CYTOLOGICAL, STATISTICAL AND TRANSMISSION ELECTRON MICROSCOPY STUDIES OF SECONDARY ASSOCIATION IN THE GENUS *PHASEOLUS*

By

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ABSTRACT

CYTOLOGICAL, STATISTICAL AND TRANSMISSION ELECTRON MICROSCOPY STUDIES OF SECONDARY ASSOCIATION IN THE GENUS *PHASEOLUS*

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Four cultivars of *Phaseolus coccineus* L., five cultivars of *P. vulgaris* L. and one collection of *P. vulgaris* var. *aborigineus* were examined cytologically for the presence of secondary association of bivalents (bivalent pairing) during metaphase I of meiosis in pollen mother cells. Statistical methods were presented for evaluating the deviation of the observed degree of association to that expected at random. The degree of secondary pairing was found to be highly significant. Transmission electron microscope techniques were modified to enable the viewing of squashed but intact pollen mother cells which showed that a physical connection could occur between bivalents secondarily associated.

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INTRODUCTION

For this study secondary association will be differentiated from meiotic synapsis using the definition of Rieger, et al. (1976).

> Secondary association or pairing of bivalents: The nonrandom distribution of bivalents at first metaphase of meiosis in some polyploid species. The bivalents occur in pairs or groups if they are related by genetic and evolutionary factors.
>
> Meiotic synapsis or pairing: The intimate association of homologues easily recognized during first prophase of meiosis and leading to bivalent or multivalent formation.... When the pairing process is viewed under the electron microscope the visibly single zygotene chromosomes form a tripartite ribbon called a synaptonemal complex which is absent in achiasmate meiosis.

The phenomenon of secondary association of bivalents or bivalent pairing during meiosis was first noted by Kuwada (1910) in *Oryza sativa*. However, it was not until the early 1930's that the phenomenon was adequately defined and characterized.

Lawrence (1929, 1931) described the occurrence of secondary association in several species in the genus *Dahlia*. He noted that secondary association was not widely accepted, many cytogeneticists believing the phenomenon to be nothing more than an artifact of fixation (Lawrence 1931). Lawrence described the

phenomenon as a result of the juxtaposition of homologous chromosomes following primary association and used three criteria to support his hypothesis of nonrandomness:

1. Secondary association was found to be constant in the best of fixations.
2. The average number of chromosomes per association and the average frequency of each kind of association was characteristic for a given species.
3. At metaphase, associated bivalents are similar in size and configuration, structurally similar with respect to the position and number of chiasmata and the position of their attachments.

Based on these postulates of nonrandomness and homology between associates, Lawrence proposed a basic number of x = 8 to explain the maximum association observed in *Dahlia merckii* of two groups of three bivalents, 2(3), and five groups of two bivalents 5(2).

A second paper (Darlington and Moffett 1930) which appeared about the same time used arguments similar to those put forth by Lawrence (1931) to hypothesize that the basic number of x = 17 in species of *Malus* was of secondary origin and that the true basic number was therefore x = 7. The authors presented a scheme to explain the maximum association observed (figure 1)[1].

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[1]In this scheme each letter represents a bivalent, the same letter used more than once indicates homologous bivalents.

To further substantiate this contention they state that the basic number of x = 7 occurs in other subfamilies of the Rosaceae.

AAA
BBB
CCC
DD
EE
FF
GG

x = 7

Figure 1. The hypothesized scheme put forth by Darlington and Moffett (1930) to explain the maximum association of bivalents seen in *Malus* species 2n = 34.

Several authors published papers describing similar occurrences of secondary association in a number of unrelated genera. Muntzing (1933) proposed a basic number of x = 6 rather than x = 12 for *Solanum tuberosum* based on the frequent secondary association of chromosomes in groups of two (38% of the chromosomes).

Catcheside (1934) employed Lawrence's (1931) postulates to hypothesize that secondary balanced polyploidy occurs within a group of *Brassica* species (2n = 18). Based on secondary pairing a basic number of x = 6 was determined for the genus.

Subsequent authors have disputed several contentions put forth in the papers by Lawrence (1931) and Darlington and Moffett (1930) which laid the ground work for the

study of the phenomenon of secondary association. Gustafsson (1934) noted a physical connection between the associated bivalents in *Taraxacum* a point which both Darlington and Moffett (1930) and Lawrence (1931) claimed did not occur nor should be seen in secondary association. Gustafsson attributed the physical contact made by bivalents in *Taraxacum* to the presence of a greater degree of homology, physical contact implying that the chromosomes were more closely related than in the case when only a juxtaposition occurred. Gustafsson (1934) attempted to explain what he thought to be the mechanics of secondary association by hypothesizing, "that the secondary associates are due to terminal affinities and to the fusion of the pellicles of chromosomes."[2]

Heilborn (1936) was one of the first to determine the percentage of associations between distinguishable bivalents. He looked at the degree of association between the two smaller chromosomes in *Carex pilulifera* and between the three larger chromosomes of *C. panicea* and found that a definite association existed. However, unlike earlier workers, Heilborn found secondary association to be as prevalent during prophase as metaphase

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[2]Electron microscope studies have not supported the presence of chromosome pellicles.

and suggested a different explanation of the mechanisms involved in secondary association: "secondary association of chromosomes results from the action of the forces of nuclear division upon chromosomes of different size and mass." However, as soon as homologous bivalents occur they become associated on account of their identity in size and mass and in this way secondary association can still be used to imply chromosome duplication (Heilborn 1935).

