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"Complement Fractionation" in Natural Diploid Orchid Species

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Summary. Four out of 10 diploid orchid species showed "complement fractionation"; a complex cytological phenomenon, hitherto reported only in polyploid plants. The manifestation of this phenomenon during meiosis is the formation of chromosome subgroups resulting eventually in cells with more than the usual four sporads; five or six being the optimum number in the investigated orchid species. No implications whatsoever can be deduced as to the genetic or genomic constitution of the end products. The presence of the phenomenon in these orchid species could perhaps indicate a polyploid ancestry or concealed hybridity. The operation of "complement fractionation", however, could be interpreted as an alternative evolutionary pathway opposed to polyploidy.

Key words: Complement fractionation – Excess sporads – Diploid orchid species – *Phaius* tribe – Evolution

Introduction

The term "complement fractionation" was first introduced by Thompson (1962) to describe the independent behaviour of subgroups of a chromosome complement in mitotic and meiotic cells in polyploid *Rubus* cultivars. Observations of this complex cytological phenomenon have been extended but confined only to natural allopolyploid plants of *Rubus* (Bammi 1965) and *Globba* (Lim 1973). In Thompson's original interpretation, "complement fractionation" had no implications whatsoever as to the genetic or genomic constitution of the end products. However, in the natural allotetraploid *Rubus* hybrid, chromosomal subgroups were suggested to represent its constitutent genomes. Furthermore, in the allopolyploid *Globba* taxa, the chromosomal composition of the subgroups could be indicative of their genomic history from which the original or ancestral basic number could be deduced. The present cytological survey of 10 naturally occurring diploid orchid species of the *Phaius* tribe shows the presence of "complement fractionation" in only four of them. The phenomenon and its implications are described and discussed in this paper.

Materials and Methods

The *Phaius* tribe is made up of six genera consisting only of terrestrial plants which are widely distributed throughout the whole of peninsular Malaya (Holttum 1964). Sixty plants, comprising ten species from four of the six genera, were analysed meiotically. The genera and species are listed in Table 1. Basic numbers of the four genera, *Plocoglottis, Calanthe, Spathoglottis* and *Phaius,* form a dysploid series viz., $\underline{x} = 19, 20, 20$ and 21, respectively. All the species are diploids with a high degree of fertility resulting from either artificial or natural pollination (Teoh and Lim 1978).

Collections of flower buds were carried out for two consecutive flowering periods (years). Young buds were fixed initially in a mixture of 95% alcohol, chloroform and glacial acetic acid according to the ratio of 1:1:2 (see Shindo and Kamemoto 1963). After 12 hours, the pollinia were treated overnight in a similar fixative but mordanted with ferric chloride. Staining was carried out in alcohol-HCl-carmine according to the procedure outlined by Snow (1963). Slides for analyses were made permanent by the alcohol exchange method (Bradley 1948).

Results

The consequences of "complement fractionation" would be cell division products with variable chromosome numbers. The manifestation of this phenomenon are cells with more than four sporads at the completion of meiosis. Table 1 illustrates the extent of formation of such cells. The most frequent types were cells with five or six sporads; the maximum number, being eight (Figs. 1 m–o). They were observed in four of the ten ta-

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Table 1. Frequency of sporad formation in the orchid species

Species	Cells with sporad numbering									
	2n	4	4+Micro-nuclei	5	6	7	8	Aborted	Others	Total
Calanthe veratrifolia	40	377	5	10	5	4	2	4	16	423
C. veratrifolia	40+1B	411	15	17	10	3	3	6	24	489
C. rubens	40+2B	331	3	6	7	3	3	2	4	359
C. aurantiaca	40	314	3	–	–	–	–	–	2	319
C. pulchra	40	360	6	4	3	–	–	2	7	382
Spathoglottis plicata	40	409	5	–	–	–	–	3	22	439
S. microchilina	40	465	5	–	–	–	–	4	23	497
S. affinis	40	450	3	–	–	–	–	3	20	476
Phaius tankervilliae	48	307	–	–	–	–	–	–	9	316
Plocoglottis lowii	38	286	5	–	–	–	–	–	4	295
P. javanica	38	455	4	2	1	–	–	–	7	469

