

Studies on reproductive biology of some ferns of Kumaon Himalayas

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The reproductive biology of nine species collected from Kumaon Himalayas has been studied through *in-vitro* raised isolate and composite populations of the gametophyte. Taxa such as *Adiantum incisum*, do not show sporophyte production through intra-gametophytic selfing probably due to constitution of the zygotic genotypes and perhaps their gene pool is charged with hundred percent genetic load. On the other hand *Lygodium japonicum*, *Cheilanthes farinosa*, *Tectaria coadunata*, *Adiantum capillus-veneris* and *Pteris vittata* produced considerable amount of homozygous sporophytes through intra-gametophytic selfing. On the basis of observation, all the taxa have been categorized into two groups, i.e. (1) those having lesser genetic diversity; and (2) those having greater genetic diversity.

Since in Kumaon Himalayas the environmental conditions are very much favourable for the growth and development of gametophytic and sporophytic generations, the results of mating system correlates with the population diversity and density of the taxa. The study shows that the taxa having mixed mating are widely distributed whereas others having inter-gametophytic mating are restricted in distribution.

Key-words - Homosporous fern, Reproductive Biology, Intra-gametophytic selfing, Inter-gametophytic selfing, Gene pool, Genetic load.

INTRODUCTION

FREE living, independent haploid and diploid generations and union of egg and sperm from the same gametophyte have made homosporous ferns ideal to study the reproductive mechanism, which also determines the genetic diversity, colonizing ability and evolutionary potential of any species.

Mating patterns are generally recognized in ferns as selfing i.e. (1) within or (2) between prothalli of the same parent sporophytes, and termed as intra-gametophytic selfing and inter-gametophytic selfing, respectively (3) out crossing, between prothalli, arising from the different sporophytes of the same species is termed as inter-gametophytic crossing (Klekowski & Lloyd 1968; Klekowski 1969). A number of morphological features such as sequence of gametangial ontogeny, their locations on the gametophyte, frequency of sexual gametophyte, longevity of bisexual stage, regeneration pattern of the gametophyte, antheridiogen activity have been found to influence significantly these mating systems (Klekowski & Lloyd 1968; Soltis *et al.* 1988;

Klekowski 1969a, 1969b, 1970; Khare 2001). Taking into consideration of the above simple mechanism, several homosporous ferns were examined for their reproductive biology in relation to ability of colonizing new habitats, genetic diversity and evolutionary potential. It has been noted that the majority of species reproduced through inter-gametophytic selfing and crossing (Hedrick 1987; Soltis & Soltis 1992; Korepelainen & Kolkkala 1996; Hooper & Haufler 1997; Khare 2001), whereas mixed mating i.e. having both intra-gametophytic as well as inter-gametophytic were also observed in several species (Khare & Kaur 1979; Klekowski 1982; Soltis & Soltis 1987).

In view of the reproductive factors vis-à-vis diversity of ferns of Kumaon Himalayas, nine species such as, *Adiantum incisum* Forsk., *A. capillus-veneris* L., *Pteris vittata* L., *Cheilanthes farinosa* Kaulf., *Dryopteris stenolepis* (Bak.) C. Chr. *et al.* *D. wallichiana* (Spreng.) Hyl., *Tectaria coadunata* (Wall. Ex Hook. Et Grev.) C. Chr. *Lygodium japonicum* (Thunb.) Sw. and *Microsorium*

membranaceum (D. Don) Ching have been studied for their reproductive biology and results are correlated with their population diversity and evolutionary pattern.

MATERIAL AND METHOD

Spores were collected from the plants growing in different localities of Kumaon Himalayas, e.g. Bhujiaghat, Kilbury (Nainital), Bageshwar, Binsar (Almora) and Cahubatia (Ranikhet) during the years 2000-2003. Collected spores were stored in a desiccator in laboratory. Surface sterilized spores were sown onto the Parkers and Thompsons's nutrient media in glass petri dishes of 80 x 17 mm size. All the cultures were kept in a culture room. The temperature of the culture room was maintained at $22 \pm 2^{\circ}\text{C}$ and light intensity was given at 220-230 ft. c.

