma etc. In the present work the reproductive biology of three *Dryopteris* species *Dryopteris caroli-hopei* Fraser.-Jenk., *D. chrysocoma* (Christ) and *D. cochleata* (Buch.-Ham. ex D.Don) are being discussed.

## MATERIALS AND METHODS

Ripe fronds of three *Dryopteris* species were collected from Uttarkhand in the month of September- October. Ripe fronds of four to five original plants were taken from each population. These fronds were stored in sealed plastic bags and brought back to the laboratory.

The bags were then opened and the fronds were then allowed to dry for several weeks. The spores were released after drying. These spores were kept separate in paper packets. The spores were sown in early November. The spores were raised on ½ Knop's nutrient solution without supplement of sugar and/or growth hormones. Sterilized petridishes containing 20 ml of ½ Knop's were taken. A small quantity of spores was evenly spread on a piece of clean paper which was inverted over the petridish and gently tapped. An almost uniform distribution of spores is obtained on the surface of the nutrient solution.

### Observation

The spores usually germinate within 4-12 days of sowing. Usually prothallial initial is the first to appear followed by a filamentous initial. In a composite population of fern gametophytes it is usually noticed that there are some fast growing gametophytes and others that are slow growing. A few meristic prothalli were then transferred to fresh Knop's solution for observations. All the prothalli were studied periodically at 10 day intervals. These fast growing gametophytes tend to leach out the antheridiogens which hinder the growth of other growing gametophytes. These slow growing gametophytes usually remain only male throughout their life.

### *Dryopteris caroli-hopei* Fraser -Jenk

Spores pale brown, bilateral 28.0-31.5 X 35-42 µm in size with a folded perine.

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The spores germinate within 6-8 days of sowing. The spore coat cracks at the laesura to give out the prothallial and rhizoidal initial. The prothallial initial further divides to form 3-4 celled filament. The filament further divides to form a spatulate prothallus which later on assumes a cordate shape. The archegonia are produced after the formation of the midrib. The percentage of male gametophytes is 69%, percentage of female gametophytes is 46% and the percentage of fast growing gametophytes is 97% (Fig. 1).



Fig. 1. *Dryopteris caroli-hopei*, various stages of development of gametophyte and position of sex organs

#### *D. chrysocoma* (Christ)

Spores are dark brown, bilateral 35-38 X 45-49  $\mu\text{m}$  in size with a granulose perine. The spores germinate within 5-7 days. The prothallial initial is the first to be cut off. The margin of the mature gametophyte is highly uneven and dissected and almost every cell gives off a unicellular papillate gland. The wings are variously curved and ruffled. The antheridia are present on midrib intermixed with archegonia and on the wings. The archegonia appear about 26-27 weeks of sowing. The percentage of male gametophytes is 64%, percentage of female gametophytes is 52% and the percentage of fast growing gametophytes is 96% (Fig. 2)

#### *D. cochleata* (Buch.-Ham. ex D. Don)

Spores are brown, bilateral, 31.5-38.5 X 42-52  $\mu\text{m}$ , perine folded. The spores germinate within 5-7 days of sowing. The prothallus becomes cordate after 15-16 weeks.

The adult prothallus has uneven margin. The antheridia are present on the midrib intermixed with the archegonia and on the wings. The archegonia appear after 26-27 weeks of sowing. The percentage of male gametophytes is 71%, percentage of female gametophytes is 50% and the percentage of fast growing gametophytes is 97% (Fig. 3).

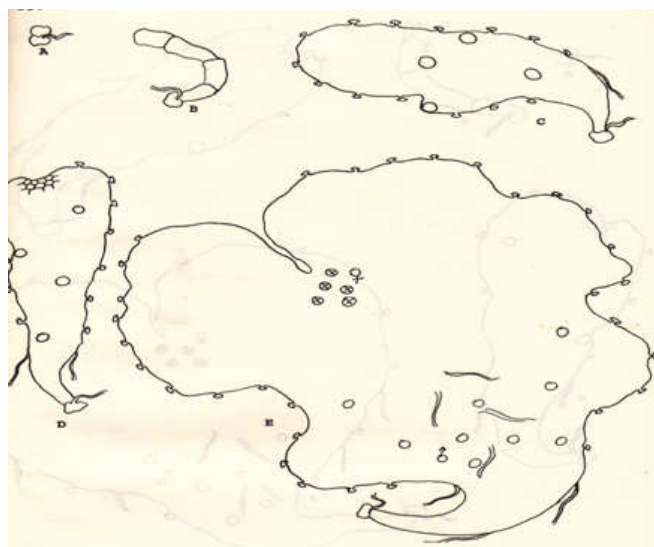


Fig. 2. *Dryopteris chrysocoma*, various stages of development of gametophyte and Position of sex organs

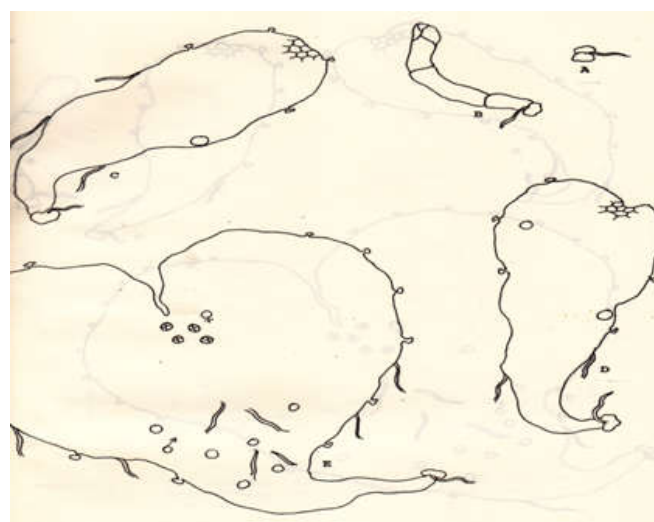


Fig. 3. *Dryopteris cochleata*, various stages of development of gametophyte and position of sex Organs

## RESULTS AND DISCUSSION

In the above three species studied antheridia were the first to appear. Bisexual prothalli originated from initially male prothalli.

S.No.	Species	Initial Sex	% of males	% of females	% of fast growing gametophytes	% of bisexual gametophytes
1.	<i>Dryopteris caroli-hopei</i>	Male	69	46	97	87
2.	<i>D. chrysocoma</i>	Male	64	52	96	89
3.	<i>D. cochleata</i>	Male	71	50	97	90

The production and persistence of males in the culture may suggest some activity of an antheridiogen system which needs to be further explored (Kurumatani *et al.*, 2001). It is now realized that not all homosporous ferns can undergo intragametophytic selfing habitually. Intragametophytic selfing promotes successful colonization in some homosporous ferns (Suter *et al.*, 2000). The ability to reproduce by intragametophytic selfing facilitates rapid spread. There are several kinds of adaptations that tend to favour intergametophytic mating like:

- different timing in the appearance of sex organs- antheridia and archegonia, at different times in the same gametophyte.
- Mutual effect of gametophytes: When a fast growing gametophyte reaches a spatulate stage certain chemicals called antheridiogens are leached out from the notch area which inhibit the growth of other gametophytes so that they grow slowly and produce sex organs a little later than the fast growing gametophytes.

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