Table 2. "Complement fractionation" (CF) in meiotic cells of *Calanthe veratrifolia* (2n=40). 200 cells were analysed for each stage

(a)	Meiotic stage	Metaphase I	Anaphase I	Telophase I	Metaphase II	Telophase II	
	No. of cells showing CF	13	20	12	13	10	
(b)		Bivalent distribution					
	Subgroups at Metaphase I	8 – 12	9 – 11	6 – 14	5 – 15	10 – 10	4 – 16	20 (Normal)
	No. of cells	3	3	3	1	2	1	187
(c)	Type	Chromosome distribution at Anaphase I	No. of cells	Total			
	Subgroup formation at one pole only	6 – 14/20	1				
		4 – 16/20	1				
		8 – 12/20	2				
		9 – 11/20	2				
		7 – 13/20	2				
		5 – 15/20	2				
		4 – 7 – 9/20	1	10			
	Subgroup formation at both poles	4 – 16/ 7 – 13	1				
		8 – 12/ 8 – 12	2				
		8 – 12/10 – 10	1				
		5 – 15/ 5 – 15	1				
		5 – 15/ 9 – 12	2				
		10 – 10/10 – 10	2				
		7 – 13/10 – 10	1	10			
	Normal	20/20	180	180			

xa, mainly in the genus *Calanthe* and in very low frequencies ranging from 0.2 to 6.8%. "Complement fractionation" occurred irrespective of the presence or absence of B chromosomes in the *Calanthe* species.

Due to the low frequencies in the production of such sporads, detailed meiotic analyses were possible only in *Calanthe veratrifolia.* Cells showing "complement fractionation" were recorded during Metaphase I, Anaphase I, Telophase I, Metaphase II and Telophase II (Table 2).

During the first meiotic division, subgroups of the chromosome complement were observed to behave as follows. (1) They could be arranged spatially near one another on the same metaphase plate and segregating in a common spindle synchronously or asynchronously (Figs. 1 a–d). (2) They could be arranged on the same