Nandi (1936) gave yet a different explanation to describe the secondary association found in the genus *Oryza*. He compares the arrangement of the chromosomes to the arrangement formed by a corresponding number of floating magnets. When related bivalents are not associated it is because they are not lying near enough to each other.

With yet a different interpretation Thomas and Revell (1946) ascribe the mechanisms of secondary association in *Cicer arietinum* to the fusion of heterochromatin noting that the fusion of heterochromatic regions was not specific even though a high degree of association does occur among morphologically similar bivalents.

They also examined the effect that the squash technique had on the arrangement of bivalents. By studying many individual cells, before and after applying pressure

to the coverslip, they showed that the degree of association in fixed unsquashed material was not altered by squashing (Thomas and Revell 1946). However, Brown (1950) arrived at a different conclusion when comparing squashed and sectioned material in *Luzula campestris* 2n = 12. He noted that the high degree of secondary association present in squashed preparations of this species suggests a secondary polyploid nature, particularly since a related species, *L. purpurea,* has a diploid complement of 2n = 6. Unfortunately, an examination of sectioned cells of the same species showed a lower incidence of secondary association and Brown (1950) concluded that the observed secondary association in squashed material was only an artifact of the technique. He did not, however, dismiss all secondary association as an artifact and believed the work of Darlington which described sectioned material, to represent a good example of true secondary pairing.

Jakob (1957) working with *Ricinus communis,* 2n = 20, postulates that the secondary association found in this species is due in part to either centromeric attraction and/or in the exchange of portions of pairing strands without the formation of chiasmata in the regions of interchromosomal contact.

Riley (1960) and Kempanna and Riley (1964) using ditelocentric *Triticum aestivum* looked at the number of

intervening bivalents which occurred between the marked bivalents and in their 1964 paper concluded that, "...secondary pairing genuinely occurs between bivalents with genetically and structurally similar chromosomes, moreover there is no association between genetically unrelated bivalents."

Hu (1962) agreeing with the findings of Kuwada (1910) and Nandi (1936), concluded that the 2n = 24 species of *Oryza* showed a maximum association of two groups of three, 2(3), and three groups of two, 3(2). Comparing the observed number of bivalents in association to that expected from a Poisson distribution, he concluded that the genus *Oryza* is of a secondary polyploid origin and "the doubling of genetic material in remote ancestry might have played an important role in the evolution of the genus" (Hu 1962).

Kempanna and Setty (1967) working with *Eleusine coracana*, 2n = 36, used a Chi^2 test to compare the observed number of bivalent groups in each cell to the expected number as defined by the equal probability of obtaining one group of 18 bivalents, two groups....18 groups of bivalents. They found that the nine group class deviated the most from the expected value and thus proposed a basic number of x = 9 for this species.

Sastrapradja and Rijanti (1972) noted secondary association in *Colorasia gigantea* and *C. esculenta*,

2n = 28, and indicated that the maximum association was 6(1) + 1(2) + 2(3) and that "the bivalents were held together by one or two chiasmata." The authors were hesitant to declare these species to be of a secondary polyploid origin and concluded that the phenomenon needed further substantiation in this genus.

Gupta and Roy (1973) noted secondary association in *Euryale ferox* Salisb. 2n = 58. With the formation of groups of 2, 3 and 4 bivalents they suggested its gametic number to have originated secondarily by means of allopolyploidy.

Mursal (1979) used the evidence of secondary association in haploids and hybrids of *Gossypium* to give additional support to the homoeology between the A and D genomes of cotton. Eighty-three percent of the associates were of the AD type and seventeen percent either of the AA type or DD type. (Here the letters represent chromosomes belonging to either the A or D genome of cotton.)

In yet another study, Rejon and Oliver (1980) combining electrophoretic studies and the presence of secondary association during meiosis point to a remote polyploid origin in the genus *Asphodelus*, 2n = 28 to 2n = 84, and hypothesize a basic number of x = 7 for the genus.

From the evidence available to date, it seems clear that the presence of secondary association can be used to denote homology between the chromosomes despite the lack of agreement on the mechanisms involved. The exact mechanism of secondary association has not yet been clarified.

The purpose of the present study was to investigate the occurrence of secondary association in two species in the genus *Phaseolus* and to statistically evaluate its significance if present. Secondary association in the genus *Phaseolus* L. has never been reported. Two authors Hussein (personal communique) and Machado (1978), working with the closely allied genus *Vigna* Savi., 2n = 22, have noted the occurrence of secondary association. Based on the occurrence of secondary association and multipolar meiosis (grouping of chromosomes of a genome) they proposed a basic number of x = 5 or 6 for the genus.

A diploid number of 2n = 22 has been reported for all species of *Vigna* and *Phaseolus* with occasional reports of 2n = 20-24 (Frahm-Leliveld 1965, Bauchan & Tai, in press). Meiosis in the genus *Phaseolus* L. has been described as highly regular except for an occasional inversion and the occurrence of precocious separation at metaphase I (Honma 1968, Sarbhoy 1977, Machado 1978, Sinha & Roy 1979).