Periodically the germlings were observed under microscope and before attainment of the sex organs, prothalli from stock culture were picked up at random and separated as follows:

1. **Isolated (A):** One prothallus per petri dish (50 replicates)
2. **Composite cultures (AXA) :** 20-25 prothalli per dish from one plant (10 replicates)
3. **Composite cultures (BxB):** 20-25 prothalli per petri dish from another plant (10 replicates)

The cultures were observed periodically and different ratio of gametophyte bearing male and female or bisexual conditions were recorded. After the initiation of gametangia all the isolate and composite populations were flooded from above with sterile distilled water twice in a week to facilitate fusion of gametes for fertilization. Percentage of sporophytes produced at each level was recorded. By the combination of cultures isolated from the stock cultures only intra-gametophytic selfing in isolates and intra- and inter-gametophytic selfing in composite populations were tested.

Five cultures of isolate and one each of composite culture were kept unwatered throughout the course of the experiment to check the sexuality of the species. These populations did not produce sporophytes suggesting the sexual nature of the species under study.

OBSERVATION

On the basis of the available data, the taxa are categorized into two groups :

A. Taxa having greater genetic diversity :

Total failure of sporophyte production in four taxa, e.g. *Microsorium membranaceum*, *Adiantum incisum*, *Dryopteris stenolepis* and *D. wallichiana* was observed in isolate population, indicative of lack of intra-gametophytic selfing. Except *M. membranaceum*, the spores of *Adiantum incisum*, *Dryopteris stenolepis* and *D. wallichiana* required light for germination and between 40 to 45 days of sowing cordate bisexual gametophyte developed. This bisexual condition remained up to 65 days. The antheridia initiated first followed by archegonia. The frequency of antheridia was lesser and formed for very short duration, i.e. spent up between 3-5 days but archegonia developed in succession (Table 1). About 50 per cent gametophytes did not produce sex organs, which is suggestive of lack of antheridiogen activity.

Spores of *M. membranaceum* germinated in dark. After 45 days of spore sowing archegonia initiated first instead of antheridia and after 60 days onward gametophytes became bisexual. In all the taxa the antheridia were located towards the rhizoidae end and the archegonia were borne below the apical notch near the midrib region.

Breeding results show absence of sporophyte production through intra-gametophytic selfing in all the taxa, only sporophyte formation was observed in composite population that varies from taxon to taxon. Sex-ontogenetic pattern favours inter-gametophytic selfing. Maximum bisexual gametophytes were observed in *Microsorium membranaceum* followed by *Dryopteris wallichiana* and *Adiantum incisum*, but sporophyte production was minimum in *Microsorium membranaceum* followed by *Adiantum incisum*, *Dryopteris stenolepis* and *D. wallichiana*. The breeding result clearly indicated inverse proportion of bisexual condition vis-à-vis sporophyte formation through inter-gametophytic selfing (Fig. 1).

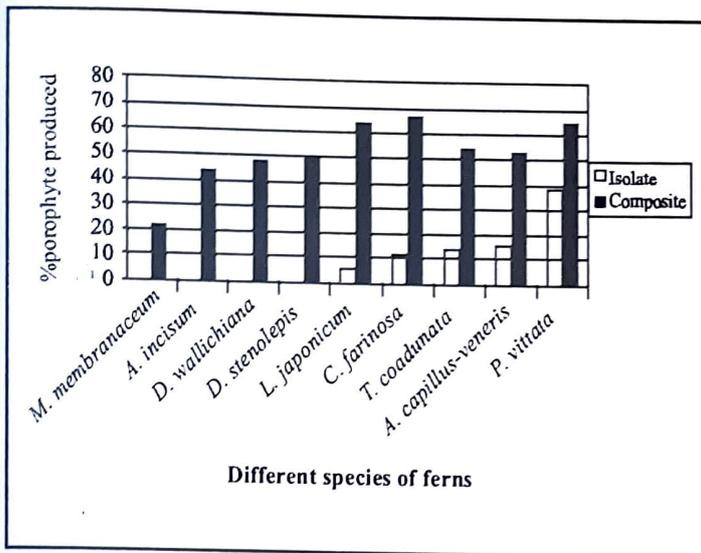


Fig. 1: Percentage of sporophyte produced in different population.