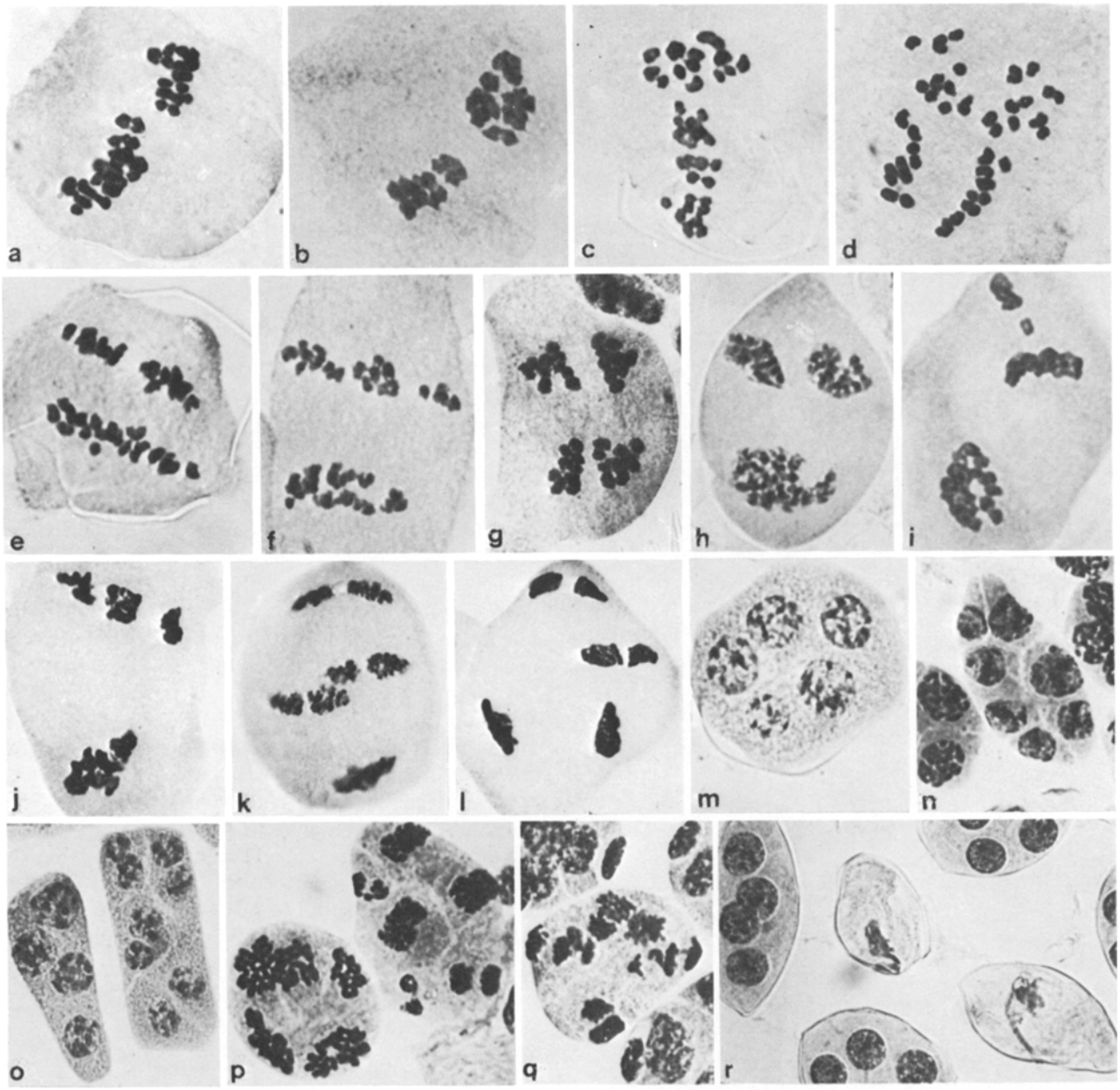

Fig. 1. a–r. "Complement fractionation" in pollen mother cells of *Calanthe veratrifolia.* **a, b** Two subgroups adjacent to each other at Metaphase I; **c, d** Asynchronous segregation of subgroups at Anaphase I; **e, f, g** Subgroups formation at only one or both poles during Anaphase I; **h** Meiotic interphase pollen mother cell with 3 nuclei; **i, j** Metaphase II cells showing subgroups; **k, l** Telophase II cells which will result in 7 and 6 sporads, respectively; **m, n, o** 5, 7 and 8 sporads respectively; **p, q** Deviant sporads undergoing the first pollen mitosis; metaphase (5), telophase (6) and anaphase (7); **r** Spores possessing less than the usual amounts of chromatin material. Magnification x 1000

metaphase plate and segregating equally or unequally in a partially split spindle (Figs. 1 e, f). (3) They could be orientated on separate metaphase plates and segregating in multiple spindles (Figs. 1 b, g).

At Metaphase I, usually not more than two subgroups were found in the deviant pollen mother cells. This was observed in less than seven per cent of cells analysed (Table 2 a). Distribution of bivalents in the subgroups did not appear to conform to any regular pattern (Table 2 b). Subgroups with less than four bivalents or more than sixteen bivalents were not recorded.

Table 2 c shows clearly that chromosome distribution to the opposing poles during Anaphase I did not adhere to any regular pattern and can be divided into

three types. The first consists of cells with segregating chromosomes in subgroups formation at one pole only (Figs. 1 e, f). Cells belonging to the second type show subgroups at both poles (Fig. 1 g). The normal ones belong to the last type. In some cells the number of chromosomes in the subgroups corresponded to those found at the earlier Metaphase I stage but in others it did not.

The results of the above subgroup bahaviour are pollen mother cells with 3 or 4 nuclei at meiotic interphase (Fig. 1 h). Presumably this condition is retained when Metaphase II is reached (Figs. 1 i, j) and regular segregation occurs resulting in Telophase II cells as illustrated in Figs. 1 k and l. A schematic representation of the pathways leading to the formation of excess sporads is presented in Fig. 2.