MATERIALS AND METHODS

Seeds of the following accessions were obtained from the W-6 Regional Plant Introduction Station of the U.S. Department of Agriculture, Pullman, WA 99164.

Phaseolus coccineus L.

175855	Collected in a market, Yozgat, Yozgat, Turkey, Sept. 20, 1948 Collector J. R. Harlan
311977	'Ayocote' Mexico, 1965 Collector H. S. Gentry No. 21318
358091[1]	Probistipski Buciste, Yugoslavia, Received Feb. 15, 1970 Collector Lazar Aladzajkov
361520	Sarhan, Gopalpur, India Collector Himachal Pradesh

P. vulgaris L.

150943	Tlalnepantla, Mexico, Received May 1, 1945, Presented by Dario L. Arrieta
226898	'Pan de Libano', Spain, Received July 11, 1955 Presented by O. H. Pearson
281996	'Pajaritos' Chile, Received July 19, 1962 Presented by the Rockefeller Foundation Santiago
311798	'Frijol negro' from market in Jutiapa Guatemala, 1965 H. S. Gentry, No. 21363

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[1]This accession was listed under *P. multiflorus* in the Bean Inventory (*Phaseolus* species) Catalog of Seed Available (Western Regional Plant Introduction Station; 1978; Pullman, Washington) but is now considered a synonym of *P. coccineus* (Bailey and Bailey 1976).

P. vulgaris L. (continued)

314729 From a market in Alma Ata. U.S.S.R., in the vicinity of Samarkand, Uzbeckistan, May 9, 1966. Quentin Jones and Wesley Keller, No. 335.

P. vulgaris var. *aborigineus* (Burk.) Baudet.

266910[2] Collected from the wild, Argentina. Received June 23, 1960 Presented by Instituto de Botanica Darwinion San Isidro.[3]

All plants were grown under greenhouse conditions. Plants were fertilized with "Ra·pid·gro"[4] approximately every two weeks, but no other chemicals were applied. Plants of *P. vulgaris* var. *aborigineus* required a short day length to promote flowering and were grown under 8 hours light and 16 hours of dark. All other taxa were grown under 16 hour days. Buds were collected between the hours of 9 and 10 a.m. and were fixed in six parts ethanol : three parts glacial acetic acid : one part chloroform for 30 hours at which time they were transferred to 70% ethanol and refrigerated until they were used.

Approximately fifty well spread metaphase I pollen mother cells were observed, photographed and scored for

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[2]This accession was listed under *P. aborigineus* in the Bean Inventory but according to a recent revision of the genus is now considered a variety of *P. vulgaris* Marechal 1978).

[3]Collection data was obtained from Plant Inventory, United States Department of Agriculture, Washington D.C.

[4]Ra·pid·gro Corporation, Dansville, N.Y.

the degree of secondary association present for each of the accessions.

Staining of chromosomes in squashed pollen mother cells was found optimum when proprionic carmine was utilized as opposed to acetocarmine which gave unsatisfactory results. All usable slides were made semi-permenant using Dental Sticky wax. In this study, cells in metaphase I were of primary importance, however, other stages were observed for irregularities. All squashed but intact metaphase I cells with 11 countable bivalents were photographed using a Zeiss Photoscope II light microscope with a Planapo 63x N.A. 1.4 objective. The recording medium was Kodak Panatomic X film with 10 minute development in undiluted Kodak Microdol X at 70^0 F.

Drawings were made during observation along with photomicrographs of each cell and each cell was scored for the degree of secondary association present. Two bivalents were only scored as associated if they were in physical contact or close together and sufficiently isolated from all other bivalents as to appear grouped.

For a representative number of slides, stage coordinates were recorded for photographed cells to aid in their relocation after treatment for transmission electron microscope studies described below.

Several slides were then processed using a modification of a technique developed by Ris (1978). The process enables one to transfer cells from glass slides to EM grids. With wax removed, the slides were placed on solid CO_2 and coverslips were removed. The slides were then immediately immersed in distilled water (DH_2O). After removal from the DH_2O the slides were covered with aqueous uranyl acetate for three hours at which time they were rinsed with 50% ethanol and run through a dehydration series: 70%, 80%, 90%, 100%, 100% ethanol. Ten minute intervals were used. Excess ethanol was then blotted from areas of the slide not containing cells and the slides were covered with a 2% parlodion solution using iso-amylacetate as a solvent. The slides were allowed to dry in a horizontal position in a dust free environment over night. The slides could then be returned to the photoscope and cells relocated using the coordinates recorded. Once a cell of interest was located a circle, approximately the size of an EM grid, was drawn around the cell using a diamond marking tool. Next a drop of water was placed over the embedded cell which was carefully lifted off of the slide and floated on the water surface. The cell was then picked up on a 100 mesh 0.5% formvar-coated grid. After blotting off excess water the grid was immersed in iso-amylacetate and left over night to remove the parlodion. The grid was then critical point

dried, carbon-coated and viewed in a Philips 201 TEM at 80 or 100 KEV. Micrographs were made using Kodak Electron Microscope film 4489 and developed in D-19 for four minutes at 70^{o} F.

