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CYTOLOGY OF PHYLLACTINIA : LITTLE KNOWN THOUGH FULL OF PROMISE

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I am, at this occasion, stricken by mixed feelings. While on one hand I feel privileged to have the invitation to deliver the First Umakant Sinha Memorial lecture, on the other I cannot contain the pain of having to deliver it in memory of a very dear friend, who, in years was my junior. We had long association spanning over many years.

When I received the invitation I was hesitant to accept it. I felt handicapped because my area of specialization is not the one that Prof. Sinha pursued throughout his career. He was a microbial geneticist. My association with microbes has been very limited. During the seventies, we undertook studies on the cytology of powdery mildews and a part of the results of these studies I propose to present before you, and dedicate to the memory of my dear friend.

Phyllactinia belongs to sub-family Phyllactinieae of family Erysiphaceae. Members of the genus are characterised by mycelium which is both external as well as internal, asexual stage is of the ovululeriopsis type and perithecia exceed 150 μ in diameter. It is a mildew genus. The term mildew is misleading because when used alone it refers as much to the pathogen as to the disease that it causes to the host. Moreover, mildews refer to sac as well as algal-fungi. To resolve this confusion, Yarwood (1957) proposed that ascomycetous mildews be called "powdery mildews" and phycomycetous ones the "downy mildews".

Powdery mildews are a group of obligate parasites, specific to angiosperms. A total of 7,609 flowering plant species are known to get infected by these fungi the world over (Hirata, 1976). They are characterised by, (i) superficial and septate mycelium, (ii) conidia borne singly or in chains, and bearing high water content that enables them to germinate in absence of free moisture, (iii) closed fruiting bodies which bear various types of appendages, and above all (iv) host specialization.

Phyllactinia, along with some other powdery mildew genera, is referred to family Erysiphaceae. Categorisation within this family does not offer any difficulty upto the generic level, based as it is on such stable characters as nature of appendages, number of asci per cleistocarp, endo-or ectophytic nature of mycelium etc. However, the distinctions are blurred thereafter; species descriptions based largely on quantitative data show great overlaps. Although, despite changes in chromosome number, the size of fruiting body and parts thereof do not fluctuate, yet such parameters do not enjoy the stability of Qualitative characters. Taxonomic idiosyncracy in powdery mildews stems from this fault.

Hirata (1966) specifies Central and South-western Asia and Central and Southern Europe as the home of powdery mildews. From here, they are believed to have dispersed in various directions to reach such other parts of the world where they flourish today. From within the Indian subcontinent, the state of Jammu and Kashmir, lying only a short distance away in the South-east, is closest to the centre of origin of powdery mildews. On account of this eco-geographic proximity, the powdery mildew fungi are expected to be quite common within the State. This possibility is further increased by the fact that angiospermic elements of the vegetation of Kashmir (which happen to be the only hosts of powdery mildews) have migrated to the valley very largely

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from Central and South-western Asia; the arboreal element in particular is almost exclusively from the neighbouring floras of Central and South-western Asia and parts of Europe. Coming as they are from the home of powdery mildews, many of these tree species are among the choice hosts of powdery mildews; the same host species is, at times, infected by more than one powdery mildew.

Of the 8 genera identified in Erysiphaceae, *Phyllactinia* and four others, namely *Podosphaera, Uncinula, Macrosphaera* and *Pleochaeta* are pathogenic only on trees. The number of hosts of these genera in Kashmir have been compared with the numbers recorded from all other parts of the country and the world at large.

Table 1: Break-up of the host range of different mildew genera parasitising the arboreal vegetation.

Area	No. of host spp. under				
	Micro-sphaera	Phyll.	Unci.	Podo.	Pleo.
World	753	522	377	180	22
India	06	49	22	03	01
J & K	01	15	10	02	01

The above table, as well as the one below reveal that whereas *Microsphaera* is the most common powdery mildew pathogen on trees the world over, in India and the State of Jammu and Kashmir, *Phyllactinia* is by far more common.