In the orchid species investigated, all the products of meiosis are contained in a tetrad. Cells with excess sporads undergo the normal pollen mitosis synchronously within the tetrad (Figs. 1 p, q).

Evidence of premeiotic instability comes from observations of some pollen mother cells which has less than the usual amounts of chromating material (Fig. 1 r). These cells ("aborted" in Table 1) were observed in extremely low frequencies. It is likely that "complement fractionation" could have been operative in some premeiotic cells to give rise to this abnormality.

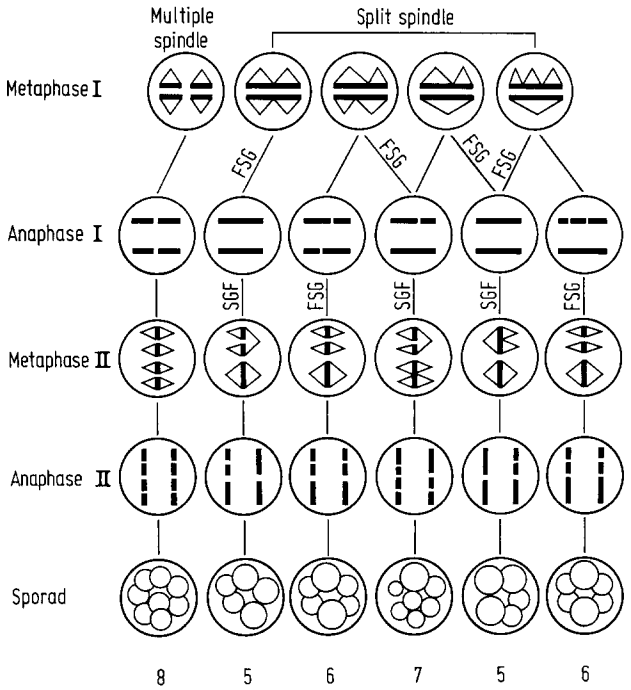

Fig. 2. Schematic representation of the pathways leading to the formation of excess sporads in *Calanthe veratrifolia*. (FSG = Fusion of subgroups; SGF = subgroup formation)

Discussion

The occurrence of "complement fractionation" in natural diploid orchid species provides convincing evidence that this phenomenon is a natural one. Furthermore its presence was observed in buds of those species involved for two consecutive flowering periods (years). Since the frequency of this phenomenon is different for different species, it is very likely to be genotypically controlled and substantiates a similar conclusion reached by Bammi (1965) on the allotetraploid *Rubus* clones. However, no consistent pattern of subgroup formation was observed in the orchid species to warrant any postulations on their genomic constitution or ancestral basic number. The present observations and results obtained concur with Thompson's original interpretation of the phenomenon.

So far this phenomenon seems to be associated with polyploid plants only. "Complement fractionation" could also be extended to explain the meiotic behaviour of artificially induced polyploids such as the colchiploid *Lycopersicum esculentum* (Gottschalk 1958) and *Ribes nigrum* (Vaarama 1949). However, this is probably the first instance of natural diploid plant species showing this phenomenon. Nevertheless, diploid artificial orchid hybrids of *Aranda* (*Arachnis* × *Vanda*) show extensive subgroup formation in meiotic cells and is one of the main contributory causes of hybrid sterility (Yip 1977). Undoubtedly "complement fractionation" also contributes significantly to the observed partial sterility in the allopolyploid plants of *Rubus* (Bammi 1965) and Globba (Lim 1973). In the diploid orchid species investigated, the plants are extremely fertile. The effect of these deviant sporads on fertility is made thoroughly insignificant by the superabundance of the normal sporads.

Thompson (1962) suggested that "complement fractionation" might be considered as a source of variation whereby the end products would be progeny with highly variable chromosome numbers. This could arise by the functioning of the resultant gametes in fertilisation to produce these unique segregants. The observation of deficient spores undergoing the first pollen mitosis in the orchid species might suggest that they are at least functional up to this stage. Whether they germinate and partake in fertilisation is not known. Besides, unique segregants in terms of highly deviant chromsome numbers have yet to be discovered in the *Orchidaceae* (Mehlquist 1974; Tanaka and Kamemoto 1974).