Pollen stainability was determined for each accession utilizing standard iodine techniques on fresh or fixed material.

RESULTS

Meiosis in PMC's of all species observed was normal with 2n = 22 except for the occurrence of precocious separation, a rare anaphase I bridge (figure 2E) and frequent secondary association. At diakinesis, two pairs of chromosomes were seen attached to the nucleolus and the loose association of bivalents was apparent (figure 2A). At metaphase I, some degree of secondary association was evident in 95.74% of all cells observed (figures 2B & C). Equal separation at anaphase I without cytokinesis was the normal case. Metaphase II again exhibited strong secondary association (figure 2D). Secondary association was also apparent during early telophase II (figure 2F). The frequency of bivalent association for *P. coccineus*, *P. vulgaris* and *P. vulgaris* var. *aborigineus* are recorded in table 1 and the mean number of configurations of $\underline{k}$ bivalents per association, where k = 1, 2-7, for each accession are recorded in table 2. Transmission electron microscopy studies of PMC's showed that a physical connection could exist between bivalents in association (figure 3 & 4). The technique enabled direct correlation of light micrographs with electron micrographs, beneficial for the study of

cytogenetic phenomena. Pollen fertility was high for all accessions (table 3).

Fig. 2. Meiosis in *Phaseolus*

A. Diakinesis showing two bivalents attached to the nucleolus in *Phaseolus vulgaris* var. *aborigeneus* (accession number 266910)