Table II:

		World	India	JK
Phyll-Macro.	ratio	0.69	8.17	15.00
Phyll-Unic.	"	1.38	2.22	1.55
Phyll-Podo.	"	2.90	16.33	7.50
Phyll-Pleo.	"	23.72	49.00	15.00

Evaluation of quantitative characters as tools in Taxonomy of genus Phyllactinia

As pointed out earlier, identification of species in powdery mildews, including those of genus *Phyllactinia*, is based on Quantitative features. The only Quantitative character that has earned acceptability is the perithecial size. About the validity of other characters, namely size of, appendage base, perithecial wall cells, asci, ascospores and conidia and the number of asci per cleistocarp, the opinion is divided.

The correlation co-efficient (γ value) for the size of perithecium and breadth of appendage base is, 0.554. Since this value exceeds the p value at 0.05 (0.482), a positive correlation significant at 5% level, is indicated between the two parameters. It follows therefrom that, larger the perithecium broader is the base of appendages that it bears. Similar positive correlation exists between perithecium size and the number of asci it contains. Correlation between the two parameters is significant at 1% level (the calculated γ value is 0.632 against 0.606 at $p = 0.01$) indicating that number of asci is directly proportional to perithecium size. Being positively correlated with perithecium size, the above two characters (size of appendage base and the number of asci) will invariably support and never alter the conclusions arrived at on basis of size of fruiting body. This fact buffers their usefulness as parameters in taxonomy.

The size of perithecial wall cells cannot be utilized for species delimitation in *Phyllactinia* because the taxa tested do not exhibit sufficient difference with regard to this trait. In contrast, in the related genus, *Sphaerotheca*, wall cell size is very specific and therefore, very significant for use in classification. Similarly, size of ascus, ascospore and conidium have limited potential for use in species delimitation in *Phyllactinia* though in related mildew genera, *Sphaerotheca* and *Erysiphe*, these characters have proved useful beyond doubt.

From what has been stated above, it follows that in *Phyllactinia* the main Quantitative character of proven taxonomic importance is perithecium size. Breadth of appendage base and number of asci per perithecium can be employed as adjuncts.

Base chromosome number in Phyllactinia

Great deal of variation has been recorded in chromosome count of the different forms of *Phyllactinia*. The numbers recorded, from time to time, are listed overleaf.

Table III: Index to chromosome numbers recorded in species of *Phyllactinia*

Mildew spp.	Host	Chr. No.	
		n	2n
P. Corylea	not mentioned	4	8
P. Corylea	"	8	16
P. Corylea	*Morus alba*	5	10
P. Corylea	*Corylus avellana*	10	20
P. Corylea	*Fraxinus americana*	8	16
(= *P. guttata*)	*Corylus americana*		
	Celastrus scandens		
	Betula lutea		
P. guttata	*Indigofera heterantha*	10	20
(= *P. corylea*)	*Robinia pseudoacacia*		
	Morus nigra		
	Crataegus monogyna	12	24
	Pyrus communis		
	Pyrus pashia		
P. yarwoodii	*Dalbergia* sps.	5	10
(= *P. dalbergiae*)			
P. dalbergiae	*Dalbergia sissoo*	10	20
(= *P. yarwoodii*)			
P. gmelinae	*Gmelina arborea*	10	20
P. fraxini	*Juglans regia*	10	20
P. kashmirensis	*Amygdalus communis*	5	10

Perusal of the table reveals that 5 counts have been recorded so far in genus *Phyllactinia*. While some species, such as *P. dalbergiae, P. yarwoodii, P. fraxini* and *P. Kashmirensis* are known by a single count each, *P. guttata* and *P. corylea* include more than one chromosome race. Of the different counts on record, n = 10 is the most common count, represented as it is in 5 species, namely *P. corylea; P. gmelinae, P. guttata, P. dalbergiae* and *P. fraxini*. The problem of base number in genus *Phyllactinia* is yet to be resolved. Kamat & Pande (1971) opine that the genus represents either a monobasic series based on x, 4 or a dibasic group with 4 and 5 as base numbers. So long as techniques are not perfected for comparing chromosome morphology, it will be hard to choose between the two alternatives. However, support for the monobasic concept, holding 4 as the primary base number comes with consideration of chromosome numbers of other genera of Erysiphaceae; x=4 is also known in species of *Erysiphe, Uncinula* and *Microsphaera*. On the contrary, x, 5 is restricted to three species of *Phyllactinia* and one of *Sphaerotheca*. Should x, 4 be treated as the sole base number for *Phyllactinia* spp, 5 will be a derived number. In that case, eu - and aneuploidy will have to be ranked as equally important factors for differentiation of species and origin of chromosome races.