2). The average ratio of both the sexes are almost same (10-12 sexes per gametophyte) in all stages of maturity. The duration of the sexual stages was longer, i.e. more than 10 days, favouring a condition for intra-gametophytic as well as inter-gametophytic selfing (Table 2). The location of the sex organs were the same, antheridia were located towards the rhizoidal end whereas archegonia towards the notch of the gametophyte. The sex-ontogenic pattern seems to be favourable for intra - as well as inter-gametophytic selfing.

The sporophyte production through intra-gametophytic selfing (isolate cultures) was only 6% in *L. japonicum*, 12% in *C. farinosa*, 14% in *T. coadunata*, 16% in *A. capillus-veneris* and 38 in

Table 1. Chronological changes in sex ratio in stock culture of different taxa having greater genetic diversity

Name of taxa	Days after sowing	Sample size	Neuter	Male	Female	Bisexual
<i>Microsorium membranaceum</i>	45	20	11	-	09	-
	50	20	06	02	08	04
	55	20	04	03	08	05
	60	20	-	02	01	17
<i>Adiantum incisum</i>	40	20	09	06	05	05
	45	20	04	11	09	09
	50	20	-	-	17	-
	55	20	-	-	20	-
<i>Dryopteris stenolepis</i>	39	25	25	02	-	-
	43	25	20	05	-	-
	47	25	07	10	08	08
	52	25	-	09	16	09
	55	25	-	-	-	-
<i>Dryopteris wallichiana</i>	39	25	22	03	-	-
	43	25	12	06	07	06
	47	25	04	10	11	10
	52	25	-	-	25	-

B. Taxa having lesser genetic diversity:

In this category, *Adiantum capillus-veneris*, *Cheilanthes farinosa*, *Pteris vittata*, *Lygodium japonicum* and *Tectaria coadunata* produced sporophytes through intra - as well as inter-gametophytic selfing. In *L. japonicum*, the spores germinate in dark and archegonia initiated first, whereas in other taxa germination occurred in light, antheridia initiated first followed by archegonia (Table

in *P. vittata*. The range of sporophyte production in composite population was 55% to 68% (Fig. 1).

DISCUSSION

By studying the reproductive biology, it is possible to screen the sporophytes for "deleterious genes" which indicates the amount of genetic load present in the gene pool of the taxon, which causes them to cross breed and make them as a poor or good colonizer on

Table 2. Chronological changes in sex ratio in stock culture of different taxa having lesser genetic diversity

Name of taxa	Days after sowing	Sample size	Neuter	Male	Female	Bisexual
<i>Lygodium japonicum</i>	39	20	-	-	06	-
	43	20	-	-	08	-
	47	20	06	08	10	08
	52	20	02	15	16	15
	55	20	-	10	07	11
<i>Cheilanthes farinose</i>	30	20	18	2	-	-
	35	20	07	07	06	06
	40	20	-	18	18	18
	45	20	-	-	-	20
	50	20	-	-	07	13
<i>Tectaria coadunata</i>	35	20	04	08	05	03
	40	20	-	05	03	12
	45	20	-	-	04	16
	50	20	-	-	20	-
<i>Adiantum capillus-veneris</i>	35	20	12	08	-	-
	40	20	04	16	16	16
	45	20	-	12	08	08
	50	20	-	06	14	06
	55	20	-	-	20	-
<i>Pteris vittata</i>	40	20	11	09	-	-
	45	20	-	12	08	08
	50	20	-	14	15	14
	55	20	-	03	16	03
	60	20	-	-	20	02

barren land and widely or restricted occurrences of the species. It seems to be one of the important trends of evolution of "adaptive gene complex" of fern taxa, which is more often highly specialized in relation to its ecological niches favourable to its growth. The "adaptive gene complex" can modify the reproductive biology and can throw light on the genetic evolution of the taxon. It would be interesting to find out how far the said trends of specialization are naturalized by taxa from different ecological niches. The reproductive biology from Kumaon Himalaya has been examined and gene pool, physiological parameters on sex-ontogeny and ecological condition for gametophytic

growth and survival have been identified.