B. Metaphase I showing the maximum secondary association of 4(2) 1(3) in *Phaseolus coccineus* (accession number 358091)

C. Metaphase I showing the maximum association of 4(2) 1(3) in *Phaseolus coccineus* (accession number 361520)

D. Anaphase I with secondary association in *Phaseolus vulgaris* var. *aborigeneus* (accession number 266910)

E. Late anaphase I with chromatin bridge in *Phaseolus coccineus* (accession number 175855)

F. Anaphase II showing secondary association in *Phaseolus vulgaris* (accession number 150943)

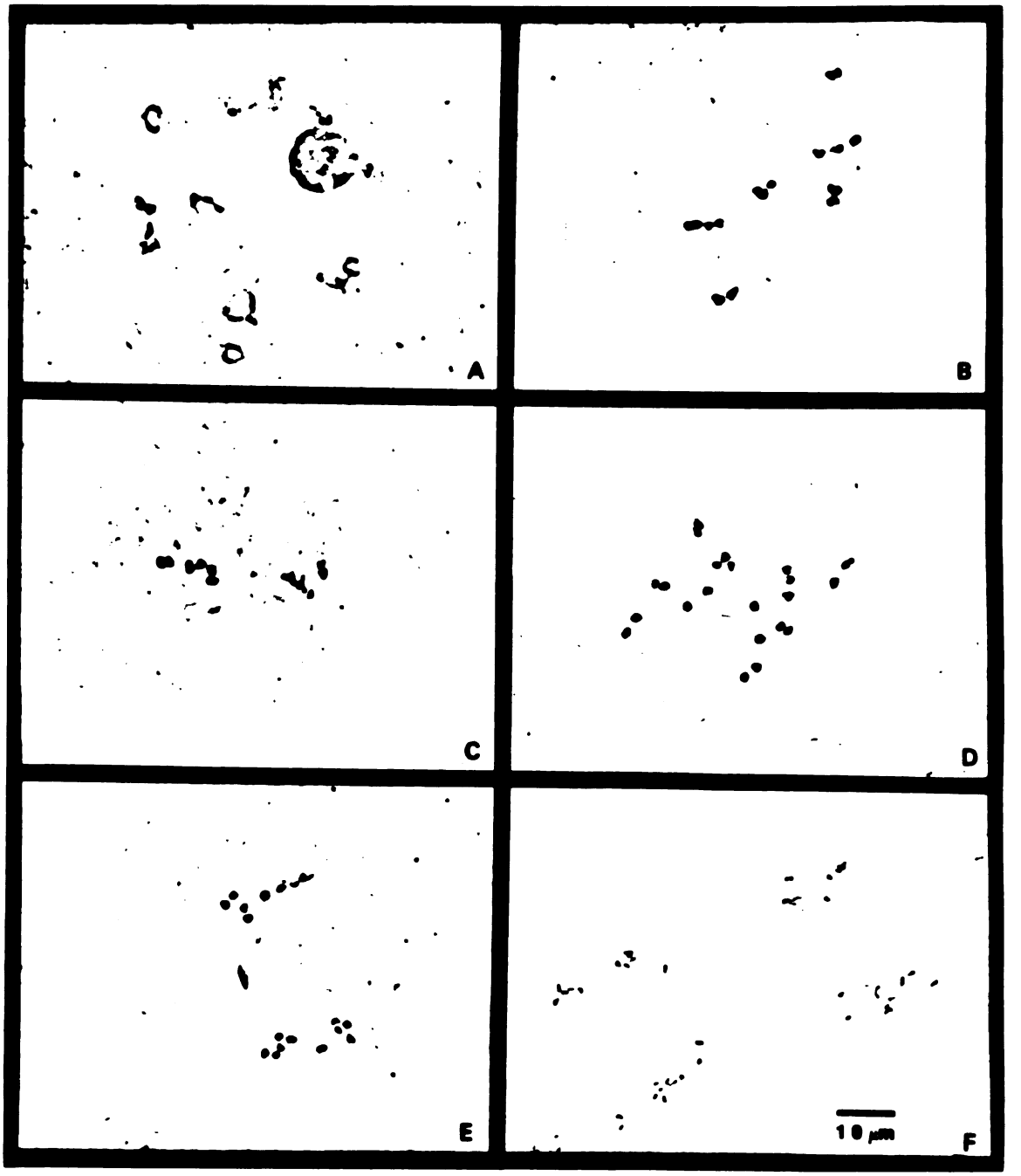


Figure 2

TABLE 1

FREQUENCY OF BIVALENT CONFIGURATIONS

Species	11(1)	1(2)	1(3)	2(2)	1(2) 1(3)	3(2)	2(2) 1(3)	4(2)	3(2) 1(3)	5(2)	4(2) 1(3)	Other	Total
P. coccineus L.													
# of cells	12	22	1	38	29	43	41	23	22	11	11	93	345
Percent	3.5	6.4	0	11.0	8.0	12.5	11.9	6.7	6.3	3.2	3.2	27.0	100
P. vulgaris L.													
# of cells	14	14	6	35	20	33	16	22	13	2	2	67	244
Percent	5.7	5.7	2.5	14.3	8.2	13.5	6.6	9.0	5.3	0.8	0.8	27.5	100
P. vulgaris var. *aborigineus*													
# of cells	4	14	4	19	10	17	9	10	6	3	0	36	132
Percent	3.0	10.6	3.0	14.4	7.6	12.9	6.8	7.6	4.6	2.3	0	27.3	100

TABLE 2

MEAN NUMBER OF ASSOCIATIONS INVOLVING k BIVALENTS

Species & Accession Number		k Number of Bivalents Per Configuration 1	2	3	4	5	6	7	Total Number of PMC Scored
P. coccineus L.									
175855	$\bar{Y}$	4.044	2.382	0.412	0.191	0.0294	0.0147		68
	$s_{\bar{Y}}$	2.555	1.168	0.557	0.435	0.172	0.122		
311977	$\bar{Y}$	5.324	1.980	0.343	0.147	0.0196			102
	$s_{\bar{Y}}$	2.691	1.200	0.560	0.385	0.140			
358091	$\bar{Y}$	4.667	1.807	0.588	0.167	0.0263	0.0263		114
	$s_{\bar{Y}}$	2.537	1.171	0.652	0.400	0.161	0.161		
361520	$\bar{Y}$	2.902	2.262	0.721	0.246	0.0656	0.0164		61
	$s_{\bar{Y}}$	2.113	1.377	0.614	0.475	0.252	0.129		
P. vulgaris L.									
150943	$\bar{Y}$	4.154	1.654	0.539	0.1923	0.1346	0.0577	0.0192	52
	$s_{\bar{Y}}$	2.420	0.956	0.646	0.449	0.348	0.227	0.140	
226898	$\bar{Y}$	3.755	2.302	0.491	0.232	0.0377			53
	$s_{\bar{Y}}$	1.028	1.181	0.581	0.438	0.195			
281996	$\bar{Y}$	5.244	2.267	0.2889	0.0889				45
	$s_{\bar{Y}}$	2.554	1.169	0.555	0.291				
311798	$\bar{Y}$	4.806	1.75	0.528	0.1944	0.0278	0.0278		36
	$s_{\bar{Y}}$	1.055	1.221	0.170	0.407	0.169	0.169		
314729	$\bar{Y}$	6.310	1.672	0.190	0.138	0.0345			58
	$s_{\bar{Y}}$	3.344	1.354	0.399	0.351	0.186			
P. vulgaris var. *aborigineus*									
266910	$\bar{Y}$	5.015	1.841	0.447	0.144	0.0379	0.0152	0.0152	132
	$s_{\bar{Y}}$	2.694	1.258	0.672	0.354	0.193	0.123	0.123	

Fig. 3. Comparison of a light micrograph of a *Phaseolus vulgaris* (accession number 150943) PMC at Diakinesis with electron micrographs of the same cell. Electron micrographs taken at 100KEV