The presence of "complement fractionation" in some of the *Calanthe* species might perhaps indicate that they are highly evolved polyploids and that their ancestral species are no longer in existence. This could be substantiated by the absence of an euploid series in all the genera investigated and the high basic numbers of 19, 20 and 21 (Teoh and Lim 1978). In fact orchid genera generally possess high basic numbers (Jones 1974; Ducan 1959). This has led Goldblatt (1979) to conclude that polyploid ancestry for most orchid genera is appa-

rent even though the *Orchidaceae* is still poorly understood cytologically. It is also probable that initially when the ancestral genomes combine in hybrid formation, "complement fractionation" might have been more marked then as is evident in the *Aranda* hybrids (Yip 1977). It could be argued that the ancestral genomes are now so well integrated that they function meiotically as one. "Complement fractionation" might well be just an indication of its concealed hybridity. Furthermore the presence of the phenomenon could be viewed as an attempt to revert to lower ploidy levels; an evolutionary alternative opposed to polyploidy.

Literature

Bammi, R.K. (1965): "Complement fractionation" in a natural hybrid between *Rubus procerus* Muell. and *R. lacinatus* Willd. Nature **208,** 608

Bradley, M.V. (1948): A method for making aceto-carmine squashes permanent without removal of the cover-slip. Stain Technol. **23,** 41–44

Duncan, R.E. (1959): Orchids and cytology. In: The Orchids, a Scientific Survey (ed. Withner, C.L.), pp. 189–260. New York: Ronald Press

Goldblatt, P. (1979): Polyploidy in angiosperms: monocotyledons. In: Polyploidy, Biological Relevance (ed. Lewis, W.H.), pp. 219–239. New York: Plenum Press

Gottschalk, W. (1958): Untersuchungen zum Problem der Herabregulierung der Chromosomenzahl in experimentell erzeugten polyploiden Pflanzen. Z. indukt. Abst. Vererbungsl. **89,** 52–79

Holttum, R.E. (1964): Flora of Malaya. Volume 1: Orchids. Singapore: Government Printing Office

Jones, K. (1974): Cytology and the study of orchids. In: The Orchids: Scientific Studies (ed. Withner, C.L.), pp. 383–392. New York: Wiley

Lim, S.N. (1973): Meiotic instability in the plant genus *Globba.* Chromosomes Today **4,** 321–334

Mehlquist, G.A.L. (1974): Some aspects of polyploidy in orchids, with particular reference to *Cymbidium, Paphiopedilum* and the *Cattleya* alliance. In: The Orchids: Scientific Studies (ed. Withner, C.L.), pp. 393–409. New York: Wiley

Shindo, K.; Kamemoto, H. (1963): Karyotype analysis of some Sarcanthine orchids. Am. J. Bot. **50,** 73–79

Snow, R. (1963): Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technol. **38,** 9–13

Tanaka, R.; Kamemoto, H. (1974): List of chromosome numbers in species of the *Orchidaceae.* In: The Orchids: Scientific Studies (ed. Withner, Cl.L.), pp. 411–483. New York: Wiley

Teoh, S.B.; Lim, S.N. (1978): Cytological studies in Malayan members of the *Phaius* tribe (*Orchidaceae*). I. Somatic chromosomes. Malaysian J. Sci. **5(A),** 1–11

Thompson, M.M. (1962): Cytogenetics of *Rubus.* III. Meiotic instability in some higher polyploids. Am. J. Bot. **49,** 575–582

Vaarama, A. (1949): Spindle abnormalities and variation in chromosome number in *Ribes nigrum.* Hereditas **35,** 136–162

Yip, Moh-Ying (1977): Cytological studies of some *Aranda* (*Arachnis* × *Vanda*) hybrids (Orchidaceae). Cytologia **42,** 565–579

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