Polyploidy : If 16, 20 and 24 represent polyploid numbers, forms with these counts should exhibit such anomalies as multivalent formation, erratic anaphases, high sterility etc., which is not the case. Lack of these polyploid attributes in species of *Phyllactinia* indicates that numerical alterations have been followed by diploidization mechanisms of the kind operative among flowering plants.

The notion that fungi have evolved in absence or comparative infrequency of polyploidy like animals has been advocated by mycologists for long. Although Wakayama (1930) disproved this by indicating existence of polyploidy in such groups where chromosome numbers represent direct multiples, yet Stebbins (1950) and Olive (1953) have stressed the lack or relative infrequency of polyploidy in fungi. Whatever little information has been generated on chromosome counts of fungi during the past few decades it has forced a change in this outlook. Rogers (1973) is emphatic in stating that polyploidy may be or may have been an important factor in the evolution of fungi. He points to existence of polyploidy in ascosporogenous yeasts, discomycetes, *Puccinia* and *Allomyces*. Burnett (1976) has suggested existence of autopolyploidy in *Puccinia* and *Cyathus stercorieus*.

The findings on cytology of *Phyllactinia* are significant for two reasons. The first, that they dispel the earlier belief of lack of polyploidy in fungi and second, that they establish existence of inter as well as intraspecific polyploidy. Polyploidy upto hexaploid level has been recorded in genus *Phyllactinia*.

Numerical chromosome changes in relation to taxonomy

Numerical alterations in chromosomes may or may not impose morphological changes. Whenever they do, the variants warrant separate taxonomic status. Tare (1955) was the first to highlight the bearing of chromosome number on taxonomy of powdery mildews. He supported splitting of *E. acaciae* from *E. polygoni*, orginally advocated on grounds of morphology, because the former has 4 and latter 8

chromosomes in the haploid complement. That, mere alteration in chromosome number does not necessarily impose a major morphological change, leading to the formation of new species, is indicated by the existence of intraspecific variants in *Erysiphe* and *Phyllactinia*. For instance, *E. graminis* var. *hordei* exists in races with n = 2 (Kimber & Wolfe, 1966) and n = 6-8 (Mckeen, 1972). Accordingly, chromosome number cannot be a dependable character for use in species delimitation. It can at best, be employed as adjunct to other conventional parameters.

Chromosome races in relation to host-range and pathogenicity

The data available on chromosome numbers reveal existence of interspecific polyploidy in different species of Erysiphaceae. Chromosome races exist in *Phyllactinia guttata, P. corylea, Erysiphe graminis* var. *hordei* etc. Existence of these races widens genetic variability of pathogens including their flexibility to infect and thrive on varied hosts. Evidence for this proposition is provided by observations on the host range of chromosome races, n, 10 and n, 12 of *P. guttata*.

The race with n = 12 is pathogenic on rosaceous trees,*Crataegus monogyna, Pyrus communis* and *P. pashia*. That, having haploid number 10 has hosts in Fabaceae (*Indigofera heterantha* & *Robinia pseudoacacia*). Although, Rosaceae and Fabaceae belong to same order, Rosales, yet the response the two chromosome races of *P. guttata* elicit from members of the two families is significantly different. Rosaceous hosts are vulnerable to race n, 12 and the legumes to race n, 10 of the fungus.