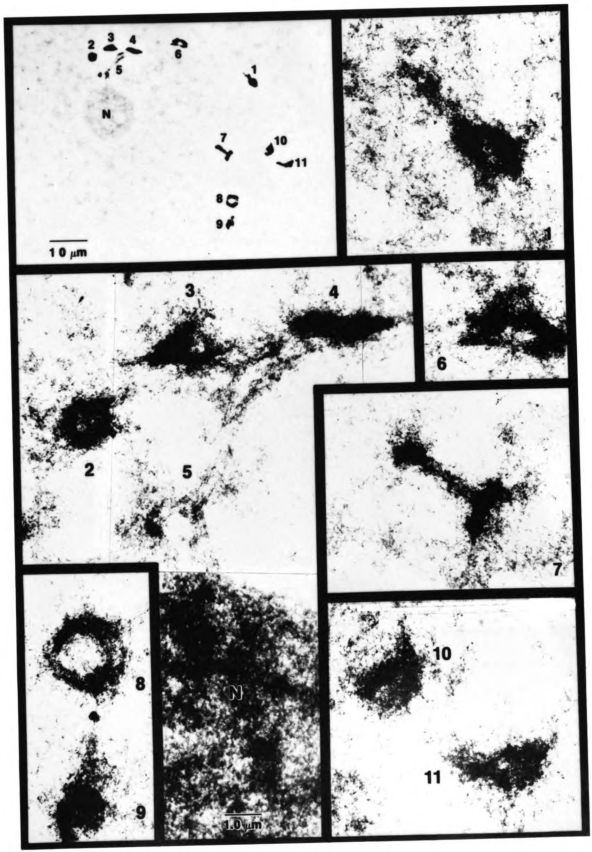


Figure 3

Fig. 4. Electron micrographs of bivalents in association.

A. Secondary association in *Phaseolus vulgaris* var. *aborigeneus* (accession number 266910) 100KEV

B. Secondary association in *P. vulgaris* var. *aborigeneus* (accession number 266910) 100KEV

C. Secondary association in *P. vulgaris* (accession number 150943) 80KEV Taken 30,000X

D. Secondary association in *P. vulgaris* var. *aborigeneus* (accession number 266910) 100KEV

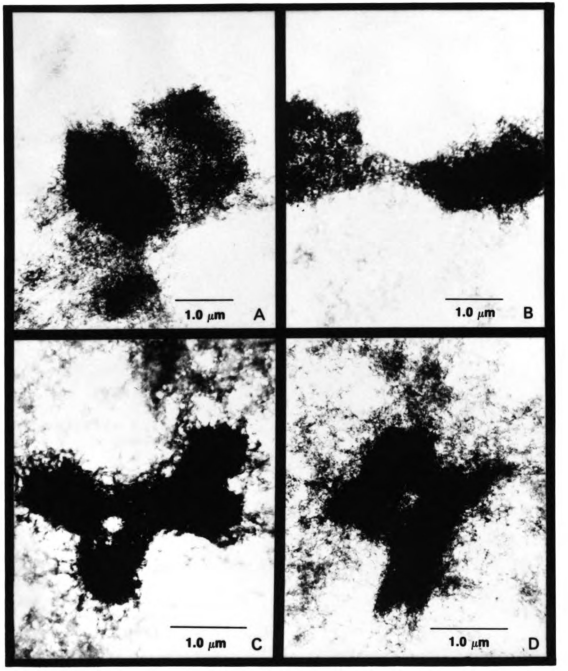


Figure 4

TABLE 3

POLLEN VIABILITY AS DETERMINED BY COUNTING I_2-KI AND UNSTAINED POLLEN GRAINS

Species & Accession Number	% Viable Pollen	Total Number of Pollen Grains Counted
P. coccineus L.		
175855	95	217
311977	99	203
358091	98	297
361520	94	409
P. vulgaris L.		
150943	81	235
226898	93	413
281996	85	799
311798	74	416
314729	84	242
P. vulgaris var. *aborigineus*		
266910	93	395

DISCUSSION

In order to analyze statistically the significance of secondary association a method of estimating the expected number of groups of bivalents given a random distribution of chromosomes was desired. It was not possible to utilize methods developed by other workers studying chromosome arrangement, because of the difficulty of distinguishing one bivalent from another, and the small size and uniform appearance of *Phaseolus* chromosomes (Barton, et al. 1967, Irwin 1967, Riley 1960, Kempanna & Riley 1964, Ferrer and Lacadena 1977, Lacadena & Ferrer 1978). Considering the limitations it was found necessary to look to other disciplines to arrive at a useful solution for purposes of the present study.

Specifically this investigation attempted to find a method of determining the random expectation of obtaining n clumps of k small particles given N points randomly distributed over an area A.

Roach (1968) developed a method of determining the number of clumps of circular laminae with radius r, where the centers of the circles are randomly distributed over a plane surface. In order for a clump to occur the

center points of two laminae had to fall within 2r of each other.

The probability that a circle is isolated is equal to the probability that no laminae center falls within a distance 2r from a point on the plane. Thus

$$P_1 = e^{(-4\pi r^2 N)} \qquad 1$$

and the probability of an isolated clump of $\underline{k}$ circles is

$$P_k = P_1(1 - P_1)^{k-1} \qquad 2$$

The mean number of isolated clumps, C_k, of size $\underline{k}$ in the area $\underline{A}$ is