Study of *Adiantum incisum*, *Dryopteris stenolepis*, *D. wallichiana* and *Microsorium membranaceum* shows that none of the gametophyte in isolate cultures is able to produce the sporophyte. In other words homozygous sporophytes never produced through intra-gametophytic selfing. A possible explanation for the absence of sporophyte production in the isolates in these cases is perhaps due to constitution of the zygotic genotype. The gene pool of these taxa is charged with high level of genetic load that would lead to inbreeding depression upon intra-gametophytic selfing. In *Adiantum incisum* apical

buds developed due to which it also propagates vegetatively and form the large population in favourable condition. The epiphytic habitat of *M. membranaceum* is one of the reasons for poor selfing. Previous studies on epiphytic ferns also indicate similar findings (Hooper & Haulfer 1997). Genetically these taxa can be characterized as a highly heterozygous species. Ganders (1972) proposed that natural selection operates to favour heterozygotes rather than homozygous sporophytes under natural conditions. The mating system of these taxa seems to corroborate the view, as they are genetically much evolved species.

Ontogeny of the gametophyte was prolonged in unisexual condition and short duration of the hermaphroditism could be another reason for inter-gametophytic mating. This could be also due to antheridiogen (an antheridia inducing hormone) activity. Since the intra-gametophytic selfing is nil, thus diminishes the capacity for colonizing the new habitat. In these taxa, the production of sporophytes is also low through inter-gametophytic selfing and crossing. The result indicated that this mating behaviour is not much suited for wide distribution, frequent occurrence and introduction in barren land. In the area of occurrence of these taxa, the frequency is not so high which also corroborates the findings.

The taxa such as *A. capillus-veneris*, *Cheilanthes farinosa*, *Pteris vittata*, *Lygodium japonicum* and *Tectaria coadunata* produced a considerable percentage of sporophytes through intra-gametophytic selfing in other words they produced a considerable number of homozygous sporophytes. Prolonged bisexual condition could be one of the reasons to increase the possibility of these mating. This type of breeding system is well suited for long distant colonization of the species through spore dispersal. The gene pool of these taxa are charged with less genetic load and resulting breeding system suggested that the area of study may be of considerable higher populations and wide occurrence of the taxa in the entire Kumaon region. The observations of distribution and density of population of studied taxa corroborates the results of their mating phenomenon.

The high yield of sporophyte in isolates in *P.*

vittata is indicative of meagre genetic load presence in gene pool. This type of mating system favours the species to be an excellent colonizer and due to this reproductive behaviour the species in particular are wider in occurrence in the foothill to the higher altitude of entire Kumaon region in different habitat (Fig. 1). In these taxa genetic load may increase with time as new spore colonize the area and a gradual increase in inter-gametophytic mating occur (Crist & Farrar 1983).

Among the studied taxa the spore germination in most of the species is light dependent. When fern spores fall to the ground from sporophyte after maturation, a portion of them will percolate into the pore space of soil or rock crevices and remains quiescent due to absence of light and in due course of time adapt for dark germination. Two species reported here, i.e. *Lygodium japonicum* and *Microsorium membranaceum* germinate in dark condition and in mature gametophyte archegonia initiate first followed by antheridia.

Present study suggests that the evolutionary status of the taxa could be ascertained by examining the genetic diversity. The taxa, which are completely heterozygous, are more advanced than the other taxa, which possess mixed mating.

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REFERENCES

- Crist, KC & Farrar, DR 1983. Genetic load and long distance dispersal in *Asplenium platyneuron*. *Can. J. Bot.* **61**: 1809-1814.
- Ganders, FR 1972. Heterozygosity for recessive lethals in the homosporous fern populations: *Thelypteris paulustris* and *Onoclea sensibilis*. *Bot. J. Linn. Soc.* **65**: 211-221.
- Hedrick, PW 1987. Genetic load and the mating system in homosporous ferns. *Evolution* **41**(6): 1282-1289.