Like other obligate parasites, powdery mildews also depend on the genetic control of their hosts. Selection pressure on the pathogen always favours such genes and gene-combinations which enhance its survival potential. On the contrary, from the stand point of host, selection stress favours those genes and gene-combinations that impart resistance against the parasite. The host-parasite relationship depends upon the interplay between genetic system of the two partners. According to the gene-for-gene system (Flor, 1971; Wheeler, 1975), for each gene conditioning resistance in a plant, there is a corresponding gene that conditions pathogenicity in the fungus. To resist the pathogen, the host plant should be able to generate combinations of resistant genes which outnumber the combinations of virulence genes in the pathogen. Parasitic fungi exercise a whole gamut of means, such as mutations, somatic and meiotic recombination, hybridization, parasexuality and heterokaryosis for generating new combinations of genes for virulence. Increase or decrease in chromosome number results in change in the number of linkage groups through independent assortment and crossing over and production of new recombinants for genes including genes controlling host resistance.

Chromosome number in members of Rosaceae is generally higher; 2n = 32 and 34 in *Crataegus monogyna* and 2n = 34 and 51 in *Pyrus communis*. In such host-parasite systems where the hosts have large chromosome complement and are regularly bred for disease resistance and improved quality, as is the case with rosaceous fruit trees, selection stress on the pathogen is very great. Chromosome size in fungi in general is very small as compared to their hosts, with the result that their recombination index is low. To compensate for this disadvantage, numerical changes assume great relevance.

That, the chromosome numbers of host and the parasite bear relationship is also brought out by the fact that the race of P. guttata infecting *C. monogyna, Pyrus communis* and *Pyrus pashia*, bearing high chromosome counts, has n = 12. On the contrary, *Amygdalus communis*, also of Rosaceae, but having comparatively low chromosome number (2n - 16), hosts *P. Kashmiriensis* which has n = 5.

The significance of numerical chromosome alterations is particularly great in those taxa which are not able to fully exploit he variation generated through sexual reproduction. In species of *Phyllactinia* three-fourths of the variation generated during ascosporogenesis is eliminated.

Harper (1905), whose work on the cytology of ascus is monumental, observed that in *P. corylea* ascospores differentiate around only two of the 8 products of meiosis. During the 87 years that have

elapsed since the observation was made first, no better insight has been gained into the precise fate of meiotic products. Stepwise details have been collected from *P. guttata* and *P. kashmiriensis* in which two poles of the ascus are easily distinguishable (Sharma, 1980) the basal pole being continuous with a narrow stalk which attaches the ascus to the perithecium. In these species, the two haploid nuclei formed through the first division of meiosis migrate to opposite poles of the ascus. They undergo two mitoses each and produce 4 nuclei. Those, at the upper pole of ascus show signs of degeneration soon after their formation. Out of the four nuclei formed from the basal haploid nucleus two (rarely one) move to the upper pole to share the fate of the other nuclei at that pole. The remaining 2 or 3 nuclei that persist in the middle, have prominent nucleoli, bear deep staining chromatin and are larger in size. Through free cell formation, the surviving nuclei differentiate as ascospores. Thus, mature ascus in *Phyllactinia* bears only two or at best three ascospores.

The cause behind wholesale degeneration of four or five nuclei at the upper ascus-end is not vivid. Following exchange of genetic material through crossing-over, the bivalents assemble at metaphase spindle in the centre of ascus. Alignment of bivalents on the spindle is mostly random but it can be directed. Preferential polarization of centromeres during megasporogenesis occurs in certain lines of maize. Similar non-random distribution can take place in all those cells which, like the megaspore mother cell of angiosperms, have distinct poles. Ascus falls in this category.

Directed orientation could also be caused by some sort of 'affinity' between the non-homologous chromosomes of one genotype which makes them align in such a way that they pass to the same pole. This kind of affinity has been inferred through genetic studies in mice and yeast.

Whatever the cause behind degeneration of nuclei at one pole of the ascus of *Phyllactinia*, the fact remains that it neutralizes, to a great extent, the effect of recombination caused by crossingover and independent assortment. The little that persists is eliminated by the degeneration of one or two nuclei of the basal pole too. The two ascospores that finally differentiate within an ascus are, more often than not, genetically alike. For this reason, species of *Phyllactinia* derive very little advantage from sexual reproduction. Therefore, numerical variations assume special significance.

Existence of intraspecific chromosome races in pathogens play dual role. On one hand they offer the parasite a mechanism for widening its host range, and on other, they provide it the means to overcome host resistance.

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