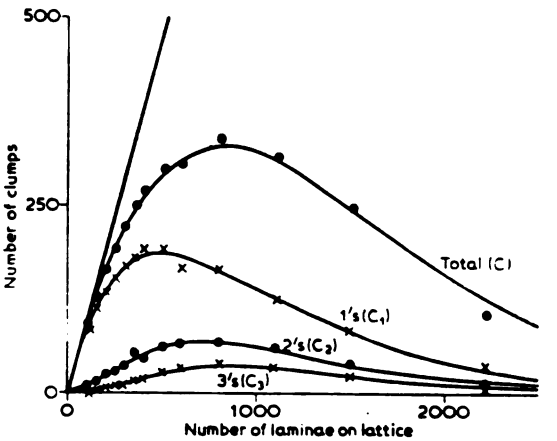
$$C_k = \frac{NP_k}{k} = \frac{NP_1(1 - P_1)^{k-1}}{k} \qquad 3$$

and the mean number of isolated clumps of all sizes, $\underline{C}$, in unit area $\underline{A}$ is

$$C = \frac{NP_1 \ln_e P_1}{P_1 - 1} \qquad 4$$

Roach found that the expected values of the number of clumps in an area of 20 cm^2 for densities of 100-2200 formed by circles of r = .25 were remarkably close to the actual value found by experiment (graph 1). For the purposes of the present study A = 88 cm^2, a conservative estimate of the area of the circle surrounding the space occupied by the chromosomes of a PMC at metaphase I as determined from photographs at 1350X magnification. In a similar manner, the radius of the chromosomes is

set at 0.15 cm and N = 11. The expected values of the mean number of associations of size k are given in table 4.



Graph 1. Comparison of the number of isolated clumps expected and the number found by experiment.[1]

TABLE 4

EXPECTED NUMBER OF CLUMPS OF SIZE k (C_k) AND MEAN NUMBER OF ISOLATED CLUMPS OF ALL SIZES (C) AS CALCULATED USING EQUATIONS 3 AND 4.

K	C_k
1	10.618
2	0.1844
3	0.004268
4	0.000111
5	0.0000031
C	10.807

[1]S. A. Roach, The Theory of Random Clumping. (London: Methuen & Co. Ltd., 1968), p. 30.

Two other authors approached the problem from a slightly different perspective than did Roach. These are presented here for sake of comparison to those values already reported.

Mack (1950, 1948) gives

$$C_k = k^2 \binom{N}{k} (\pi R^2)^{k-1} \qquad 5$$

where $\underline{R}$ must be defined as the radius of the circle enclosing the clumped points divided by $\sqrt{A}$. For our purposes C_k will be calculated for the two extremes for which the radius of the circle enclosing the chromosomes of radius .25 cm can take (table 5). The enclosing circle radius $\underline{R}$ must be between .15 cm, when the chromosomes completely overlap one another and 0.3 cm for the case when the two chromosomes just come into contact with each other.

TABLE 5

RANGE OF THE EXPECTED NUMBER OF CLUMPS OF SIZE k (C_k) AS CALCULATED USING EQUATION 5.

k	
2	$0.176 \leq C_2 \leq 0.707$
3	$0.000958 \leq C_3 \leq 0.0153$
4	$0.0000027 \leq C_4 \leq 0.00175$

The values found in table 4 as calculated using the equation reported in Roach (1968) fall between the extremes reported in table 5.

Mack (1948) also gives an equation for calculating the probability of obtaining $\underline{m}$ simultaneous $\underline{k}$ aggregates

$$P_m(k,N) = \frac{(C_k)^m e^{-C_k}}{m!} \qquad 6$$

which could be used for calculating the probability of any chromosome configuration of $\underline{m}$ equal sized clumps.

A third paper by Berg (1945) gives yet another set of equations for the calculation of C_k and P_m. As with Mack (1948), Berg is concerned with the number of points likely to be contained within a small area $\underline{a}$ of a larger area $\underline{A}$ containing $\underline{N}$ randomly distributed points. Berg presents his argument for square areas only; however, he notes that the result is not dependent on the shape of the area. For calculating C_k he first gives the Poisson distribution which takes a similar form to that presented by Roach with some modifications for dealing with the area encircling the laminae rather than with the area of the laminae themselves. Therefore

$$P_k = \frac{(Na)^k e^{-Na}}{n!} \qquad 7$$

and C_k can be calculated using

$$C_k = P_k \frac{k}{a} \qquad 8$$

where $a = \frac{\pi R^2}{A} (k-1)$.

Using our data where R takes the values discussed in the previous example one gets values of C_k as found in table 6.

TABLE 6

RANGE OF THE EXPECTED NUMBER OF CLUMPS OF SIZE k (C_k) USING EQUATION 8.

k	
2	$0.0963 \leq C_2 < 0.375$
3	$0.00169 < C_3 < 0.0256$
4	$0.0000333 \leq C_4 < 0.00197$

Again the values found in table 4 are within the range of those in table 6. The values of table 5 are comparable to those in table 6, except that the former overestimates C_2 when compared with the latter.

It can be seen that the three papers presented give results comparable to one another even though they approach the problem differently. It is therefore felt that any of the three methods reported could be used to calculate the mean expected number of clumps containing k chromosomes. For convenience, equation 3 which gives single values for C_k will be used to evaluate our data. The mean number of chromosomes X_k involved in an association of k chromosomes per cell can be calculated for both the expected and observed values using

$$X_k = C_k(k) \qquad 9$$

To test the null hypothesis of H_0: chromosomes randomly distributed versus the alternate hypothesis H_1: chromosomes not randomly distributed, the Kolmogorov-Smirnov test for goodness of fit will be utilized to compare X_k observed with X_k expected for each accession (table 7) (Zar 1974). All observed values of X_k for $k \geq 5$ are lumped in the X_5 class.

Considering the results of table 7, it can be seen that secondary association seems to play a definite role in the positioning of chromosomes on the metaphase plate. The maximum association present as determined by observation appears to be 4(2) + 1(3) from which a basic number of X = 5 or 6 might be hypothesized using the argument that secondary association implies homology (Darlington and Moffett 1930, Lawrence 1931, Gustafsson 1934, Riley 1960, Kempanna and Riley 1964). A possible diploid chromosome formula might be:

AAA
BB
CC
DD
EE

My findings support the work of Machado (1968) and Hussein (personal communique) on members of the genus *Vigna* Savi. which is closely aligned to the genus *Phaseolus*.

From the evidence compiled at present, it is impossible to determine the sequence of events which gave

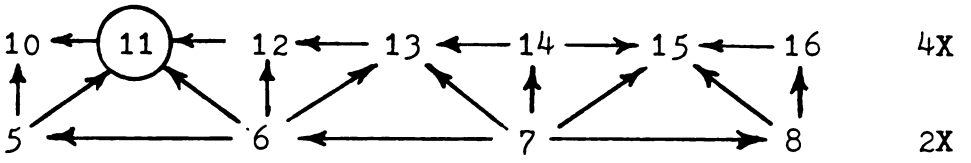
TABLE 7

THE KOLMOGOROV-SMIRNOV VALUES (D) AND THEIR SIGNIFICANCE, CALCULATED USING THE EXPECTED X_k AND OBSERVED X_k FOR EACH ACCESSION.

Species & Accession Number	k					D	α
	1	2	3	4	≥5		
	Observed X_k						
P. coccineus L.							
175855	4.044	4.764	1.236	0.764	0.192	0.598	<.001
311977	5.324	3.96	1.029	0.588	0.189	0.481	<.01
358091	4.667	3.614	1.764	0.668	0.287	0.541	<.002
361520	2.902	4.524	2.163	0.984	0.427	0.701	<.001
P. vulgaris L.							
150943	4.154	3.308	1.617	0.7692	1.152	0.588	<.001
226898	3.755	4.604	1.473	0.928	0.24	0.624	<.001
281996	5.244	4.534	0.867	0.3556	0.00	0.489	<.01
311798	4.806	3.5	1.584	0.778	0.332	0.528	<.005
314729	6.310	3.344	0.57	0.552	0.224	0.392	<.05
P. vulgaris var. *aborigineus*							
266910	5.015	3.682	1.341	0.576	0.386	0.509	<.005
Expected X_k	10.618	0.3688	0.0128	0.000444	1.55×10^{-5}		

rise to the haploid number of this group. The group could represent a complex hybridization of segmental allotetraploids having been derived from the two individuals with different chromosome numbers (Grant 1971) in which case the probable basic numbers are 5 and 6, or from the polyploidization of probable diploid hybrids with a basic number of x = 5, 6 or 7 followed by secondary aneuploidy.

Stebbins (1971) gives the following chart as a probable means of deriving a haploid number of n = 11.



From Stebbins 1971, in part.

Indirect support for the hypothesis of polyploidy in the genus *Phaseolus* comes from the aneuploidy present in some members of the *Phaseolus-Vigna* complex, 2n = 20-24 (Frahm-Leliveld 1965, Bauchan and Tai, in press). A polyploid possessing duplicated genes and chromosomes is more likely to survive and be maintained when one chromosome or even a pair of chromosomes is missing than would a diploid (Elliott 1958).

Additional evidence of polyploidy lies in the proposed basic number for angiosperms in general and for members of the Fabaceae in particular. Several authors report this number to be in the range of x = 6, 7 or 8

(Wanscher 1934, Senn 1938, Stebbins 1950, Atchinson 1951, Turner and Fearing 1959, Bandel 1974, and Sands 1975). Based on his proposed basic number of the Fabaceae, Raven (1975) states that the tribe Phaseoleae Brongn. might have a basic number of x = 7 with n = 11 possibly resulting through aneuploidy from n = 14.

Similar cases of polyploidization might have occurred in two other tribes of the subfamily Faboideae, Hebysareae D.C. (sensu lat.) where genera with a basic number of x = 10 or 11 and others with x = 7, 6 or 5 exist and in the tribe Galegeae B & H (sensu lat.) with genera of both x = 10 or 11 and x = 8, 7 or 6. Turner and Fearing (1959) suggest that it might be possible that the n = 10, 11 complex of the Hedysareae might have arisen from the n = 5, 6 or 7 group.

Considering the absence of n = 5, 6 or 7 members in the Phaseoleae it is necessary to look to other groups for a possible connection to a group with this chromosome number.

The genus *Abrus* Adans., n = 11, is typically placed in the tribe Vicieae Adans. but thought by Senn (1938) more closely aligned to the Phaseoleae. Based on chromosome number and anatomical features, Senn proposed *Abrus* as an intermediate between the Vicieae and Phaseoleae. Hutchinson (1964) recognizes *Abrus* as belonging to its own tribe,Abreae Wight & Arne,intermediate between the

Phaseoleae and Vicieae. Based on this relationship the Phaseoleae might have been derived from the x = 5, 6 or 7 Vicieae or its now extinct ancestor, a hypothesis which might warrant further investigation.

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